European Cell Death Organization (ECDO)

University of Cagliari
Sardinia, Italy

14th ECDO Euroconference on Apoptosis

September 29-October 4, 2006, Chia, Sardinia, Italy

Program and Book of Abstracts

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Local Organisation

Amedeo Columbano
University of Cagliari
Department of Toxicology
09124 Cagliari
Sardinia, Italy
E-mail:columbano@unica.it
+39-070-675 8345

Mauro Piacentini
University of Rome "Tor Vergata"
Department of Biology
00133 Rome
Italy
E-mail:mauro.piacentini@uniroma2.it
+39-0672594234

ECDO Scientific Committee

Wilfried **Bursch**
Institute of Cancer Research, Medizinische Universität, Vienna, Austria
Amedeo **Columbano**
University of Cagliari, Cagliari, Sardinia
Klaus-Michael **Debatin**
University Children's Hospital, Ulm, Germany
Wim **Declercq**
Department for Molecular Biomedical Research, Ghent University, Belgium
László **Fésüs**
University of Debrecen, Debrecen, Hungary
Marie-Lise **Gougeon**
Institut Pasteur, Paris, France
Marja **Jäättelä**
Institute for Cancer Biology, Danish Cancer Society, Copenhagen, Denmark
Mauro **Piacentini** (ECDO President)
University of Rome "Tor Vergata", Rome, Italy
Hans-Uwe **Simon**
University of Bern, Bern, Switzerland
Boris **Turk**
Jozef Stefan Institute, *Ljubljana*, Slovenia
Peter **Vandenabeele**
Department for Molecular Biomedical Research, Ghent University, Belgium
Zsuzsa **Szondy**
University of Debrecen, Debrecen, Hungary
Boris **Zhivotovsky**
Karolinska Institutet, Stockholm, Sweden

ECDO Secretariat

Veronique **Vandevoorde**
Department for Molecular Biomedical Research,
VIB-University of Ghent, Ghent (Zwijnaarde), Belgium
http://www.ecdo.eu

Supported by EC within the framework of the Marie Curie
Conferences and Training Courses

Table of Contents

Welcome

Dear Colleagues,

It is a great pleasure and an honor to welcome all the participants of the 14th Euroconference on Apoptosis in this beautiful location in Sardinia, Chia Laguna.

This is the second time that a Euroconference is organized in Italy, the previous one was organized 10 years ago by Gerry Melino and Mauro Piacentini in Capri. However, we would like to mention that already in 1989 Amedeo and Vanna Columbano organized a cell death meeting in Hotel Capo Boi in Sardinia which was the second meeting ever organized in our field after the Bambury meeting which was held in Cold Spring Harbour in 1988. The meeting in Sardinia was attended by the pioneers of the cell death such as John Kerr, Andrew Wyllie, Richard Lockshin, Zahra Zakeri, David Tomei and many others. The meeting was really the occasion for many of them to meet for the first time and to exchange ideas and information about the molecular mechanisms regulating this complex biochemical event. It is unforgettable the image of these, now very famous and recognized scientists, drawing dying cells on the sand of the white beach next to Capo Boi Hotel. At that time only few genes were known to be modulated during cell death and if we compare with what we know now about the apoptosis pathways and their implications in diseases, it is difficult to think that the meeting took place only 17 years ago.

In these years the Cell Death community has grown up exponentially and the meeting in Chia Laguna is an additional proof of this. In fact, the 14th Euroconference will host about 360 scientists coming from 4 different continents. We are sure that during this conference they will have the possibility of exchanging ideas and to get proficous inspirations from the interactions with colleagues and friends sharing different experiences. As you may notice, we decided to draw the programme by utilizing most of the time for scientific discussing (*cell death*), but leaving some free time in the early afternoon, before the poster sessions, in order to enjoy the beauty of the Sardinia's beaches (*survival*). It would be very nice to see again scientists discussing science on the white sand of Chia Laguna.

The ECDO Scientific Board has attempted to cover most of the hottest topics of cell death giving space to various aspects of cell death and its regulation. We will learn about new cell death pathways as well as the role of the different cell compartments in the regulation of the various cell death types. As always during the Euroconferences many young scientists will have the chance to present their data, as short talks, during the scientific sessions. This is in line with the ECDO spirit aimed to promote this field at all levels by encouraging young scientists to interact with the most experienced ones and to challenge themselves in a stimulating atmosphere.

We would to finish saying that this 14th Euroconference could have never be possible without the advice of the ECDO Scientific Board members, which met in December at this site to draw the scientific programme, and the invaluable work of ECDO Secretary Veronique Vandevoorde. We would also like to express our gratitude to the University of Cagliari for its financial support.

Dear Friends and Colleagues!
I hope you will leave Sardinia with the idea to come back soon for its beauty, but also with the certainty that this trip was very important in development of your work.

September 30, 2006

Amedeo Columbano
Mauro Piacentini

Evaluation form

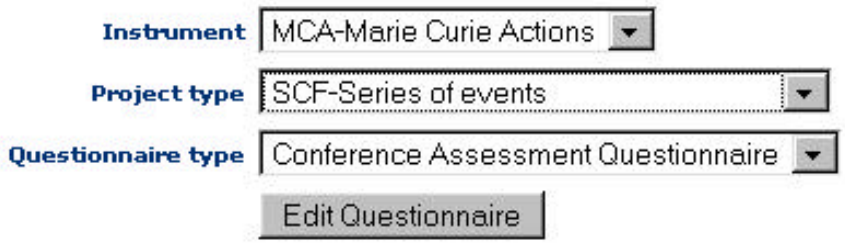
Also this year's Euroconference is organised within the framework of the EC Marie Curie Programme 'Europtosis'. Thanks to the financial support of the EC, a substantial number of participants received a grant to attend this meeting.

In view of this European programme, ECDO would appreciate it very much if you could fill in the **evaluation questionnaire online** upon your return. This will only take about 5 minutes of your time and the total procedure is outlined on the next page.

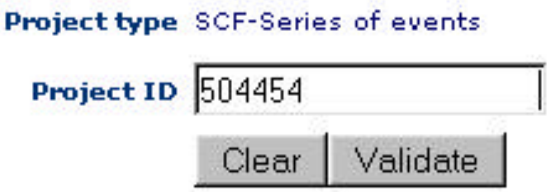
Thank you for your cooperation!

The ECDO board

1 Choose the URL: http://webgate.cec.eu.int/sesam/index.do
2. In the left banner, select "**MCA Questionnaires**"
3. The " **Select questionnaire type**" page is presented below. The "Instrument", "Project type" and "Questionnaire type" options you need to select are filled in on the example



4. "Edit questionnaire"
5. The '**Project identification**" page is presented. The "Project ID' number you have to fill in is **"504454".**



6. After pressing the "**Validate**" button, you will reach the "**MCA Conferences and Training Courses Assessment Questionnaire**".
7. Please read carefully the instructions, complete the questionnaire, and submit your evaluation by pressing the "submit" button.

CONFERENCE INFORMATION

CONFERENCE VENUE:

Le Méridien Chia Laguna
Località Chia · Domus de Maria, Sardinia · Cagliari 09010 · Italy
Phone: (39)(070) 92391
fax: (39)(070) 9230141

SCIENTIFIC CORRESPONDENCE (CHAIRS)

Amedeo Columbano
University of Cagliari
Department of Toxicology
09124 Cagliari
Sardinia, Italy
E-mail:columbano@unica.it
+39-070-675 8345

Mauro Piacentini
University of Rome "Tor Vergata"
Department of Biology
00133 Rome
Italy
E-mail:mauro.piacentini@uniroma2.it
+39-0672594234

ECDO SECRETARIAT

Veronique Vandevoorde
Technologiepark 927
9052 Gent (Zwijnaarde)
Phone: +32-9-3313682
Fax: +32-9-3313511
E-mail: Veronique.Vandevoorde@dmbr.ugent.be
http://www.ecdo.be

REGISTRATION

To collect the conference materials you are kindly requested to register at the Conference Secretariat in Le Méridien Chia Laguna.

Opening hours of the registration desk

September 29 (Friday)	14:00 – 18.00
September 30 (Saturday)	8.00 – 18.00
October 1 (Sunday)	8.30 – 18.00
October 2 (Monday)	8.30 – 18.00
October 3 (Tuesday)	8.30 – 18.00

Participants are kindly requested to wear their name badges during all events of the meeting.

CONFERENCE SECRETARIAT

If you need any help during the conference you can find the local organisers and the ECDO secretary at the registration desk. They will help you in all practical aspects of conference participation.
In case of emergency please call one of these mobile phone numbers:
+39-340 2844299 or **+39-349 5360013**

LECTURE ROOMS

The conference will take place in the **CENTRO CONGRESSI** of the Chia Laguna Hotel.
All lectures will be given in the conference room (**SALA CHIA**).
Poster presentation will take place in the **SALA TORRE**.
Please look at the information boards at the registration desk for further information on the poster sessions.

INTERNET CONNECTION

During the conference, wireless Internet access will be available at the lobby and centro congressi for those participants who have their laptops with them. A card to be bought from the hotel is needed. Additionally, a few PCs will also be provided at the lobby.

LUNCH AND REFRESHMENT

Organised lunches will be served at the beach for all participants staying in the conference hotel. Refreshments are included in the registration fee for all registered participants.

INCOMING MESSAGES AND MESSAGE BOARD

Messages received by the desk will be posted on the message board located at the registration desk. Participants may also use this board to leave messages to other delegates.

INSTRUCTIONS FOR SPEAKERS

We support Powerpoint 2000 or XP. If you want to use your own laptop, or you have a Macintosh, be sure that there is a VGA connector for an external monitor.
We prefer Powerpoint presentations. Your Powerpoint presentation should be on USB stick or CD.
Slide presentations are not supported.
Short orals take 15 minutes (discussion included). Hence, try to prepare a talk of 10-12 min.

You will be able to upload your presentation in the lecture room before your presentation. A technician will be of your assistance for the file upload in the conference hall.

INSTRUCTIONS FOR POSTER PRESENTERS

To allow maximal viewing of the posters, we kindly ask the presenting authors to exhibit their poster **during the entire meeting**.

Poster should be made in portrait and the measurements are **100 cm (height) x 80 cm (width)**.
Posters should be readable by viewers one meter away. The poster should contain the title of the submitted abstract, the author(s)' name(s) and affiliation at the top. The organisers will provide mounting materials to fix posters.

Posters should be mounted at the beginning of the meeting and removed by 19:00 on October 4 (Tuesday). We cannot take responsibility for the posters not removed by evening time on Tuesday October 4.

Three fixed poster sessions are scheduled during the meeting (see final program). Presenting authors are requested to be near their poster during one of the three sessions (division will be announced on site).

This year, **Nature Reviews Molecular Cell Biology** donates a poster prize consisting of a 1-year print and online subscription to the journal.

SOCIAL PROGRAMME

The scientific programme of the conference will be completed with social events, allowing some time for informal discussions for the participants.

Welcome Reception
The Welcome reception will be held at the Chia Laguna hotel at 20:30 on Saturday, September 30.
Social Dinner
The dinner on Monday evening, October 2 will be held on the beach, where a Sardinian group will sing traditional Sardinian folk songs.

SPONSORS

Poster Prize is sponsored by **Nature Reviews Molecular Cell Biology**, and consists of a 1-year print and online subscription to the journal.

ACCOMPANYING PERSONS' PROGRAMMES

Accompanying persons' programmes can be organized with the staff of the Hotel.

SMOKING

Smoking is allowed only at the especially dedicated area of the hotel. Please smoke preferably outside the building.

PRACTICAL INFO

TRANSPORT TO/FROM THE AIRPORT

Taking a taxi

The tariffs of the taxi companies may differ to some extent, but the one way trip from Cagliari-Elmas airport to the conference hotel will cost approximately 85-95 euro.

TELEPHONE SERVICE

Public telephones at the conference site are available at the Centro Congressi. Prepaid phone cards should be purchased before reaching the hotel.

INSURANCE

Participants are strongly advised to make their own insurance arrangements. The organisers cannot accept any liability for personal injuries sustained, or for loss or damage to property belonging to participants and accompanying persons, either during or as a result of the conference.

FOREIGN EXCHANGE AND BANKING FACILITIES

The Italian currency is the Euro. Currency exchange booths are available at the airport terminals.

3rd Training Course on

Concepts and Methods in Programmed Cell Death

"Role of organelles in cell death"

Cagliari, Sardinia, Italy, September 30, 2006

Programme

Chairs: Amedeo Columbano and Zahra Zakeri

9:00-10:00	**Jiri Bartek (Copenhagen, Denmark)** *DNA damage checkpoints and cell death*
10:00-11:00	**Sharad Kumar (Adelaide, Australia)** Caspase biology and function
11:00-11:30	Coffee
11:30-12:30	**Jean-Claude Martinou (Geneve, Switserland)** *The role of mitochondria in cell death*
12:30-13:30	**Randal J. Kaufman (Ann Arbor, USA)** *The role of ER stress and translation in cell death*
13:30-15:00	Lunch
15:00-16:00	**Kris Gevaert (Ghent, Belgium)** *Proteomics of dying cells*
16:00-17:00	**Eric Baehrecke (Maryland, USA)** *Surviving and dying by autophagy*

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Conference Programme

14th EUROCONFERENCE ON APOPTOSIS

September 29- October 4, Cagliari, Sardinia, Italy

"Death or Survival? Fate in Sardinia"

Saturday evening September 30

Chairs: **Amedeo Columbano (Cagliari, Italy) and Mauro Piacentini (Rome, Italy)**

18:30 **Official Opening**

19:00-20:00: **Keynote lecture**

Paolo Comoglio (Turin, Italy)
Invasive Growth: A Genetic Program Linking Cell Migration and Survival.

20:30 **Welcome reception and dinner**

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

October 1: Sunday morning

Session 1: p53 family

Chair: Marie-Lise Gougeon (Paris, France)

9:00-9:30 **Gerry Melino (Leicester, UK)**
Role of p63 in epidermal development

9:30-10:00 **Zhao-Qi Wang (Jena, Germany)**
Role of DNA damage response molecular NBS1 in neuronal disorders

10:00-10:15 **Maria Balakireva (Paris, France)**
Ral promotes apoptosis via the JNK pathway through its effector, the exocyst complex

10:15-10:30 **Carla O'Connor (Dublin, Ireland)**
Physiological concentrations of XIAP do not restrict effector caspase activity but delay effector caspase activation in single living cells

10:30-11:00 Coffee break

Session 2: DNA damage and cancer

Chair: Amedeo Columbano (Cagliari, Italy)

11:00-11:30 **Peter Chumakov (Cleveland, USA)**
Pro- and anti-oxidant functions of the p53 tumor suppressor

11:30-12:00 **Manuela Baccarini (Vienna, Austria)**
Raf-1 and apoptosis: lessons from conditonal knock-outs

12:00-12:15 **Anna Maria Porcelli (Bologna, Italy)**
Mitochondrial DNA mutations in relation to cancer: the model of thyroid oncocytoma

12:15-12:30 **Wim Declercq (Ghent, Belgium)**
Role of caspase-14 in terminal differentiation of keratinocytes

12:30-16:30 Lunch on the beach

October 1: Sunday afternoon

16:30-18:00 Poster session

Session 3: BH3 proteins

Chair: Boris Turk (Ljubljana, Slovenia)

18:00-18:30 **Andreas Strasser (Parkville, Australia)**
BH3-only proteins are essential initiators of programmed cell death and stress-induced apoptosis in normal and cancer cells

18:30-19:00 **Atan Gross (Rehovot, Israel)**
The Role of Mitochondrial Carrier Homolog 2 in tBID-Induced Apoptosis

19:00-19:15 **Lorraine Kerr (Edinburgh, United Kingdom)**
The molecular chaperone nucleophosmin binds to activated Bax.

19:15-19:30 **Patricia Rigou (Paris, France)**
Positive regulation of the Apaf-1 apoptosome by AAP, a novel APAF-1-Interacting protein

20:30 Dinner

October 2: Monday morning

Session 4: ROS and mitochondria

Chair: Peter Vandenabeele (Ghent, Belgium)

9:00-9:30 **Piergiuseppe Pelicci (Milan, Italy)**
p66Shc oxidative stress and longevity

9:30-10:00 **Guido Kroemer (Villejuif, France)**
Organelle crosstalk in the apoptotic response

10:00-10:15 **Tomasz Rudka (Leuven, Belgium)**
Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1 dependent cristae remodeling

10:15-10:30 **Christian Frezza (Padova, Italy)**
OPA1 Controls Apoptotic Cristae Remodelling Independently from Mitochondrial Fusion

10:30-11:00 Coffee break

Session 5: Cell death in neurodegeneration

Chair: László Fésüs (Debrecen, Hungary)

11:00-11:30 **Pierre Vanderhaeghen (Brussels, Belgium)**
Ephrin/Eph signaling during brain development: at the crossroads of neural cell guidance and apoptosis

11:30-12:00 **Ruth Slack (Ottowa, Canada)**
Dissociating the dual roles of AIF in the regulation of apoptosis after acute neuronal injury

12:00-12:15 **Viktor Takács (Budapest, Hungary)**
Detection of autophagy in neurodegeneration mutant and transgenic Drosophila strains

12:15-12:30 **Michael Blank (Tel Aviv, Israel)**
Condensin I recruitment and uneven chromatin condensation precede mitotic cell death in response to DNA damage

12:30-16:30 Lunch on the beach

October 2: Monday afternoon

16:30-18:00 Poster session

Session 6: Survival pathways in cancer

Chair: Peter Krammer (Heidelberg, Germany)

18:00-18:30 **John Blenis (Boston, USA)**
Modulating the battle between survival and pro-death signaling pathways in cancer cells

18:30-19:00 **A. Thomas Look (Boston, USA)**
Upstream and Downstream Modifiers of the BCL-2 Survival Pathway in Cancer

19:00-19:15 **Delphine Mérino (Dijon, France)**
Differential inhibition of TRAIL-mediated DR5-DISC formation by Decoy Receptor 1 and 2

19:15-19:30 **Silvia Cursi (Rome, Italy)**
Src kinase phosphorylates Caspase-8 on Tyr380: a novel mechanism of apoptosis suppression

20:30 Dinner

October 3: Tuesday morning

Session 7: Cell death pathways

Chair: Richard Locksin (New York, USA)

9:00-9:30 **Frank Madeo (Graz, Austria)**
Apoptotic death of ageing yeasts

9:30-10:00 **Guillermo Velasco (Madrid, Spain)**
Cannabinoids induce apoptosis of tumor cells via activation of the endoplasmic reticulum stress pathway

10:00-10:15 **Thomas Simmen (Edmonton, Canada)**
Novel PACS-2 Interactors on the endoplasmic reticulum

10:15-10:30 **Diana Brust (Frankfurt am Main, Germany)**
Involvement of a putative apoptotic machinery in lifespan control of the filamentous ascomycete Podospora anserina

10:30-11:00 Coffee break

Chair: Hans-Uwe Simon (Bern, Switzerland)

11:00-11:30 **Sharon Reef (Rehovot, Israel)**
The road map to programmed cell death: Switching between apoptosis and autophagic cell death.

11:30-12:00 **Mauro Piacentini (Rome, Italy)**
Ambra-1 regulates autophagy during development of the nervous system in mammals

12:00-12:15 **Saska Ivanova (Ljubljana, Slovenia)**
MAGUKs, scaffolding proteins at cell junctions, as substrates for the caspases and cathepsins during apoptosis

12:15-12:30 **Helena Paidassi (Grenoble, France)**
C1q recognizes phosphatidylserine on apoptotic cells and likely acts as an early, multi-ligand bridging molecule in apoptotic cell clearance

12:30-16:30 Lunch on the beach

October 3: Tuesday afternoon

16:30-18:00 Poster session

Session 8: Cell death and inflammation

Chair: Klaus-Michael Debatin (Ulm, Germany)
Boris Zhivotovsky (Stockholm, Sweden)

18:00-18:30 **Naohiro Inohara (Ann Arbor, USA)**
Host defense mechanism mediated by Nod proteins

19:00–20:00 ***ECDO honorary lecture*:**
Jurg Tschopp (Lausanne, Switserland)
Gout-associated uric acid crystals activate the NALP3 inflammasome

20:30 Closing remarks and dinner

Lecture Abstracts:

Invited speakers and short communications

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Invasive Growth: a Genetic Program Linking Cell Migration and Survival

Paolo M. Comoglio

Institute for Cancer Research and Treatment, University of Turin School of Medicine, Candiolo (Turin) – Italy E-mail: paolo.comoglio@ircc.it

Invasive growth is a genetic program in which cell proliferation combines with cell-cell dissociation, migration, and apoptosis protection. It occurs under physiological conditions -during epithelial organ development, angiogenesis, wound healing- and in cancer progression toward malignancy [(1)]. Recent evidence suggests that, in post-natal life, invasive growth is a program for stem and progenitor cells. The program is triggered by MET, a proto-oncogene whose expression is regulated in response to unfavorable microenvironment conditions, such as hypoxia. The encoded tyrosine-kinase receptor, activated by HGF, (a protein closely related to blood coagulation factors) triggers cell motility and displacement toward more favorable tissue environments. MET activates -concomitantly- signal transduction pathways impinging on cell proliferation (MEK-Ras) and motility (PI3K-Rac). Apoptosis protection is mediated by sustained activation of a PI3K-Akt pathway, as well as by recruitment of BAG-1, a co-chaperon of Hsp70, acting as functional partner of BCL2. Microarray analysis showed that 27 genes involved in the control of apoptosis are trascriptionally regulated by MET. Interestingly, MET turns on also hemostasis genes, generating a fibrin path for invasion and a pericellular nest preventing apoptosis caused by loss of adhesion (*Anoikis*). Targeting the Met kinase receptor by recombinant "decoy" proteins, ligand antagonists or antibodies successfully blocks tumor onset and progression toward metastasis, mainly by induction of apoptosis.

(1) Carla Boccaccio and Paolo Comoglio
NATURE REVIEWS | CANCER VOLUME 6 | AUGUST 2006 | 637-645

Role of p63 in epidermal development

Gerry Melino

Medical Research Council, Toxicology Unit, Leicester, UK. University Tor Vergata, Rome, Italy. Email: gm89@le.ac.uk

Here show a major role for p63 in the development of both for cornified (skin) and non cornified (thymus) epithelia formation. Therefore it is also pivotal for normal education of T-cells in the thumus.

Development of the epidermis, the outer layer of the skin formed of keratinocytes, requires the activity of the transcription factor p63, since p63-/- mice are born without epidermis and consequently die at birth. The TP63 gene contains two distinct promoters, which drive the expression of two proteins, one with an amino-terminal trans-activation domain (TAp63) and one without (ΔNp63), although their relative contribution to epidermal formation and homeostasis is not known. To address this issues, we reintroduced TAp63α and/or ΔNp63α into p63-/- mice by in vivo genetic complementation. While extremely rare patches of differentiated keratinocytes were detected in p63-/- and p63-/-; TA mice, there were significant signs of epidermal basal layer formation in p63-/-;ΔN mice. When both TAp63α/ΔNp63α were re-introduced in p63-/- mice, a still greater number of patches of differentiated skin appeared. In addition, microarray analysis indicates that ΔNp63α induces the expression of basal layer markers, while TAp63α drives the expression of upper layer markers. Indeed, ΔNp63α directly transactivates the keratin 14 promoter; we also show that TAp63α action is mediated at the molecular level by direct transactivation of IKKα. The action of p63 is not restricted to the epidermis, as the p63-/- mice have an abnormally small thymus with very low numbers of thymocytes, although the expression of K5/K8 differentiation markers appears normal in thymic epithelial cells. In p63-/-;ΔN mice, and even more in p63-/-;ΔN;TA, but not in p63-/-;TA, the thymus was bigger, with a significantly increased cellularity in comparison to p63-/-. The data presented are consistent with a role for ΔNp63α in controlling the expansion of epithelial cells from progenitor precursors in epidermis and thymus, to allow TAp63α, acting subsequently and synergistically to ΔNp63α, to control epithelial differentiation via IKKα.

p63 regulate apoptosis during DNA damage, thus supporting the p53/p73 pathway. The activity of p63 depends on its protein levels, and evidence suggests that post-transcriptional regulation plays a major role in p63 response. However, the molecular mechanisms underlying the regulation of p63 protein stability remain largely unknown. Here we report that p63 stability is directly regulated by the ubiquitin/proteasome pathway and p63 is degraded through specific mechanisms, different from that of p53. Upon DNA damage, p63 is degraded through a NEDD4-like-mediated mechanism and PML protein modulates p63 half-life by inhibiting its degradation in a nuclear body (NB)-dependent way. As a result, PML significantly increases the ability of p63 to transactivate promoters of the *bax* and *p21* genes and potentiates p63-dependent apoptosis and tumor suppressive activity. In turn, p63 pro-apoptotic function is markedly impaired in *PML*$^{-/-}$cells. Thus, our findings demonstrate that PML plays a crucial role in modulating p63 function. A C-terminal PY motif of p63 binds to a specific E3 ligase of the NEDD4-like family, resulting in ubiquitination and degradation of p63. Consistent with the defect described in KO mice, TAp63/ΔNp63 differently regulate epithelial development; TAp63/ΔNp63 are both crucial also for the development of the thymus.

Role of DNA damage response molecular NBS1 in neuronal disorders

Zhao-Qi Wang

Leibniz Institute for Age Research - Fritz Lipmann Institute (FLI) Jena, Germany

Nijmegen Breakage Syndrome (NBS), belonging to a group of chromosome instability syndromes, exhibits distinct clinical features, including microcephaly, cerebellar degeneration, chromosomal instability, radiosensitivity, immunodeficiency and cancer predisposition. Molecule and genetic studies propose that DNA damage response pathways, including DNA repair, apoptosis and cell cycle control, play an important role in these phenotypes. To study the molecular pathway by which the DNA damage response machinery controls cellular processes and initiates pathogenesis, we have generated "conditional" knockout mice in which the key DNA damage response molecule Nbs1 (mutated in NBS) is deleted using the Cre-*lox*P technique, which allows the introduction of a null mutation of Nbs1 in specific tissues and cell types. When Nbs1 is deleted in neural tissues, these mice show a combination of the neurological anomalies of NBS, including microcephaly, growth retardation, cerebellar defects and ataxia. The cerebellar defects is due to proliferation defects and enhanced apoptosis in *Nbs1*-deficient neurons, which are mostly caused by ATM-mediated p53 overactivation triggered by a high degree of chromosome breaks in *Nbs1*-deficient neuroprogenitors. Importantly, introduction of p53 mutations significantly rescue the neurological defects of *Nbs1* mutant mice. However, these double mutant mice develop brain tumors in later life. We have further characterized the cause of microcephaly of these mice and found a great reduction of cerebral cortex and corpus callosum, reminiscent of human NBS neuronal phenotype. In *Nbs1*-deficient neuronal progenitors, activation of the ATR pathway is severely impaired in response to DNA replication blockage. Finally, inactivation of the *Nbs1* gene in the mouse lens causes progressive cataract due to disruption of lens epithelial cells and incomplete denucleation of fibre cells, revealing a novel function for Nbs1 in cataractogenesis. This study demonstrates that early DNA damage response molecule Nbs1 plays an important role in controlling neurogenesis by regulating activation of its downstream targets including p53.

Ral promotes apoptosis via the JNK pathway through its effector, the exocyst complex

Maria Balakireva[1], Carine Rossé[1], Yu-chen Chien[2], Geneviève Gonzy-Treboul[3], Michel Gho[4], Stéphanie Voegeling-Lemaire[1], Sandra Aresta[5], Jean-Antoine Lepesant[3], Michael White[2] and Jacques Camonis[1*]

[1] Institut Curie, Inserm U528, 26 rue d'Ulm, 75005 Paris, France; [2] Dept. Cell Biology, UTSW, Dallas, USA; [3] Institut Jacques Monod, UMR 7592 CNRS et Universités Paris 6 et Paris 7, Paris, France; [4] UMR7622, Paris, France; 5. Hybrigenics, Paris, France

The Ral pathway is an essential component of physiological Ras signalling as well as Ras-driven oncogenesis. Ral GTPase activity appears a crucial cell-autonomous factor supporting tumour initiation and progression.
We have undertaken a genetic and cell biology approach using *Drosophila melanogaster* as an *in vivo* model. Drosophila has a single *Ral* gene and the Ral-centered protein network appears conserved in human and flies. We have generated null and hypomorphic alleles of the *Ral* locus. *Ral* null animals were unviable, showing that *Ral* is an essential gene. In hypomorph Ral mutants, reduced Ral expression in cells of the sensory organ lineage produced a loss-of-bristle phenotype, which was due to a post-mitotic cell-specific apoptosis during sensory organ development.
We have explored the various pathways that lead to apoptosis in *Drosophila* for their interactions with Ral. Our results showed that the loss-of-bristle phenotype in Ral mutants was caused by an activation of TRAF1–JNK signalling and is independent of caspase-8. Genetic epistasis and immuno-fluorescence imaging revealed that Ral activity suppresses JNK activation and induces activation of the p38 MAP kinase pathway. Genetic analysis showed that the exocyst executes Ral function in apoptosis. HGK (MSN in flies), a MAP4K that activates the JNK pathway, was found binding *in vivo* the exocyst complex, one of Ral effectors. This interaction finds its functional justification in the epistasis between mutants of *Ral* and of *msn*. The Ral-dependent restriction of JNK activation is conserved in mammalian cells, as is the exocyst/HGK relationship. This suggests the Ral/exocyst/JNK regulatory axis may represent a key component of both developmental regulatory programs and oncogenic transformation.
We conclude that Ral counters apoptotic programs to support cell fate determination by acting as a negative regulator of JNK activity and a positive activator of p38. The exocyst complex appears as Ral executioner in the JNK pathway. A pathway from Ral to the exocyst to HGK appears as the molecular basis of Ral action on JNK. Cancer cells have to sustain proliferative signals and to relieve pro-apoptotic signal, and Ral *via* the exocyst complex might be in charge at least of this latter task in oncogenesis.

Physiological Concentrations of XIAP do not restrict Effector Caspase Activity but delay Effector Caspase Activation in Single Living Cells

Carla O'Connor[1], Heinrich Huber[2], Markus Rehm[1], and Jochen HM Prehn[1]

[1] Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, York House, York Street, Dublin 2
[2] Siemens Medical Research, Siemens Ireland, Leeson Close, Dublin 2, Ireland

Apoptosis via the mitochondrial pathway induces Mitochondrial Outer Membrane Permeabilization (MOMP) and the release of cytochrome c (cyt-c) into the cytosol. Cyt-c triggers the sequential activation of initiator caspase-9, and effector caspases-3/-7. Inhibitor of apoptosis proteins (IAPs) inhibit these caspases, with x-linked IAP (XIAP) being the most potent and best characterised representative. A direct association between over-expression of XIAP and tumour malignancy has been reported in several studies. However, at physiological concentrations XIAP's role in the regulation of apoptotic signalling remains controversial as (i) XIAP deficient mice develop normally and (ii) XIAP deficient or parental cells respond very similar to activation of the mitochondrial pathway in classical biochemical analyses.

To analyse the molecular function of XIAP within the complex intracellular signalling system, we designed a computational model of the mitochondrial pathway. The model included concentrations, reaction and inhibitory constants for all key molecules involved and enabled us to follow all proteins and protein complexes over time. The model predicted that physiological XIAP concentrations may delay caspase activation after MOMP but not caspase activity.

To test the hypotheses generated with the computational approach, we examined XIAP deficient and parental colon cancer cells. The influence of XIAP on both effector caspase activation and activity was analysed at physiological conditions within single living cells by the help of a microscopic monitoring system. A FRET substrate, comprised of cyan fluorescent protein (CFP), a linker containing a caspase-3 specific cleavage site (DEVD), and yellow fluorescent protein (Venus) enabled us to monitor the temporal profiles of caspase activation and activity at a time resolution of less than one minute.

Following MOMP, physiological XIAP concentrations were sufficient to delay the onset of effector caspase activation by several minutes. Once activated, however, effector caspases rapidly cleaved the FRET substrate within 10 min, regardless of XIAP being present or not. Thus, as the model predicted, we found that physiological concentrations of XIAP do not restrict effector caspase activity but delay effector caspase activation in single living cells.

This research was supported by a grant from Cancer Research Ireland to JHM Prehn.

Pro- and Anti-oxidant Functions of the p53 Tumor Suppressor

Peter M. Chumakov

Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, USA and Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia.

The p53 tumor suppressor is a major factor that enforces genetic stability of animal cells. It is widely accepted that p53 restricts proliferation of abnormal cells by induction of cell cycle arrest, or by triggering apoptosis. Some of the p53-induced genes are associated with the increase in intracellular levels of reactive oxygen species (ROS), which is associated with pro-apoptotic function of p53. In addition, the excessive release of ROS in response to activation of p53 is implicated in the development of senescent phenotype. However, there are evidences that p53 can enforce genetic stability through alternative mechanisms that are linked to antioxidant defense. Recently we found that the p53-regulated sestrins (SESN1 and SESN2) are important modulators of peroxiredoxins, which participate in decomposition of intracellular H_2O_2. In addition, studies from other laboratories indicate that a function of p53 is required for regulation of mitochondrial respiration, which may result in decreased production of intracellular ROS. We found that physiological levels of p53 are sufficient for maintaining relatively low intracellular levels of ROS in unstressed or mildly stressed cells. Inhibition of p53 function by genomic knockout, RNAi, expression of dominant-negative p53 mutants, or by overexpression of MDM2 is associated with significantly increased intracellular ROS levels, excessive oxidation of DNA, increased mutation rate, and karyotype instability, which are prevented by incubation with antioxidant N-acetylcysteine (NAC). Dietary supplementation with NAC improves substantially karyotype stability in p53 knockout mice and prevents the development of characteristic lymphomas. In a xenograft model NAC supplementation reduced growth rate of xenografts derived from p53-negative cells. Our results suggest that the antioxidant function of p53 plays substantial role in its tumor suppressor function.

Raf-1 and apoptosis: lessons from conditonal knock-outs

Manuela Baccarini

Max F. Perutz Laboratories, University of Vienna, Doktor-Bohr-Gasse 9, 1030 Vienna (Austria)

The serine/threonine kinase Raf-1 has been mainly studied as the link between the small GTPase Ras and the MEK/ERK kinase module. ERK activation, in turn, has been implicated in a variety of biological processes, including proliferation, survival, and differentiation. We are using conditional ablation to determine the essential function(s) of Raf-1 in vivo. During the past two years, tissue-restricted ablation has allowed us to define kinase-independent functions of Raf-1 in migration and apoptosis, in particular Fas-induced apoptosis. These novel functions of Raf-1 are based on its ability to physically interact with, and inhibit, other serine-threonine kinase. Raf-1 can repress Fas signaling by counteracting the activation of the proapoptotic kinase MST2 or restrict the surface expression of Fas by modulating Rok-a, which regulates Fas internalization. In contrast, in erythroblasts, Raf-1 regulates terminal differentiation by inhibiting the premature expression of Fas at the pretranslational level. To date, the control of Fas expression in erythroblasts is the only essential function of Raf-1 that dependes on its ability to activate the ERK pathway. Fas, once expressed, antagonizes erythroblast proliferation by exerting a negative feedback on Raf-1 expression and ERK activation, and simultaneously induces caspase activation, thereby precipitating differentiation. These results identify Raf-1 and Fas as key molecules whose expression finely tunes both apoptosis and erythropoiesis.

Mitochondrial DNA mutations in relation to cancer: the model of thyroid oncocytoma

Anna Maria Porcelli, Anna Ghelli, Martha Hoque, Luisa Iommarini, Claudia Zanna, Michela Rugolo

Dip. di Biologia, Via Irnerio 42, Università degli Studi, Bologna, Italy

A variety of mtDNA mutations has been associated with various types of cancer: renal adenocarcinoma, colon, breast and prostate cancer, and thyroid tumors. However, in most cases the pathophysiological significance of these mutations still remains unclear. Our attention has recently been focused on thyroid oncocytomas, which are tumors characterized by cells with an abnormal abundance of mitochondria. Previous studies suggest that a mitochondrial dysfunction might underlie thyroid oncocytoma and a high frequency of mtDNA variants in complex I and IV genes has been described. However, no evidence was available on the relevance of these mtDNA mutations with regards to the biochemical phenotype. To investigate the energetic efficiency of the XTC.UC1 cells derived from a thyroid oncocytoma, we used a growth medium where glucose was substituted by galactose, i.e. a condition which forces the cells to rely solely on mitochondria for ATP production. In contrast to other non oncocytic cell lines, XTC.UC1 cells exhibited a significant decrease in both cell viability and ATP levels when incubated in galactose medium. Furthermore, mitochondrial ATP synthesis driven by complex I was also significantly reduced, thus confirming the occurrence of a severe coupling defect in oxidative phosphorylation. Sequencing of XTC.UC1 mtDNA indeed identified a heteroplasmic, non-conservative substitution in cytochrome b and a frameshift mutation in ND1, leading to production of a truncated protein undergoing degradation (Bonora et al., Cancer Res. in press). In the present study, we have investigated the mechanism of XTC.UC1 cell death in galactose medium. Although the nuclei exhibited a remarkable chromatin alteration, we were unable to detect any DNA fragmentation and protection by pan-caspase inhibitors, suggesting that a caspase-independent pathway was involved. Furthermore, western blot analysis revealed that several cellular proteins underwent a time-dependent proteolytic cleavage during incubation in galactose medium. This protein degradation pathway was not affected by incubation with inhibitors of different proteases, but was abolished by Bcl-2 overexpression. The mechanism of cell death and its relevance in tumor progression will be discussed.

This work was supported by an AIRC grant and by "L'Oreal Italia Per la Donne e la Scienza" fellowship.

Role of caspase-14 in terminal differentiation of keratinocytes

Denecker, Geertrui[1]; Hoste, Esther[1]; Gilbert, Barbara[1]; Hochpied, Tino[1]; Lippens, Saskia[1], Ovaere, Petra[1]; Hachem, Jean-Pierre[2], VandenBroucke, Caroline[3]; Vandamme, Petra[4]; D'Herde, Katharina[5]; Gevaert, Kris[4]; Vandekerckhove, Joël[5]; Schoonjans, Luc[6]; Libert, Claude[1]; Vandenabeele, Peter[1]; Declercq, Wim[1]

[1]Department of Molecular Biomedical Research, University of Ghent - VIB, Ghent, Belgium.
[2]Department of Dermatology, Free University of Brussels, Belgium.
[3]Department of Pathology, University of Ghent, Ghent, Belgium.
[4]Department of Biochemistry, University of Ghent – VIB, Ghent, Belgium.
[5]Department of Anatomy, Embryology, Histology, Medical Physics, University of Ghent, Ghent, Belgium.
[6]Department of Transgene Technology, University of Leuven, Leuven, Belgium.

Terminal differentiation of keratinocytes can be considered as a special form of cell death during which the dead corpses persist. In the skin caspase-14 activation occurs in the uppermost layers of the epidermis and correlates with epidermal cornification. During terminal keratinocyte differentiation the classical apoptotic caspases are not activated. So this skin specific expression and processing of caspase-14 is intriguing. To unravel the possible role of caspase-14 in skin differentiation, we have generated caspase-14-deficient mice. Histological sections of the back skin and thymus of newborn wild-type and caspase-14-deficient mice revealed no macroscopic differences. Immunohistochemical analysis of other early and late differentiation markers demonstrated a normal expression pattern of K1, K10, K14, loricrin, involucrin and filaggrin. However, the skin of caspase-14-deficient mice had a more shiny appearance and showed a licheniform phenotype. Electron microscopic analysis indicated the presence of high numbers of composite keratohyalin granules in the caspase-14-deficient epidermis. These granules are normally used as profilaggrin stores. In accordance, caspase-14 mice show an altered profilaggrin-processing pattern when analyzed by western blotting. These data argue for a role of caspase-14 in the terminal differentiation of keratinocytes. We will report on phenotypes, which are related with the improper functioning of the stratum corneum as barrier in caspase-14 deficient mice.

BH3-only proteins are essential initiators of programmed cell death and stress-induced apoptosis in normal and cancer cells

A Strasser[1]; A Villunger[1,2]; P Bouillet; EM Michalak[1]; LA O'Reilly[1]; DCS Huang[1]; PN Kelly; L Coultas[1]; E Naik[1], M Erlacher[2]; J Kuroda; T Kaufmann[1]; P Hughes[1]; H Puthalakath[1], JM Adams[1]

[1]The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia and [2]Institute for Pathophysiology, University of Innsbruck, Austria

Genetic and biochemical experiments have demonstrated that BH3-only proteins are essential for initiation of programmed cell death and stress-induced apoptosis and that Bax/Bak-like proteins are required for this process, probably functioning downstream. Different BH3-only proteins are required for cell death induced by different stimuli and they can also function in a cell type-specific manner. Bim is required for the death of many cell types triggered by growth factor withdrawal, for deletion of autoreactive lymphocytes and for termination of cytotoxic T cell (CTL) immune responses. The apoptosis provoked by DNA damage requires the p53 tumor suppressor and this death is dependent on the BH3-only protein Puma and to a lesser extent also Noxa. Surprisingly, Puma was found to also be essential for apoptosis induced by several p53-independent stimuli, including cytokine withdrawal or treatment with glucocorticoids or phorbol ester. Experiments with non-transformed cells and tumour cells have demonstrated that BH3-only proteins are essential for anti-cancer therapy-induced cell killing. Puma is required for apoptosis induced by γ-radiation or several widely used chemotherapeutic drugs, including etoposide or dexamethasone. Bim, on the other hand is needed for the death of chronic myelogenous leukemia (CML) cells triggered by the BCR-ABL kinase inhibitor Gleevec.

The Role of Mitochondrial Carrier Homolog 2 in tBID-Induced Apoptosis

Yehudit Zaltsman, Michal Schwartz, Liat Shachnai and Atan Gross

Department of Biological Regulation, The Weizmann Institute of Science, Rehovot 76100, Israel, E-mail: atan.gross@weizmann.ac.il

BCL-2 family members are pivotal regulators of apoptosis, and mitochondria are a major site-of-action for these proteins. BID, a BH3-only pro-apoptotic BCL-2 family member plays an essential role in the TNFα/Fas death-receptor pathway *in vivo* by triggering a mitochondrial apoptotic program. Activated BID, tBID, triggers/regulates this program by inducing the activation of BAX and BAK, however the exact mechanism(s) by which tBID triggers the activation and regulates the activity of both proteins is poorly understood. We recently revealed that in cells signaled to die by TNFα, tBID interacts with mitochondrial carrier homolog 2 (Mtch2). Mtch2 is a novel and previously uncharacterized protein, which is related to a family of mitochondrial carriers that catalyze the transport of metabolites across the inner membrane. Mtch2 is an integral membrane protein exposed on the surface of mitochondria, and tBID and BAX form a complex with Mtch2 in cells signaled to die by TNFα. Thus, Mtch2 is an excellent candidate for the regulation of apoptosis that is triggered by tBID at the mitochondria.

The molecular chaperone nucleophosmin binds to activated Bax.

David C. MacDonald, Jane Thompson, John Sharkey & Lorraine E. Kerr.

Astellas CNS Research, The University of Edinburgh, The Chancellor's Building, 49 Little France Crescent, Edinburgh EH16 4SB, UK, email: Lorraine.Kerr@ed.ac.uk

Bax-mediated apoptosis has been implicated in the pathophysiology of various neurological disorders such as stroke and Alzheimer's disease. However, the underlying mechanisms and protein co-factors involved in the activation and translocation of this apoptogenic member of the Bcl-2 family to the mitochondrial surface have yet to be fully identified and characterised. We have identified the molecular chaperone, nucleophosmin (B23, numatrin), as a novel Bax-binding molecule. The interaction between Bax and nucleophosmin was confirmed by reciprocal co-immunoprecipitation studies in whole cell lysates prepared from SH-SY5Y human neuroblastoma cells and using mixtures of recombinant proteins. In order to characterise the interaction further, an amplified luminescent proximity homogeneous assay was developed. Bax and nucleophosmin were separately conjugated to donor and acceptor beads. When excited by laser (680nm), the donor beads release singlet state oxygen molecules which subsequently leads to acceptor bead excitation and emission only when the beads are brought into close proximity (ie by binding of the two proteins of interest). Full length recombinant Bax and nucleophosmin failed to interact in 150mM NaCl, 10mM HEPES-KOH pH7.4 buffer containing 0.01% BSA. However a strong proximity assay signal was evident upon addition of 0.1% NP-40 or 0.1% Tween-20. This corresponded to the detergent induced conformational change in Bax as demonstrated by immunoprecipitation with the conformationally-sensitive Bax antibody 6A7. Bax-nucleophosmin binding could be inhibited by an antibody directed against the C-terminus of Bax but not one raised against the N-terminus. Furthermore binding was observed in the proximity assay using a peptide corresponding to the last 21 amino acids at the Bax C-terminus in conjunction with nucleophosmin. Taken together these data demonstrate that nucleophosmin binds to activated Bax specifically to the C-terminus of Bax which is normally sequestered within the inactive molecule.

Positive regulation of the Apaf-1 apoptosome by AAP, a novel APAF-1-Interacting protein

Valeria Piddubnyak[1], Patricia Rigou[1], J. Hickman[2], O. Genest[2], Jean-Christophe Rain[3], Laurence Michel[4], Alain Mauviel[4] and Jean-Luc Poyet[1]

[1]INSERM U716, Laboratoire de Pharmacologie, Institut de Génétique Moléculaire, 27 rue Juliette Dodu, Paris 75010, France ; [2]Institut de Recherche Servier, Croissy, France ; [3]Hybrigenics SA, Paris, France ; [4]INSERM U697, Pavillon Bazin, Hôpital Saint-Louis, 1 Avenue Claude Vellefaux, Paris 75010, France ;

Apoptosis, a major form of cell death, depends on the activation of caspases, a group of cysteine proteases. Two main activation pathways (intrinsic and extrinsic) of the initiator procaspases have been identified. The intrinsic pathway, which recognizes developmental cues, genomic stress or cytotoxic damage, involves the formation of a large caspase-processing complex termed the apoptosome. While it is clear that the core of the apoptosome complexes comprises Apaf-1 and caspase-9, other cellular proteins may modulate the formation or the activity of the apoptosome. In fact, in the view of several observations, it has long been suspected that Apaf-1-mediated activation of caspase-9 has additional regulatory mechanisms. To further investigate the regulation of the apoptosome at the level of Apaf-1, we developed a yeast two-hybrid strategy using the WD40-deleted Apaf-1 (residues 1-591) as bait to screen a placenta cDNA library. 34 interacting cDNA clones were identified from 60 million yeast transformants. Among them, we studied a new Apaf-1-interacting protein that we designed AAP (Apaf-1-Associating Protein). AAP binds specifically to the CED4 domain of Apaf-1 and was able to drastically potentiate Apaf-1-dependend, but not Apaf-1-independent apoptosis. An isoform of AAP representing the binding domain of AAP to Apaf-1 acted as a dominant negative of the full length protein, inhibiting Apaf-1-mediated cell death. siRNA directed against AAP significantly prevented etoposide-, as well as cisplatin-induced cell death, but had no effect on Fas-induced cell death. However, silencing of the *AAP* gene blocked neither cytochrome *c* release from mitochondria into cytosol nor the reduction of the mitochondrial membrane potential following etoposide or cisplatin treatment. Thus, our findings strongly suggest a role for AAP in the apoptotic process and that AAP specifically modulates the Apaf-1-mediated pathway at the level of, but not above, Apaf-1.

p66Shc oxidative stress and longevity

Piergiuseppe Pelicci

Organelle crosstalk in the apoptotic response

Guido Kroemer

CNRS-FRE2939, Institut Gustave Roussy, Villejuif, France

Apoptosis can be induced by different types of stress affecting distinct organelles including the nucleus, the endoplasmic reticulum, lysosomes, the autophagic machinery and mitochondria. It is an ongoing conundrum which particular sensors are activated in response to which kind of stress and how organellar stress is streamlined to cause an apoptotic (rather than an adapative) response. It is also an ongoing conundrum how different proteins that have been involved in the DNA damage response, in the autophagic regulation or in apoptosis execution act in a strictly compartmentalized and functionally hierarchized manner. Our present work is designed to break this dogma, and I will present evidence suggesting that components of the DNA damage response, the telomerase and the apoptosome may have unexpected functions. As an example, it appears that the telomerase protein subunit hTERT regulates the mitochondrial (intrinsic) apoptotic pathway. Recently, we have also found that the apoptosome component Apaf-1 participates in the DNA damge response. These examples reveal a new type of interorganellar crosstalk in the response to cellular stress.

Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling

Tomasz Rudka[1§], Sara Cipolat[2§], Dieter Hartmann[1§], Veronica Costa[2], Lutgarde Serneels[1], Katleen Craessaerts[1], Christian Frezza[2], Wim Annaert[3], Luca Scorrano[2*] and Bart De Strooper[1*]

[§] These authors contribute equally to this study
[1]Neuronal Cell Biology and Gene Transfer, [3]Membrane Trafficking Laboratories, Center for Human Genetics, Flanders Interuniversity Institute for Biotechnology (VIB4) and K.U.Leuven, Leuven, Belgium; [2]Dulbecco-Telethon Institute, Venetian Institute of Molecular Medicine, Padova, Italy.

Rhomboids, evolutionarily conserved integral membrane proteases, participate in crucial signaling pathways. Presenilin associated rhomboid like (PARL) is an inner mitochondrial membrane rhomboid of unknown function, whose yeast orthologue is involved in mitochondrial fusion. We have generated $Parl^{-/-}$ mice and found that they display normal intrauterine development, but from the 4th postnatal week undergo progressive multisystemic atrophy leading to cachectic death. Atrophy is sustained by increased apoptosis, both in and ex vivo. $Parl^{-/-}$ cells display normal mitochondrial morphology and function, but are no longer protected against intrinsic apoptotic death stimuli by the dynamin-related mitochondrial protein OPA1. $Parl^{-/-}$ mitochondria display reduced levels of a soluble, intermembrane space (IMS) form of OPA1 and OPA1 specifically targeted to IMS complements $Parl^{-/-}$ cells, substantiating the importance of PARL in OPA1 processing. $Parl^{-/-}$ mitochondria undergo faster apoptotic cristae remodeling and cytochrome c release. These findings implicate regulated intramembrane proteolysis in controlling apoptosis.

OPA1 Controls Apoptotic Cristae Remodelling Independently from Mitochondrial Fusion

Christian Frezza[1], Sara Cipolat[1], Olga Martins de Brito[1], Massimo Micaroni[2], Galina V. Beznoussenko[2], Tomasz Rudka[3], Davide Bartoli[1], Roman S. Polishuck[2], Nika N. Danial[4], Bart De Strooper[3] and Luca Scorrano[1]

[1]Dulbecco-Telethon Institute, Venetian Institute of Molecular Medicine, Padova, Italy; [2]Department of Cell Biology and Oncology, Consorzio "Mario Negri Sud", Santa Maria Imbaro, Italy; [3]Neuronal Cell Biology and Gene Transfer Laboratory, Center for Human Genetics, Flanders Interuniversity Institute for Biotechnology (VIB4) and K.U. Leuven, Belgium; [4]Department of Cancer Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA.

Mitochondria amplify activation of caspases during apoptosis by releasing cytochrome c and other cofactors. This is accompanied by fragmentation of the organelle and remodeling of the cristae. Molecular mechanisms governing the latter remain unclear. Optic Atrophy 1 (OPA1), a pro-fusion dynamin-related protein of the inner mitochondrial membrane mutated in dominant optic atrophy protects from apoptosis by preventing cytochrome c release. This is independent from mitochondrial fusion but depends on the oligomerization of two forms of OPA1, the soluble, intermembrane space and the inner membrane integral one. The pro-apoptotic BCL-2 family member BID disrupts OPA1 oligomers, while high levels of OPA1 stabilize them and prevent mobilization of cytochrome c. OPA1 does not interfere with activation of the mitochondrial “gatekeepers” BAX and BAK, but controls shape of mitochondrial cristae during apoptosis. Thus, OPA1 has genetically and molecularly distinct functions in mitochondrial fusion and in cristae remodelling during apoptosis.

Ephrin/Eph signaling during brain development: at the crossroads of neural cell guidance and apoptosis

Vanessa Depaepe, Nathalie Suarez, Lara Passante, Audrey Dufour, and Pierre Vanderhaeghen

University of Brussels (ULB)- IRIBHM -ULB Campus Erasme B-1070 Brussels-BELGIUM

The ephrin/Eph gene family has been implicated in an array of developmental processes, from gastrulation to axon guidance, from angiogenesis to synaptic plasticity. In most systems studied so far, ephrins mainly act as potent guidance factors, enabling selective cell repulsion or adhesion through modulation of cytoskeletal dynamics. Recently we uncovered a role for ephrins in the control of the size and shape of the brain, through the unexpected regulation of apoptosis of neural progenitors, both in vitro and in vivo. Together with recent work from other groups that have shown unexpected effects of ephrins on cell survival and proliferation, these findings suggest that ephrins, like neurotrophins, have evolved as pleiotropic factors that can control very different functions depending on the cellular context. While the mechanisms allowing ephrin/Eph signaling to control guidance, apoptosis, or proliferation in a cell-specific manner remain unknown, the identification of ephrin/Eph genes as positive regulators of apoptosis during forebrain neurogenesis uncovers a novel signaling pathway potentially involved in other aspects of developmental and stem cell biology, and in the course of oncogenesis.

Dissociating the dual roles of AIF in the regulation of apoptosis after acute neuronal injury

RS Slack[1], ECC Cheung[1], N Joza[3], NAE Steenaart[5], KA McClellan[1], M Neuspiel[2], JG MacLaurin[1], P Rippstein[2], DS Park[1], GC Shore[4], HM McBride[2], JM Penninger[3]

[1]Ottawa Health Research Institute-Department of Cellular and Molecular Medicine, [2]Ottawa Heart Institute, University of Ottawa, Ottawa, Ontario, Canada; [3]IMBA, Institute of Molecular Biotechnology of the Austria Academy of Sciences, Vienna, Austria, [4]Department of Biochemistry, McGill University, Montreal, Quebec, Canada [5]Gemin X Biotechnologies Inc., Montreal, Quebec, Canada

The mitochondria release multiple proteins that propagate both caspase dependent and caspase independent apoptotic cascades. Apoptosis Inducing Factor (AIF) is an important regulator involved in multiple neuronal injury pathways. Neurons with reduced levels of AIF exhibit enhanced protection against caspase dependent and caspase independent cell death. AIF is involved in DNA-damage mediated apoptosis as well as excitoxicity induced by activation of glutamate receptors. The role of AIF in apoptosis is obscured however, since AIF plays an essential role in maintaining cell survival. To dissociate the dual roles of AIF we used a mitochondrially anchored AIF that cannot be released during apoptosis. Forebrain-specific AIF null mutation (tel. *Aif*$^{\Delta}$) mice have defective cortical development and reduced neuronal survival due to defects in mitochondrial respiration. Mitochondria in AIF deficient neurons are fragmented with aberrant cristae, indicating a novel role of AIF in controlling mitochondrial structure. Expression of mitochondrially-anchored AIF in tel. *Aif* $^{\Delta}$ *Apaf1*$^{-/-}$ neurons significantly enhanced survival after injury. AIF mutants that cannot translocate into the nucleus failed to induce cell death. Despite the presence of mitochondrially anchored AIF to maintain organelle integrity, we show that the presence of endogenous AIF is a critical determinant in the induction of apoptosis. These results indicate that the pro-apoptotic role of AIF can be dissociated from its essential physiological function. Cell death induced by AIF is through its pro-apoptotic activity once it is translocated to the nucleus, not due to the loss of AIF from the mitochondria.

Supported by CIHR to RSS, and a Marie Curie Excellence Grant and the Austrian National Bank to J.M.P.

Detection of autophagy in neurodegeneration mutant and transgenic Drosophila strains

Viktor. Takács and Miklós Sass

Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest, Hungary, e-mail: takacsvik@gmail.com

Autophagy plays an essential role in many biological events such as tissue remodeling, differentiation, adaptation to changing environmental conditions or cellular death at certain physiological and pathological situations. This process is highly conserved in eukaryotes. Our studies focus on the functions of hormonal and genetic regulation of autophagy during the postembryonic development of *Drosophila melanogaster*. We also want to use this model organism to examine neuronal degeneration. There are known mutant and transgenic flies with characteristic neurondegeneration. The common features of these models are pronounced cell death in CNS, accumulation of intracellular aggregates and shortened life span. These phenotypes resemble many human neurodegenerative disorders, like Alzheimer's, Huntington's and Parkinson's diseases. Since the effects of the mutations and the expressed transgenic proteins has been investigated mostly at light microscopic level the cellular mechanisms of the neuronal death remained unclear.

Therefore, a detailed electron microscopic survey was performed on the available mutant strains to disclose the possible participation of autophagy during the neuronal loss.

The ultrastructure of neurodegenerative strains including wild type and mutant alfa-synuclein, APP and different polyglutamine transgenes, loechrig, blue cheese, bubblegum and SwissCheese mutants were investigated in various age and the characteristic electron microscopic features of neuronal and glial cells has been determined. We show autophagic vacuolization in loechrig and blue cheese mutants and Spinocerebellar Ataxia 3 protein transgenic. In the case of the other mutants severe mitochondrial disintegration, formation of membrane-whirles and accumulation of intracellular aggregates were observed. We demonstrate that these features in the adult brain ganglia are age-dependent, and become more sever during aging.

It isn't clear yet, that upregulated autophagy is the reason of cellular death, or quite the contrary the failure of this process hinder the cell to effectively protect itself against toxic effects. We identified some loss-of-function P-element induced mutants with inhibited autophagic activity, which could be used to modulate the neurodegenerative phenotype of flies mentioned above. These experiments help to use *Drosophila* as a model organism to understand the basic molecular mechanisms of brain related diseases and get closer to disclose the role of autophagy in the CNS under different pathological conditions.

Condensin I recruitment and uneven chromatin condensation precede mitotic cell death in response to DNA damage

Michael Blank[1], Yaniv Lerenthal[1], Leonid Mittelman[2] and Yosef Shiloh[1]

[1]The David and Inez Myers Laboratory for Genetic Research, Department of Molecular Genetics and Biochemistry, and [2]Interdepartmental Core Facility, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel, e-mail: blankmic@post.tau.ac.il

Cell cycle progression following DNA damage is rapidly halted by checkpoint controls, which are relaxed after the damage has been assessed and processed. Cells with misrepaired or unrepaired DNA lesions are eliminated by different cell death mechanisms. One such mechanism is mitotic cell death (MCD), also known as "mitotic catastrophe", a prominent but poorly defined form of cell death that is described mainly in morphological terms. MCD is an outcome of aberrant mitosis that results in formation of giant multimicronucleated cells. MCD has been shown to prevail in cells with impaired in the G1, G2, prophase and mitotic spindle checkpoint functions and has been reported to be a major form of tumor cell death following treatments with ionizing radiation (IR) or certain chemotherapeutic agents. One of the early steps in the chain of events that culminates in MCD is cell entry into premature mitosis. To date, evidence of premature mitosis in damaged cells relies primarily on the appearance of uneven chromatin condensation (UCC) - formation of hypercondensed chromatin aggregates. Although damage-induced UCC appears at least morphologically to differ from other types of chromatin condensation in eukaryotic cells such as mitotic or apoptotic chromatin condensation, the question arises whether the mechanisms operative in mitotic or apoptotic chromatin condensations are involved in MCD-related UCC. We established a functional link between the induction of DNA damage and such mitotic abnormalities and have provided some of the missing components of this process. We found that the induction of double strand breaks (DSBs) in the DNA in cells with compromised p53-mediated G2/M checkpoint triggers unscheduled activation of Cdk1 and chromatin loading of Condensin I complex, followed by UCC and subsequent appearance of multimicronucleated cells – a hallmark of MCD. We show that these processes engage some of the players of normal mitotic chromatin packaging, but not those that drive the apoptotic chromatin condensation. Moreover, using a panel of tumor cell lines, we demonstrate that defective damage-induced, p53-mediated G2/M checkpoint is an important but not a sole requirement for the activation of this pathway.

Modulating the battle between survival and pro-death signaling pathways in cancer cells

John Blenis, Jeffrey P. MacKeigan, Rana Anjum and Leon O. Murphy

Department of Cell Biology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115

Evasion from apoptosis is a hallmark of cancer, and recent success using targeted therapeutics underscores the importance of identifying anti-apoptotic survival pathways. We have utilized RNA interference (RNAi) to systematically screen the kinase and phosphatase component of the human genome. In addition to known kinases, we identified several new survival kinases. Interestingly, numerous phosphatases and associated regulatory subunits contribute to cell survival, revealing a previously unrecognized general role for phosphatases as negative regulators of apoptosis. We have also identified a subset of phosphatases with tumour-suppressor-like activity. Finally, RNAi targeting of specific protein kinases sensitizes resistant cells to chemotherapeutic agents. The development of inhibitors that target these kinases or phosphatases may lead to new anti-cancer strategies.

Upstream and Downstream Modifiers of the BCL-2 Survival Pathway in Cancer

A. Thomas Look

Differential inhibition of TRAIL-mediated DR5-DISC formation by Decoy Receptor 1 and 2

Delphine Mérino [1], Najoua Lalaoui [1], Alexandre Morizot [1], Pascal Schneider [2], Eric Solary [1] and Olivier Micheau [1]

[1] INSERM, U517, Univ. Bourgogne, Dijon, F-21000, [2] Biochemistry Department, University of Lausanne, Boveresses 155, CH-1066 Epalinges, Switzerland.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor (TNF) family that induces cancer cell death by apoptosis with some selectivity. TRAIL-induced apoptosis is mediated by the transmembrane receptors DR4 (also known as TRAIL-R1) and DR5 (TRAIL-R2). TRAIL can also bind DcR1 (TRAIL-R3) and DcR2 (TRAIL-R4) that do not mediate cell death, due to absent or truncated cytoplasmic death domain, respectively. In addition, DcR1 and DcR2 inhibit DR4- and DR5-mediated, TRAIL -induced apoptosis and we demonstrate that this effect occurs through distinct mechanisms. While DcR1 prevents the assembly of the death-inducing signaling complex (DISC) by titrating TRAIL within lipid rafts, DcR2 is co-recruited with DR5 within the DISC where it inhibits initiator caspase activation. In addition, DcR2 prevents DR4 recruitment within the DR5 DISC. The specificity of DcR1 and DcR2-mediated TRAIL inhibition reveals an additional level of complexity for the regulation of TRAIL signaling.

Src kinase phosphorylates Caspase-8 on Tyr380: a novel mechanism of apoptosis suppression

Silvia Cursi (1), Alessandra Rufini (2),Venturina Stagni (1), Ivano Condò (2), Roberto Testi (2) and Daniela Barilà (1,2)

(1) Laboratory of Cell Signaling, Fondazione Santa Lucia, 00179, Rome, Italy
(2) Laboratory of Immunology and Signal Transduction, Department of Experimental Medicine and Biochemical Sciences, University of Rome "Tor Vergata", Via Montpellier,1 00133 Rome, Italy.

We identified Caspase-8 as a new substrate for Src kinase. Phosphorylation occurs on Tyr380, situated in the linker region between the large and the small subunits of human Procaspase-8, and results in down-regulation of Caspase-8 proapototic function. Src activation triggers Caspase-8 phosphorylation on Tyr380 and impairs Fas-induced apoptosis. Accordingly, Src failed to protect Caspase-8-defective human cells in which a Caspase-8-Y380F mutant is expressed from Fas-induced cell death.
Remarkably, Src activation upon EGF-receptor stimulation, triggers endogenous Caspase-8 phosphorylation and prevents Fas-induced apoptosis. Tyr380 is phosphorylated also in human colon cancers where Src is aberrantly activated. These data provide the first evidence for a direct role of tyrosine phosphorylation in the control of caspases and reveal a new mechanism through which tyrosine kinases inhibit apoptosis and participate in tumor progression.

Apoptotic death of ageing yeasts

Buttner, Sabrina, Eisenberg, Tobias, Carmona-Gutiettez, Didac, Madeo Frank

IMB, Universitaetsplatz 2,8010 Graz, Austria

Endonuclease G and AIF, both mitochodrially located nucleases, have been suggested to play a crucial role in genuine mitochondrial function but also in apoptosis during a plethora of degenerative disorders such as noise induced hearing loss, neurodegeneration and muscle degeneration during ageing.
We describe the yeast and fly orthologues of Endo G (Nuc1p and EndoG) whose overexpression triggers apoptotic cell death. Nuc1p displays mitochondrio-nuclear localization and induces apoptosis in yeast independently of caspases or AIF. Instead, we found other biochemical interactors necessary for Nuc1 mediated cell death. We thus picture for the first time a pathway for EndoG mediated death.
Yeasts mutated in the ER residing calcium pump PMR1 (whose mutation in mammalian cells is responsable for hereditary Haily-Haily disease) die during chronological ageing in a caspase-independent but Aif1-dependent fashion. In contrast, during heat stress, Aif1p pretects against cell death. This means, we can dissect the lethal and vital functions of AIF as well as caspase-dependent and -independent pathways in a single yeast mutant.

Cannabinoids induce apoptosis of tumor cells via activation of the endoplasmic reticulum stress pathway

Guillermo Velasco

Department of Biochemistry and Molecular Biology I, School of Biology, Complutense University, Madrid, Spain

Cannabinoids, the active components of marijuana and their derivatives exerts a wide variety of biological effects by mimicking endogenous substances - the endocannabinoids anandamide and 2-arachidonoylglycerol - that bind to and activate specific cannabinoid receptors .(CB1 and CB2)

One of the most exciting areas of research in the cannabinoid field is the study of the potential application of cannabinoids as therapeutic agents. Among these possible applications, cannabinoids are being investigated as antitumoral drugs. Thus, cannabinoid administration has been shown to curb the growth of several models of tumor xenografts in rats and mice. These antitumoral actions of cannabinoids rely, at least in part, on the ability of these compounds to induce apoptosis of tumor cells. However, the molecular mechanisms responsible for these effects had remained unravelled. Analysis of gene expression of several tumor cell lines (sensitive and resistant to cannabinoid induced apoptosis) has led us to identify the stress-regulated protein p8 (also designated as candidate of metastasis 1) as an essential mediator of cannabinoid pro-apoptotic and anti-tumoral action. p8 mediates its apoptotic effect via up-regulation of the endoplasmic reticulum stress-related genes ATF-4, CHOP and TRB3. The potential therapeutic implications of the activation of this pathway by cannabinoids in tumor cells will be discussed.

Novel PACS-2 Interactors on the endoplasmic reticulum

Thomas Simmen[1)], Timothy J. Cooper[1)], Nathan Myhill[1)], Anastasia D. Blagoveshchenskaya[2)], Katia Carmine Simmen[4)], Gary Thomas[3)]

1) University of Alberta, Department of Cell Biology, Edmonton, Canada; 2) Division of Nephrology and Hypertension, Oregon Health & Science University, Portland, OR 97239, USA; 3) Vollum Institute, Oregon Health & Science University, Portland, OR 97239, USA. 4) University of Alberta, Department of Biochemistry, Edmonton, Canada

Phosphofurin acidic cluster sorting proteins (PACS) are involved in the localization of cytosolic and transmembrane proteins with acidic clusters to the trans Golgi network (TGN), mitochondria and the endoplasmic reticulum (ER). The family member PACS-2 can interact with the Bcl-2 protein Bid on a phosphorylatable acidic cluster close to its caspase cleavage site (Simmen et al., 2005). Through this interaction, PACS-2 mediates the translocation of Bid to the mitochondria during cellular stress. This is consistent with a delayed apoptosis onset in the absence of PACS-2. As the PACS-2-Bid interaction increases during the onset of apoptosis and PACS-2 itself translocates onto mitochondria, PACS-2 could reside at the center of a stress-sensing pro-apoptotic pathway. Unpublished work indicates that a high number of novel PACS-2 interactors are found on the ER. A subgroup of these corresponds to protein folding chaperones, such as calnexin or members of the TMX protein family that are related to protein disulfide isomerase (PDI). Similar to Bid, calnexin interacts at a cytosolic acidic cluster with PACS-2. This interaction, independent of cellular stress, mediates localization of calnexin to the ER. PACS-2 depletion leads to leakage of calnexin to the cell surface. PACS-2 also regulates ER-localization of BAP31, whose p20 caspase cleavage product promotes mitochondrial fragmentation. Both interactions suggest that the interaction of PACS-2 with ER chaperones could be a missing link between ER protein folding and apoptosis induction during ER stress. They also provide an explanation for the so far unclear role of calnexin in the onset of apoptosis.

Involvement of a putative apoptotic machinery in lifespan control of the filamentous ascomycete *Podospora anserina*

Diana Brust, Andrea Hamann, Heinz D. Osiewacz

Institute of Molecular Biosciences, J.W. Goethe-Universität, Max-von-Laue Straße 8, 60438 Frankfurt, Germany, e-mail: brust@em.uni-frankfurt.de

In contrast to most other fungi, the filamentous ascomycete *Podospora anserina* does not show indefinite growth. After about 30 days of linear growth, the growth rate of the wild type strain s declines and the morphology and physiology of cultures changes dramatically, ending up with the death of the hyphal tips. This senescence syndrome has a clear mitochondrial etiology. It was repeatedly demonstrated that defective mitochondria accumulate during ageing leading to respiratory deficiency. The role of mitochondria in ageing of cultures suggests the involvement of apoptosis-like processes in the final stage of development of this fungus. In order to unravel the role of such processes, an *in-silico* analysis was performed resulting in the identification of several putative apoptosis factors: two metacaspases, two AIF homologs (apoptosis-inducing factor) and two AMID homologs (AIF homologous mitochondrion-associated inducer of death). The deletion of the metacaspase gene *PaMca1* was found to have a remarked effect on lifespan: Deletion strains are characterized by a 2.5 fold lifespan extension. Deletion of *PaMca2* and *PaAmid1* also results in lifespan extension but to a lower extent. A *PaAif1* deletion strain is under investigation. Currently, we favour the following model: during ageing of cultures reactive oxygen species (especially hydrogen peroxide) accumulate due to mitochondrial deficiency. This leads to the activation of an apoptotic machinery. This idea is supported by the following findings: (1) senescent mycelium releases high amounts of hydrogen peroxide; (2) hydrogen peroxide treatment leads to the pronounced induction of caspase activity in the wild-type but not in the *PaMca1* deletion strain; (3) wild-type protoplasts are more susceptible to hydrogen peroxide treatment than protoplasts of the *PaMca1* deletion strain.
Future investigations will focus on the role of the mitochondrion-associated AIF and AMID homologs in the senescence process. For this purpose, currently new deletion strains are constructed. In addition, the effect of manipulating different apoptotic pathways (caspase-dependent and independent) in double and triple deletion strains will be investigated with respect to lifespan. Also the age-dependent localization of the *P. anserina* AIF and AMID homologs will be analysed using fluorescence microscopy of transgenic strains carrying GFP fusion constructs.

The road map to programmed cell death: Switching between apoptosis and autophagic cell death.

Sharon Reef, Einat Zalckvar, Shani Bialik, and Adi Kimchi.

Department of Molecular Genetics. Weizmann Institute of Science, Rehovot 76100, Israel.

The complexity of molecular networks underlying programmed cell death is reflected by the finding that a few independent roads utilizing different sets of genes can lead to the final execution of cell death. In addition to the well known caspase-dependent pathways causing apoptosis, autophagic genes can be also recruited to the process generating a second road of caspase-independent autophagic cell death. In this work we discovered a novel isoform of p19ARF, named smARF, which induces autophagic cell death. smARF is generated by a mechanism of internal initiation of translation resulting is a short isoform which lacks the nucleolar targeting signals and the p53 activating domain present in the longer form. smARF is a short lived protein, which is rapidly degraded by the proteasome, but accumulates after inappropriate proliferative signals generated by oncogenes. Surprisingly, smARF translocates to the mitochondria, impairs the structure of the mitochondria, and dissipates the mitochondrial membrane potential in a p53 and Bcl-2 family independent manner. Ultimately, smARF induces massive autophagy in cells, as identified by molecular markers and by electron microscopy. The knock down of Beclin-1 and ATG5 protected to some extent from smARF-induced cell death, proving that smARF induces caspase-independent autophagic cell death. Thus in principle, two completely different molecular pathways can be activated by the p19ARF isoforms. The full length nucleolar isoform of p19ARF can activate a pathway from within the nucleolus which involves p53 activation, followed by caspase-dependent type I apoptotic cell death. smARF, on the other hand, can induce a second pathway that initiates within the mitochondria, involves the dissipation of mitochondrial membrane potential, and ultimately will induce type II autophagic cell death. Both forms are elevated in response to oncogenes and may serve as fail safe mechanisms against cancer development, backing -up each other.

Reef, S., Zalckvar, E., Shifman, O., Bialik, S., Sabanay, I., Oren, M. and Kimchi, A. (2006) A short mitochondrial form of p19ARF induces autophagy and caspase-independent cell death. Mol Cell 22, 463-475.

Ambra-1 regulates autophagy during development of the nervous system in mammals

Anastassia Stoykova[1], Gian Maria Fimia[2], Alessandra Romagnoli[2], Luigi Giunta[3], Sabrina Di Bartolomeo[3], Roberta Nardacci[2], Claudia Fuoco[3], Peter Gruss[1], Kamal Chowdhury[1], Mauro Piacentini[2,3] and Francesco Cecconi[3,4]

[1]Department of Molecular Cell Biology, Max-Planck Institute of Biophysical Chemistry, 37077 Göttingen, Germany
[2] National Institute for Infectious Diseases IRCCS L. Spallanzani, 00149 Rome (Italy)
[3]Department of Biology, University of Tor Vergata, 00133 Rome (Italy)
[4] Laboratory of Molecular Neuroembriology, IRCCS Fondazione Santa Lucia, 00143 Rome (Italy); e-mail: francesco.cecconi@uniroma2.it

Autophagy is a highly conserved process in eukaryotic cells by which long-lived proteins, cytoplasmic organelles as well as entire cells are degradated. During the autophagic process, excess or aberrant cellular materials are sequestered into double-membrane vesicles and delivered to the degradative organelle, the lysosome/vacuole, for breakdown and eventual recycling of the macromolecules. This process has an important role in various biological events such as adaptation to changing environmental conditions, cellular remodeling during development and differentiation, and determination of lifespan. Moreover, a number of human diseases, as cancer, cardiomyopathy and some neurodegenerative disorders are associated with an increase of autophagic activity.

Ambra-1 (Activating molecule of beclin regulated autophagy) is a novel gene identifyed in our lab by the "gene trapping" technique in mouse. The expression of Ambra-1 is prominent in many parts of the nervous system during embryogenesis and in adult tissues. Ambra-1gt/gt embryos die at embryonic day 16.5 and show a peculiar phenotype. Focusing on the nervous system, we observe a prominent exencephaly due to an enhanced cell proliferation in comparison with wild type (wt) embryos. In addition, mutant embryos show some defects in patterning and closure of the neural tube. A yeast two-hybrid assay showed a specific binding of Ambra-1 protein with several proteins involved in vesicle trafficking and autophagy as, in example, the yeast Atg6 mammalian homolog Beclin1. To investigate a possible role of Ambra-1 protein in autophagy, in vivo and in vitro studies have been addressed. An in vivo approach we are using consists in the ultrastructural analysis of mutated and wt embryos at different embryonic stages by electron and confocal microscopy. In parallel, in vitro experiments have been conducted on murine embryonic fibroblasts (MEFs) isolated from wt and Ambra-1 embryos. Unlike wt MEFs, Ambra-1gt/gt MEFs are not able to respond to the autophagic stimulus rapamycin. Furthermore, we observe that overexpression of Ambra-1 gene in a human fibroblast cell line results in an enhanced autophagic response to rapamycin whereas downregulation of the gene by siRNA drastically reduces this response, thus confirming the involvement of Ambra-1 in autophagy.

MAGUKs, scaffolding proteins at cell junctions, as substrates for the caspases and cathepsins during apoptosis

Saška Ivanova[1], Uroš Gregorc[1], Lawrence Banks[2], David S. Bredt[3], Ron Javier[4], Rok Romih[5], Peter Vandenabeele[6], Vito Turk[1], Boris Turk[1]

[1]*Department of Biochemistry, Jožef Stefan Institute, Ljubljana, Slovenia*
[2]*International Centre for Genetic Engineering and Biotechnology, Trieste, Italy*
[3]*Department of Physiology, University of California San Francisco, San Francisco, USA*
[4]*Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston,USA*
[5]*Institute of Cell Biology, Faculty of Medicine, Ljubljana, Slovenia*
[6]*Department of Molecular Biomedical Research, VIB-University of Ghent, Ghent, Belgium*

Membrane-associated guanylate kinases (MAGUKs) are often found in different cell junction structures, where they have a general role in creating and maintaining specialized membrane domains and act as molecular scaffolds for signaling pathway components. MAGUK proteins have a common multidomain organization including several PDZ domains, an SH3 or WW domain, and one guanylate kinase (GUK) domain. All these domains act as a protein-protein interacting domains enabling MAGUKs to assemble a combination of cell adhesion molecules, cytoskeletal proteins, receptors, ion channels and their associate signaling components at specific membrane sites. Since loss of the cell-cell contacts and isolation of a dying cell is one of the important morphological features of apoptosis, it is believed that in order to ensure a fast and efficient detachment of a dying cell, components of cell-cell contacts have to be cleaved, among them the MAGUKs.

Our in vitro experiments showed that all MAGUKs tested were cleaved by at least one of the executioner caspases and degraded by several cathepsins. By inducing the apoptosis with UV and staurosporine in different epithelial cell lines, we showed that caspases can also cleave MAGUKs in different cell models. This cleavage could be prevented by zVAD-fmk, a pan-caspase inhibitor. Using a selective lysosomal disrupting agent LeuLeuOMe, which induces apoptosis through lysosomal pathway, we have further shown that MAGUKs are cleaved and/or degraded in HaCaT and CaCo-2 cells. This cleavage/degradation could be prevented by E-64d, an inhibitor of papain-like cysteine proteases, but not by zVAD-fmk. Immunohistological stainings in HaCaT, MDCK and CaCo-2 cells showed that MAGUKs are localized to cell membrane and that after induction of apoptosis with various agents this localization is disrupted, which correlates with the loss of cell-cell contacts. In the case of the dominant negative mutant of MAGI-1 (D761A), we observed a few hours delay in detachment of dying cells, suggesting that cleavage of MAGI-1 is important for proper cell detachment. Furthermore, cells pretreated with zVAD-fmk were also dying (relatively high percentage of annexin V positive cells and compromised morphology under light microscopy), but had no DEVDase activity, suggesting that these cells undergo some kind of caspase-independent cell death. Preliminary TEM pictures showed increased amount of double-membrane vesicles in these cells, a key feature of autophagy. In contrast to apoptotic cells, majority of these cells were still attached and MAGUKs were intact, further supporting the idea that cleavage of MAGUKs is an important step in fast and efficient cell detachment.

C1q recognizes phosphatidylserine on apoptotic cells and likely acts as an early, multi-ligand bridging molecule in apoptotic cell clearance

Helena Païdassi[1], Pascale Tacnet-Delorme[1], Virginie Garlatti[2], Claudine Darnault[2], Berhane Ghebrehiwet[3], Christine Gaboriaud[2], Gérard J. Arlaud[1] and Philippe Frachet[1].

[1]Laboratoire d'Enzymologie Moléculaire, [2]Laboratoire de Cristallographie et Cristallogénèse des Protéines, Institut de Biologie Structurale, 41 rue J. Horowitz, 38027 Grenoble cedex 1, France, and [3]Department of Medicine, University of Stony Brook, New York, USA, e-mail : philippe.frachet@ibs.fr

Clearance of apoptotic cells is critical for development, tissue remodelling and maintenance of homeostasis, and is crucial to control the immune responses mediated by macrophages and dendritic cells. Phosphatidylserine (PS) exposure at the surface of apoptotic cells is a universal indicator of apoptosis and a key element of apoptotic cell recognition. Surprisingly, little is known about how PS recognition is mediated and leads to apoptotic cell internalization by phagocytes. C1q, which is well known as the recognition unit of the C1 complex of complement, also senses altered structures from self and is a major actor of immune tolerance. Hela cells were rendered apoptotic by UV-B treatment and a variety of cellular and molecular approaches were used to investigate the nature of the target(s) recognized by C1q. Using surface plasmon resonance (SPR), C1q binding was shown to occur at early stages of apoptosis, well before cell permeabilization, and to involve recognition of a cell membrane component. C1q binding and PS exposure, as measured by annexin V labelling, proceeded concomitantly, and annexin V inhibited C1q binding in a dose-dependent manner. As shown by co-sedimentation, SPR and X-ray cristallography analyses, C1q recognized PS specifically and avidly (K_D = 3.7-7 x 10^{-8} M), through multiple interactions between its globular domain and the phosphoserine group of PS. Confocal microscopy demonstrated that the majority of the C1q molecules were remarkably distributed in membrane patches where they co-localized with PS. In summary, this study demonstrates that PS is a major C1q ligand on apoptotic cells. The C1q-PS interaction takes place at early stages of apoptosis, likely in newly organized membrane patches. Given the known versatility of its recognition function, these data suggest that C1q has the unique ability to sense different ligands which collectively provide strong “eat me” signals. These observations shed new light on the role of C1q as a major bridging molecule in apoptotic cell recognition and clearance.

Host defence mechanism mediated by Nod proteins.

Naohiro Inohara

Department of Pathology, University of Michigan Medical School. Ann Arbor, MI, USA, e-mail:ino@umich.edu

Nods are intracellular proteins that play in key regulatory roles in pathogen responses and cell death. Nod1 and Nod2, two Nod family members, are involved in host recognition of bacterial components. Genetic variations in the Nod1 and Nod2 genes are associated with susceptibility to several human diseases including Crohn's disease and allergic diseases. Whereas Nod1 is ubiquitously expressed in multiple tissues including intestinal epithelial cells, Nod2 is expressed highly in APCs and Paneth cells. Mouse models showed that Nod2 provides the first defence line against intestinal Listeria infection, whereas Nod1 appears to be critical at a later stage during bacterial infection. However, the mechanisms by which Nods protect hosts from Listeria infection are poorly understood. Here we show that both Nod1 and Nod2 stimulation induce innate immune genes in intestinal epithelial cells. Intraperitoneal Nod1 stimulation induced recruitment of acute inflammatory cells, which was abolished in Nod1-/- mice. Nod1 stimulation induced massive production of chemokines but poorly TNF, IL-1, IL-12 and IFNs, suggesting that the primary role of Nod1 is to induce the recruitment of immune cells. Nod1 stimulation with a T-cell dependent antigen enhanced acquired immune responses, although no direct induction of co-stimulatory molecules in APCs or lymphocyte proliferation by Nod1 stimulation was detected. The adjuvant activity of Nod1 ligand was absent in Nod1-deficient mice. These results indicate that Nod1 functions as a pathogen recognition molecule to induce expression of molecules involved in the early stages of the innate immune response. Furthermore, Nod1 can act as an adjuvant for secondary IgG responses to T-cell dependent antigens through a mechanism that appears to be different from those for TLRs. Together with these findings, the role of Nod molecules in local and systemic immunity as well as in human diseases will be discussed.

Gout-associated uric acid crystals activate the NALP3 inflammasome

Jurg Tschopp

Department of Biochemistry, University of Lausanne, 1066 Epalinges, Switzerland

Development of the acute and chronic inflammatory responses known as gout and pseudogout are associated with the deposition of monosodium urate (MSU) or calcium pyrophosphate dihydrate (CPPD) crystals, respectively, in joints and periarticular tissues. Although MSU crystals were first identified as the aetiological agent of gout in the eighteenth century and more recently as a 'danger signal' released from dying cells, little is known about the molecular mechanisms underlying MSU- or CPPD-induced inflammation. We will present data that show that MSU and CPPD engage the caspase-1-activating NALP3 (also called cryopyrin) inflammasome, resulting in the production of active interleukin (IL)-1beta and IL-18. Macrophages from mice deficient in various components of the inflammasome such as caspase-1, ASC and NALP3 are defective in crystal-induced IL-1beta activation. Moreover, an impaired neutrophil influx is found in an in vivo model of crystal-induced peritonitis in inflammasome-deficient mice or mice deficient in the IL-1beta receptor (IL-1R). These findings provide insight into the molecular processes underlying the inflammatory conditions of gout and pseudogout, and further support a pivotal role of the inflammasome in several autoinflammatory diseases.

Poster Abstracts

Supported by EC within the framework of the Marie Curie
Conferences and Training Courses

Accumulation of oxidative damage is responsible for autophagic programmed cell death of senescent cells

Gosselin Karo, Bouali Fatima, Martien Sébastien, Vercamer Chantal, Abbadie Corinne

UMR8161 CNRS/Institut Pasteur de Lille/Université Lille1/Université Lille2. Institut de Biologie de Lille, 1 rue Calmette, BP447, 59021 Lille Cedex, France

Senescence is the state reached by cells with time and population doublings. Two mains mechanisms were shown to promote senescence: telomere erosion and cumulative oxidative damage. After a while at the senescent growth plateau, senescent cells ultimately die by a process we wanted to characterize.
We first investigated for death by apoptosis by searching for the occurrence of phosphatidyl serine externalization, release of cytochome C or AIF from mitochondria, cytoplasm and chromatin condensation, DNA double-strand breaks, cytoskeleton degradation, Bid degradation and caspase-3 activation. None of these changes were evidenced in senescent cells.
We actually observed that senescent cells display numerous vacuole-like structures, suggesting they could die by autophagic programmed cell death. Their nuclei display a multi-foci-condensed chromatin and numerous single-strand breaks, and their mitochondria were swelled and clustered around the nuclei. Eight-oxo-guanines and amino-imino-propene bridges, two types of oxidative damage, were detected inside altered nuclei and mitochondria of senescent cells, as well as inside the vacuole-like structures, suggesting these vacuoles to be autophagosomes having engulfed the oxidatively-altered mitochondria and nuclei. We indeed provide evidence for an increase in masses of lysosomes and autophagic vacuoles with senescence, and a preservation of the cytoskeleton necessary for vesicle trafficking during autophagy. We also document an increase in the expression of Beclin-1, a protein involved in the autophagosome formation, and a total degradation of Bcl-2, recently described to interact with and inhibit Beclin-1. By triple staining of mitochondria, lysosomes and nuclei, we show that died cells are virtually resumed to a big autophagolysosome having engulfed all altered mitochondria and nuclei. Finally, we show that three-methyladenine, an inhibitor of autophagosome formation, but zVAD, an inhibitor of caspases, was able to maintain senescent cells alive for a while.
We conclude that senescent cells die by autophagy, not by apoptosis, because of accumulation with time of oxidative damage into nucleus and mitochondria. Considering that most tumours or transformed cell lines display a reduced autophagic activity, our results suggest that escaping autophagic cell death could be an initial event for cell transformation.

Dexamethasone induces apoptotic cell death through NMDA receptor activation in cultured cerebellar granule cells, but does not act through NMDA receptor subunits 2a or B or L-type Ca channels.

Petra Aden[1], Chris Jacobs[2], Minh D. Trinh[2], Terje Rootwelt[3], Jon Lømo[1], Ragnhild E. Paulsen[2]

[1]Department of Pathology, Ullevål University Hospital, Oslo Norway
[2]Department of Pharmaceutical Biosciences, University of Oslo, Norway
[3]Department of Pediatrics, Rikshopitalet, University of Oslo, Norway

Potent glucocorticosteroids are used in premature infants to prevent respiratory distress syndrome. Recently their use has been questioned by reports indicating a negative effect on the developing brain. We showed earlier that DEX (dexamethasone) increased cell death assayed by trypan blue in cultured rat pup cerebellar granule cells. Cell death was of apoptotic nature as it could be blocked by caspase III inhibitor, and AIF translocation from the cytoplasm to the nucleus could be demonstrated. DEX caused cell death could not be blocked by magnesium, but was effectively inhibited by MK 801, a non-competitive antagonist of the NMDA receptor, and CGP 39551, a competitive antagonist of the NMDA receptor. At the same time DEX triggered cell death was blocked by RU 28318, arguing for a crosstalk between the steroid receptor and the NMDA receptor.
To further elucidate the mechanisms which would allow for crosstalk between the NMDA receptor and the steroidreceptor we tested the effect of different NMDA receptor subunit antagonists against DEX induced cell death in rat granule cell cultures. Ifendprodil (10 µM), a selective NMDAR 2B antagonist, and zinc, a potent NMDAR 2A antagonist in concentrations of 1 µM, did reduce cell death in glutamate-treated neurons but not in DEX treated neurons. We further applied the L-type Ca-channel inhibitor verapamil together with DEX, as DEX could potentially cause high Ca influx through other channels than NMDA receptors. Verapamil showed no effect on DEX triggered cell death.
We conclude that DEX toxicity may be blocked by general NMDA receptor blocker as well as corticoid receptor blockers. In rat pup cerebellar granule cells neither the NMDAR 2A or NMDAR 2B subunit of the NMDA receptor nor the L-type Ca channel were involved.

HAMLET (Human alpha-lactalbumin made lethal to tumor cells) triggers rapid macroautophagy in tumor cells

Sonja Aits[1], Oskar Hallgren[1], Lotta Gustafsson[1], Patrick Brest[1], Ann-Kristin Mossberg[1], Matthias Mörgelin[2] and Catharina Svanborg[1]

[1]Institute of Laboratory Medicine, Section of Microbiology, Immunology and Glycobiology, Lund University, Lund, Sweden. [2]Department of Clinical Sciences, Section for Clinical and Experimental Infectious Medicine, Lund University, Lund, Sweden. E-mail: Sonja.Aits@med.lu.se

HAMLET, a complex of partially unfolded alpha-lactalbumin and oleic acid, selectively kills tumour cells while leaving healthy differentiated cells unaffected. So far all of more than 40 tested tumour cell lines, derived from different tissues and species, were found to be sensitive to HAMLET. In addition, HAMLET is also effective *in vivo*. Cells dying after HAMLET treatment show features of apoptosis including cytochrome c release, caspase activation and DNA fragmentation. Nevertheless, HAMLET-induced cell death does not only depend on classical apoptosis as caspase inhibition fails to prevent cell death. Furthermore, cell death is not influenced by *p53* status or Bcl-2 expression level. Therefore, the role of macroautophagy as an alternative mechanism of cell death in HAMLET-treated cells was examined. After HAMLET treatment tumor cells undergo dramatic morphological changes. Electron microscopy of A549 lung carcinoma cells revealed extensive formation of cytoplasmic vacuoles, damaged mitochondria and double membrane vesicles. This was accompanied by a rapid decrease in ATP levels. Organelle damage and loss of energy are major causes of stress that may trigger macroautophagy. Macroautophagy is characterized by the formation of double membrane vesicles (autophagosomes) as seen in HAMLET-treated cells. Macroautophagy has also been reported to be accompanied by an increase in staining with monodansylcadaverine (MDC) which, even though recent reports have doubted its specificity for autophagic vacuoles, is widely used as a marker for macroautophagy. HAMLET changed the MDC staining in A549 cells from a weak staining to an intense granular staining. This was blocked by 3-methyl adenine (3MA), an inhibitor of class III phosphatidylinositol 3-kinase and autophagosome formation. A similar autophagic response was observed in LC3-gfp-transfected A549 cells, where HAMLET caused a change from uniform (LC3-I) to granular staining (LC3-II) reflecting LC3 translocation to autophagosomes. This response to HAMLET was also inhibited by 3MA. However, HAMLET-induced cell death was not prevented by 3MA, even though it could inhibit the autophagic response. The results show that macroautophagy is part of the complex tumor cell response to HAMLET but represents more likely a defense mechanism in response to organelle damage than the crucial mechanism of cell death.

Role of presenilin-dependent g-secretase activity in the control of p53-mediated cell death in Alzheimer's disease.

Cristine Alves da Costa[1], Claire Sunyach[1,], Raphaëlle Pardossi-Piquard[1,], Jean Sévalle[1], Bruno Vincent[1], Toshitaka Kawarai[2], Nadège Girardot[3], Jie Shen[4], Peter St George-Hyslop[2], and Frédéric Checler[1]

1-Institut de Pharmacologie Moleculaire et Cellulaire, IPMC-CNRS, Valnonne, France
2-Center for Research in Neurodegenerative diseases, University of Toronto, Toronto, Canada
3- Center for Nuerologic Diseases, Harvard Medical School, Boston,USA

Presenilins (PS) are part of the γ-secretase complex that produces the amyloid β-peptide (Aβ) from its precursor (βAPP). Mutations in PS that cause familial Alzheimer's disease (FAD) increase Aβ production and trigger p53-dependent cell death. We demonstrate that PS deficiency, catalytically inactive PS mutants, γ-secretase inhibitors and βAPP or APLP2 depletion, all reduce the expression and activity of p53, and lower the transactivation of its promoter and mRNA expression. p53 expression was also diminished in the brains of PS- or βAPP-deficient mice. The γ- and ε-secretase-derived C-terminal fragments (AICDC59 and AICDC50, respectively) of βAPP trigger p53-dependent cell death, and increase p53 activity and mRNA. Finally, PS1 mutations enhance p53 activity in HEK293 cells and p53 expression in FAD-affected brains. Thus, our study shows that AICDs control p53 at a transcriptional level, in vitro and in vivo, and that FAD mutations increase p53 expression and activity in cells and human brains.

Mechanisms of doxycycline-induced cytotoxicity and anoikis on human bronchial epithelial cells

Matthieu Sourdeval[1], Karine Andreau[1], Christophe Lemaire[2], Catherine Brenner[2], Emmanuelle Boisvieux-Ulrich[1] and Francelyne Marano[1]

[1]Laboratoire de Cytophysiologie et Toxicologie Cellulaire, Université Paris 7 Denis Diderot, 2 Place Jussieu, 75251 Paris Cedex 05, France ; [2]Laboratoire de Génétique et Biologie Cellulaire, Université de Versailles/St Quentin, CNRS FRE 2445, 45 av. des Etats-Unis, 78 035 Versailles Cedex, France

Doxycycline (DOX), a synthetic tetracycline, may have potential utility in the treatment of chronic inflammatory diseases and in the management of cancers due to its role in growth, invasion and metastasis of many tumors, on cell proliferation and as inducer of apoptosis. In the present study, the effect of DOX was investigated in a human bronchial epithelial cell line. DOX induced a time- and concentration-dependent cell proliferation inhibition, associated with a cell cycle arrest in S phase, a decrease in viability due to apoptosis and necrosis, and anoikis. This latter was partly correlated with early activation of caspase-3 before detachment, and with mitochondrial alteration. Cell transfection with a Bcl-2 encoding vector showed a decrease both in mitochondrial depolarization and cell detachment. DOX-induced apoptosis included decrease in Bcl-2 expression, increase in Bak expression and caspase-3 and -9 activation but appeared to be p53- and Bax-independent. A better comprehension of the DOX-induced apoptotic pathway could allow to abolish its toxic effects, improving the therapeutic efficiency of DOX.

Collagen VI muscular dystrophies from animal model to human therapy: mitochondria as targets for therapeutic intervention in muscle cell death

Alessia Angelin[1], Tania Tiepolo[2], Cristina Golfieri[1], Paolo Grumati[2], Patrizia Sabatelli[3], Valeria Petronilli[1], Nadir M. Maraldi[3], Luciano Merlini[4], Paolo Bonaldo[2] and Paolo Bernardi[1]

Departements of [1]Biomedical Sciences and [2]Histology, Microbiology and Medical Bioechnologies, University of Padova (Italy); [3]ITOI-CNR and University of Bologna (Italy); [4]Department of Medical Genetics, University of Ferrara (Italy)

Inherited mutations of Collagen VI (ColVI) genes have been linked to two human muscle diseases, Bethlem Myopathy (BM) and Ullrich Congenital Muscular Dystrophy (UCMD). We identified the pathogenic mechanism underlying muscular defects in *Col6a1*$^{-/-}$ mice, an animal model of both BM and UCMD. In skeletal muscle fibers isolated from *Col6a1*$^{-/-}$ mice, a deregulated opening of the mitochondrial permeability transition pore (PTP) is the causative event of cell death. Cyclosporin A (CsA), a PTP inhibitor, rescued the myophatic alterations of *Col6a1*$^{-/-}$ mice, suggesting a pharmacological strategy to treat patients affected by congenital ColVI deficiencies (1). Here we show that addition of oligomycin, an ATP synthase inhibitor, or of rotenone, a respiratory chain inhibitor, caused a decrease of mitochondrial membrane potential and induced apoptosis in myoblasts prepared from *Col6a1*$^{-/-}$ mice and UCMD patients but not from healthy donors. Both these events could be prevented either by plating cells onto purified ColVI, or by adding calcium chelators or CsA. These findings demonstrate (i) that the mitochondrial defect is not restricted to muscle fibers and (ii) that inappropriate PTP opening may play a role in the pathogenesis of human ColVI myopathies as well. These findings open new perspectives for pharmacological intervention in human ColVI deficiencies. We are also investigating the signal transduction pathways that connect ColVI to mitochondria (see Grumati et al., this meeting). Consistent with an involvement of GSK-3β, we show that insulin and Li^+ prevent the depolarizing effects of oligomycin on mitochondria from ColVI-deficient cells.

1.Irwin, W. A., Bergamin, N., Sabatelli, P., Reggiani, C., Megighian, A., Merlini, L., Braghetta, P., Columbaro, M., Volpin, D., Bressan, G. M., Bernardi, P., and Bonaldo, P. (2003) *Nat.Genet.* **35,** 367-371

Bcl-2 and Bcl-XL display differential pro-survival effects in different cell compartments in response to different cytotoxic stimuli.

Anguissola S, Reimertz C, Prehn JHM.

Department of Physiology & Medical Physics, Royal College of Surgeons in Ireland (RCSI), Dublin 2, Ireland.

Bcl-2 family proteins are key players in the control of cell survival versus cell death decisions; they are classified into two subfamilies according to anti- or pro-apoptotic function. Bcl-2 and Bcl-XL are the principal anti-apoptotic members of the family and they are over-expressed in different malignancies conferring resistance to chemotherapy .

Endoplasmic Reticulum (ER) stress induced by ER homeostasis perturbation has been described to cause apoptosis; inhibition of the protein degradation machinery, the proteasome, also leads to apoptosis, and these newly discovered apoptosis pathways have been suggested as promising anticancer approaches .

The aim of the project is to determine the pro-survival effect of Bcl-2 and Bcl-XL to drugs currently used in cancer treatment and to new pharmacological approaches to estimate the therapeutic benefit for tumors with a defined molecular background, i.e. Bcl-2 or Bcl-XL over-expression.

To assess the role of Bcl-2 and Bcl-XL in cell death regulation and their ability to induce resistance to chemotherapy the two proteins have been stably transfected, in the wild type form, or targeted specifically to the mitochondrial outer membrane or to the endoplasmic reticulum, into breast carcinoma cells. The transfected cells have been exposed to genotoxic drugs (etoposide, oxaliplatin) and proteasome inhibiting-drugs (epoxomicin) used in cancer therapy, or to ER Stress-inducing agents (thapsigargin, tunicamycin). Cell death has been analyzed by flow cytometry by measuring propidium iodide uptake into the cells.
Our results show that Bcl-2 and Bcl-XL share some functional similarities but also display some non redundant properties: both the proteins improve survival to the ER stress agent thapsigargin and to the genotoxic drug etoposide but they show a different response to oxaliplatin, endoplasmic reticulum-targeted Bcl-XL induces a survival response while the Bcl-2 counterpart does not.
Surprisingly Bcl-XL rescued cells from proteasome inhibition-induced cell death only when over-expressed at the mitochondria, and not in the wild type form; moreover Bcl-2 partially rescued cells from tunicamycin-induced cell death while Bcl-XL did not. These data suggest that proteasome inhibition- and ER Stress-based chemotherapy could more efficiently target tumors with a characterized molecular background of Bcl-2 or Bcl-XL over-expression compared to classical genotoxic drugs-based approaches.

Supported by a grant from the Higher Education Authority PRTLI Cycle 3 (Program of Human Genomics).

Modulation of ceramide metabolism as a possible strategy for N-(4-hydroxyphenyl)retinamide resistance tumor cells

Apraiz A., Pérez-Yarza G., Nieto-Rementería N., MD. Boyano, A. Asumendi

Department of Cell Biology and Histology, School of Medicine and Dentistry, University of the Basque Country, Leioa-48940 Bizkaia, Spain
e-mail: aintzane.asumendi@ehu.es

N-(4-hydroxyphenyl)retinamide (4HPR), a synthetic retinoid, is a promising chemopreventive and antineoplasic agent for cancer. Previous studies described the strong apoptotic effect of this retinoid on human leukemia cell lines and the sequence of the early events, related mainly to the mitochondrial apoptotic pathway. 4HPR induced ceramide accumulation (both by sphyngomyelin hydrolysis and *de novo* synthesis) is one of the first events in sensitive CEM human acute lymphoblastic leukemia (ALL) cells and ceramide is accepted as the mitochondrial oxidative stress inductor. It is also clear the effect of ceramide metabolism inhibitors such as *d, l-threo*-1-phenyl-2-hexadecanoylamino-3-morpholino-1-propanol (PPMP; glucosylceramide synthase and 1-acylceramide synthase inhibitor), *d,l-threo*-dihydrosphingosine (DHS; sphingosine kinase inihibitor) and L-*threo*-dihydrosphingosine (safingol; sphingosine kinase and protein kinase C alpha inhibitor) as 4HPR induced apoptotic enhancers.

Acquired or secondary quimioresistance is one of main and common problems to be faced to. Related to this, we have established 4HPR-resistante CEM cell lines (CEM R) and studied their response to ceramide metabolism modulators. Comparing with original CEM cells, CEM R cells show lower proliferation rate and a higher sensitivity to DHS and safingol but no early ROS production after 4HPR treatment. We have also tested other tumor cell lines (E1, Bcl-2 overexpressing CEM cells; Jurkat, ALL; A375, melanoma; HeLa, cervix carcinoma) with higher natural resistance to 4HPR and safingol is also specially citotoxic on them. When treating cells (48h) with 4HPR(1μM)+ PPMP(10μM)+ DHS(5μM) or 4HPR(1μM)+ PPMP(10μM)+ safingol(4μM), cell viability decreases from 50-60 to 90-95% in all mentioned cell lines except for E1 and A375 cells, which are more resistant to 4HPR(1μM)+ PPMP(10μM)+ DHS(5μM). Nevertheless, observed strong viability fall is no related to a early ROS production .

Those results lead our study to search for molecules modulated by ceramide or derivatives, related to multidrug resistance (MDR), involved in the mitochondrial apoptotic pathway and specially affected by safingol, such is the case of the protein kinase C alpha (PKC a).

Mcl-1 is a Tightly Regulated Key Player in Neuronal Cell Death

Nicole A. Arbour[1], Grace O. Iyirhiaro[2], Eric C. C. Cheung[2], Jason G. MacLaurin[2], David S. Park[2] and Ruth S. Slack[2]

Ottawa Health Research Institute – Neuroscience Centre, [1]Department of Biochemistry, Microbiology and Immunology, [2]Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario K1H 8M5 Canada. Email: narbo081@uottawa.ca

Anti-apoptotic Bcl-2 family member, Mcl-1 is believed to play a critical role in response to swiftly changing environmental cues or acute cellular stress in many systems; in part because of rapid transcription, and post translational modification. The study of Mcl-1, however, in the nervous system is limited. Here we show the importance Mcl-1 in the inhibition of neuronal cell death; as well as its differential regulation in response to two distinct models of neuronal injury. Following DNA damage induced injury; we find that Mcl-1 is down-regulated at both the transcriptional and post-translational levels. Sustained expression of Mcl-1 protects against DNA damage induced neuronal cell death, as well as neuronal cell death initiated by BH3-only proteins PUMA and BMF. In this system, the post-translational down-regulation of Mcl-1 occurs as a result of caspase activity, and proteosome degradation. In contrast to DNA damage induced injury, following hypoxic injury, very little regulation of Mcl-1 is seen due to post-translational modification by caspases or degradation by the protoesome, suggesting that its role in hypoxic injury may be less critical in this context. Further, we show a requirement for Mcl-1 in neuronal survival. Our results demonstrate a context dependant regulation of Mcl-1 in neuronal cell death and suggest its direct involvement in regulating the balance of survival and death in response to acute brain damage.

This work was supported by Heart and Stroke Foundation of Ontario (HSFO) grant to RSS, and Canadian Institutes of Health Research (CIHR) studentships to NAA and ECCC.

The effect of 5-FU on of Jurkat E6 cells with respect to bcl-2 family proteins

Dina Aresvik[1], Monica Åsegg-Atnoesen[1], Rolf D. Pettersen[1], Tore G. Abrahamsen[2] and Marianne S. Wright[1]

Pediatric research Institute, Dept. of Pediatrics, Rikshospitalet-Radiumhospitale Medical Center, Oslo, Norway. e-mail: diana.aresvik@studmed.uio.no

Programmed cell death or apoptosis is a mechanism that is involved in all aspects of life; including tissue homeostasis, the clearance of damaged cells and the immune defense. The use of chemotherapeutic agents in cancer patients is intended to induce stress and DNA damage in the cancer cells, hopefully leading to cell death. Apoptosis can occur in two ways: either by the extrinsic pathway and direct signaling of membrane death receptors or by the intrinsic path through regulation of bcl-2 family proteins, causing a loss of mitochondria membrane potential, thereby leading to apoptosis. Both paths involve a cascade of activated caspases which lead to DNA fragmentation and apoptosis. DNA damage is often followed by a cell cycle arrest and DNA repair mechanisms, but a substantial dose of chemotherapeutic agents will lead eventually to apoptosis. In addition, cancer cells often become resistant to anticancer drugs.

The purpose of this study is to delineate the signal transduction pathway for 5-Fluorouracil (5-FU) in Jurkat E6 cells, a T cell line. 5-FU attacks directly nucleic acids and affects the cell cycle. First we have examined whether Jurkat T cells can be used to establish a model system in which to analyze these effects. We have treated them with 5-FU with increasing doses and time. By using flow-cytometry we have established death curves. We have further analyzed the expression of all possible pro- and anti-apoptotic proteins and verified the most relevant ones by Western blot. So far we observed that bak is present as a multimer after stimulation with Staurosporine and 5-FU.

Induction of apoptosis in rat prostate cancer cells following treatment with difluoromethylornithine and roscovitine

Elif Damla Arisan, Narcin Palavan Unsal

Halic University, Molecular Biology and Genetics Department, 34280 Findikzade-Istanbul email: damlaarisan@gmail.com

Roscovitine is one of the newly discovered cyclin-dependent kinase (CDK) activity inhibitor, which was derived from plant cytokines. It upregulates the bax activity and suppress the anti-apoptotic proteins such as Bcl-2. However only CDK inhibition is not enough to rescue from cancerous cell type. Polyamines are very essential molecules in cell life. Especially in highly proliferating cell types, the content of polyamines increase in correlation with proliferation rate. Therefore, in this study we first treated two different prostate cell lines, low metastatic AT-2 and high metastatic Mat-Lylu with difloromethyornithine (DFMO) to suppress intracellular polyamine content and then treat with roscovitine. The potential sensitization of prostate cell lines against roscovitine was examined with bax:Bcl-2 ratio which is accepted as resistance marker against chemo/radiotherapy. AT-2 and Mat-Lylu rat prostate cell lines were treated with 5mM DFMO for 24h, and then 20uM roscovitine treated for 24h. The cell proliferation rate was determined by MTT assay. Apoptosis determination was done by PI staining and DNA fragmentation assay. Bax:Bcl-2 ratio was determined by real-time PCR. The DFMO treatment has no cytotoxic effect on both cell line, but only roscovitine treatment was decreased proliferation rate by 45% and 23% in AT-2 and Mat-Lylu cell lines, respectively. DFMO in combination with roscovitine for 24 h caused an inhibition in cell proliferation by 56% and 26% in AT-2 and Mat-Lylu cell lines. Bax:Bcl-2 ratio in roscovitine treatment was found as 2,1 and 1,3, these ratios were correlated with proliferation rate after drug treatment. Bax:Bcl-2 ratio for AT-2 and Mat-Lylu were found as 1,2 and 0,98. This ratio was established for DFMO in combination with roscovitine in both cell lines were determined 2,5 and 1,9 fold. In conclusion, the inhibition of intracellular polyamines with DFMO pretreatment more efficient in low metastatic prostate cancer cell line than in high metastatic ones in correlation with proliferation rate. Bax:Bcl-2 ratio determination was functional to understand the cytotoxic and sensitization responses of both cell line. Metastatic profile of cell lines was the major point of drug efficiency.

New derivatives of doxorubicin are potent inducers of erythroid differentiation of human Chronic Myelogenous Leukemia (CML) cell line K562

Szulawska Agata, Arkusinska Justyna & Czyz Malgorzata

Department of Molecular Biology of Cancer, Medical University, Lodz, Poland

Human chronic myelogenous leukemia (CML) cell line K562 can be chemically induced to differentiate towards erythrocytes. We have previously shown that K562 cell line responds to the treatment with newly synthesized anthracycline derivatives. They inhibit K562 cell line proliferation and some of them induce differentiation into more mature hemoglobin-containing cells. DOXM, morpholine derivative of doxorubicin was the most potent cytostatic agent and inducer of differentiation when compared with other anthracycline derivatives. In cell cycle analysis we have demonstrated that those anthracycline derivative that exert the greatest cytostatic potential caused G_2/M arrest which in turn might contribute to the development of a differentiating phenotype [Czyz M, et all., Biochem Pharmacol 2005].

As an extension of this work, to gain further evidence that K562 cells in the presence of some of those newly synthesized compounds differentiate towards erythroid lineage, in the present study we examined γ-globin expression, and GATA-1 expression and its DNA-binding activity. Since cytoplasmic level of mRNA, and hence protein level and its activity, depends not only upon rate of the synthesis, but the mRNA degradation rate should also be considered, we explored the possibility that anthracycline derivatives might increase mRNA stability of erythroid genes. The results clearly indicate that the anthracycline derivatives closely related in their structure might induce differentiation process by distinct mechanisms. In respect that DOXM is the most efficient inducers of differentiation we have also studied the influence of DOXM and STI571 used in combination on differentiation and apoptosis of K562 cell lines. We have found that DOXM in combination with STI571 inhibited proliferation and blocked cell cycle progression as well as induced expression of erythroid genes in K562 cell line more efficiently that each compound alone. We have also shown that DOXM alone was not able to induce apoptosis in this refractory to apoptosis cell line. In combination with STI571, however, already low concentration of DOXM caused significant increases in the number of apoptotic cells.

Noxa mediates p53-independent ER stress-induced apoptosis of neuroectodermal tumours

Jane L Armstrong[1], Gareth J Veal[1], Christopher P F Redfern[1], Penny E Lovat[1+2]

[1]Northern Institute for Cancer Research and [2] Dermatological Sciences, School of Clinical and Laboratory Sciences, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK

Neuroectodermal tumours, such as neuroblastoma and melanoma, are derived from primitive neural crest cells and present a significant therapeutic challenge. The synthetic vitamin A analogue fenretinide, a potent inducer of apoptosis, has emerged as a novel agent for both chemoprevention and chemotherapy in neuroectodermal tumours. Mechanisms of fenretinide-induced apoptosis are complex and involve reactive oxygen species (ROS), NF-kappa B, mitochondrial cytochrome c release and caspase activation. Furthermore, our recent data indicates that fenretinide induces apoptosis through ROS-dependent endoplasmic reticulum (ER) stress; however the precise mechanisms by which this triggers apoptosis are unknown. The aims of this study were to investigate mechanisms of ER-mitochondrial crosstalk in response to ER stress-inducing agents in comparison with conventional chemotherapeutics.

Studies of expression levels of the pro-apoptotic BH3-only protein Noxa in a panel of neuroectodermal cell lines suggested a correlation between Noxa up-regulation and fenretinide sensitivity. To test the hypothesis that Noxa mediates ER stress-induced apoptosis, neuroblastoma (SH-SY5Y) and melanoma (A375) cell lines were transfected with control, p53 or Noxa siRNA oligonucleotides for 24h, or SH-SY5Y cells were stably transfected with an inducible dominant-negative construct for Ikappa Balpha to abrogate NF-kappa B activity. Cells were then treated with thapsigargin (1.5 or 7.5 μM), a known inducer of ER stress, or fenretinide (5 or 10 μM), cisplatin (SH-SY5Y, 5μM) or temozolomide (A375, 1mM) for 18h.

In both SH-SY5Y and A375 cells, fenretinide-induced Noxa expression was dependent on ROS generation. In both cell lines, induction of Noxa expression by fenretinide or thapsigargin was not dependent on p53, while cisplatin- or temozolomide-induced Noxa expression was largely p53-dependent. Cisplatin- and temozolomide-induced apoptosis was inhibited in cells treated with p53 siRNA, whereas fenretinide and thapsigargin-induced apoptosis was unaffected. Conversely, fenretinide and thapsigargin-induced apoptosis was inhibited by siRNA to Noxa whilst there was no effect on cisplatin- or temozolomide-induced apoptosis. Furthermore, SH-SY5Y cells exhibit NF-kappa B-dependent repression of inhibitor of apoptosis protein XIAP expression in response to ER stress.

These data suggest that the p53-independent induction of Noxa and the NF-kappa B-dependent inhibition of XIAP are both required for apoptosis in response to ER stress in neuroectodermal tumours.

Bax and the mitochondrial permeability transition cooperate in the release of cytochrome C during thapsigargin induced apoptosis

Dawei Zhang and Jeffrey S. Armstrong

Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore. Email: bchjsa@nus.edu.sg

Endoplasmic reticulum (ER)-stress induces apoptosis by mechanisms that are not fully clear. Here we show that ER-stress induced by the Ca2+-ATPase inhibitor thapsigargin (THG) activates cytochrome c-dependent apoptosis through cooperation between Bax and the mitochondrial permeability transition (MPT) in human leukemic CEM cells.
Pharmacological inhibition of the MPT as well as interference RNA knockdown of the MPT core component cyclophilin D (CyD) blocked cytochrome c release and caspase dependent apoptosis but did not prevent Bax translocation, oligomerization or Nterminal exposure in mitochondria. Interference RNA knockdown of Bax also blocked THG-mediated cytochrome c release and apoptosis, but did not prevent MPT activation and converted caspase-dependent cell death to caspase independent cell death. Our results indicate that Bax and the MPT cooperate in the regulation of ER-stress induced apoptosis and in the absence of Bax, the MPT leads to cell death by necrosis.

VDAC1-based peptides interaction with Hexokinase I as a new approach for cancer therapy

Laetitia Arzoine and Varda Shoshan-Barmatz

Ben-Gurion University of the Negev, Department of Life Sciences, Beer Sheva, Israel,
e-mail:arzoinel@bgu.ac.il

Tumour cells exhibit a high rate of glycolysis and high expression levels of mitochondria-bound Hexokinase I and II (HK-I and HK-II, 100-fold higher than in normal cells). It is well documented that HK-I and HK-II interact with the mitochondrial voltage-dependent anion channel (VDAC). Our recent studies showed that HK-I specifically interacts with bilayer-reconstituted VDAC and induced its closure. In addition, HK-I prevented the release of cytochrome c from mitochondria, suggesting the blockade of the gateway to apoptosis. Furthermore, over-expression of HK-I in the tumour-derived cell line U-973 protects against staurosporine-induced apoptotic cell death. Interestingly, single point mutations in two of the four cytosolic loops of VDAC1, according to its proposed topological model, abolished the anti-apoptotic effect of HK-I on cell death induced by both STS and VDAC1 over-expression. Based on these results, we suggested that HK-I over-expression in cancer cells not only assures energy supplies, but also reflects an anti-apoptotic defence mechanism involving its direct interaction with VDAC. In the present study, the interaction of synthetic peptides with purified HK-I is demonstrated using Surface Plasmon Resonance technology. We found that the first and the third cytosolic loops and the N-terminal VDAC1-peptides bind to immobilised HK-I in a specific manner. No binding of the second cytosolic loop and of the loop exposed to the mitochondrial intermembranal space to immobilised HK-I was observed. Similar results were obtained when the effect of the VDAC1-peptides on the detachment of mitochondria-bound HK-I from isolated rat brain mitochondria, was studied. Since HK-I protect cancer cells against apoptosis; these results suggest that VDAC1-derived peptides, which interfere with HK-I binding to mitochondria, can be used to induce apoptosis in cancer cells. Thus, targeting VDAC1-derived peptides to tumour cells over-expressing anti-apoptotic proteins, such as HK-I and Bcl-2, would minimize their self-defense mechanisms, thereby promoting apoptosis and increasing their sensitivity to chemotherapy.

Lysosomal mechanisms of death in HTC rat hepatoma cells

Riccardo Autelli[1], Elisa Prigione[1], Gabriella Bonelli[1], Nicola Schiavone[2], Sergio Capaccioli[2] and Francesco M. Baccino[1]

[1]Department of Experimental Medicine and Oncology, Corso Raffaello 30, Torino, Italy and [2]Department of Experimental Pathology and Oncology, Firenze, Italy, e-mail: riccardo.autelli@unito.it

HTC rat hepatoma cells undergo apoptotic-like death following treatment with TNF/cycloheximide (CHX). This death was caspase-independent and involved the endo-lysosomal compartment, as it was prevented by ammonium chloride, a lysosomotropic agent, or bafilomycin A1, an inhibitor of the vATPase responsible of vacuolar acidification. Lysosomal involvement in both caspase-dependent and independent death processes, though to be fully elucidated as yet, has been often related to the activation of acidic sphingomyelinase, a key enzyme for ceramide generation in the endo-lysosomal compartment. TNF treatment, on the other hand, has been demonstrated to act also by bringing about ceramide generation, eventually leading to cell death.

To investigate whether ceramide generation plays a role in this model of death, HTC cells have been treated with TNF/CHX in the presence of inhibitors of the ceramide pathway such as desipramine, which selectively inhibits acidic sphingomyelinase, and fumonisin B_1, an inhibitor of ceramide synthase. Only desipramine effectively protected the cells from TNF, but also induced deep changes in cell morphology. The cytoplasm of desipramine-treated cells was filled with many variously sized vacuoles, similarly to what was observed in cells treated with ammonium chloride. Thus, the protective effect of desipramine was associated to morphological changes that might suggest an impairment in the proper functioning of the acidic vacuolar system. To further analyze ceramide involvement in this death, the protective role of Bcl-2 was evaluated. This molecule has been demonstrated to effectively protect from many kinds of non-necrotic cell death, including those that rely on ceramide generation. HTC cells were then transfected with a plasmid encoding human Bcl-2 and stable transfectants were treated with TNF/CHX or C_2-ceramide. Preliminary experiments indicate that Bcl-2 affords a better protection from C_2-ceramide than from TNF/CHX, suggesting as well that TNF- and C_2-ceramide-related death mechanisms diverge in these hepatoma cells.

The results obtained so far support the view that ceramide has only a minor role in the death of TNF-treated HTC cells and that the protective effect of desipramine is possibly related to an action on the endo-lysosomal compartment.

Role of reactive oxygen and nitrogen species in stimulation of inflammatory response and Fas mediated programmed cell death of polymorphonuclear leukocytes in epidemic dropsy patients

Ch.Kishore Babu[1], Satyanand Patel[2], Madhu Dixit[2] and Mukul Das[1]

[1]*Food Toxicology Laboratory, Industrial Toxicology Research Centre, Po Box#80, M.G. Marg, Lucknow-226001, India*

[2]*Pharmacology Division, Central Drug Research Institute, Post Box #173, Chattar Manzil Palace, Lucknow 226001, India*

Epidemic Dropsy is an acute food adulterant toxic syndrome caused by the consumption or body massage of mustard oil adulterated with argemone oil (AO) even for short duration. Interconvertible alkaloids, sanguinarine and dihydrosanguinarine, are the toxic etiological agents present in AO. Our earlier studies have shown that consumption of mustard oil adulterated with AO causes oxidative stress in the plasma of dropsy patients. Since, polymorphonuclear leukocytes (PMNs) are recognized to play an important role in host defense by producing reactive oxygen and nitrogen species (ROS, RNS) during phagocytosis and their migration from blood to inflammatory tissues following noxious insult, the present study was aimed to investigate the generation of ROS/RNS in the stimulation of inflammatory mediators and the course of molecular events associated with apoptosis of PMNs in dropsy patients during March 2005 outbreak at Lucknow, Uttar Pradesh, India. Superoxide and nitrite radical formation in PMNs was found to be increased significantly ($P<0.05$) in dropsy patients (137-138%). Dichlorofluorescein mean fluorescence for H_2O_2 measurement was significantly enhanced in resting, arachinidonic acid and *E.Coli* stimulated PMNs of patients (103-429%). Increased activities of superoxide dismutase and glutathione peroxidase (47-79%) and decreased in activities of catalase (56%) and glutathione reductase (57%) were noticed in dropsy patients. Lipid and protein oxidation in PMNs (159-161%), nitrotyrosine formation in plasma proteins and 8-OHdG excretion in urine (133-187%) were found to be enhanced along with concomitant depletion of glutathione (GSH) levels (67%) in PMNs of patients. Enhancement of phagocytosis (107%) in PMNs and elevation of interleukin-6, interleukin-8 and tumor necrotic factor-α (107, 406 & 68%) in plasma were observed in patients. In addition, apoptosis of PMNs was significantly enhanced (1.47 folds) which may be related to the caspases pathway as caspases 3, 8 and 9 activities were dramatically enhanced in patients (2.03-3.6 folds). Enhancement of caspase 3 activity may also be responsible for DNA fragmentation (119%) along with diminished (42%) total antioxidant capacity (TAC) in plasma. Enhanced production of ROS and RNS in PMNs of dropsy patients may cause depletion of antioxidants including GSH and TAC, which may cause damage to PMNs macromolecules including lipids, proteins and DNA. Enhancement of caspase 8 and other sequential events in PMNs of patients suggests that AO induces apoptosis via Fas mediated pathway and that enhanced secretion of inflammatory mediators may be responsible for pulmonary edema and the other cardiac manifestations as observed in dropsy patients.

Activation of caspase-8 by Tumor Necrosis Factor Receptor 1 (TNFR1) is necessary for OGD-mediated apoptosis in cultured cortical cells

Nahuai Badiola, Cristina Malagelada, Juan Hidalgo, Josefa Sabriá and José Rodríguez-Alvarez

Instituto de Neurociencias, Universidad Autónoma de Barcelona, 08193 Cerdanyola del Valles, Barcelona, Spain, e-mail: nahuai.badiola@uab.es

Several evidences indicate that apoptosis is involved in ischemia-mediated neuronal damage. By using the oxygen and glucose deprivation (OGD) model in cortical neurons cultures, we had previously described that 50% of death was apoptotic (Malagelada *et al*., 2005). Here we show that blocking TNF-α action with an antibody against this cytokine produced a significant inhibition of OGD-mediated caspase-3 activation and chromatin condensation. Accordingly, we have observed an increase in TNF-α release by OGD. On the other hand, treatment with TNF-α did increase caspase-8 activity in cortical cultures. Similar activation of caspase-8 was observed in OGD-treated cultures. However, we did not observe caspase-8 activation when anti-TNF-α antibody was added to OGD-treated cultures, indicating that TNF-α mediated caspase-8 activation. A time-course analysis of caspase-8 and -3 activities demonstrates that caspase-8 is activated earlier than caspase-3. Moreover, selective inhibition of caspase-8 did reduce OGD-mediated caspase-3 activation and cell death, indicating that caspase-8 activation is determinant for a later proteolysis of caspase-3. Next we wanted to know which TNF-α receptor was involved in its pro-apoptotic effect in OGD. Studies in cortical cultures obtained from TNF-α receptor 1 knock-out mice show that in TNFR1 knock-out cultures there is no activation of caspase-8 after OGD. Also, a clear decrease in OGD-mediated caspase-3 activity was observed in TNFR1 knock-out cultures. In summary, we show that OGD-mediated apoptosis is mainly mediated by TNF-α through TNFR1 and caspase-8 activation.

Mechanisms of cell death induced by adenoviral E1A deletion mutants in ovarian cancer

S.K. Baird, J.L. Aerts, N.R. Lemoine, I.A. McNeish

Molecular Oncology Unit, Cancer Research UK Clinical Centre, Barts and The London School of Medicine and Dentistry, Charterhouse Square, London EC1M 6BQ, UK, sarah.baird@cancer.org.uk

Selectively replicating adenoviral mutants have great therapeutic potential in ovarian cancer. Vectors deleted in E1A CR2 are able to replicate selectively within malignant cells and can extend survival substantially in human ovarian carcinoma xenograft models in mice. A greater understanding of the mode of cell death induced could allow viral efficiency to be improved further. Adenoviral cell death was long thought to utilise a classical apoptotic mechanism, since viral proteins such as E1B-19K interact with the apoptotic machinery. We transfected IGROV1 and OVCAR4 ovarian carcinoma cell lines with the vectors *dl*922-947 and *dl*CR2 and found low levels of early caspase-3 and PARP cleavage, whilst cell death was not enhanced by co-expression of Smac/DIABLO. The general caspase inhibitor zVAD.fmk caused only a small decrease in levels of cell death, but mitochondrial membrane integrity was breached concurrently with propidium iodide uptake, suggesting mitochondrial involvement. However, inhibition of the mitochondrial permeability transition with cyclosporin A caused only a very modest reduction in viral cytotoxicity. As the natural viral life cycle involves driving the infected cell into S phase followed ultimately by lysis, our finding of very little hypodiploid DNA and minimal levels of Annexin V exposure may simply reflect this. Co-treatment with general cysteine and aspartic protease inhibitors E64 and pepstatin suggested that cathepsins do not play an important role. The addition of virus appeared to stabilise the lysosomal membrane and initially increase the number or size of lysosomes. These carcinoma cell lines were less susceptible to oxidative stress-driven cell death than other ovarian cell lines. Increased mitochondrial permeability may increase levels of autophagy, in cells with an endo-lysosomal system already stimulated by the uptake of virus. Combinations with cisplatin and paclitaxel showed true synergy *in vitro* and *in vivo* experiments are ongoing. Adenovirus-mediated cell death may occur via a programme that involves some apoptotic and some autophagic features, which would be helpful in increasing the efficiency of therapies against ovarian carcinomas that have developed resistance to apoptosis.

Endoplasmic reticulum-dependent apoptosis in acetaminophen-induced liver injury

Gábor Nagy[a], Lívius Wunderlich[a], András Szarka[b], Gábor Bánhegyi[a], and József Mandl[a]

[a]*Hungarian Academy of Sciences - Semmelweis University, Endoplasmic Reticulum Research Group, and* [b]*Budapest University of Technology and Economics, Dept. of Biochemistry and Food Technology, Budapest, Hungary*

Overdosage of the antipyretic acetaminophen (AAP) leads to acute hepatocellular injury. Glutathione depletion and oxidative challenge both have pivotal roles in the pathomechanism. The endoplasmic reticulum (ER) via its phase I & II enzymes of biotransformation plays a direct role in metabolizing AAP. This, together with the fact that ER functions are sensitive to redox imbalance, led to the presumption that ER-dependent non-mitochondrial apoptotic pathways might be initiated upon AAP overdose. We, therefore, investigated alterations in ER redox status and important markers of ER stress in the early phase of AAP-induced liver injury in male CD-1 mice. Microsomal free reduced glutathione decreased by 90% after AAP administration. AMS-labeling of thiol-groups of ERp72, a PDI family oxidoreductase showed that ERp72 is present only in its oxidized form after AAP-treatment. CHOP/GADD153 and the ER-associated procaspase-12 were found to be robustly induced 3 hours after the treatment. Interestingly, cleavage (activation) of caspase-12 was much less evident than its induction. Another ER stress-responsive protein, the activating transcription factor 6 (ATF6), was activated upon AAP-treatment, as was shown by immunoblot detection of its 50 kDa cleaved fragment. On the contrary, when examining expression of ER-resident chaperones (protein disulfide isomerase, ERp72, GRP78, GRP94) we found no induction upon AAP-treatment. Caspase-3, a downstream caspase with effector function, was also found to be cleaved. The Fas-ligand-induced caspase-8, on the other hand, was not activated upon AAP-administration. Our in vivo data suggest that AAP-treatment causes redox imbalance in the ER lumen. The ER stress response is then initiated with the dominance of pro-apoptotic factors. However, the apoptotic scenario is masked by necrotic events.

TRAIL is Required for Virus Elimination in Chronic HCV Infection

Xandra Volkmann[1], Michael P. Manns[1], Klaus Schulze-Osthoff[2], and Heike Bantel[1]

[1]Dept. of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, [2]Institute of Molecular Medicine University of Duesseldorf, Duesseldorf, Germany

Hepatitis C Virus (HCV) infection is characterized by chronic inflammatory liver injury and fibrotic alterations which is associated with the risk of developing liver cirrhosis and hepatocellular carcinoma. We have recently demonstrated that caspase activation is considerably elevated in chronic HCV infection and that caspase activation is required for a sustained response to antiviral therapy. However, the sustained response rate of patients with HCV genotype I to usual combination therapy with interferon and ribavirin is only about 50 % and we demonstrated that non-responding patients showed lower serum caspase activity. The molecular mechanisms of this unsufficient caspase activation and virus elimination are unknown. Since the TRAIL system has been implicated to play a pivotal role in the induction of apoptosis of virus-infected cells, we investigated the role of TRAIL in chronic HCV infection. We first analyzed mRNA and protein expression of TRAIL and TRAIL receptors in healthy and HCV-infected liver by RT-PCR and immunohistochemistry. We could show that compared to healthy liver tissue TRAIL as well as TRAIL-R1 and TRAIL-R2 are significantly upregulated in HCV-infected liver. Compared to untreated HCV-infected liver biopsies, treatment of HCV-liver with different versions of recombinant human TRAIL considerably increased caspase activity as measured by a luminometric enzyme assay. To investigate whether TRAIL is required for HCV clearance, TRAIL activity was measured by ELISA in sera from HCV-infected patients either responding or non-responding to antiviral therapy. In patients responding to antiviral therapy TRAIL activity closely correlated with virus elimination following interferon treatment. Responding patients showed significantly higher Interferon-induced TRAIL activity levels in serum compared to non-responding patients. Thus, patients who did not respond to antiviral therapy might fail an effective TRAIL-induced caspase activation and therefore apoptosis of virus-infected hepatocytes. These data open new insights into the molecular mechanisms of HCV clearance and the unresponsiveness to HCV therapy. Measurement of TRAIL activity in sera of patients with chronic HCV-infection might provide a diagnostic tool for early evaluating the efficacy of antiviral therapy.

Alphavirus and apoptosis: The apoptotic pathways triggered in mammalian cells as a response to Semliki Forest virus infection.

Gerald M. Barry and John K. Fazakerley

Centre for Infectious Diseases, College of Medicine and Veterinary Medicine, University of Edinburgh, Edinburgh, UK

In a multicellular organism, virally infected cells have a predilection towards undergoing apoptosis. In response to virus infection, this reaction may be viewed as an arm of the innate immune system. This can be seen as a utilitarian act if the cell succeeds in committing suicide before viral particles can be released. Its effectiveness is underlined by the existence of many anti-apoptotic strategies that are employed by large DNA viruses to combat this defence mechanism.

Semliki Forest virus (SFV) is in the *Alphavirus* genus of the *Togaviridae* family. It is closely related to Chikungunya virus, Eastern, Western and Venezuelan equine encephalitis and serves as a small animal model to study the pathogenesis of encephalitis. Mammalian cells infected with SFV, rapidly undergo cell death. The virus infection is detected by a number of different cellular defence proteins which can induce an inflammatory response, the establishment of an anti-viral state and the initiation of cell death in the infected cell.

Using a variety of methods including wst-1 assay, colourimetric caspase activation assays and western blot analysis as well as genetically modified forms of the Semliki Forest virus, a pathway and time course of cell death has been established. Cell death is shown to be by apoptosis and involves endoplasmic reticulum stress and the intrinsic pathway of caspase activation.

Overexpression of both catalytically active and -inactive cathepsin D by cancer cells enhances apoptosis-dependent chemo-sensitivity

Mélanie Beaujouin[1], Stephen Baghdiguian[2], Guy Berchem[3], Emmanuelle Liaudet-Coopman[1]

[1]INSERM U540 'Molecular and Cellular Endocrinology of Cancers', University of Montpellier 1, France.
[2]CNRS UMR 5554, University of Montpellier 2, France.
[3]CRP-Santé, Laboratory of Experimental Hemato-Cancerology, Luxembourg.
E-mail: beaujoin@montp.inserm.fr; liaudet@montp.inserm.fr

The aspartic protease cathepsin D (cath-D) is a key mediator of induced-apoptosis and its proteolytic activity has been generally involved in this event. During apoptosis, cath-D is translocated to the cytosol. Since cath-D is one of the lysosomal enzymes which requires a more acidic pH to be proteolytically-active relative to the cysteine lysosomal enzymes such as cath-B and -L, it is therefore open to question whether cytosolic cath-D might be able to cleave substrate(s) implicated in the apoptotic cascade. Here we have investigated the role of wild-type cath-D and its proteolytically-inactive counterpart over-expressed by 3Y1-Ad12 cancer cells during chemotherapeutic-induced cytotoxicity and apoptosis, as well as the relevance of cath-D catalytic function. We demonstrate that wild-type or mutated catalytically-inactive cath-D strongly enhances chemo-sensitivity and apoptotic response to etoposide. Both wild-type and mutated inactive cath-D are translocated to the cytosol, increasing the release of cytochrome c, the activation of caspases-9 and -3 and the induction of a caspase-dependent apoptosis. In addition, pre-treatment of cells with the aspartic protease inhibitor, pepstatin A, does not prevent apoptosis. Interestingly therefore, the stimulatory effect of cath-D on cell death is independent of its catalytic activity. Overall, our results imply that cytosolic cath-D stimulates apoptotic pathways by interacting with a member of the apoptotic machinery rather than by cleaving specific substrate(s).

Effect of the anthracycline Sabarubicin on the mitochondrial compartment of the human ovarian tumor cell line A2780

Daniela Bellarosa, Federica Nardelli, Alessandro Bressan, Mario Bigioni, Giuseppe Sacco, Stefano Manzini, Monica Binaschi.

Cell Biology and Molecular Cell Biology Laboratory, Department of. Pharmacology, Menarini Ricerche S.p.A, Pomezia, Italy

Mitochondria are central integrating organelles in apoptosis: they house approximately 1000 proteins that are mainly encoded by nuclear DNA; mitochondrial DNA encodes only 13 proteins including four enzymes complexes of the respiratory chain. In some studies, mitDNA seems to have an important role for cell death triggered by some antitumoral drugs.
Genotoxic stress induced by some chemotherapeutic drugs such as anthracyclines has been shown to activate apoptosis in a number of different cell types. Following DNA damage, anthracyclines activate nuclear events, and more late mitochondrial dysfunctions, whose kinetics is peculiar of this class of antitumoral agents. However, the effective contribution of some mitochondrial alterations to apoptotic cell death induced by anthracyclines and their correlation with nuclear signalling remains to be elucidated.
In this study our aim was to evaluate the effect of the new anthracycline Sabarubicin on the mitochondrial compartment of A2780 ovarian tumor cells. Experiments of drug uptake showed a significative accumulation of Sabarubicin into the mitochondrial fraction. Further Sabarubicin proved to cause DNA strand breaks in mitochondrial DNA (mtDNA). However an unspecific blockade of mitochondrial genes transcription was not observed. To further investigate the role of mitochondrial genome in the apoptotic pathway, we selected A2780 cells depleted of mtDNA (rho°). We found that A2780 rho° were as sensitive as A2780 wt to Sabarubicin induced apoptosis. Nevertheless, Sabarubicin evoked alterations of the mitochondrial membrane potential (early hyperpolarization followed by a late depolarization) both in A2780 wt and in A2780 rho°. The hyperpolarization event precedes, with a peak at 18 hours, the onset of apoptosis induced by Sabarubicin.
Together, these data suggest that Sabarubicin affects mitochondria altering their DNA and membrane potential, but a direct link between mitochondrial DNA damage and apoptosis cannot be demonstrated.

Acute apoptosis by cisplatin requires induction of reactive oxygen species but is not associated with damage to nuclear DNA

Maria Berndtsson, Maria Hägg, Theocharis Panaretakis, Aleksandra Mandic Havelka, Maria C. Shoshan and Stig Linder

Cancer Center Karolinska, Department of Oncology and Pathology, Karolinska Institute and Hospital, S-171 76 Stockholm, Sweden

Cisplatin is a broad-spectrum anticancer drug that is also widely used in experimental studies on DNA damage-induced apoptosis. Induction of apoptosis within 24 – 48 hours requires cisplatin concentrations that are at least one order of magnitude higher than the IC_{50}. We here show that such high, apoptosis-inducing cisplatin concentrations induce cellular superoxide formation, and that apoptosis is inhibited by superoxide scavengers. The same concentration limit and the requirement for superoxide are true also for induction of caspase activation in enucleated cells (cytoplasts), showing that cisplatin-induced apoptosis occurs independently of nuclear DNA damage. In contrast, lower cisplatin concentrations, which do not induce acute apoptosis, are sufficient for induction of DNA damage signaling. We propose that the anti-proliferative effects of cisplatin at IC_{50} doses involve premature senescence and secondary, non-stress induced apoptosis. The higher doses currently used in *in vitro* studies lead to acute, stress-induced apoptosis which involves induction of superoxide but is largely DNA damage-independent.

Protection from apoptosis, arrest at the G_2/M checkpoint and stimulation of DNA repair are main mechanisms underlying drug resistance induced by the *BCR/ABL* oncogene

Artur Slupianek[1], Grazyna Hoser[2], Ireneusz Majsterek[3], Janusz Blasiak[3] and Tomasz Skorski[1]

[1]Temple University, School of Medicine, Department of Microbiology and Immunology, Philadelphia, PA 19140, USA; [2]Medical Center for Postgraduate Education, Department of Clinical Cytobiology, 01-813 Warsaw, Poland; 3University of Lodz, Department of Molecular Genetics, Banacha 12/16, Poland, e-mail januszb@biol.uni.lodz.pl

Drug resistance is one of the main obstacle of anticancer therapy, therefore understanding its mechanisms may be of importance in fighting cancer. BCR/ABL is a fusion tyrosine kinase present in chronic myelogenous leukemia and reported to induce therapeutic drug resistance. Since apoptosis and DNA repair are fundamental cellular reactions to the DNA-damaging agents, they are expected to be involved in therapeutic resistance. We used murine myeloid and lymphoid cell lines and their BCR/ABL-transformed clones, as well as human leukemic cells expressing BCR/ABL, which were treated with cisplatin, idarubicin, mitomycin C and gamma radiation. All BCR/ABL-expressing cells displayed resistance to drugs and radiation. Treated BCR/ABL+ cells expressed increased levels of anti-apoptotic protein Bcl-xL, displayed decreased extent of apoptosis-related DNA fragmentation and lower activity of caspase-3, compared to BCR/ABL-cells. BCR/ABL-expressing cells repaired also damage to their DNA with higher efficacy and displayed G2/M cell cycle arrest upon drug or radiation treatment. BCR/ABL-transformed cells expressed enhanced level of RAD51, which is essential for the repair of double-strand DNA breaks. Therefore, protection from apoptosis by the increased expression of Bcl-xL, RAD51-dependent stimulation of DNA repair and arrest at the G2/M checkpoint may underline BCR/ABL-induced resistance in leukemic cells.

Defective Apoptosis of Peripheral Blood Lymphocytes in Hyper-IgD and Periodic Fever Syndrome

Evelien J. Bodar[1], Jeroen C.H. van der Hilst[1], Waander van Heerde[2], Jos W.M. van der Meer[1], Joost P.H. Drenth[3], Anna Simon[1]

Department of [1]General Internal Medicine, [2]Haematology and [3]Gastroenterology, Division of Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Introduction: Hereditary periodic fever syndromes are characterized by incapacitating attacks of fever and generalized inflammation. Three of these syndromes are the hyper-IgD syndrome (HIDS), caused by mutations in the mevalonate kinase gene, TNF-Receptor Associated Periodic Syndrome (TRAPS), caused by mutations in the type 1 TNF receptor gene and Familial Mediterranean Fever (FMF), caused by mutations in the MEFV gene encoding pyrine. Their pathogenesis remains unclear.
Aim of the study: To investigate apoptosis in periodic fever patients as a possible pathogenic factor.
Methods: We measured anisomycin-induced apoptosis with annexin V flowcytometry in peripheral blood lymphocytes from symptom-free patients with HIDS (n=7), TRAPS (n=7) and FMF (n=2).
Results and conclusion: HIDS lymphocytes showed a decreased percentage of apoptosis during remission compared to controls (12 vs 50%, p=0,0023), whereas no difference was observed in TRAPS or FMF lymphocytes. This decreased apoptosis of lymphocytes may be a central pathogenic mechanism in HIDS since dysfunction of one of the inhibitory mechanisms to curtail the immunological response to trivial insults could lead to the excessive generalized inflammation seen during a HIDS attack.

Survivin splice variant roles in caspase-3 dependent apoptosis

Romain Boidot[1,2], Frédérique Vegran[1,2], Arlette Hamman[2], Anabelle Sequeira-Legrand[2], Eric Solary[2] and Sarab Lizard-Nacol[1]

[1]Molecular Genetics Laboratory, Centre Georges-François LECLERC, 1, rue du Professeur Marion, 21079 Dijon, France, e-mail: r-boidot@club-internet.fr
[2] INSERM U517, Faculté de Médecine, 7, boulevard Jeanne d'Arc, 21000 Dijon, France

Survivin is one of the Inhibitor of Apoptosis Proteins family members, and is active, at least in part, by inhibition of Caspase-3 activity. *Survivin* gene encodes four protein variants: Survivin-ΔEx3, Survivin-3B, Survivin-2B and Survivin-2α. Some studies have indicated that Survivin, Survivin-ΔEx3 and Survivin-3B have anti-apoptotic activities, whereas Survivin-2B and Survivin-2α have pro-apoptotic properties. However, the influence of these proteins was not compared in caspase-3 dependent apoptosis.

In this study, we analyzed the role of each variant in response to etoposide that induced a caspase-3 dependent apoptosis. Each GFP-coupled variant was stably transfected in HBL100 mammary cancer cell line, known to have an active caspase-3 protein. The transfection efficiency was checked by GFP-specific PCR and by flow cytometry. The overexpression of each variant was quantified by real-time quantitative PCR. Cells were treated 48 hours with 1.5 µg/ml of etoposide. The treatment-induced apoptosis was assessed by flow cytometry and compared with parental cells.

Etoposide treatment induced 35% of apoptosis in control parental cells, 30% in Survivin transfected cells, and 32% in Survivin-3B one's. In contrast, transfection with Survivin-ΔEx3 or Survivin-2B induced 54% and 41% of apoptosis, respectively.

Thus, while Survivin and Survivin-3B have no effect on etoposide-induced apoptosis, Survivin-ΔEx3 and Survivin-2B have pro-apoptotic activity in response to etoposide. These preliminary results showed that Survivin variants have differential apoptotic activities in response to etoposide, and indicated for the first time that Survivin-ΔEx3 variant may act as pro-apoptotic protein.

Stable transfections of the five Survivins are currently performed in MCF-7 and MCF-10A cells. These results will be completed with Survivin-2α and confirmed on MCF-7 and MCF-10A transfected cells. Other drugs will be tested such as vinblastine, cyclophosphamide, and 5-fluorouracil on all transfected cells. The study of possible interactions between each protein variant and caspase-3 will be also performed.

"Lethal kiss" on a dielectrophoresis (DEP) based Lab-on-a-chip device: two-color real-time determination of cytotoxic T-lymphocyte activity

Monica Borgatti[1,2], Mélanie Abonnenc[3], Enrica Fabbri[1], Irene Mancini[1], Riccardo Gavioli[1], Cinzia Fortini[1], Luigi Altomare[4], Nicolò Manaresi[3], Gianni Medoro[3], Aldo Romani[4], Marco Tartagni[4], Roberto Guerrieri[4] and Roberto Gambari[1,2]

[1]Department of Biochemistry and Molecular Biology, University of Ferrara, Italy; [2]ER-GenTech, Department of Biochemistry and Molecular Biology, University of Ferrara, Italy;
[3]Silicon Biosystems, Bologna, Italy; [4]Center of Excellence on Electronic Systems (ARCES), University of Bologna, Italy.

Cytotoxic T-lymphocytes (CTL) are of great importance for the control of the immune response against tumor onset and progression (1). It is well known indeed that CTL of cancer patients can exhibit the ability to lyse tumor cells. This feature is of great relevance for the development of immunotherapy of tumors. Accordingly, the analysis of the cytolytic activity of isolated CTL populations displaying cytotoxic activity against tumor cells is of great clinical impact, since the isolation of highly cytoxic CTL clones is a complex and long procedure needed of step by step validation of the enrichment achieved. In this respect, dielectrophoresis (DEP) based Lab-on-a-chip technology could represent a very appealing approach, since it allows the manipulating of large numbers of single cells or cell populations. Here we present two dielectrophoresis-based Lab-on-a-chip platforms useful for the analysis of CTL-mediated cell lysis. One platform (the SmartSlide) displays parallel electrodes and generated cylinder-shaped cages within which CTL and target cells are entrapped (2). The second platform (the DEParray) generates spherical DEP cages that can entrap clusters composed of several CTLs and a single (if present) target cell (3). We present a two-color staining approach to label target cells to the aim of a real-time determination of the cytotoxic activity of T-lymphocyte precursors in these two (DEP)-based Lab-on-a-chip platforms.
We demonstrate that these devices can be used to manipulate CTL clustered to target cells with the aim to develop an efficient methodology to quantify CTL-mediated cytolysis and identification of CTL clusters or clones exhibiting high cytotoxic activity. The proposed approach is not radioactive, fast and is suitable with the use of low numbers of target cells.

(1) Blair A. *et al.* Immunotherapeutic strategies in acute lymphoblastic leukaemia relapsing after stem cell transplantation. *Blood Rev.* 19, 289-300 (2005); (2) Medoro, G. *et al.* A Lab-on-a-chip for individual cell separation based on the moving-cages approach. *Eurosensor,* September (2002); (3) Manaresi, N. *et al*. A CMOS Chip for individual cell manipulation and detection. *IEEE Journal of solid-state circuits* 38 (12), 2297-2305 (2003).

Work supported by C.I.B., Fondazione Cariparo, MIUR-FIRB, MIUR-COFIN, Spinner, AIRC.

Dissecting the pro-apoptotic function of the caspase substrate HDAC4

Gabriela Paroni, Alessandra Fontanini, Carmela Foti, Nadia Cernotta, and Claudio Brancolini

Dip. Scienze e Tecnologie Biomediche Università degli Studi di Udine, p.le Kolbe 4 33100, Udine ITALY, email cbrancolini@makek.dstb.uniud.it

Caspases show the exquisite ability to target important regulators of the survival-death fate in order to enhance the death's verdict. Hence, caspases substrates are instrumental to understand the cellular pathways that govern survival and death. In principle caspase substrates can be used as picklocks to open the doors keeping secret important survival pathways. HDACs (histone deacetylase) are chief regulators of gene expression as part of transcriptional co-repressor complexes that influence chromatin remodelling. Among the different HDACs, HDAC4 is specifically target of caspase regulation during apoptosis. In healthy cells HDAC4 is under intense nuclear-cytoplasmic shuttling control. Under apoptosis caspase-2 and caspase-3 cleave HDAC4 and generate an amino-terminal fragment, which accumulates into the nuclei, where it acts as transcriptional repressor. When ectopically expressed the HDAC4 caspase-cleaved nuclear fragment induces cell death through a mitochondrial pathway of caspase activation, which is under the control of Bcl-2 and Akt.
Although HDAC4 has principally been studied in relation to muscle and chondrocyte differentiation, recent evidences indicate that this enzyme lies at the crossroad of different options, including the control of neuronal cell death. In this work we have investigated the proapoptotic function of HDAC4 with the aim to unveil import pro-survival pathways silenced by HDAC4. As a first step, different HDAC4 mutants and the strictly related enzymes HDAC5, were evaluated for the capability to accumulate in a signal-independent manner into the nucleus. In contrast to the wt, which is cytosolic, all the mutants generated and HDAC5, accumulated into the nuclei at similar levels. Moreover, even though they repressed transcription at similar extents, only the caspase-cleaved fragment and a point mutant in the NES (nuclear export sequence) induced apoptosis when ectopically expressed. We have noted that phosphorylation of the 14-3-3 binding sites in HDAC4 can modulate the apoptotic potential independently form the subcellular localization. FRAP experiments have shown that the dynamic of HDAC4-chromatin interaction is important in the activation of the cell death program. We have also attempted to clarify, which among the different transcription factors regulated by HDAC4, are important for cell survival. The pro-survival roles of MEF2C, Runx2 and SRF, three well-known partners of HDAC4 have been investigated by expressing the wt and the dominant negative forms or by expressing fusions with the transcriptional activation domain VP16. Finally, the ability of HDAC4 to repress Bcl-2 expression will also be discussed.

The role of calpains in Feline Calicivirus-induced apoptosis

Ben Brennan, Alessandro Natoni, Lisa O. Roberts and George E. N. Kass

School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, UK.
Email: g.kass@surrey.ac.uk / l.roberts@surrey.ac.uk

Caliciviruses are responsible for many important diseases of man and animals yet we still know relatively little about the molecular mechanisms of pathogenesis, especially the events involved in cell damage. Noroviruses cause gastroenteritis or "projectile vomiting" in humans and outbreaks are often seen in hospitals, cruise ships and in military camps, while feline calicivirus (FCV) causes cat 'flu'. Our previous work has shown that FCV infection of cells induces apoptosis[1]. Furthermore, we have shown that apoptosis proceeds through the mitochondrial pathway, involving bax translocation to the mitochondria, cytochrome *c* release and a drop in mitochondrial membrane potential[2].

In attempting to find out what triggers these events, our recent data showed that the removal of extracellular calcium through use of the chelator EGTA prevented the induction of apoptosis. This led us to hypothesise that there is a role for calcium and possibly the calpains in FCV-induced apoptosis. A role of calpains in FCV-induced apoptosis was suggested by the inhibitor N-acetyl-Leu-Leu-Nle-CHO (ALLN) which prevented phosphatidyl serine exposure and cell death. Investigation into the mechanism revealed that calpain activation as demonstrated by the cleavage of fodrin and Suc-Leu-Leu-Val-Tyr-AMC preceded caspase-3 activation. Moreover, ALLN inhibited cytochrome *c* release from the mitochondria.

We therefore propose that calpains pay a key role in the early apoptotic events occurring after infection with FCV. The activation of the calpains is calcium dependent and occurs prior to the activation of the downstream events such as cytochrome *c* release from the mitochondria and the activation of the effector caspases-3 and -9.

[1]Al-Molawi, N., Beardmore, V.A., Carter, M.J., Kass, G.E.N., and Roberts, L.O., Caspase-induced cleavage of the feline calicivirus (FCV) capsid protein during apoptosis of infected cells. J. Gen. Vir. 84, 1237-1244, 2003.

[2]Natoni, A., Kass, G.E.N., Carter, M.J., and Roberts, L.O., The mitochondrial pathway of apoptosis is triggered during feline calicivirus infection. J. Gen. Vir. 87, 357-361, 2006.

CD95L-independent activation-induced cell death of primary lymphocytes is mediated by cleaved HPK1

Dirk Brenner[1], Alexander Golks[1,3], Mareike Becker[1], Friedemann Kiefer[2], Peter H. Krammer[1], and Rüdiger Arnold[1*]

[1]Tumor Immunology Program, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany
[2]Max-Planck-Institute for Molecular Biomedicine, Von-Esmarch-Strasse 56, D-48149
Münster, Germany
[3]Present Address: Novartis Pharma AG, 4002 Basel, Switzerland

T cell receptor stimulation in activated T cells induces activation-induced cell death (AICD) via the extrinsic cell death pathway involving death receptors, like CD95 (Fas/Apo-1). In addition, primary lymphocytes can also use the intrinsic cell death pathway, which depends on the mitochondria. The molecular mechanism controlling the ratio between extrinsic and intrinsic cell death pathway in AICD is not clear. In primary lymphocytes we find that cleaved hematopoietic progenitor kinase 1 (HPK1) can induce AICD via the intrinsic cell death pathway independent of CD95. During expansion of primary T cells HPK1 is cleaved by a non-apoptotic caspase-3 resulting in release of HPK1-C. AICD of primary T cells depends on HPK1-C, which suppresses induction of anti-apoptotic Bcl-2, Bcl-X_L and Bcl-2A1 and leads to cell death execution by pro-apoptotic Bcl-2 family members, e.g. Bim. Consequently, T cells of HPK1-C transgenic mice show increased AICD *in vivo*. Our results define HPK1-C as a novel physiological regulator between the extrinsic and the intrinsic cell death pathway in activated primary lymphocytes.

Overexpression of $a_v b_3$ integrin protects human laryngeal carcinoma cell line from cisplatin-induced apoptosis

Anamaria Brozovic[1], Dragomira Majhen[1], Jochen Zisowsky[2], Sanjica Jakopec[1], Maja Osmak[1], Andreja Ambriovic-Ristov[1]

[1]*Division of Molecular Biology, Ruder Boškovic Institute, Bijenicka 54, 10000 Zagreb, Croatia, e-mail:brozovic@irb.hr*
[2]*Institut of Pharmacy, An der Immenburg 4, 53121 Bonn, Germany*

Drug resistance of tumor cells is the major obstacle for successful treatment of cancer patients. Different molecular mechanisms have been recognized as the cause of resistance: reduced drug accumulation, increased drug inactivation, increased ability to repair and/or tolerate DNA lesions, altered activity of nuclear enzymes and inhibition of apoptosis. Recently a novel mechanism of multidrug resistance has been identified that is based on integrin-mediated adhesion to extracellular matrix. As a model for determination of the role of $\alpha_v\beta_3$ integrin in drug resistance, we used $\alpha_v\beta_3$ integrin negative human laryngeal carcinoma cell line (HEp2), and three HEp2-derived cell clones with graded expression of $\alpha_v\beta_3$ integrin. We showed that $\alpha_v\beta_3$ integrin expression protects cells from cisplatin induced apoptosis. Using western blot analysis we determined increased expression of Bcl-2 protein in HEp-$\alpha_v\beta_3$ integrin expressing cells in comparison to HEp2 cells. In addition, we found increased level of glutathione (GSH) in HEp2-$\alpha_v\beta_3$ integrin expressing cells as compared to HEp2 cells. However, there was no significant difference in the extent of platination between HEp2 cells and HEp2-$\alpha_v\beta_3$ integrin expressing cells. Pre-treatment of HEp2-$\alpha_v\beta_3$ integrin expressing cells, with specific inhibitor of glutathione synthesis, buthionine sulfoximine (BSO), decreased level of GSH and abrogated integrin-mediated cisplatin-resistance to the level observed for HEp2 cells. However, the BSO treatment did not influence the expression of Bcl-2. To determine the involvement of Bcl-2 in resistance we established several clones from HEp2 with increased expression of Bcl-2. Increased expressions of Bcl-2 did not conferee cisplatin resistance nor induce increased level of GSH. Therefore, our results suggest that $\alpha_v\beta_3$ integrin mediated cisplatin resistance in HEp2 cells is dependent on increased GSH level, which contributes to cell survival by mechanisms independent of cisplatin inactivation or inhibition of DNA adduct formation. Since the up-regulation of $\alpha_v\beta_3$ integrin has been found in cisplatin-resistant cells, obtained by repeated cisplatin treatment of HEp2 cells, this phenomenon may be one of the mechanisms of resistance development.

Oxidative stress by ascorbate/menadione association kills K562 human chronic myelogenous leukaemia cells and inhibits its tumour growth in nude mice.

Verrax Julien, Stockis Julie, Tison Aurélie, Taper Henryk and Buc Calderon Pedro

Unité de Pharmacocinétique, Métabolisme, Nutrition et Toxicologie; Faculté de médecine, Université Catholique de Louvain, Belgium, calderon@pmnt.ucl.ac.be

Cancer cells usually exhibit a poor antioxidant status, thus raising the possibility to kill cancer cells through an oxidative stress. To do that, we used a synergistic association of ascorbate and menadione. Actually, menadione is reduced by ascorbate to form the dehydroascorbate and the semiquinone free radicals. The semiquinone radical is rapidly reoxidized to its quinone form by molecular oxygen thus generating reactive oxygen species (ROS) among them hydrogen peroxide (H_2O_2) likely to be the major oxidizing agent involved in the cytolytic process. The effect of this oxidative stress was therefore examined in K562 cells, a human erythromyeloid leukaemia cell line. The results show that ascorbate enhances menadione redox cycling, leading to the formation of intracellular reactive oxygen species (as shown by dihydrorhodamine 123 oxidation). The incubation of cells in the presence of both ascorbate/menadione and aminotriazole, a catalase inhibitor, resulted in a strong decrease of cell survival, reinforcing the role of H_2O_2 as the main oxidizing agent killing K562 cells. This cell death was not caspase-3 dependent. Indeed, neither procaspase-3 nor PARP were processed and only a weak cytochrome c release was observed. Moreover, we observed only 23 % of cells with depolarized mitochondria. In ascorbate/menadione-treated cells, DNA fragmentation was observed without any sign of chromatin condensation (DAPI and TUNEL tests). The cell demise by ascorbate/menadione is consistent with a necrosis-like cell death confirmed by both cytometric profile of annexin-V/propidium iodide labeled cells and by light microscopy examination. Finally, we showed that a single i.p. administration of the association of ascorbate and menadione is able to inhibit the growth of K562 cells by about 60 % (in both tumour size and volume) in an immune-deficient mice model. Taking together, these results reinforced our previous claims about a potential application of the ascorbate/menadione association in cancer therapy.

Glucocorticoid-induced apoptosis in ALL cells is mediated through lowered glucose uptake and utilization

Eva Buentke (1), Edward Laane (1), Katja Pokrovskaja (1), Claes-Göran Östensson (2), Anna Porwit-MacDonald (1) and Dan Grandèr (1)

(1) Department of Oncology-Pathology, Karolinska Institute, 171 76 Stockholm, Sweden
(2) Department of Molecular Medicine, Karolinska Institute, 171 76 Stockholm, Sweden
E-mail: eva.buentke@ki.se

Glucocorticoids (GCs) are central in treatment of leukemia, and the initial response to these substances is also predictive of the response to other treatments. GCs induce apoptosis in lymphocytes through activation of pro-apoptotic Bcl-2 family proteins Bak and Bax, down-regulation of anti-apoptotic Bcl-2 and Bcl-xL, and eventually loss of mitochondrial membrane potential. The detailed mechanism of GC-induced apoptosis is, however, not fully established. The present study aimed at investigating upstream signaling pathways that mediate GC-induced programmed cell death. We showed that the GC dexamethasone affect both glucose uptake and glucose utilization, and thus cellular bioenergetics, in a pre-B ALL cell line. To assess if this contributes to GC-induced cell death we used di-methylsuccinate, a substance that feeds into the Krebs cycle, and theoretically should substitute for the lowered glycolysis in GC-treated cells. Interestingly, di-methylsuccinate rescued cells from GC-induced cell death by inhibiting Bcl-2 and Bcl-xL downregulation, and Bak and Bax activation, thus leading to reduced annexin V positivity and reduced loss of mitochondrial membrane potential. Using a GC-resistant cell line, we found that the effect of dexamethasone on glucose uptake and utilization correlates to the cells sensitivity to GC-induced cell death. Further, we found that dexamethasone lowers the cell-surface expression of glucose transporters.
In conclusion, these studies will assist in the identification of novel signaling pathways induced by GCs, connecting cellular metabolism and induction of cell death. Importantly, increased detailed knowledge of the signaling pathways involved will help to define reasons for GC resistance in ALL cells.

A novel plant toxin, persin, with *in vivo* activity in the mammary gland, induces Bim-dependent apoptosis in human breast cancer cells

Alison J. Butt[1], Caroline G. Roberts[1], Tracy Y.E Liaw[2], Maria Kavallaris[2], Colin K.W. Watts[1] and Robert L. Sutherland[1]

[1]Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, New South Wales, Australia and [2]Children's Cancer Institute Australia for Medical Research, Randwick, New South Wales Australia

Lactating livestock browsing avocado leaves develop a non-infectious mastitis and loss of milk production, associated with necrosis of the secretory epithelium of the mammary gland. Similar lesions occur in the mammary glands of lactating mice fed dried avocado leaves, and this model was used to isolate the active toxin, persin. This *in vivo* specificity for mammary epithelial cells suggests that persin could be developed as a novel therapeutic for breast cancer. Here we have examined the effects of persin on the growth of human breast cancer cells *in vitro*.
MCF-7, T-47D and MDA-MB-231 were treated with increasing concentrations of persin (6.9 – 27.6 μM), then viable cell numbers determined. MCF-7 and T-47D cells were significantly growth inhibited ($p < 0.01$) compared to untreated controls. MDA-MB-231 cells were relatively resistant to these inhibitory effects. Flow cytometric analysis of cell cycle progression demonstrated a significant accumulation of MCF-7 and T-47D cells in G_2/M phase of the cell cycle compared to controls. This effect was associated with specific changes in cell cycle regulatory proteins.
Persin-treatment of MCF-7 and T-47D cells resulted in a significant decrease in survival and induction of caspase-dependent apoptosis compared to controls ($p < 0.001$). An induction in expression of the BH-3 only proapoptotic protein, Bim was observed in cells sensitive to persin with evidence that this is regulated transcriptionally by persin. Down-regulation of Bim expression with specific siRNA abrogated persin-induced cytochrome *c* release and induction of apoptosis. Bim mediates apoptosis following cytoskeletal stress, and persin-induced apoptosis was associated with specific changes in microtubules indicative of increased polymerisation. Extension of these studies to a panel of human breast cancer cell lines demonstrated that the apoptotic response to persin correlates with expression of Bim.
In summary, we have demonstrated that the inhibitory effects of persin in sensitive breast cancer cell lines are mediated *via* specific effects on cell cycle progression and an induction of Bim-dependent apoptosis. Further delineation of the mechanism of action of persin may lead to the development of new therapeutic targets with high specificity for breast cancers.

Funded by US Army Medical Research & Materiel Command (DAMD17-03-1-0668)

ApoJ/clusterin is a suppressor of the metastatic phenotype in neuroblastoma and a novel effector of apoptosis in immortalized and prostate cancer cells

Alessandro E. Caccamo[2], Giorgia Santilli[1], Rebekka Schwab[1], Neil J. Sebire[1], John Anderson[1], Maurizio Scaltriti[2], Saverio Bettuzzi[2] and Arturo Sala[1]

[1] Molecular Haematology and Cancer Biology Unit, Institute of Child Health, 30 Guilford street, London WC1N 1EH, UK; and [2] Dipartimento di Medicina Sperimentale, Sezione di Biochimica, Biochimica Clinica e Biochimica dell'Esercizio Fisico, Università degli Studi di Parma, Parma, Italy.
e-mail: saverio.bettuzzi@unipr.it and a.sala@ich.ucl.ac.uk

ApoJ/clusterin is an enigmatic hetero-dimeric protein found within cells, extracellular spaces and body fluids. Whilst it is generally accepted that ApoJ/clusterin is involved in the regulation of cell survival and apoptosis, controversy remains as to whether it is pro- or anti-apoptotic. Being expressed both in normal and tumour cells, we have investigated whether ApoJ/clusterin is expressed in primary neuroblastomas and whether it can affect neuroblastoma metastatic behaviour. In this study we have established, by immunohistochemical analysis of 100 neuroblastoma biopsies, that expression of the extracellular and cytoplasmic form is significantly associated with differentiating, localised disease, with the majority of undifferentiated, metastatic aggressive neuroblastomas showing absence of cytoplasmic or secreted ApoJ/clusterin expression. Instead, nuclear staining was associated with localised necrotic or apoptotic cells within tumour mass. Since cytoplasmic localisation of ApoJ/clusterin could be important in inducing differentiation of neuroblasts into ganglion cells, which are found most commonly in localised disease or neuroblastoma following chemotherapy, and considering that nuclear ApoJ/clusterin is pro-apoptotic, we have also studied the metastatic potential of neuroblastoma cells following up- or down-regulation of its expression. Exogenous over-expression of ApoJ/clusterin reduced the metastatic ability of neuroblastoma cells *in vitro* and *in vivo*. In contrast, siRNA depletion of ApoJ/clusterin enhanced invasive behaviour of neuroblastoma cells. These results suggest that ApoJ/clusterin is involved in suppressing neuroblastoma metastasis. In addition, we found that a nuclear form of clusterin accumulated by post-translational modifications in response to Ca^{2+} deprivation or by over-expression of a truncated form of its cDNA lacking the secretion signal led to cell growth inhibition and cell-detachment induced apoptosis (anoikis) of prostate epithelial cells. In conclusion, our study has established that ApoJ/clusterin is a suppressor of the metastatic phenotype suggesting that inducing its expression in neuroblastoma patients could potentially be of therapeutic value.

Selective targeting of cyclin D1 by bile acids influences their ability to modulate apoptosis in primary rat hepatocytes

R.E. Castro,[1,2] J.D. Amaral,[1] P.M. Borralho,[1] S. Solá,[1] B.T. Kren,[2] C.J. Steer,[2,3] C.M.P. Rodrigues[1]

[1]Centro de Patogénese Molecular, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal, Departments of [2]Medicine and [3]Genetics, Cell Biology, and Development, University of Minnesota Medical School, Minneapolis, Minnesota, USA

Ursodeoxycholic acid (UDCA) and its taurine-conjugated derivative, tauroursodeoxycholic acid (TUDCA), act as general modulators of cell death by inhibiting classic mitochondrial pathways of apoptosis. In addition, UDCA may also control cell cycle regulators such as members of the E2F-1/p53 pathway. In contrast, deoxycholic acid (DCA) induces apoptosis both *in vitro* and *in vivo*. The precise mechanistic pathways underlying bile acid effects are not fully understood. The aims of this study were to characterize specific cell cycle/death control targets regulated by bile acids, and determine their role in the modulation of apoptosis. Global gene expression of primary rat hepatocytes incubated with either vehicle or 100 µM UDCA or TUDCA for 24 h was determined using microarrays. Cell cycle protein levels were determined by immunoblot analysis of extracts from cells incubated with UDCA, TUDCA, DCA or DCA plus UDCA for 1-30 h. In functional studies, hepatocytes were either infected with an adenovirus vector expressing a *cyclin D1* gene or transfected with a cyclin D1 reporter plasmid, in the presence or absence of bile acids. Further, cells were transfected with a plasmid containing a Bax promoter sequence reactive to p53. Apoptosis was assessed by Hoechst staining. Microarray analysis indicated that both UDCA and TUDCA modulate cell cycle-related genes, including cyclin D1. Transcriptional activation and expression of cyclin D1 were decreased in cells treated with either UDCA or TUDCA, confirming microarray data, but not in cells exposed to DCA. Hydrophobic DCA resulted in increased levels of apoptosis, while UDCA and TUDCA were able to prevent it. Furthermore, cyclin D1 overexpression resulted in increased p53 transcriptional activation and apoptosis induced by DCA. In contrast, UDCA and TUDCA effects were reduced in cyclin D1 overexpressing cells. These results suggest that cyclin D1 is involved in DCA-induced apoptosis. More importantly, inhibition of cyclin D1 by UDCA and TUDCA modulates their ability to prevent apoptosis.

(Supported by POCTI/SAU-FCF/62479/04 from FCT, Portugal)

Daxx-Brg1/BAF complexes participate in the regulation of transcription and apoptosis

Lukas Cermak[1], Jan Svadlenka[1], Paolo Salomoni[2], Cristian Bellodi[2] and Ladislav Andera[1]

[1]Laboratory of Cell Signaling and Apoptosis, Institute of Molecular Genetics AS CR, Prague, Czech Republic; [2]Medical Research Council, Toxicology Unit, Leicester LE1 9HN, United Kingdom

Death domain-associated protein 6 (Daxx) was initially described as an adaptor in the intracellular Fas (CD95) signaling, that activates JNKs and apparently enhances Fas-induced apoptosis. Since then, Daxx became known as an adaptor/co-regulator of both apoptosis and transcription. Daxx is predominantly a nuclear protein, but in a response to various, mainly stress stimuli it can shuttle between the nucleus and cytoplasm. Besides its putative role in Fas-induced apoptosis, Daxx also participates in TGFβ- and stress-induced apoptosis (UV irradiation, reactive oxygen species). In contrary, massive apoptosis in mouse embryos with deleted Daxx suggests that during the development Daxx could have an anti-apoptotic function.

To elucidate a bit controversial function of Daxx we performed yeast two-hybrid screening for novel Daxx-interacting proteins and we obtained a set of mainly nuclear proteins with a potential role in transcription and chromatin remodelling. One of them is chromatin-remodelling ATPase Brg1 (Brahma-related gene 1, SMARCA4) which is an essential subunit of SWI/SNF chromatin remodelling complex. Brg1, closely related to Brahma (Brm) protein, could act as a tumour suppressor and is mutated in various cancer cell lines. Daxx interacts with Brg1 both in the yeasts, various tumor cell lines and primary fibroblasts. Concurrent expression with PML caused Daxx-mediated sequestration of Brg1 into PODs. After fractionation of HEK293FT nuclear extract on Superose 6 we could distinguish three different nuclear Daxx-containing multiprotein complexes, one with Brg1 and its associated factors (BAFs), the second with ATRX protein and the third complex with PML protein and HDAC1. In SW13 cells, which lack the expression of Brg1, both Daxx overexpression and down-regulation by siRNA inhibits Brg1-mediated transactivation of several genes. Brg1 apparently also cooperates with Daxx in UV-induced apoptosis of primary fibroblast and potentially participates together with Brg1 complex in fine tuning of p53-mediated signaling.

Mitochondria are involved in development of oxidative stress.

Boris V. Chernyak, Denis S. Izyumov, Konstantin G. Lyamzaev, Olga Y. Pletjushkina

A. N. Belozersky Institute, Moscow State University, 119992 Moscow, Russia
e-mail: bchernyak@yahoo.com

Mitochondria can be a source of (ROS) and a target of oxidative damage during oxidative stress. Treatment of HeLa cells with hydrogen peroxide initiates a delayed accumulation of reactive oxygen species (ROS). It is accelerated by inhibitors of the respiratory chain (piericidin, rotenone and myxothiazol) and inhibited by diphenyleneiodonium (an inhibitor of flavin enzymes), indicating that flavin of Complex I is involved in the ROS production. Mitochondria-targeted antioxidant (MitoQ) prevents accumulation of ROS suggesting that the site of ROS production is located in the matrix of mitochondria. Apoptosis caused by H2O2 is augmented by the inhibitors of respiration and suppressed by MitoQ. Oxidative stress mediated by mitochondria was also induced in HeLa cells after photodynamic treatment with Mitotracker Red as a mitochondria-targeted photosensitizer. The effects of inhibitors and MitoQ on ROS production are similar to that described after H2O2 treatment. Severe photodynamic treatment causes necrosis that is prevented by MitoQ. It is concluded that the initial segments of the respiratory chain can be an important source of ROS, which are targeted to mitochondria, determining the fate of the cell subjected to oxidative stress.

Alternative mediators of proapoptotic events ; new approach in toxic liver injury

Eleonora Codorean, C.Tanase, C.Iosif, E.Raducan, D. Popescu, V.Barca

"Victor Babes" National Institute of Pathology, Bucharest, ROMANIA

Background The study was focused on the investigation of apoptosis, considered an important molecular control point in generating toxic/immunotoxic effects of chemicals. In a wide variety of inflammatory liver diseases, TNF-α has been implicated as a key cytokine triggering apoptosis through TNF-α receptor expressed by liver cells. Recent observations indicate that TNF-α might mediate apoptosis through alternative pathway involving cathepsin B, representing an apoptotic noncaspase mediator acting as a dominant execution protease in the mitochondrial pathway.

Aims To evaluate the incidence of apoptosis in liver toxic injury and their modulation by cathepsin B

Material and methods. Male Wistar rats were intoxicated with alcohol, with or without pretreatment of a cathepsin B inhibitor. After 2 - 4h the rats were sacrificed and fragments of the liver routinely prepared for histological/ imunohistochemical analysis and to evaluate DNA fragmentation by puls field gel electrophoresis.

Results TNF-α expressing cells and incidence of apoptosis were compared in intoxicated liver with or without cathepsin B inhibitor and in control lot. The incidence of TNF-α positive cells was higher than the cells undergoing apoptosis in liver of the rat receiving cathepsin B inhibitor, associated with lower morphological changes – reduced area with hepatocyte injury, minimal necrotic area and absence of fibrotic area. DNA fragmentation values, a sensitive indicator of apoptosis were also decreased in synergic action of alcohol and cathepsin B inhibitor compared to alcohol intoxication.

Conclusions Our preliminary results indicate that the alcoholic liver injury was reduced in presence of cathepsin B inhibitor suggesting that cathepsin B may play a pivotal role in TNF-α induced hepatocyte apoptosis.

Expression of cytokine (TNF-α) triggering apoptosis and DNA fragmentation (CHEF) offer complementary data about dying cells.

The lysosomal alternative mediators of apoptosis might be potential new pharmacological targets for inhibiting TNF-α mediated toxic liver injury.

The experimental system testing apoptosis may represent an important way to explore hepatotoxicity and it may account as suitable test for predicting and monitoring toxic potential of novel compounds.

Cathepsin D is a key initiator protease during neutrophil apoptosis

Sébastien Conus,[1] Shida Yousefi,[1] Martin F. Fey,[2] Thomas Reinheckel,[3] Christoph Peters,[3] and Hans-Uwe Simon[1]

[1]Department of Pharmacology, University of Bern, CH-3010 Bern, Switzerland, [2]Institute of Medical Oncology, Inselspital, University of Bern, CH-3010 Bern, Switzerland, [3]Institute of Molecular Medicine and Cell Research, University of Albert-Ludwigs, D-79106 Freiburg-in-Breisgau, Germany

In the absence and in the resolution of inflammatory responses, neutrophils rapidly undergo apoptosis. Here we report about a new proapoptotic pathway in these cells that requires cathepsin D translocation and is essential for the enzymatic activity of the initiator caspase-8. Pharmacological or genetic inhibition of cathepsin D delayed spontaneous apoptosis of mature neutrophils. Neutrophil death was associated with a caspase-independent translocation of cathepsin D from the azurophilic granules into the cytosol, where it targets mitochondria and triggers cytochrome c and Smac/DIABLO release. Cathepsin D translocation was blocked under inflammatory conditions *in vivo* and appeared to be an early event following apoptosis induction, since the cathepsin D inhibitor pepstatin A delayed caspase-8 activation and Bid/Bax cleavage. These findings were confirmed by a cell-free assay in which cathepsin D recombinant enzyme cleaved directly cytosolic caspase-8. Moreover, cytosolic extracts from immature neutrophils exhibited delayed cleavage of caspase-8 compared to blood neutrophils, explaining at least partially why neutrophils are short-lived upon complete maturation. Taken together, cathepsin D plays a key role in the initiation of neutrophil apoptosis.

The NF-kB Pathway Mediates Fenretinide-Induced Apoptosis In SH-SY5Y Neuroblastoma Cells.

Marco Corazzari[1], Penny Lovat[2], Gian Maria Fimia[1], Serafina Oliverio[3], John Catterall[2], Christopher Redfern[2] and Mauro Piacentini[1+3]

I.N.M.I. "L. Spallanzani", Rome[1], Italy; Northern Institute for Cancer Research[2], School of Clinical Medical Sciences, University of Newcastle upon Tyne, UK and Department of Biology[3], University of Rome, 'Tor Vergata'[3], Italy

Fenretinide induces apoptosis in SH-SY5Y neuroblastoma cells via a signaling pathway involving the production of reactive oxygen species (ROS), ganglioside GD3, 12-lipoxygenase activity and the induction of Bak and the transcription factor GADD153. It has been suggested that NF-κB may play a pro-apoptotic role in SH-SY5Y cells and be linked to lipoxygenase activity. The aim of this work was to test the hypothesis that NF-κB activity mediates fenretinide-induced apoptosis in SH-SY5Y cells. Apoptosis, but not the induction of ROS, in response to fenretinide was blocked by abrogation of NF-κB activity with an inducible dominant-negative construct for IκBα (dnIκBα) transfected into SH-SY5Y cells. Gel-shift and measurements of NF-κB activity confirmed the abrogation of NF-κB activity in cells transfected with the inducible dnIκBα construct. Apoptosis induced by GD3, a signalling intermediate of fenretinide-induced apoptosis, was also blocked by induction of dnIκBα. In addition, abrogation of NF-κB by induction of dnIκBα blocked the fenretinide-dependent induction of Bak and GADD153. SH-SY5Y cells can undergo apoptosis in response to inducers of Endoplasmic Reticulum (ER) stress such as thapsigargin, Brefeldrin A and Tunicamycin, and this was also blocked by abrogation of NF-κB activity. In parental SH-SY5Y cells, fenretinide induced NF-κB activity and IκBα phosphorylation. These results suggest that NF-κB activity links fenretinide-induced ROS to the induction of apoptosis in SH-SH5Y cells, and supports the idea that fenretinide induces apoptosis in SH-SY5Y neuroblastoma cells as a result of ER stress. Therefore, this pathway presents additional targets for the development of new drugs to treat neuroblastoma.

Insight into the role of Elongator in DNA-damage induced apoptosis

Cornez, Isabelle; Creppe, Catherine; Close Pierre and Chariot Alain.

Laboratory of Medical Chemistry, CTCM, Center for Biomedical Integrative Genoproteomics, University of Liege, Sart-Tilman, 4000 Liege, Belgium

IKAP/hELP1 is a subunit of the human Elongator complex needed for the transcriptional elongation of many but still poorly characterized target genes. The critical role played by Elongator is exemplified by the Familial Dysautonomia (FD) disease, a recessive sensory and autonomic neuropathy caused by a splice mutation of the *IKBKAP* gene. This mutation causes tissue-specific exon skipping and the translation of a truncated and unstable protein and consequently disruption of Elongator function. FD patients have some complex clinical characteristics and have among others a lower ganglionic and neuronal density.
We recently showed that Elongator depleted cells do not migrate properly, which may underlie the neuropathology in FD patients. The fact that neuronal density is lower in FD patients may be the result of a defect in cell motility during embryogenesis but may also be due to increased sensitivity of neuronal cells to pro-apoptotic signals. Therefore, the goal of this study is to determine to which extend Elongator is required for the transcription of pro or anti-apoptotic genes.
As an experimental model, we used colon cancer-derived HCT116 cells and addressed the potential role of Elongator in DNA damage-induced apoptosis by establishing the gene expression profile of IKAP/hELP1 depleted versus control cells, stimulated or not with daunomycin through micro-array analyses. We now report the genes whose expression are Elongator-dependent and will provide insights into the mechanism by which Elongator modulates the expression of selected pro and anti-apoptotic genes.

AOPP and RCOs as indicators of Oxidative Stress in Hemodialysis and Peritoneal Dialysis Patients

de Cal M, Corradi V, Cruz D and Ronco C.

Nephrologia, Ospedale San Bortolo, Vicenza, Italy

Oxidative stress (OS) has long been incriminated in the development of the dialysis-related pathology, because it reduces the survival rate and quality of life in Hemodialysis patients (HD) and Peritoneal Dialysis patients (PD). Inflammation induces OS by production of oxidants.
Advanced Oxidation Protein Products (AOPP) are markers of OS. Carbonyl stress is also present; carbohydrate, lipids and amino acids are the precursors for Reactive Carbonyl Compounds (RCOs). The carbonyl content of plasma proteins is an important tool in the study of protein OS.
The aims of this study were: 1) to measure protein oxidative stress in HD and PD patients, 2) compare them to healthy volunteers/controls (C), and 3) investigate the correlation between AOPP and RCO in dialysis patients.
We collected blood samples from 81 HD , 51 PD patients and 20 C. In HD patients, blood was collected just before the start of dialysis; in PD patients, blood was taken at the time of routine laboratory investigations.
Measurement of AOPP: we performed spectrophotometer (340nm); concentrations were expressed as µmol/L of chloramines T equivalents.
Measurement of RCO: we performed spectrophotometer (370nm) using dinitrophenylhydrazine-binding; concentrations were expressed as nmol/mg of proteins.

parameters	patients	N	Mean ± SD	*p* C vs HD	*p* C vs PD	*p* HD vs PD
AOPP	**C**	20	69.38 ± 39.72	p<0.001	p<0.001	p<0.001
	HD	81	237.63 ± 36.66			
	PD	52	146.19 ± 57.00			
RCO	**C**	20	0.35 ± 0.09	p<0.001	p<0.001	p<0.001
	HD	81	0.99 ± 0.55			
	PD	52	1.75 ± 0.78			

Among HD patients, AOPP was significantly correlated with RCO (R=0.382, p<0.001). However, this correlation was not seen among PD patients.
AOPP and RCO were significantly higher in both HD and PD compared to controls. In addition, AOPP was higher in HD compared to PD. However, RCO was higher in PD compared to HD: the glucose content of PD solution may play a role. Nevertheless the value of RCO in HD was still much higher than controls. In summary, dialysis patients had evidence of higher OS, which may be indicative of the severity of the inflammatory state.

Involvement of nucleolin in Tumor Necrosis Factor-induced cell death

Anneleen Cottyn and Katia Vancompernolle

Department of Medical Protein Research, Molecular and Metabolic Signalling Unit, Faculty of Medicine and Health Sciences, Ghent University and Flanders Interuniversity Institute for Biotechnology (VIB), Albert Baertsoenkaai 3, B-9000 Ghent, Belgium;
Email: presenting author: anneleen.cottyn@ugent.be and corresponding author: katia.vancompernolle@UGent.be

Engagement of TNF-R1 by TNFa in the fibrosarcoma cell line L929 activates a caspase-independent cell death pathway that requires the generation of oxidative stress in the mitochondria. We have recently shown that TNF induces a substantial increase in the cytotoxic glycolytic metabolite methylglyoxal, which, together with the phosphorylation of glyoxalase I, leads to the formation of specific methylglyoxal-derived Advanced Glycation End products (AGEs). AGEs are generally formed as a consequence of oxidative stress and are implicated in a number of pathophysiological conditions, including the development of diabetes, tissue damage caused by ischemia/reperfusion and neurodegenerative diseases.

Through affinity purification with an anti-MG-AGE antibody, we identified nucleolin as a differential protein among TNF-treated versus control cells. Nucleolin is a multifunctional protein that is mostly expressed in rapidly dividing cells and also in tumour cells. Besides its nuclear localization, it is also found in the cytoplasm and even extracellular on the plasma membrane. It is composed of a number of functional domains, including a RNA-binding domain and a C-terminal GAR (Glyc- and Arg-rich segment) domain. The latter domain is involved in facilitating binding of RNA to the RNA-binding domain and also in protein-protein interactions.
Induced overexpression of WT nucleolin promotes TNF-induced cell death in L929 cells, while overexpression of a mutant that lacks the GAR domain has no significant effect on TNF-induced cell death. By contrast, induced overexpression of a mutant of nucleolin that lacks the N-terminal domain and the NLS sequence inhibits TNF-induced cell death.
Furthermore, upon TNF-treatment nucleolin undergoes complex intracellular translocations. Within 10 min of TNF treatment, nucleolin translocates from the cytoplasm to the nucleus, which is then rapidly followed by a translocation back to the cytoplasm. Before the cells die, nucleolin withdraws from the plasma membrane and retrotranslocates into a peri-nuclear area.

In summary, these data indicate that nucleolin plays a role in TNF-induced cell death and that the nuclear localization of nucleolin is required during the cell death process. However, the exact functional role of nucleolin in TNF-induced cell death remains to be determined.

Endoplasmic Reticulum Stress induced Caspase activation

Lorraine Cuffe, Eva Szegezdi, Susan Logue and Afshin Samali

Cell Stress and Apoptosis Research Group, Department of Biochemistry and National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland.

Endoplasmic Reticulum (ER) stress is a condition induced by imbalance in the homeostasis of the ER. To restore balance, a highly conserved mechanism referred to as the unfolded protein response (UPR) is initiated. Failure to reinstate cellular homeostasis induces a switch from pro-survival to pro-apoptotic signalling. However, the signalling events between the initial activation of the UPR and the final demise of the cell remain largely undetermined. In this study we have examined caspases processed in response to ER stress-mediated apoptosis. Caspase-12, an ER-localised protease, is thought to be activated during ER stress-mediated apoptosis. We investigated the importance of this caspase in the cell death mechanism. Our results demonstrate that pro-caspase-12 is amongst the first caspases processed followed by sequential processing of pro-caspases-3, -7 and -9 respectively. By using caspase inhibitors and mouse embryonic fibroblasts deficient for caspase-2, -3, -9 and -12 we aimed to identify the caspase cascade activated during lethal ER stress. Given the potential role of ER stress-mediated apoptosis in numerous disease states this represents the first step in understanding the contribution of caspases in the propagation of ER originating stress signals.

Inhibitor of Apoptosis proteins (IAPs) and apoptotic signalling in Malignant Mesothelioma

Inez Czerwionka[1], Simon Fox[2], Arun Dharmarajan[1]

[1]School of Anatomy and Human Biology, The University of Western Australia; Crawley,Australia, [2]School of Pharmacy, Curtin University of Technology and West Australian Biomedical Research Institute, Bentley, Australia, e-mail: iczerwionka@anhb.uwa.edu.au

Malignant mesothelioma (MM) is an aggressive malignancy that originates from mesothelial cells and is highly resistant to conventional forms of anti-cancer therapy. Defects in apoptotic pathways are believed to play a major role in determining resistance to chemotherapy and characterization of these pathways in mesothelioma is required in order to develop more effective therapies. The inhibitor of apoptosis proteins (IAPs) are a family of proteins that regulate apoptosis and have been implicated in this resistance. There is evidence that upregulation of specific IAP molecules can influence tumour progression and response to chemotherapy. In this study we examined the apoptotic signaling in MM cells and the potential role of IAPs in this signalling. We examined expression of six IAP genes in both human mesothelioma and mesothelial cells. Results showed that XIAP, IAP-1, IAP-2, survivin and bruce were expressed in all four MM cell lines and four primary mesothelial cultures. There was no evidence for differential expression of these genes between MM and mesothelial cultures. Livin expression was detected in only one MM cell line. Various aspects of apoptotic signaling in response to the chemotherapeutic drugs (cisplatin and docetaxel) were also analyzed including: the mitochondrial integrity, caspase activation, cell viability and phosphatidylserine translocation. In order to further characterize the role of IAPs we next examined the transcriptional regulation of these genes in response to cisplatin using real time RT-PCR. These experiments indicated that there was no significant regulation of IAPs at the transcriptional level in these cells during cisplatin-induced apoptosis. This data indicates that both cisplatin and docetaxel induce apoptosis in MM cells via intrinsic pathway signalling with a concentration dependent response which varied between cell lines. Regulation of IAP expression was not seen at the RNA level as has been described in other tumour types but may occur through protein interactions. This and other aspects of IAP regulation are the subject of ongoing investigation in our laboratory.

Apoptotic neutrophils release macrophage migration inhibitory factor upon death receptor stimulation

Arezoo Daryadel, Remo Filippo Grifone, Hans-Uwe Simon, and Shida Yousefi

Department of Pharmacology, University of Bern, Bern, Switzerland
e-mail: shida.yousefi@pki.unibe.ch

Macrophage migration inhibitory factor (MIF) is an important cytokine involved in the regulation of innate immunity and present at increased levels during inflammatory responses. Here, we demonstrate that mature blood and tissue neutrophils constitutively express MIF as a cytosolic protein not associated with azurophil granules. MIF expression was increased in blood neutrophils from patients suffering from cystic fibrosis and in joint neutrophils from rheumatoid arthritis patients compared to normal blood neutrophils. Functionally active MIF, but not proteases stored in azurophil granules, was released from neutrophils following short-term tumor necrosis factor (TNF)-α or agonistic anti-Fas mAb stimulation in a caspase-dependent manner. MIF release correlated with the redistribution of phosphatidyl serine in the plasma membrane without concurrent uptake of propidium iodine, demonstrating that neutrophils secrete this cytokine as a consequence of apoptosis and not due to necrotic cell death. Although MIF was also generated by immature bone marrow neutrophils, stimulation of these cells with TNF-α or anti-Fas mAb was not associated with apoptosis induction and consequently not followed by MIF release. Taken together, apoptotic mature neutrophils release MIF upon death receptor stimulation, suggesting that apoptosis may not always occur without the induction of pro-inflammatory mechanisms.

The cationic host defence peptide LL-37 mediates contrasting effects on apoptotic pathways in different primary innate immune cells

Peter G. Barlow[1], Yuexin Li[2] , Thomas S. Wilkinson[1], Christopher Haslett[1], A. John Simpson[1], Robert E. W. Hancock[2], Donald J. Davidson[1]

[1]MRC / University of Edinburgh Centre for Inflammation Research, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, Scotland. [2] Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada.

LL-37 is a cationic host defence peptide (CHDP) of the cathelicidin family, expressed primarily by neutrophils and epithelial cells. This CHDP is upregulated under conditions of inflammation, and has both immunomodulatory and antimicrobial activities. The importance of LL-37 to innate immunity is indicated by the increased susceptibility to infection of neutrophil cathelicidin-deficient morbus Kostmann patients. Additionally, studies utilising knockout mouse models and gene therapy augmentation in the murine lung have clearly demonstrate the significance of this CHDP (and its murine homologue CRAMP) to host defence against infection. However, the key mechanisms involved, and the relative significance of immunomodulatory and direct microbicidal activities remain unknown. We hypothesised that LL-37 could modulate the initiation and resolution of inflammation through effects on host cell apoptosis, and studied primary innate immune effector cells *in vitro*. We demonstrate that LL-37 is a potent inhibitor of human neutrophil apoptosis, signalling through $P2X_7$ receptors and G-protein coupled receptors. This process involved modulation of Mcl-1 expression, inhibition of BID and pro-caspase-3 cleavage, and the activation of PI 3-kinase and NF-κB, but not ERK1/2 MAPK pathways. In contrast to the inhibition of neutrophil apoptosis, LL-37 induced apoptosis in primary and immortalised airway epithelial cells, involving caspase-dependent mechanisms. These data demonstrate alternate consequences of LL-37-mediated modulation of apoptotic pathways in different human primary cells of the innate immune system. We propose that these novel immunomodulatory properties of LL-37 contribute to peptide-mediated enhancement of innate host defences against acute infection, and are of considerable significance in the development of CHDP and their synthetic analogues as potential immunomodulatory antimicrobial therapeutics.

Supported by The Wellcome Trust, The Salvesen Trust and Genome British Columbia.

Reduced tumor growth in vivo and increased c-Abl activity in PC3 prostate cancer cells overexpressing the Shb adapter protein.

Padideh Davoodpour[1], Maréne Landström[2] and Michael Welsh[1]

Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden
Ludwig Institute of Cancer Research, Uppsala Branch, Uppsala, Sweden

In order to understand the molecular mechanisms regulating apoptosis in prostate cancer cells upon treatment with 2-methoxyestradiol (2-ME), human PC3 cells were transfected to obtain cells overexpressing the Shb adapter protein. Shb is a Src Homology 2 domain protein that previously has been shown to promote apoptosis in several other cell types. PC3 clones overexpressing Shb exhibited increased rates of apoptosis, both under basal conditions, and in the presence of 2-ME. Elevated apoptosis in Shb cells was not a consequence of increased p38 mitogen activated protein kinase (MAPK) activity. However, the Shb cells failed to increase their activation of survival mechanisms through ERK (extracellular signal-regulated kinase) and Akt in response to 2-ME. In addition, the Shb cells displayed increased activity of the pro-apoptotic kinase c-Abl. Pre-treatment with p38 MAPK (SB203580) or c-Abl (STI-571) inhibitors completely blocked the apoptotic response to 2-ME. On the other hand, inhibition of the ERK and Akt pathways promoted cell death. The PC3-Shb cells displayed reduced tumor growth in vivo. It is concluded that Shb promotes 2-ME-induced PC3 cell apoptosis by a combination of increased pro-apoptotic signaling and decreased defence responses to these apoptotic cues. Activation of both the p38 MAPK and c-Abl pathways appears to be required for 2-ME induced prostate cancer cell apoptosis.

Inflammation Markers and Oxidative Stress Markers in Hemodialysis Patients

de Cal M, Corradi V, Cruz D and Ronco C.

Nephrologia, Ospedale San Bortolo, Vicenza, Italy.

The high prevalence of coronary disease and infections among Hemodialysis (HD) patients is thought to be related to Oxidative Stress (OS). The majority of reactive oxygen species are formed during cellular respiration and by activated phagocytic cells. Carbonylation of proteins in vitro may lead to protein dysfunction, including enzyme inactivation, decrease in immunoglobulin receptor binding. HLA-DR expression may be an important parameter to study the function of immunocompetent cells.
We performed a study to evaluate the relationship between inflammatory and OS markers in HD.
We evaluated 81 HD patients (mean age 62.23 yrs, 59M/22F, DM 20%, mean time on dialysis 4.7 yrs) and collected blood samples before HD for several inflammatory markers [CRP high sensitivity, plasma apoptogenic potential (Apo), antigenic Mean Fluorescence Intensity of HLA-DR (MFI), percentage of monocytes HLA-DR+ (%DR+)] and OS markers [Advanced Oxidative Protein Products (AOPP), Reactive Carbonyl compounds (RCOs)].
AOPP and RCO were measured by spectrophotometry at absorbance readings of 340nm and 370nm, respectively. MFI and %DR+ were evaluated by flow cytometric analysis. Apo was measured using a cultured monocytic cell line (U937), and was assessed by fluorescence microscopy after 96h. Correlations among the various markers were analyzed by Pearson correlation.
We found a significant positive correlation between %DR+ and Apo ($R=0.230$; $p=0.039$), %DR+ and AOPP ($R=0.256$; $p=0.021$), %DR+ and MFI ($R=0.487$; $p=<0.001$); and a significant negative correlation between CRPhs and %DR+ ($R=-0.363$; $p=0.001$).
There appears to be a close correlation between HLA-DR and AOPP. This suggests that AOPP acts as a mediator of oxidative stress and monocyte respiratory burst, and that monocytes are a potential cellular target. In addition the positive correlation founded between HLA-DR and APO could explain how plasma full of AOPP has an apoptogenic potential on monocytes.

AMPA receptor activation can induce both apoptotic and necrotic cell death in primary cultured motoneurons.

M. De Paola*, P. Bigini*, D. Curti°, T. Mennini*.

* *Department of Molecular Biochemistry and Pharmacology, "Mario Negri" Institute for Pharmacological Research, 20157 Milan, Italy.*
° *Department of Molecular and Cellular Physiological and Pharmacological Sciences, University of Pavia, Piazza Botta 11, 27100 Pavia, Italy.*

The selective degeneration of motor neurons is, by an histo-pathological point of view, the main feature of the devastating neurodegenerative disorder Amyotrophic Lateral Sclerosis (ALS).
Glutamate mediated excitotoxicity plays an important contribution to motor neuron loss in this disease and it seems to be mainly mediated by AMPA receptors.
In this study we have characterized the neurodegenerative patterns induced by the glutamatergic agonists kainate and AMPA in primary cultured motor neurons, through the activation of the common AMPA receptor.
As evidenced by immunocytochemical assays, different intracellular pathways are induced depending on the intensity of the initial excitotoxic stimulus mediated by AMPA receptor activation. Low AMPA and kainate concentrations (0.3µM and 5µM respectively) induce typical apoptotic-like intracellular mechanisms such as the activation of caspase 3 and 9, the expression of phosphatidylserine on the membrane's outer leaflet (revealed by annexin-V binding), finally leading to DNA fragmentation. On the contrary, higher doses (AMPA 1µM and kainate 15µM) induce membrane permeabilization and cell swelling, leading to a non-apoptotic motoneuron degeneration.
Our study demonstrate that AMPA receptor activation can trigger apoptosis or necrosis in cultured motor neurons depending on the intensity of the stimulus (concentration, time of exposure). Thus, a careful examination of the intracellular mechanisms induced by excitotoxic stimuli is needed in order to obtain accurate analysis of the events involved in cellular neurodegeneration and possible strategies for new therapeutic approaches.

Effects of Cisplatin and Quercetin combination in N-ras transformed myoblast cells.

Asuman Demiroglu-Zergeroglu

Gebze Institute of Technology, Department of Biology/Istanbul Yolu. No:101 41400 Çayirova Gebze/Kocaeli

Platinium drug Cisplatin (CP) is widely used for the treatment of many malignancies. Although CP is an effective agent, its clinical utility is limited. This is mostly due to CP's toxicity that links to the production of free radicals. On the other hand, Flavonoid Quercetin (QC) is known as a strong antioxidant and scavenger of free radicals. Hence, using QC with CP might reduce the CP induced toxicity. This study analyses the effects of QC, CP and their combinations in CO25 myoblast cell line that was grown in 10% HS with dexamethasone to activate N-ras gene or without dexamethasone to allow differentiation. Initial experiments demonstrated that QC and CP individually inhibited proliferation of cells at dose and time dependent fashion in growth medium and in ras-activating medium. The combination of 10mikrogram/ml CP and 1-10mikroM doses of QC prevents the cytotoxic effect of CP, cells grown in both culture mediums. However, the same QU doses were not enough to overcome 50mikroM CP toxicity in ras inactivated cells. On the other hand, in ras activated cells CP toxicity were reduced after 96 hours. These initial results may be an indication for the ras activation involvement in QU's protective mechanisms. Nevertheless this mechanisms need to be investigate further at molecular level.

Target genes to sensitize ovarian cancer cells to cisplatin

Dettori Daniela, Cossu Costanza, Nano Elisa, Olivero Martina and Di Renzo Maria Flavia

Laboratory of Cancer Genetics, Institute for Cancer Research and Treatment, University of Torino School of Medicine, Candiolo (Turin), Italy.
e-mail: daniela.dettori@ircc.it

In women ovarian carcinoma is the seventh most common cancer and the fourth most frequent cause of death by cancer. 70% of ovarian cancer patients have an advanced ovarian carcinoma at presentation, which is treated with cytoreductive surgery and first-line chemotherapy with platinum-based compounds (e.g. Cisplatin) and taxanes (e.g. Paclitaxel). Although these treatments prolonged patient survival, the overall cure rate of the disease has not changed dramatically: sooner or later, responsive patients manifest resistance to chemotherapy. Resistance to apoptosis is one of the hallmarks of cancer cells: an important aim of cancer therapy is to target or bypass the mechanisms of apoptosis protection in particular when the impaired apoptotic pathway is the same that confers drug resistance.
We recently showed that Hepatocyte Growth Factor (HGF), known as a survival factor, unexpectedly enhances apoptosis in human ovarian cancer cells treated with cisplatin (CDDP) and paclitaxel (PTX). We demonstrated that this effect depends on the p38 mitogen-activated kinase: its activity is in fact stimulated by HGF and further increased by the combined treatment with HGF and drugs.

In this work we integrated genome-wide expression profiling, in silico data survey and functional assays to identify transcripts regulated in ovarian cancer cells made more responsive to CDDP by HGF. By means of oligonucleotide microarrays we found that HGF pre-treatment changes the transcriptional response to CDDP. Quantitative RT-PCR not only validated all the fifteen most differentially expressed genes, but also confirmed that they were primarily modulated by the combined treatment with HGF and CDDP and reversed by suppressing p38 MAPK activity. Among the differentially expressed genes, we focused functional analysis on two regulatory subunits of the protein phosphatase 2A (PP2A), which were down-modulated by HGF plus CDDP. Decrease of each subunit by RNA interference made ovarian cancer cells more responsive to CDDP, mimicking the effect of HGF. In conclusion, we demonstrate that HGF and CDDP modulate transcription in ovarian cancer cells and that this transcriptional response is involved in apoptosis regulation. We also provide the proof-of concept that the identified genes might be targeted to increase the efficacy of chemotherapeutics or to revert chemotherapy resistance.

Autophagic cell death by photodamage to the endoplasmic reticulum

Michael Dewaele, Esther Buytaert and Patrizia Agostinis

Dept. Molecular & Cell Biology, Faculty of Medicine, Catholic University of Leuven, Belgium

Photodynamic therapy (PDT) is a paradigm of anticancer therapy utilizing the generation of reactive oxygen species to kill the cancer cells[1]. Recently, we have identified the photodamage to the sarco(endo)plasmic-reticulum Ca^{2+}-ATPase (SERCA) pump and consequent loss in the ER-Ca^{2+} homeostasis as the most apical molecular events leading to cell death in hypericin-photosensitized cells[2]. Downstream of the ER-Ca^{2+} emptying, both caspase-dependent and -independent pathways are activated to ensure cell demise. The induction of apoptosis as a cell death modality is dependent on the availability of proapopototic Bax and Bak proteins, which are essential effectors of the mitochondrial outer membrane permeabilization (MOMP) and subsequent caspase activation. Antagonists of autophagy protect *Bax*$^{-/-}$*/Bak*$^{-/-}$ murine embryonic fibroblasts (MEF) cells from photokilling, whereas they do not prevent apoptotic cell death in the wild type MEF. On the other hand, caspase-3/-7 inhibitors partially protect wild type cells from PDT-mediated apoptosis while they do not affect autophagic cell death in the photosensitized *Bax*$^{-/-}$*/Bak*$^{-/-}$ MEF, thus arguing that these two pathways are concurrently promoted and do not actively suppress each other. Preliminary examination of apoptotic and autophagy parameters in ER-photodamaged cancer cells suggests that these processes are initiated jointly although with different kinetics and that the inability of the pan-caspase inhibitor zVAD-fmk to rescue the cells from photokilling may be due to enduring autophagy. In conclusion, while Bax/Bak-dependent MOMP is the preferred cell death route in hypericin-photosensitized cells ensuring the fast caspase-dependent disassembly of the cells, the concomitant induction of autophagy builds up a default cell death pathway, which is utilized to kill cells with defects in the apoptotic pathway. Although the exact contribution of autophagy as a tumor suppression mechanism *in vivo* should be carefully evaluated, the ability of hypericin-PDT to engage different lethal routes of cell destruction, encourages further studies to establish the molecular mechanisms turning autophagy into a cell death pathway in cancer cells exposed to this anticancer strategy.

[1]Dolmans *et al*., (2003) Nat Rev Cancer 3:380-7
[2]*Buytaert* et al*., (2006) FASEB J. 20:756-8*

Random mutagenesis screen in yeast reveals novel residues required for Bak oligomerization and pro-apoptotic activity

Grant Dewson, Huiyan Sim, Tobias Kratina, Hamsa Puthalakath, Peter Colman†, Ruth Kluck

Molecular Genetics of Cancer Division, †Structural Biology Division, Walter & Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Melbourne, Victoria 3050, Australia

While Bax and Bak are essential for mitochondrial permeabilization during apoptotic cell death, the mechanism by which they mediate apoptosis and the functionally requisite domains responsible remain unclear. As an unbiased approach to identify all Bak pro-apoptotic domains and their functions, we randomly mutated Bak and screened for mutants that failed to kill yeast. This screen identified Bak LOF mutations within the BH3 domain supporting previous evidence that the BH3 domain is important for Bak pro-apoptotic function. LOF mutations were also identified throughout putative helices α2 to α5, which, based on the solution structure of Bax, constitute a hydrophobic binding cleft. In each case, yeast LOF Bak mutants also lacked pro-apoptotic activity in mammalian cells, as stable expression in *bax*$^{-/-}$*bak*$^{-/-}$ mouse embryonic fibroblasts failed to re-sensitise these cells to apoptotic stimuli. In contrast, transient expression of most Bak mutants in cells that contained endogenous Bax and Bak, still induced apoptosis, indicating that previous analyses of Bak mutations in Bax/Bak-expressing cells may not represent physiological Bak function. Biochemical analysis of the newly identified LOF Bak mutants revealed that conformation change was not sufficient for apoptosis and that Bak homo-dimerisation correlated with apoptotic function. Furthermore, targeted second site mutagenesis partially rescued one LOF Bak mutant, providing novel information regarding Bak homo-dimerisation.

Ambra-1 regulates autophagy during development of the nervous system in mammals

Anastassia Stoykova[1], Gian Maria Fimia[2], Alessandra Romagnoli[2], Luigi Giunta[3], Sabrina Di Bartolomeo[3], Roberta Nardacci[2], Claudia Fuoco[3], Peter Gruss[1], Kamal Chowdhury[1], Mauro Piacentini[2,3] and Francesco Cecconi[3,4]

[1]Department of Molecular Cell Biology, Max-Planck Institute of Biophysical Chemistry, 37077 Göttingen, Germany
[2] National Institute for Infectious Diseases IRCCS L. Spallanzani, 00149 Rome (Italy)
[3]Department of Biology, University of Tor Vergata, 00133 Rome (Italy)
[4] Laboratory of Molecular Neuroembriology, IRCCS Fondazione Santa Lucia, 00143 Rome (Italy); e-mail: francesco.cecconi@uniroma2.it

Autophagy is a highly conserved process in eukaryotic cells by which long-lived proteins, cytoplasmic organelles as well as entire cells are degradated. During the autophagic process, excess or aberrant cellular materials are sequestered into double-membrane vesicles and delivered to the degradative organelle, the lysosome/vacuole, for breakdown and eventual recycling of the macromolecules. This process has an important role in various biological events such as adaptation to changing environmental conditions, cellular remodeling during development and differentiation, and determination of lifespan. Moreover, a number of human diseases, as cancer, cardiomyopathy and some neurodegenerative disorders are associated with an increase of autophagic activity.

Ambra-1 (Activating molecule of beclin regulated autophagy) is a novel gene identifyed in our lab by the "gene trapping" technique in mouse. The expression of Ambra-1 is prominent in many parts of the nervous system during embryogenesis and in adult tissues. Ambra-1gt/gt embryos die at embryonic day 16.5 and show a peculiar phenotype. Focusing on the nervous system, we observe a prominent exencephaly due to an enhanced cell proliferation in comparison with wild type (wt) embryos. In addition, mutant embryos show some defects in patterning and closure of the neural tube. A yeast two-hybrid assay showed a specific binding of Ambra-1 protein with several proteins involved in vesicle trafficking and autophagy as, in example, the yeast Atg6 mammalian homolog Beclin1. To investigate a possible role of Ambra-1 protein in autophagy, in vivo and in vitro studies have been addressed. An in vivo approach we are using consists in the ultrastructural analysis of mutated and wt embryos at different embryonic stages by electron and confocal microscopy. In parallel, in vitro experiments have been conducted on murine embryonic fibroblasts (MEFs) isolated from wt and Ambra-1 embryos. Unlike wt MEFs, Ambra-1gt/gt MEFs are not able to respond to the autophagic stimulus rapamycin. Furthermore, we observe that overexpression of Ambra-1 gene in a human fibroblast cell line results in an enhanced autophagic response to rapamycin whereas downregulation of the gene by siRNA drastically reduces this response, thus confirming the involvement of Ambra-1 in autophagy.

Parvovirus H-1 triggers a type II cell death in permissive gliomas through a Bcl-2 resistant and cathepsin B dependent mechanism

Matteo Di Piazza, Laurent Deleu and Jean Rommelaere

Program in Infection and Cancer, Division F010 and INSERM Unit 701, German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany. e-mail: m.dipiazza@dkfz.de

Gliomas are the most common brain cancers, characterized by an exceptionally wide cellular heterogeneity and extreme migratory features. These tumors are often resistant to the induction of apoptotic cell death as a result of the onset of survival mechanisms occurring during astrocytes malignant transformation. In particular, the overexpression of Bcl-2 family members interferes with both apoptosis induction by DNA damaging agents (i.e. cisplatin) or soluble death ligand (i.e. TRAIL) and starvation-induced autophagic death pathways. Autonomous parvoviruses are small, non enveloped, single-stranded DNA viruses endowed, either naturally or through appropriate engineering, with the capacity to kill malignant cells while being non-cytopathic towards healthy tissues. Using low passage cultures of gliomas we have shown that parvovirus H-1 (H-1PV) induces death in cells resistant to either TRAIL or cisplatin or both. H-1PV triggers cell death by the formation of acidic vesicles and the cytosolic activation of lysosomal cathepsins B and L even when Bcl-2 is overexpressed. Moreover, H-1PV reduces the levels of Stefin B and Cystatin C, two cathepsins inhibitors that interfere with the lytic effect of the virus. This killing mechanism, which resembles type II cell death, is in contrast not induced by parvovirus H-1 infection of normal human astrocytes. Furthermore, H-1PV-induced glioma regression *in vivo* is also associated with cathepsin induction. In a rat syngenic glioma model, H-1PV intracerebral injection results in a striking and irreversible tumor regression and shows an enhanced cathepsin B activity which is limited to the glioma-bearing hemisphere. These observations suggest that parvovirus H-1 is especially relevant to the treatment of gliomas through its ability to efficiently hijack the lysosomal/cathepsin pathway and kill these tumor cells irrespective of their resistance to conventional death inducers.

In vitro and *in vivo* effects of Trolox on arsenic mediated toxicity

Zuanel Diaz, Anna Laurenzana, Hyman M. Schipper and Wilson H. Miller Jr.

Lady Davis Institute for Medical Research, McGill University. Montreal, Quebec, Canada. e-mail: zuanel.heredia@mail.mcgill.ca

Arsenic trioxide (As_2O_3) has its major clinical activity in acute promyelocytic leukemia (APL), but its use in other malignancies is limited by the toxicity of concentrations required to induce apoptosis in most non-APL tumor cells. We have reported that trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a hydrophilic analogue of a-tocopherol with increased antioxidant capacity and cell permeability, enhances As_2O_3-mediated apoptosis in APL, chronic myelogenous leukemia, lymphoma, myeloma and breast cancer cells. The addition of trolox increases intracellular oxidative stress, as evidenced by HO-1 and HSP70 protein levels, JNK activation, and protein and lipid oxidation. We also observed a decrease in mitochondria membrane potential and the release of cytochrome c to the cytoplasm. The synergistic effects of trolox may be specific to As_2O_3, as trolox does not add to toxicity induced by other chemotherapeutic drugs. Contrary to the pro-apoptotic effects of this combination in cancer cells, trolox protected non-malignant cells from As_2O_3-mediated cytotoxicity. We performed *in vivo* experiments and found that administration of trolox to BDF_1 mice considerably reduced As_2O_3-induced hepatomegaly, as well as serum alanine and aspartate transaminases, which are indicators of hepatocellular death. Oxidative stress markers were also upregulated significantly more in the As_2O_3-treated group than in the As_2O_3+trolox-treated group. The enzymatic activity of cytochrome c oxidase as well as hepatic ATP levels, were decreased in the animals treated with As_2O_3 but not significantly changed in the group receiving the combination. Hematoxilin and eosin staining on liver tissues demonstrated that trolox decreased the presence of binuclated cells and lymphocyte infiltration that resulted from As_2O_3 treatment. Additionally, we studied anti-tumor effects of this combination *in vivo*. BDF_1 mice bearing P388 ascitic tumors received similar treatments for 20 days. A significant increase in life span was observed in the As_2O_3+trolox group compared to As_2O_3 alone. Our data suggest that trolox might prevent some of the clinical manifestations of As_2O_3-related toxicity and increase its pro-apoptotic capacity and therapeutical potential in hematological malignancies.

Notch regulates the cytokine-dependent survival of activated T-cells

P. Divya, B. Geetha and Apurva Sarin

National Centre for Biological Science, Bellary Road, Bangalore 560065, Karnataka, India

Peripheral T-cell homeostasis results from a balance between factors promoting survival and those triggering deletion of antigen-reactive cells. Cytokines such as interleukin-2 (IL-2) promote T-cell survival whereas reactive oxygen species (ROS) sensitize T-cells for apoptosis. The cytokine Interleukin-2 (IL-2) functions not only as a growth factor, promoting T-cell proliferation and survival, but also primes T-cells activated by self-antigens for Activation Induced Cell Death (AICD). The transmembrane receptor Notch promotes IL-2 responsiveness and proliferation by regulating cytokine receptor expression during primary encounter with antigen. Since IL-2 can drive both survival and death of antigen-stimulated T-cells, here we ask if Notch biases IL-2 signaling towards either one of these outcomes in activated T-cells. Disrupting Notch signaling (by inhibitors of gamma-secretase) in activated T-cells maintained in IL-2, reduces Akt/PKB activity and expression of the anti-apoptotic protein Bcl-x_L triggers the accumulation of ROS and consequent apoptosis. Apoptosis is inhibited by a broad-spectrum ROS scavenger and does not depend on Fas - Fas Ligand signaling. Ectopic expression of Notch intracellular domain protected activated T-cells from apoptosis triggered by cytokine-deprivation. Thus, in addition to the functions described for Notch in promoting antigen-dependent T-cell proliferation and differentiation, we suggest that Notch may augment immune function by modulating the cytokine-dependent survival of antigen-stimulated T-cells.

GILZ (Glucocorticoid Induced Leucine Zipper) is a mediator of glucocorticoid-induced apoptosis

Valerio Donato, Enrico Velardi, Stefano Bruscoli, Rosa Di Virgilio, Cristina Marchetti, Michela Massetti and Carlo Riccardi

Sect of Pharmacology, Dept of Clinical and Experimental Medicine, School of Medicine, University of Perugia, Via del Giochetto, 06122 Perugia, Italy

It is well known that glucocorticoid hormones receptor (GR) is involved in regulation of cell death and survival. In particular, in T lymphocytes glucocorticoid hormones (GCH) induce or counteract apoptosis activated by other stimuli such as TCR-triggering or heat shock.
We previously identified a number of non-genomic (transcription-independent) signal involved in GC-induced thymocyte apoptosis. Results indicated that GCH-activated thymocytes apoptosis requires a sequence of non-genomic events, mediated by GCH/GR interaction, such as GR-associated Src kinase release, PI-PLC phosphorylation/activation, aSMase activation and ceramide generation, cytochrome-c release, caspase-9, -8 and –3 activation. GCH-induced caspase activation is a non-genomic signal but is inhibited by mRNA and protein synthesis inhibitors. Since GCH do not influence caspases gene transcription, these results suggest that gene transcription of unknown genes is required for caspases activation.
GCH modulate transcription of numerous genes including GILZ (Glucocorticoid Induced Leucine Zipper). GILZ is a gene rapidly induced by dexamethasone (DEX) able to modulate thymocyte apoptosis. We evaluated the possible role of GILZ in GC-induced apoptosis. For that purpose we evaluated the role of GILZ in caspase activation using different experimental approaches including TAT-GILZ fusion protein, GILZ over-expression by transient transfection, and siRNA to evaluate the actual role of GILZ in GC-activated apoptosis. Results indicate that GILZ is required for GCH-induced caspases activation, and thymocyte apoptosis.
In conclusion these results indicate that GCH-activated thymocyte apoptosis requires both genomic and non-genomic signals and that GILZ can participate to GCH-induced caspases pathway activation.

A new caspase-independent pathway to apoptosis that affects lysosomes is triggered by the proapoptotic Bcl-2-related protein Nbk/Bik in human melanoma cells

Malte Oppermann, Lothar F. Fecker, Christoph C. Geilen, Jürgen Eberle

Charité - Universitätsmedizin Berlin, Department of Dermatology and Allergy, Skin Cancer Center Charité, 12203 Berlin, Germany

Proteins of the Bcl-2 superfamily located at the membranes of cellular organelles are central regulators of apoptosis. The Bcl-2 family includes antiapoptotic and proapoptotic factors; the proapoptotic proteins further subdivide in BH3-only and multi-domain proteins. BH3-only proteins are regarded as sensors for proapoptotic signals, and they may subsequently activate multi-domain proteins such as Bax or Bak. In a previous study, we have shown that the basic expression of Nbk was only weak in melanoma cells at the mRNA and protein level. Its conditional expression by applying inducible tetracycline-responsive expression systems, triggered apoptosis and enhanced sensitivity for proapoptotic stimuli such as agonistic CD95 activation or chemotherapeutic treatment. In vivo, the effects of Nbk have been investigating by stably transfected melanoma cells, which were subcutaneously injected into nude mice. Significantly delayed melanoma growth was the result, when mice received doxycycline for induction of Nbk expression in the tumor cells.
In the present study, we addressed the mechanism of Nbk-induced cell death in melanoma cells. Typical hallmarks of apoptosis such as DNA fragmentation, nuclear fragmentation, shift of the sub-G1 peak and TUNEL positivity were seen after induction. On the other hand, common characteristics of apoptosis such as cytochrome c release and caspase activation were not detected, when Nbk was induced solely by doxycycline in a Tet-On system. Reinforced expression of Nbk by using an enhancer of the Tet-On system also led to caspase activation, which may however indicate a secondary effect. Interestingly, inhibition of lysosomal cathepsins significantly decreased apoptosis induction, whereas selective caspase inhibition remained without effect. Moreover, lysosomal staining revealed dramatic changes in the lysosomal pH, indicating permeability of the membrane. Finally, we could demonstrate a release of lysosomal cathepsins into the cytosol after Nbk induction.
These data demonstrate the high proapoptotic potential of BH3-only proteins as Nbk/Bik in melanoma cells and, they may indicate a new, lysosome-dependent, proapoptotic pathway activated in melanoma cells. In this model, cathepsins may substitute for caspase activation often missing in melanoma. New pathways also imply the hope for new therapeutic options against this so far deadly cancer.

Critical roles of PUMA and A1 in the regulation of mast cell apoptosis and survival

Maria Ekoff, Andreas Strasser, Gunnar Nilsson

Department of Medicine, Clinical Immunology and Allergy Unit, Karolinska Institutet, Stockholm, Sweden and The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, email: maria.ekoff@ki.se

Mast cells are effector cells in inflammation and play a critical role in IgE-dependent immediate hypersensitivity reactions. This is facilitated by their capacity to rapidly increase in numbers, release inflammatory mediators and to undergo activation-induced survival upon FcεRI crosslinking. Once the inflammation resolves, mast cell numbers return to baseline. Still, knowledge of the mechanisms regulating mast cell survival and apoptosis is limited.
The objective was to investigate the role of Bcl-2 family members in the regulation of mast cell apoptosis after growth factor deprivation and FcεRI crosslinking. Due to their heterogeneity, mast cells can be divided into two major groups: connective-tissue mast cells and mucosal mast cells. We have previously described that IL-3 dependent bone marrow-derived mast cells can undergo FcεRI activation-induced survival dependent on the anti-apoptotic A1/Bfl-1 gene.
In this study, murine progenitor cells were differentiated into two populations referred to as mucosal-like mast cells (MLMC) and connective tissue-like mast cells (CTLMC). To investigate the involvement of BH3-only proteins in mast cell apoptosis following growth factor withdrawal, mast cells were developed from wt, bim-/-, bad-/-, bid-/-, bmf-/-, puma-/- and noxa-/- mice. We have previously shown that absence of pro-apoptotic Bim delays growth factor deprivation-induced apoptosis but over-expression of anti-apoptotic Bcl-2 protected mast cells against apoptosis more potently, indicating that other BH3-only proteins might be involved. Our results show that puma-/- MLMC and CTLMC had 50% increased viability compared to wt after 48 hrs. Using puma+/- we also show a gene dosage-dependent effect. We also compared the capacity of MLMC and CTLMC to survive an allergic reaction in vitro. Strikingly, FcεRI crosslinking promoted survival of CTLMC, but not MLMC. This coincided with a slight upregulation of A1 mRNA in MLMC. In contrast, FcεRI crosslinking of CTLMC caused a profound upregulation of A1. This difference among mast cell subpopulations might explain the slower turnover of connective tissue mast cells in IgE-dependent reactions in vivo.
Altogether, this study demonstrates for the first time that Puma is involved in the induction of mast cell death following growth factor withdrawal and highlights differences among mast cell populations to undergo activation-induced survival following FcεRI crosslinking.

Photochemically mediated delivery of AdhCMV-TRAIL augments the TRAIL-induced apoptosis in colorectal cancer cell lines.

Birgit Ø. Engesæter[1], Anette Bonsted[2], Trine Lillehammer[1], Olav Engebraaten[1], Kristian Berg[2], Gunhild M. Mælandsmo[1]

[1]Department of Tumor Biology, [2]Department of Radiation Biology, Rikshospitalet-Radiumhospitalet Medical Center, Montebello, N-0310 Oslo, Norway;

Tumor necrosis factor-related apoptosis inducing ligand (TRAIL) has been shown to induce apoptosis in cancer cells. In this study we have evaluated the induction of apoptosis after adenoviral gene delivery of TRAIL. Tumor targeting is an important issue in cancer gene therapy, and in this work we present a light-specific transduction method, named photochemical internalization (PCI), that enhance gene expression from adenoviral vectors selectively in illuminated areas. The potential of PCI to enhance transgene expression from AdhCMV-TRAIL and its impact on apoptotic induction in the two human colorectal cancer cell lines HCT116 and WiDr were evaluated. PCI-mediated delivery of AdhCMV-TRAIL enabled an increased expression of TRAIL, induced a synergistic reduction in cell viability compared to the individual action of AdhCMV-TRAIL and photochemical treatment, and enhanced the induction of apoptosis demonstrated by an increase in cytoplasmic histone-associated DNA fragments, caspase-8 and caspase-3 activation, PARP cleavage and a decrease in the mitochondrial membrane potential. The synergistic effect could be related to the enhanced TRAIL expression in PCI-treated samples and a modest sensitization of the cancer cells to TRAIL induced apoptosis due to the photochemical treatment. Furthermore, an increased cleavage of Bid and a cell line dependent reduction in the expression level of anti-apoptotic Bcl-2 family members were observed and could possibly contribute to the enhanced apoptotic level in samples exposed to the combined treatment. The presented results indicate that photochemically mediated delivery of AdhCMV-TRAIL allows a selective enhancement in cell killing, and suggest that PCI may be relevant and advantageous for therapeutic gene delivery *in vivo*.

Mitochondrial signalling pathway involved in monocytic oxidized LDL-induced apoptosis

N. Ermak[1,3], B. Lacour[1,3], T.B. Drüeke[2], S. Vicca[1,2]

Laboratory of Biochemistry A[1], INSERM U507[2], Necker Hospital, Paris, France
Laboratory of Physiology[3], UMR 1154, Faculty of Pharmacy, Châtenay-Malabry, France

Oxidized low-density lipoprotein (oxLDL) plays a key role in atherogenesis. We investigated the pro-apoptotic effect of oxLDL, generated in vitro by HOCl treatment, on normal fresh human monocytes isolated from peripheral blood (PBMs), on human monocyte-derived macrophages and on human monocytic U937 cell line.
We analysed apoptosis using annexin V-7AAD double labelling by flow cytometry, mitochondrial membrane potential ($\Delta\psi m$) using the fluorochrome $DiOC_6$, the expression of caspase-9, caspase-3, and cytochrome c and the modulation of Bcl-2 family proteins by western blotting.
We observed that HOCl-oxLDL (200 µg/ml) induced a significant phosphatidylserine exposure in both human PBMs (12.48 ± 1.81 % *versus* 1.47 ± 0.99 % with native LDL) and U937 cells (14.14 ± 4.57 % *versus* 2.03 ± 1.47 %) after 18h-exposure. In human monocyte-derived macrophages, we observed a total resistance to oxLDL-induced apoptosis. Moreover, involvement of mitochondrial apoptotic pathway was demonstrated by: *i)* significant $\Delta\psi m$ transition after 30 min treatment with oxLDL in human PBMs and U937 cells, *ii)* cytosolic liberation of cytochrome c after 4h treatment with oxLDL and subsequent activation of caspase-9 and caspase-3 in U937 cells, and *iii)* total resistance to oxLDL-induced apoptosis of U937 cells overexpressing Bcl-2. At the same time, we observed Bid cleavage in association with a decrease of Mcl-1 expression, whereas no change in total Bcl-2 or Bax expression occurred in U937 cells.
In conclusion, HOCl-oxLDL is able to induce human monocyte apoptosis, involving the caspase-dependent mitochondrial apoptotic pathway. This type of monocytic cell death could limit the extent of atherosclerotic areas. In contrast, human monocyte-derived macrophages are able to resist to oxLDL apoptosis. A better understanding of the activation of upstream proapoptotic signalling by HOCL-oxLDL should permit modulation of apoptosis and prevention of plaque instability.

Engineering NAIP-BIR3 to interact with SMAC type peptides

Ali Es-haghi[1], Azadeh Mohammad Gholi[1], Alex MacKenzie[2], Jamshid Davoodi[1]

[1]Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
[2]Apoptosis Research Center, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada

Eukaryotic cells produce a class of proteins known as Inhibitor of Apoptosis Proteins (IAPs) in order to inhibit the activity of caspases, which are involved in programmed cell death. Extensive studies have been conducted on the X-Linked Inhibitor of Apoptosis Protein (XIAP), the most distinguished member of this family. XIAP, a 57 kDa protein, contains three repeating BIR domains, which are involved in inhibition of caspases. The first two BIR repeats inhibit caspase-3 and -7 and the third BIR inhibits caspase-9. This domain in addition to caspase-9 interaction interacts with N-terminal AXPX sequence containing proteins like SMAC. Amino acid sequence alignment of BIR3 domains of IAPs points to the conserved tryptophan 323 residue involved in both caspase-9 interaction as well as AXPX sequence interaction. Unlike XIAP, Neuronal Apoptosis Inhibitory protein (NAIP) does not interact with SMAC, presumably due to the presence of cysteine instead of tryptophan at this position. Therefore we decide to determine whether or not AVPF binding ability can be restored to BAIP-BIR3 by mutating cysteine to tryptophan. We also sought to investigate the structural effects of the mutation of Tryptophan to cysteine in Xiap-BIR3. Thus, recombinant XIAP-BIR3 and its W323S mutant, NAIP-BIR3 as well as its C334W mutant were produced in E. coli and purified by Glutathion resin. In order to assess the effect of these mutations on protein structure, circular dichroism spectroscopy was applied. Introduction of these mutations caused some changes to their secondary structure such that XIAP-BIR3 lost its caspase-9 inhibitory activity. However C334W mutation of NAIP-BIR3 led to a more potent caspase inhibition. The interaction of the proteins with AVPF was also studied using fluorescence and circular dichroism spectroscopy. The titration experiments revealed a diminished AVPF binding for XIAP-BIR3 (W323) variant, while NAIP-BIR3 (C334W) mutant gained AVPF binding ability. These results indicate that W323 is a very important residue for caspase-9 inhibition of XIAP-BIR3 and that introduction of this residue alone is sufficient to render AVPF binding ability to NAIP-BIR3.

Stromal-derived factor-1 (SDF-1) blocks constitutive apoptosis of WHIM syndrome neutrophils

Duangmanee Sanmun, Daniel Garwicz and Bengt Fadeel

Division of Biochemical Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; e-mail: bengt.fadeel@ki.se

WHIM (warts, hypogammaglobulinemia, infections, myelokathexis) syndrome, a rare combined immunodeficiency condition, is clinically heterogeneous, the only invariant feature being the characteristic neutropenia. Despite the low levels of neutrophils in peripheral blood, the bone marrows of affected individuals contain abundant mature neutrophils, presumably as a result of the defective egress of these cells from the marrow. Recent studies of WHIM patients have revealed mutations in the gene encoding the chemokine receptor CXCR4, and it was suggested that the resulting mutant receptors display gain-of-function properties, including enhanced chemotactic responsiveness to stromal-derived factor-1 (SDF-1; also known as CXCL12), the sole cognate ligand for CXCR4. In the present study, we aimed to evaluate whether the administration of recombinant SDF-1 could block constitutive apoptosis of peripheral blood neutrophils from congenital neutropenia patients and/or controls. Our studies show, for the first time, that SDF-1 is a potent anti-apoptotic factor for WHIM neutrophils harboring a truncating *CXCR4* mutation, but not for neutrophils from control individuals, thus supporting the notion that such mutations may confer enhanced, pathological functional responses. The fact that SDF-1 suppresses apoptosis may also serve to explain, at least in part, the increased numbers of mature neutrophils in the bone marrow of these patients.

Gtdap-1 **monitors the disappearance of the gonads during stress-inducing processes in planarians**

Daniel A. Felix, Cristina González-Estévez & Emili Saló

Departament de Genètica. Facultat de Biologia. Universitat de Barcelona
Av. Diagonal 645, Edifici Annex 1a Planta 08028 Barcelona, Spain
email: dafex@tiscali.ch

Planarian tissues consist of several differentiated non-proliferating cell types and a somatic stem-cell type called neoblast representing approximately 15-25% of total cell number in the parenchyma which can give rise to any cell type.

Planarians can regenerate along any body axis. When a planarian is amputated, the epithelium around the wound closes up and neoblasts aggregate below to form a blastema. A process of tissue remodelling occurs at the same time as the whole animal reorganises so that all its structures are scaled to its new size, eventually completely restoring symmetry and proportion. Asexual worms have been used in most studies on planarian regeneration, although sexual worms also possess the capacity for regeneration. Interestingly, during regeneration of sexual worms, there is a regression of the ovaries, testes, yolk glands, and copulatory apparatus which has been observed on histological level in several species. Moreover most planarians allow long periods of starvation, and during this time they shrink from an adult size to, and sometimes beyond, the initial size at hatching, growing back to adult size when fed. In sexual planarians, first the gonads get reabsorbed before shrinking.

In contrast to commonly studied model invertebrates, planarians do not appear to segregate their germ cell lineage during early embryogenesis. Rather, the germ cells arise post-embryonically and require inductive interactions for their specification. Under stress-inducing situations the sexual structures disappear and are believed to be formed *de novo* when the conditions become optimal again. Apoptosis was thought to be the main cell death process involved in the destruction of those sexual structures. Our studies on *Gtdap-1*, the planarian ortholog of human death-associated protein-1 or *DAP-1,* show that autophagy and autophagic cell death are the main processes involved. We think that autophagy would provide the necessary energy for the survival of a part of the germ cells which would work like a positional clue for the rebuilding of the sexual structures while the rest would undergo autophagic cell death providing energy to the other cells.

Cell stress and death response in animal and cellular models of Alzheimer Disease

Elisabetta Ferraro[1], Daniela De Zio[1], Marcello D'Amelio[1], Francesca Navoni[1], Simona di Martino[1] and Francesco Cecconi[1]

Dulbecco Telethon Institute at IRCCS Fondazione Santa Lucia, 00143 Rome, Italy
e-mail: francesco.cecconi@uniroma2.it

By using a cell line devoid of Apaf1 (ETNA$^{-/-}$ cells) and treating them with amyloid-β (Aβ) peptide we have previously found that apoptosome-mediated apoptosis is involved in Alzheimer Disease (AD).
To define in detail the role of each component of the apoptosome and the possibility and modality of recovery of cells receiving neurodegenerative stimuli in the absence of Apaf1 we are now comparing Apaf1$^{-/-}$ ETNA cells with ETNA cells overexpressing a dominant negative form of caspase-9 (DNC9-ETNA cells), which binds the apoptosome but does not cleave effector caspases. We are analyzing the mitochondrial respiration and other metabolic aspects of these cells induced to die but rescued by the absence of the apoptosome to understand if their viability is supported by mitochondrial respiration or by glycolysis.
Since the amount of the Aβ we used to treat cells is much higher compared to low nanomolar levels of natural Aβ found in the brain and cerebrospinal fluid in patients, we wanted to confirm our findings in a different model of AD, i.e. ETNA cells overexpressing a mutant form of APP (Amyloid Precursor Protein), which is associated with familial AD, the Swedish mutation (APPswe, KM670/671NL). Proneural ETNA cells are only slightly affected by APPswe overproduction. By contrast, ETNA cells are highly sensitive to APPswe once they differentiate in mature neurons.
Also, to understand the role of Apaf1 in AD we want now to check the effect of the absence of Apaf1 in a murine model of this disease: a transgenic mouse that expresses APPswe (Tg2576). To do this we generated the conditional knockout of *Apaf1* by means of the Cre-loxP strategy. We designed a targeting vector containing two loxP sites, which was integrated in homologous condition within the *Apaf1* locus. We are using, to specifically recombine the locus, a Cre mouse line (αCamIIK-Cre) that expresses Cre under control of an adult neuronal specific promoter. The phenotype analysis of the conditional Tg2576-*Apaf1*$^{-/-}$ mice will elucidate the involvement of the apoptosome in AD disease.

Characterization of a RIP1-containing high molecular weight complex following oxidative stress

Nele Festjens, Kathleen D'Hondt, Lieselotte Vande Walle, Isabel Vanoverberghe, Tom Vanden Berghe and Peter Vandenabeele

Molecular Signalling and Cell Death Unit, Department for Molecular Biomedical Research, VIB and Ghent University, Fiers-Schell-Van Montagu Building, Technologiepark 927, B-9052 Ghent, Belgium,
E-mail: Nele.Festjens@dmbr.Ugent.be.

As RIP1 is known to integrate several different upstream signals to initiate a limited number of cellular responses, it is tempting to speculate that particular RIP1-containing complexes will act as crucial signalling centres conveying signals that lead to either inflammation or cell death. Recently, we described the spontaneous formation of a large RIP1-containing complex in HEK293T cells that apart from RIP1 also contains TNF receptor associated factor 2 (TRAF2) and caspase-2 and is able to induce NF-kB and p38 MAPK activation. We independently described a similar RIP1/caspase-2/TRAF2 complex in Jurkat cells following incubation of cell extracts at 37°C. Further characterization led to the identification of IKKa, Hsp90 and PKR in the complex, which are also main players during the initiation of an inflammatory response. Besides, we demonstrated the involvement of RIP1 in both oxidative stress-induced caspase-independent death and anti-apoptotic signalling pathways in Jurkat cells. Regardless of the outcome, oxidative stress also seems to induce the formation of a RIP1-containing complex, which led us to hypothesize that this complex might act as a central signalling centre that integrates the regulation of processes of inflammation, apoptosis or necrosis, depending on the type of insult.

p66Shc promotes T-cell apoptosis by inducing mitochondrial dysfunction and impaired Ca^{2+} homeostasis

Francesca FINETTI ,[1]Michela PELLEGRINI, [1] Valeria PETRONILLI,[2] Cristina ULIVIERI,[1] Fabiola GIUSTI,[1] Pietro LUPETTI,[1] Marco GIORGIO,[3] Pier Giuseppe PELICCI,[3] Paolo BERNARDI,[2] and Cosima T. BALDARI[1]

[1]Department of Evolutionary Biology, University of Siena, I-53100 Siena, Italy
[2]Department of Biomedical Sciences and CNR Institute of Neuroscience, University of Padova, I-35121 Padova, Italy
[3]Department of Molecular Oncology, European Institute of Oncology, I-20141 Milano, Italy
e-mail: finetti4@unisi.it

p66Shc, a redox enzyme that enhances ROS production by mitochondria, promotes T-cell apoptosis. We have addressed the mechanisms regulating p66Shc-dependent apoptosis in T-cells exposed to supraphysiological increases in $[Ca^{2+}]_c$. p66Shc expression resulted in profound mitochondrial dysfunction in response to the Ca^{2+} ionophore A23187, as revealed by dissipation of mitochondrial transmembrane potential, cytochrome-c release and decreased ATP levels. p66Shc expression also caused a dramatic alteration in the cells' Ca^{2+}-handling ability, that resulted in Ca^{2+} overload after A23187 treatment. The impairment in Ca^{2+} homeostasis was ROS-dependent and caused by defective Ca^{2+} extrusion due at least in part to decreased PMCA expression. Both effects of p66Shc required Ca^{2+}-dependent serine-36 phosphorylation. The mitochondrial effects of p66Shc were potentiated by but not strictly dependent on the rise in $[Ca^{2+}]_c$. Thus, Ca^{2+}-dependent p66Shc phosphorylation causes both mitochondrial dysfunction and impaired Ca^{2+} homeostasis, which synergize in promoting T-cell apoptosis.

Identification of PP1alpha as a caspase-9 regulator in IL-2 deprivation induced apoptosis

Frederic Dessauge[1], Xavier Cayla[2], Aarne Fleischer[1,4], Juan Pablo Albar[3], Ata Ghadiri[1], Marianne Duhamel[1] and Angelita Rebollo[1]

1/ Laboratoire d'Immunologie Cellulaire et Tissulaire, Hôpital Pitié-Salpêtrière, U543 Inserm, Bâtiment CERVI, 83, Bd de l'Hôpital, 75013 Paris, France.
2/ Equipe Hypophyse, UMR6175, INRA-CNRS, Université de Tours, Haras-Nationaux, Physiologie de la Reproduction et des Comportements 37380 Nouzilly, France.
3/ Centro Nacional de Biotecnologia, Campus de Cantoblanco, UAM, 28049 Madrid, Spain.
4/ Present address: Centro de Investigación del Cancer, CSIC, Campus Miguel de Unamuno, 37007 Salamanca, Spain

One of the mechanisms regulating cell death is the reversible phosphorylation of proteins. ERK/MAPK phosphorylates caspase-9 at Thr125 and this phosphorylation is crucial for caspase-9 inhibition. Until now, the phosphatase responsible of Thr125 dephosphorylation has not been described yet. Here, we demonstrate that in IL-2 proliferating cells, phosphorylated PP1a associates with phosphorylated caspase-9. IL-2 deprivation induces PP1a dephosphorylation, which leads to its activation and as a consequence, dephosphorylation and activation of caspase-9 followed by subsequent dissociation of both molecules. In cell-free systems supplemented with ATP caspase-9 activation is triggered by addition of cytochrome *c* and we show that in this process PP1a is indispensable for triggering caspase-9, as well as caspase-3 cleavage and activation. Moreover, PP1a associates with caspase-9 *in vitro* and *in vivo* suggesting that this phosphatase is responsible for caspase-9 dephosphorylation. Finally, we describe two novel phosphatase-binding sites different to the previously described PP1a consensus motifs and we demonstrate that these novel sites mediate the interaction of PP1a with caspase-9.

p400 function is important for the ability of E1A to downregulate EGFR and to induce apoptosis in tumour cells

Marcella B. Flinterman[1], Poramaporn Klanrit[1], Ahmed F. Yousef[2], Joe S. Mymryk[2] and Mahvash Tavassoli[1]

[1]Head and Neck Oncology Group, King's College London, UK; [2]Departments of Oncology and Microbiology & Immunology, University of Western Ontario, Canada. mahvash.tavassoli@kcl.ac.uk

Epidermal growth factor receptor (EGFR) is a member of the tyrosine kinase family (Type 1) of cell surface receptors. Overexpression of EGFR has been associated with increased tumour growth, metastasis, and adverse outcome in many epithelial cancers, particularly squamous cell carcinomas of lung and head and neck (HNSCC). We have recently shown that early region 1A (E1A) protein of human adenovirus downregulates endogenous EGFR expression and induces apoptosis in HNSCC cells independently of their p53 status. It has been shown that E1A mediated suppression of EGFR occurs at the transcriptional level and requires amino acid residues 38-65 within conserved region (CR1) domain. These findings identify EGFR as a potential target for virally induced apoptosis. E1A-based strategies could therefore be developed for killing of EGFR overexpressing cancers. E1A has 5 different isoforms and the major isoforms E1A12S and E1A13S regulate transcription of several cellular proteins by binding to transcriptional modulators such as pRB, CtBP, p300 and p400. In order to determine the region/s of E1A important for EGFR suppression and induction of apoptosis, we have used a panel of different adenovirus type 2 E1A isoforms as well as E1A deletions/mutants unable to bind p300, p400 and pRB. E1A12S isoform showed the strongest effect on the suppression of EGFR protein expression. E1A12S expressed from human adenovirus types 3, 9 and 12 suppressed EGFR similarly to E1A12S of adenovirus types 2, whereas E1A12S of the adenovirus type 4 and 40 had no effect on EGFR expression. Luciferase reporter assays showed that E1A was not directly able to suppress transcription from the EGFR-promoter. Using deletion mutants of E1A12S, it was shown that interaction of E1A with p400, but not p300 or pRB plays an important role in EGFR suppression by E1A. Using siRNA strategy to knock down p400, it was confirmed that HNSCC cells with reduced p400 expression were less sensitive to E1A-induced suppression of EGFR and apoptosis. p300 function seems to be dispensable as both E1A mutants unable to bind p300 as well as p300 knock out HTC-116 colon cancer cells were highly sensitive to E1A-induced cell death as compared to wild type cells. In summary this study identifies p400 but not p300 or pRB as an important mediator of E1A-induced apoptosis and EGFR downregulation.

The BH3-only protein Bim in the mitochondrial apoptotic pathway

G. Forro[1], B. Gillissen[1], A. Richter[1] and P.T. Daniel[1,2]

[1]Clinical and Molecular Oncology, University Medical Center Charité, Campus Berlin-Buch and [2]Max Delbrück Center for Molecular Medicine, Germany

Members of the Bcl-2 family, consisting of anti- and pro-apoptotic proteins, are crucial regulators of programmed cell death. Release or post-translational modification of BH3-only proteins by distinct upstream signals leads to activation of the pro-apoptotic proteins Bax and Bak and, consequently, to induction of apoptosis. Bim, a BH3-only protein, is expressed in three main isoforms, BimS, BimL and BimEL. Bim that act in the intrinsic apoptotic pathway. To investigate the function of BimL and BimS, we used a regulatable adenoviral vector based on the Tet-off system. To study the role of Bak and Bax in Bim-induced apoptosis, we overexpressed BimL and BimS in Bax-negative DU145 cancer cells. These cells were resistant to apoptosis induced by both Bim isoforms tested. DU145 cells stably re-expressing Bax were sensitized for BimL- and BimS-induced apoptosis. In contrast to the BH3-only protein Nbk, Bax-negative, Bak-overexpressing DU145 cells underwent apoptosis upon BimL and BimS overexpression. These results indicate that BimL and BimS exert their apoptotic function via both Bak- and Bax-dependent pathway. Bak and Bax activation by Bim is followed by the breakdown of the mitochondrial membrane potential and cytochrome c release. Furthermore, we showed that the effector caspase-9 and the executioner caspase-3 are similarly important for Bim-induced cell death.

OPA1 Controls Apoptotic Cristae Remodelling Independently from Mitochondrial Fusion

Christian Frezza[1], Sara Cipolat[1], Olga Martins de Brito[1], Massimo Micaroni[2], Galina V. Beznoussenko[2], Tomasz Rudka[3], Davide Bartoli[1], Roman S. Polishuck[2], Nika N. Danial[4], Bart De Strooper[3] and Luca Scorrano[1]

[1]Dulbecco-Telethon Institute, Venetian Institute of Molecular Medicine, Padova, Italy; [2]Department of Cell Biology and Oncology, Consorzio "Mario Negri Sud", Santa Maria Imbaro, Italy; [3]Neuronal Cell Biology and Gene Transfer Laboratory, Center for Human Genetics, Flanders Interuniversity Institute for Biotechnology (VIB4) and K.U. Leuven, Belgium; [4]Department of Cancer Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA.

Mitochondria amplify activation of caspases during apoptosis by releasing cytochrome c and other cofactors. This is accompanied by fragmentation of the organelle and remodeling of the cristae. Molecular mechanisms governing the latter remain unclear. Optic Atrophy 1 (OPA1), a pro-fusion dynamin-related protein of the inner mitochondrial membrane mutated in dominant optic atrophy protects from apoptosis by preventing cytochrome c release. This is independent from mitochondrial fusion but depends on the oligomerization of two forms of OPA1, the soluble, intermembrane space and the inner membrane integral one. The pro-apoptotic BCL-2 family member BID disrupts OPA1 oligomers, while high levels of OPA1 stabilize them and prevent mobilization of cytochrome c. OPA1 does not interfere with activation of the mitochondrial “gatekeepers” BAX and BAK, but controls shape of mitochondrial cristae during apoptosis. Thus, OPA1 has genetically and molecularly distinct functions in mitochondrial fusion and in cristae remodelling during apoptosis.

Identification and characterization of novel PTP inhibitors

Alberto Gambalunga[1], Eva Milanesi[2], Marco Giorgio[3], Saverio Minucci[2], Pier Giuseppe Pelicci[3], Valeria Petronilli[1] and Paolo Bernardi[1]

From the [1]Department of Biomedical Sciences, University of Padova (Italy), [2]Congenia s.r.l., Via Adamello 16, Milano (Italy), [3]Department of Experimental Oncology, European Institute of Oncology and FIRC Institute of Molecular Oncology, Milano (Italy).

The mitochondrial permeability transition pore (PTP) is a high conductance channel whose importance in the mitochondrial pathways leading to cell death is increasingly recognized. Despite major efforts, however, its molecular nature remains undefined, a state of affairs which considerably hampers progress in the field [1]. In order to develop drugs acting on the PTP and to search for molecules that could be used for target identification we have carried out a high throughput screening of a chemical library for PTP inhibitors. We have identified three classes of novel high affinity inhibitors, and we have thoroughly characterized them on isolated mitochondria for the presence of toxic effects on respiration and on energy-linked transport functions. A structure-function study is under way which is defining compounds for treatment of PTP-dependent disease models and target identification.

1. Bernardi P, Krauskopf A, Basso E, Petronilli V, Blachly-Dyson E, Di Lisa F & Forte MA (2006) The mitochondrial permeability transition from in vitro artifact to disease target. *FEBS J* 273**,** 2077-2099.

Promotion of TRAIL-induced apoptosis in HeLa cells expressing Bcl-2.

Marine Gasparian[1], Alexey Lomakin[2], Ekaterina Popova[2], Lidia Domnina2, Olga Ivanova[2], Olga Pletjushkina[2], Dmitry Dolgikh[2], and Boris Chernyak[2]

[1]Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow
[2]*A.N. Belozersky Institute, Moscow State University*
e-mail: marine_gasparian@yahoo/com

TRAIL (TNF-Related Apoptosis Inducing Ligand) selectively induces apoptosis in various transformed cells and is a potent anti-tumor cytokine. Recombinant trimeric TRAIL induces apoptosis, which depends on cytochrome c release from mitochondria and is strongly inhibited by expression of Bcl-2 in HeLa cells. Inhibitors of microtubules (nocadazol, taxol), of actin cytoskeleton (cytochalasin D) and of actomyosin related kinases (HA 1077, H7) do not cause apoptosis in HeLa-Bcl-2 but stimulate TRAIL-induced apoptosis in these cells. Caspase-8 is activated to the same extent by TRAIL alone and in combination with the inhibitors. The following cytochrome *c* release is strongly stimulated, so the bypass of Bcl-2 block via direct activation of caspase-3 by caspase -8 seems not probable explanation of the effect. Bcl-2 is not completely inactivated by the inhibitors of cytoskeleton since staurosporin-induced apoptosis and apoptosis caused by energy deprivation are still inhibited in HeLa-Bcl-2. TRAIL-induced apoptosis in HeLa cells is stimulated by inhibitors of protein synthesis (emetine, cycloheximid) indicating significant (probably NFkB-dependent) stimulation of expression of antiapoptotic proteins by TRAIL. In the presence of emetine TRAIL-induced apoptosis remains sensitive to Bcl-2 and the inhibitors of cytoskeleton augment apoptosis in HeLa-Bcl-2. These inhibitors also released inhibition of TNF-induced apoptosis and cytochrome *c* release by Bcl-2. It is suggested that structure and activity of cytoskeleton modulate mitochondria-related steps of apoptosis caused by TRAIL and TNF. The combined therapy with TRAIL and the inhibitors of cytoskeleton could be very promising for tumors with high level of Bcl-2 resistant to various chemotherapeutic agents.

Engagement of alpha2beta1 integrin reduces doxorubicin-induced apoptosis in leukemic T cell

Steve Gendron, Julie Couture, Genevieve Pelletier and Fawzi Aoudjit.

Centre de Recherche en Rhumatologie et Immunologie, CHUQ.Pavillon CHUL 2705 Blvd Laurier, local T1-49, Ste-Foy, Quebec, Canada G1V 4G2

Purpose: The resistance of cancer cells against drug-induced apoptosis in chemotherapies is a major cause of defect in anti-cancer therapies. Cell adhesion to extracellular matrix protein (ECMp) can be a mechanism that contributes to this resistance. Type I collagen (Coll I) is a major constituent of ECMp and it is also found in leukemic T cell microenvironment. In this study, we aimed to determine if leukemic T cells adhesion to Coll I can protect them from doxorubicin (Dox)-induced apoptosis, a drug widely used in chemotherapies, and contribute to their resistance.
Methods: We used leukemic T cell lines Jurkat, CEM and HSB-2 as model of leukemic T cells. Surface expression of alpha2beta1 integrin and the membrane form of RANKL was measured by flow cytometry analysis. Apoptosis was measured by Annexin V/7-AAD staining followed by flow cytometry analysis. mRNA expression of RANKL and its receptor RANK was measured by RT-PCR. JNK phosphorylation was determined by western blot.
Results: We found that Dox induces apoptosis in leukemic T cell in part by activating the RANKL/RANK signaling system. Furthermore, we show that alpha2beta1/Coll I signaling protect leukemic T cell from Dox-induced apoptosis by inhibiting RANKL expression. We also show that Dox-induced apoptosis is dependent of JNK activation that is significally reduced by Coll I signaling. Conclusion: Our results suggest that by reducing RANKL expression, adhesion of leukemic T cells to Coll I can contributes to their resistance against drug-induced apoptosis. This suggest that blockade of alpha2beta1 integrin could be a therapeutic approach to turn down leukemic T cells resistance against chemotherapies.

Autophagy as a backup mechanism for resveratrol-induced cell death in apoptosis-incompetent cells

Francesca Scarlatti, Roberta Maffei, and Riccardo Ghidoni.

Laboratory of Biochemistry and Molecular Biology, San Paolo University Hospital, School of Medicine, University of Milan, 20142 Milan, Italy.

Autophagy can contribute to tumor suppression and defects in autophagy may promote oncogenesis. Moreover autophagy can represent an alternative way to cell death when apoptosis is impeded. In cells that resist to apoptosis and in those that cannot be removed by engulfment cells, the autophagy machinery may be used for self-destruction.
Here we wish to explore the hypothesis that resveratrol, a natural polyphenol derived from fruits and grape, potentially interesting for breast cancer therapy, uses autophagy as a mechanism alternative to apoptosis to induce death in cells defective in caspase-dependent apoptotic pathways. This hypothesis is based on preliminary data from our laboratory where we observed that resveratrol induces autophagy cell death in caspase-3 negative MCF-7 breast cancer cells, but is unable to induce caspase-dependent apoptotic cell death. Otherwise, in caspase-3-transfected MCF-7 breast cancer cells resveratrol induces caspase-3 activation, PARP-cleavage and nuclear DNA damage.
In caspase-3 negative MCF-7 breast cancer cells resveratrol stimulates autophagy by increasing the formation of LC3-positive autophagosomes and the rate of proteolysis sensitive to the autophagy inhibitor 3-methyladenine. In addition, in this cell line, autophagic cell death is under the control of Atg7 protein and Akt/PKB signaling.
We suggest that resveratrol utilizes autophagy as an alternative strategy to accomplish cell death in breast cancer cells with an incompetent caspase-dependent apoptotic pathway: In perspective, resveratrol could be proposed as an agent to elicit autophagy in those cancer cells unresponsive to drugs that specifically target caspase-dependent apoptosis.

p53-dependent antiapoptotic function of synphilin-1 is mediated by its caspase-3 derived C-terminal product

Giaime Emilie[1], Sunyach Claire[1], Herrant Magalie[2], Grosso Sébastien[2], Auberger Patrick[2], McLean Pamela J. [3], Checler Frédéric[1,4] and Alves da Costa Cristine[1,4].

1- IPMC du CNRS, UMR6097, Equipe labellisée Fondation pour la Recherche Médicale, 660 Route des Lucioles, 06560, Valbonne, France.
2- Faculté de Médicine, INSERM U526, Equipe labellisée Ligue Nationale contre le Cancer, Avenue de Valombrose, 06107 Nice Cedex 2, France.
3- Alzheimer Disease Research Unit, Department of Neurology, Massachusetts General Hospital, Charlestown, Massachusetts 02129.

Parkinson's disease (PD) is the second most frequent neurodegenerative disorder after Alzheimer's disease. PD is characterized by the presence of intracytoplasmic inclusions, named Lewy bodies and by a massive loss of dopaminergic neurons in the substantia nigra. A subset of genetic forms of PD has been attributed to α-synuclein, a synaptic protein with remarkable chaperone properties. A yeast two-hybrid approach has led to the identification of a novel α-synuclein cellular partner named synphilin-1 a cytoplasmic protein. Mutation analysis of the synphilin-1 gene in familial and sporadic German PD patients allowed the identification of the R621C mutation in two sporadic PD patients, suggesting a putative role of synphilin-1 as a genetic susceptibility factor for the disease. According to the implication of synphilin-1 in PD and to the modulation of cell death by α-synuclein and parkin, two privileged binding partners of synphilin-1. We investigated the role of synphilin-1 in cell death control. We have established transient and stable transfectants overexpressing wild-type synphilin-1 in different cell systems and we show that all cell systems display lower responsiveness to staurosporine and 6-hydroxy-dopamine by decreasing caspase-3 activity and poly-ADP ribose polymerase and by down-regulating the p53-dependent pro-apoptotic pathway. Interestingly, we demonstrate that synphilin-1 catabolism is enhanced by staurosporine and blocked by caspase-3 inhibitors. *In silico* examination of synphilin-1 sequence revealed a consensus site for a caspase-3 cleavage and we show by transcription/translation assay that recombinant caspase-3 hydrolyze synphilin-1. Accordingly, we demonstrate the cleavage of synphilin-1 by cellular and purified caspase-3 and the abolishment of its anti-apoptotic function by site-directed mutagenesis of the caspase-3 site in its sequence. Finally, we demonstrate that the C-terminal fragment of synphilin-1 generated by caspase-3 is indeed responsible for the antiapoptotic phenotype of synphilin-1. In conclusion, we show for the first time that synphilin-1 displays an antiapoptotic function via p53 and that this phenotype is mediated by its C-terminal caspase-3 derived product.

Induction of apoptosis by Nbk via an ER pathway depends on Bax but is independent of Bak

Bernhard Gillissen[1], Philipp Hemmati[1], Dilek Güner[1], Gaby Forro[1], Frank Essmann[3], Antje Richter[1], Anja Richter[1], Peter T. Daniel[1,2]

[1]Clinical and Molecular Oncology, University Medical Center Charité, [2]Max-Delbrück-Center for Molecular Medicine, Berlin, Germany, [3]Institute of Molecular Medicine, University of Düsseldorf, Germany

BH3-only proteins have recently been recognized as essential initiators of apoptosis whereas multidomain pro-apoptotic Bax and Bak are executioners of death orders relayed by the BH3-only proteins. We have recently shown that Bax deficiency protects cells from apoptosis induction by the BH3-only protein Nbk/Bik. Nevertheless, it has been assumed that life-death decisions rest simply on the balance of the abundance of all proapoptotic versus antiapoptotic family members. Thus, we could not rule out that loss of Bax protects cells just by decreasing the amount of Bax/Bak-like molecules under a critical threshold necessary for Nbk to induce apoptosis. We therefore studied the influence of Bak knock-down in the Bax-proficient cell line HCT116. Bak knock-down by shRNA did, however, fail to protect these cells from Nbk induced apoptosis, whereas Bax knock-out resulted in complete resistance to Nbk enforced cell death. Additionally, re-expression of Bax in Bax deficient and Nbk-resistant DU145 cells markedly sensitized DU145 cells to Nbk induced apoptosis whereas Bax negative, Bak-overexpressing DU145 cells remained resistant. Expression of Nbk induced clustering of GFP-Bax but not of GFP-Bak fusion in DU145 cells. These results indicate that Nbk acts via an entirely Bax dependent pathway as opposed to Bcl-x_S that triggers cell death via a Bax-independent, Bak-dependent pathway. Notably, and in contrast to the BH3-only protein Noxa, Nbk increases the expression level of the antiapoptotic Bcl-2 family protein Mcl-1, which in turn sequesters and inactivates Bak. Although activation of Bax and induction of apoptosis by Nbk coincides with loss of mitochondrial membrane potential, Nbk does not colocalize with mitochondria but with the endoplasmic reticulum (ER). Consequently, targeting of Bcl-2 to either of these subcellular organelles protects cells from Nbk induced apoptosis. Bcl-2 targeted to the ER inhibits dissipation of the mitochondrial membrane potential and release of cytochrome c. Therefore, in Nbk-induced apoptosis, crosstalk between ER and the mitochondria is crucial. Thus, Nbk induces apoptosis via an ER initiated, Bax-dependent and Bak-independent dependent signalling pathway. This results in downstream triggering of the mitochondria and cell death execution via the mitochondrial apoptosis machinery.

The N-terminal conformation of Bax regulates cellular commitment to apoptosis.

John-Paul Upton, Anthony J. Valentijn and Andrew P. Gilmore

Wellcome Trust Centre for Cell Matrix Research, Faculty of Life Sciences, A.3034 Smith Building, The University of Manchester, United Kingdom. agilmre@manchester.ac.uk

A hallmark of invasive breast cancer is that cells that are normally restricted to a specific tissue compartment metastasise and repopulate diverse sites. This event is the major cause of mortality in patients with advanced breast cancer. In normal mammary cells, interactions with the extracellular matrix (ECM) provide positional information required for maintaining the integrity of the epithelia. In the absence of the correct adhesion signals, normal cells undergo apoptosis (termed anoikis), a process which is abrogated in invasive breast cancer.

We have previously shown that anoikis in mammary epithelial cells requires the pro-apoptotic Bcl-2 protein, Bax. Bax normally resides in the cytosol, but during apoptosis it translocates to mitochondria where it is responsible for releasing apoptogenic factors such as cytochrome *c*. Bax undergoes an N-terminal conformational change that has been suggested to regulate this redistribution. Using anoikis as a model, we have shown that Bax translocation does not commit cells to apoptosis, and they can be rescued by reattachment to ECM within a specific time, resulting in Bax redistributing back to the cytosol. Prolonged loss of the correct ECM signals results in cells becoming committed to anoikis. We have now investigated the molecular basis for this commitment. We show that the Bax N-terminus regulates commitment and OMM permeabilisation, but not its translocation to mitochondria. We identify Pro13 within the N-terminus of Bax as critical for this regulation. The subcellular distribution of Pro13 mutant Bax was identical to wild type Bax in both healthy and apoptotic cells. However, whereas cells expressing WT Bax could be rescued from anoikis by reattachment, Pro13 mutant Bax could not. Pro13 mutant Bax induced rapid progression to commitment, mitochondrial permeabilisation and death. Our data identify changes in Bax controlling commitment that are mechanistically distinct from those controlling its subcellular localisation. Together, they indicate that multiple regulatory steps are required to fully activate Bax on mitochondria.

Inspected by p53

Michal M. Godlewski[1]✉, Natalia Hallay[1], Monika Slupecka[2], Jaroslaw Wolinski[2], Romuald Zabielski[1,2]

[1]Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Nowoursynowska 159, 02-776 Warsaw, Poland, e-mail: mickgodl@hotmail.com
[2]The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jablonna, Poland.

Intensive growth of the small intestine starts several weeks before parturition and is characterized by a faster growth rate than the fetal body as a whole. Small intestine growth further accelerates after birth following the first administration of colostrum. Besides intensive growth, in the early postnatal period the small intestinal epithelium undergoes an essential maturation process. Enhanced proliferation of crypt stem cells results in increased susceptibility and frequency of replication errors and mutations, which trigger the p53-dependent cell cycle arrest and, in case the error cannot be repaired, the p53- and Bcl-2 family-dependent death pathways. Intensive animal production preferred nowadays tend to decrease of the sow-rearing time to the minimum and piglets are switched to milk formula earlier. This results in greater susceptibility to disease, slower growth and increased mortal rate. To cope with these problems research now aims at finding a way to supplement milk formula with bioactive substances to match colostrum and milk composition. Supplementation with the leptin resulted in an increase of mitosis to apoptosis ratio in newborn piglets in comparison to milk formula fed. The mitotic index was higher even than in sow-reared ones. Surprisingly the number of cells expressing p53 remained elevated. Similar results were observed when other promitotic factors were involved. Latest studies performed on newborn piglets showed that during periods of intensive growth and remodeling of intestinal mucosa all of the p53-positive cells expressed also active caspase 3. This suggests that all of them were diverted to the apoptosis pathways, rather then towards repair. The possible cause of this phenomenon is the fast rate of enterocyte turnover which leaves no time for time-consuming and energy-requiring process of DNA repair.

Gtdap-1 is involved in autophagy and autophagic programmed cell death in planarians

Cristina González-Estévez, Daniel A. Felix & Emili Saló

Departament de Genètica. Facultat de Biologia. Universitat de Barcelona
Av. Diagonal 645, Edifici Annex 1a Planta 08028 Barcelona, Spain
email: crisgonzalez@ub.edu

Planarians have a great morphological plasticity supported by continuous growth and degrowth of the body and a high regenerative capacity. In cellular terms, growth and degrowth are tightly coupled to the rates of cell proliferation and cell death, and its balance is influenced by food availability, temperature, body size and the genetic characteristics of the planarian. During starvation planarian decrease in size due to an increase of the cell death rates since the proliferation rates are maintained. On the other hand planarian regeneration requires wound healing and an increase of the proliferation rates of its somatic stem cells called neoblasts. The mitotic activity is higher in regions close to the wound in order to produce the new tissue or blastema (epimorphosis), while the rest of the planarian fragment adjusts to its new proportions by the production of new cells and by eliminating superfluous cells by programmed cell death (morphallaxis). Such cell death has not been demonstrated up to now.

We have studied the gene *Gtdap-1*, the planarian ortholog of human death-associated protein-1 or *DAP-1*. DAP-1 together with DAP-kinase has been identified as a positive mediator of programmed cell death induced by gamma-interferon in HeLa cells. While the function of DAP-kinase is well characterised the role of DAP-1 had not been studied in detail. We find that *Gtdap-1* is involved in autophagy and in autophagic programmed cell death dependent on caspase-3. The gene functions at the interface between survival and cell death during stress-inducing processes like regeneration, starvation and gamma-irradiation in sexual and asexual races of planarians. Our findings provide insight into the complex interconnections between cell death, cellular proliferation and cell homeostasis in planarians and new perspectives for understanding neoblast stem cell dynamics.

YopP of *Yersinia enterocolitica* induces apoptotic and necrosis-like cell death in murine dendritic cells

Sabine Gröbner[1], Stella E Autenrieth[1], Irena Soldanova[1], Dani S J Gunst[1], Martin Schaller[2], Erwin Bohn[1], Steffen Müller[1], Martin Leverkus[3], Sebastian Wesselborg[4], Ingo B Autenrieth[1], Stefan Borgmann[1]

Institute of Medical Microbiology and Hygiene, Eberhard-Karls-University of Tübingen[1], Department of Dermatology, Eberhard-Karls-University of Tübingen[2], Laboratory of Experimental Dermatology, Department of Dermatology and Venerology, Otto-von-Guericke-University of Magdeburg[3] and Department of Internal Medicine I, Eberhard-Karls-University of Tübingen[4]

Yersinia outer protein P (YopP) is a virulence factor of *Yersinia enterocolitica* that is injected into the cytosol of host cells where it targets MAP kinase kinases (MKKs) and inhibitor of κB kinase (IKK)-β resulting in inhibition of cytokine production as well as induction of apoptosis in macrophages. Here we show that YopP induces cell death of dendritic cells (DC). DC death was only partially prevented by the broad spectrum caspase inhibitor zVAD-fmk, indicating simultaneous caspase-dependent and caspase-independent mechanisms of cell death induction by YopP. Microscopic analyses and measurement of cell size demonstrated necrosis-like morphology of caspase-independent cell death. Application of zVAD-fmk prevented cleavage of procaspases and Bid, decrease of the inner transmembrane mitochondrial potential $\Delta\Psi_m$ and mitochondrial release of cytochrome c. From these data we conclude that YopP-induced activation of the mitochondrial death pathway is mediated upstream via caspases. In conclusion, our results suggest that YopP simultaneously induces caspase-dependent apoptotic and caspase-independent necrosis-like cell death in DC mediated by a yet unknown mechanism.

Characterization of molecular pathways involved in the apoptotic defects of collagen VI knockout mice, a model of human muscular dystrophies

Paolo Grumati[1], Tania Tiepolo[1], Natascha Bergamin[1], Alessia Angelin[2], Paolo Bernardi[2], Patrizia Sabatelli[3], Nadir M. Maraldi[3], Luciano Merlini[4], Paolo Bonaldo[1]

Departments of [1]Histology, Microbiology and Medical Biotechnologies, and [2]Biomedical Sciences, University of Padova (Italy); [3]ITOI-CNR and University of Bologna (Italy); [4]Department of Medical Genetics, University of Ferrara (Italy)

Collagen VI (ColVI) is an extracellular matrix protein forming a microfilamentous network in skeletal muscles and other organs. The protein is made of three distinct alpha-chains coded by separate genes. To investigate the function of collagen VI in vivo, we generated knockout mice by targeted inactivation of the *Col6a1* gene. *Col6a1*$^{-/-}$ mice display an early-onset myophatic phenotype affecting diaphragm and other skeletal muscles. Inherited mutations of genes encoding ColVI in humans cause two muscle diseases, Bethlem myopathy and Ullrich congenital muscular dystrophy.
Col6a1$^{-/-}$ muscles have a loss of contractile strength associated with ultrastructural alterations of sarcoplasmic reticulum and mitochondria and spontaneous apoptosis. Skeletal myofibers show a latent mitochondrial dysfunction, with loss of mitochondrial membrane potential, abnormal calcium homeostasis and increased sensitivity to apoptotic stimuli. These defects are due to increased opening of the permeability transition pore, a mitochondrial protein complex playing a key role in apoptosis. Lack of ColVI is the cause of these defects since they could be normalized by plating *Col6a1*$^{-/-}$ myofibers on purified ColVI [1].
Signaling pathways regulated by ColVI are largely unknown. A recent study on breast cancer demostrated that ColVI promotes cells survival and proliferation by promoting b-catenin stability and increased cyclin D1 levels [2]. My PhD project is aimed at obtaining a better insight into the molecular pathways involved in the transduction of ColVI signals within the cells and responsible for the mitochondrial dysfunction and apoptosis in myopathic muscles from *Col6a1*$^{-/-}$ mice and Ullrich patients (see also Angelin et al., this meeting). Primary myoblasts dissociated from ColVI-deficient muscles display spontaneous apoptosis and mitochondrial dysfunction. Studies on *Col6a1*$^{-/-}$ myoblasts indicate that lack of ColVI affects the activity of focal adhesion components and of Akt/PKB protein kinase. Moreover, lack of ColVI seems also to trigger Bax and initiator caspases, an essential gateway to mitochondrial dysfunction and cell death. It is well established that Akt promotes cell survival by targeting phosphorylation of GSK3β and other proapoptotic factors, such as Bad and FoxO. Therefore, we are currently investigating whether there is any correlation between ColVI and Akt pathways promoting cell survival.

[1] Irwin W.A., Bergamin N., Sabatelli P., Reggiani C., Megighian A., Merlini L., Braghetta P., Columbaro M., Volpin D., Bressan G.M., Bernardi P. and Bonaldo P. (2003). Nature 35, 367-371.
[2] Iyengar P., Espina V., Williams T., Lin Y., Berry D., Jelicks L.A., Lee H.K., Temple K., Graves R., Pollard J., Chopra N., Russell R.G., Sasisekharan R., Trock B.J., Lippman M., Calvet V.S., Petricoin III E.F., Liotta L., Dadachova E., Pestell R.G., Lisanti M.P., Bonaldo P. and Scherer P.E. (2005). J Clin Invest. 115, 1163-1176.

Interaction of apoptosis signal transduction and capacitation in human spermatozoa

Sonja Grunewald, Uwe Paasch, Hans-Juergen Glander

European Academy of Andrology Training Center, University of Leipzig, Germany
sonja.grunewald@medizin.uni-leipzig.de

Capacitation of sperm is a prerequisite for successful fertilization, determined by hyperactivated motility, markedly increased tyrosine phosphorylation levels and membrane changes previous to the acrosome reaction. However, the exact molecular mechanism is not fully clarified. The calpain-calmodulin-system is essential for membrane fusion during this process and recently cross talks to the caspase (CP) signalling cascade were postulated. Activation of caspases is a main feature of apoptotic cells. The objective of our study was to examine interactions of apoptosis signalling and the calpain-calmodulin-system during capacitation. Semen samples of healthy donors (n=14) were washed by density gradient centrifugation. Four aliquots were incubated in BWW at 37°C 5% CO2 for 3h without additives (control), with 3% BSA (capacitation), 10µM calpain-inhibitor III or 20µM calmodulin-inhibitor (Ophiobolin A). Capacitation was monitored by computer assisted sperm motion analyser (hyperactivation), chlortetracycline (CTC)-assay (CTC-B - pattern is specific for membrane changes during capacitation) and Western blot analyses of tyrosine phosphorylation. FACS analyses were performed to evaluate the activation of CP-9 and -3 and the integrity of transmembrane mitochondrial potential (TMP). Capacitation proved by CTC-assay, increased levels of tyrosine phosphorylation and hyperactivation resulted in inactivation of CP9, CP3 and improved integrity of the TMP. The amount of spermatozoa showing active CP9 decreased by 9.8±6.3% and those with active CP3 by 4.8±8.3%, spermatozoa with intact TMP increased by 17.5±7.7% compared to control ($p<0.05$). Inhibition of calpain during capacitation had no further impact on CP9, CP3 and TMP, but diminished significantly the capability for capacitation. In contrast, inhibition of calmodulin resulted in blocking of physiological changes associated with capacitation and in induction of apoptosis: CP9+ sperm increased by 68.0±25.7%, CP3+ sperm by 67.8±25.2%, sperm with intact TMP decreased by 68.3±20.5% compared to the capacitated aliquot without inhibitors ($p<0.01$).
Capacitated sperm are characterized by lowest caspase activation and preserved mitochondrial potential integrity. Inhibition of calpain during capacitation reduced significantly the capacitation-related parameters, but did not lead to apoptosis. Inhibition of calmodulin resulted in blocking of physiological changes seen during capacitation as well as in stimulation of apoptosis. Interaction of both signalling systems seems to enable the capacitation process by prevention of apoptosis.

Anti-apoptotic properties and localization of different isoforms of cytoskeleton-related Hax-1 protein

Ewa A.Grzybowska, Maria Szwarc, Elzbieta Sarnowska and Janusz A. Siedlecki

Cancer Center Institute, Roentgena 5, 02-781 Warsaw, Poland, email:ewag@coi.waw.pl

Hax-1 is a potent anti-apoptotic protein, a substrate for Omi protease, localized in mitochondria. It is also associated with cytoskeletal proteins and is involved in cell motility regulation. Additionally, Hax-1 exerts mRNA-binding properties, which was demonstrated for vimentin transcript. Our own results suggest that Hax-1 binds not only vimentin mRNA but also the mRNA of DNA polymerase β, an enzyme involved in DNA repair (Base Excision Repair). We have also identified several splice variants of the rat Hax-1, encoding for putative protein isoforms, which might help to elucidate different functions of this multitask regulatory protein. Although there is no exact homology between Hax-1 and proteins from Bcl2-family, there are several common features, like the presence of the two BH domains (BH1 and BH2) and the transmembrane domain. One of the modifications introduced by alternative splicing is an internal deletion of 61 amino acids, encompassing the two BH domains. We have analyzed the influence of this modification on apoptosis, by measuring caspase-9 levels in stressed cells overexpressing different Hax-1 isoforms. We have also analysed localization of all isoforms, biochemically and by fluorescence microscopy, using EGFP fusions of all splice variants. Mitochondrial localization of Hax-1 was demonstrated, although biochemical evidence also suggests the possibility of nuclear localization. This last finding, coupled with Hax-1 transcript-binding properties indicates that it might constitute a part of the machinery responsible for mRNA export from the nucleus, especially given Hax-1 interactions with cytoskeletal proteins. Taken together, these data suggest that Hax-1 has a broad spectrum of functions, including regulation of apoptosis and control (at the posttranscriptional level) of the expression of at least two genes involved in cell motility and DNA repair.

c-FLIP-mediated resistance to death receptor-induced apoptosis in pancreatic cancer cell lines

Christian Haag, Klaus-Michael Debatin and Simone Fulda

University Children´s Hospital, Eythstr. 24, D-89075 Ulm, Germany, email: christian.haag@uniklinik-ulm.de

Pancreatic cancer cells are naturally resistant against current chemotherapy and radiation therapy. Furthermore they show a high resistance against death receptor-mediated apoptosis although almost all pancreatic cancer cell lines express death receptors in an amount sufficient for mediating an apoptotic signal. The antiapoptotic protein c-FLIP (FLICE–inhibitory protein) protects cells from CD95(APO-1/Fas)- and TRAIL-mediated apoptosis by inhibiting cleavage and activation of procaspase-8 at the death inducing signaling complex (DISC). In this study we investigate the role of c-FLIP in inhibiting the apoptotic signaling pathway mediated by death receptor stimulation in pancreatic carcinoma cells.
We found that downregulation of c-FLIP by retroviral shRNA leads to increased cell death in pancreatic cancer cell lines of up to 40% after treatment with TRAIL and of up to 20% after treatment with an agonisitic anti-Fas antibody. To confirm these findings we either downregulated c-FLIP on the translational level using Cylcloheximide (CHX) or on the transcriptional level using 5-FU. In contrast to c-FLIP, expression levels of other known components of the DISC, like procaspase-8 or FADD, were not markely reduced after CHX or 5-FU treatment. Western Blot analysis showed that downregulation of c-FLIP after CHX or 5-FU treatment is accompanied by cleavage and activation of procaspase-8, procaspase-3, Bid and RIP after stimulation with TRAIL, while cleavage products of these molecules can not be detected in non-pretreated cells after stimulation with TRAIL. Activation of these proteins strongly suggests that the observed cells death is caused by death receptor-mediated apoptosis.
Furthermore we found that depending on the cell line 5-FU treatment leads to a preferential downregulation of c-FLIP(S). Presently we are investigating the mechanism of c-FLIP(S) downregulation by 5-FU.
Our data indicate that downregulation of c-FLIP is sufficient to overcome resistance to death receptor-mediated apoptosis in pancreatic cancer cell lines. The findings for 5-FU indicate that not only c-FLIP(L) but also c-FLIP(S) might play a major role in the resistance of pancreatic cancer cell lines against death receptor stimuli.

Epigenetic control of programmed cell death in lung carcinoma cells resistant to conventional anticancer treatments

Hajji Nabil; Karolina Wallenborg; Pinelopi Vlachos; Ulrika Nyman; Ola Hermanson; Bertrand Joseph.

Institute of Environmental Medicine, Karolinska Institute, Sweden.

Acetylation and deacetylation of nucleosomal histones play an important role in the modulation of chromatin structure, chromatin function and in the regulation of gene expression. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are two opposing classes of enzymes, which tightly control the equilibrium of histone acetylation. An imbalance in the equilibrium of histone acetylation has been associated with carcinogenesis and cancer progression.
Histone acetylation decreases chromatin compaction and promotes the access of transcription factors to their binding sites, and thereby regulates gene expression. The pan-HDACs inhibitor trichostatin A (TSA) and valproic acid (VPA), which has been shown to selectively inhibits class I HDACs, induce both chromatin relaxation and promote the effect of the DNA-damaging agent, etoposide in H157 NSCLC cells. However, only TSA is able to sensitize H157 cells to etoposide induced apoptotic cell death.

Die and let live – mechanisms of apoptosis in postnatal development of intestinal mucosa

Natalia Hallay[1], Michal M. Godlewski[1✉], Marzena Biernat[2], Jaroslaw Wolinski[2], Romuald Zabielski[1,2]

[1]Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Nowoursynowska 159, 02-776 Warsaw, Poland, e-mail: mickgodl@hotmail.com
[2]The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jablonna, Poland.

Apoptosis plays a key role in exchange of enterocyte populations during development of gastrointestinal tract in newborns, but the mechanisms involved are not fully understood. With every meal the conditions inside the gut change, as food and microorganisms introduce several stress factors to previously sterile environment. Our studies confirmed the major role of TGF-ß1 and TNFa as inducers of apoptosis. In the first day of life the major proapoptotic factor was TGF-ß1 as it was responsible for death induction in more than 3/4 of apoptotic enterocytes. Furthermore the expression of TGF-RII was abundant, especially in the basal layer of the enterocytes. In day 7 of life percentage of TGF-ß1-positive cells was on the similar level, regardless to the fact that apoptotic index was higher. The number of TNFa-positive cells doubled during first 7 days of life, suggesting increasing role of this cytokine in more and more hostile environment of the gut. Packets of dieing cells were observed only sporadically in the 1st day of life, but in 7th day their number was abundant. We confirmed TGF-ß1 responsible for "packet" pattern of cell death. Autophagy plays a significant role in the remodelling of intestinal mucosa, but only during 1st day of life. MAP I LC-3-positive dieing cells were abundant, present mostly in the crypts. MAP I LC-3 expression colocalized with caspase 3, suggesting that this form of programmed cell death is complementary rather than an alternative to apoptosis. It's interesting that MAP I LC-3 expression was observed in the basal part of nearly every enterocyte, but only on the villi. It was probably associated with so called large lysosomal transport vacuoles, present until the closure of gut barrier. On 7th day the role of autophagy diminished and a few MAP I LC-3-positive cells were scattered among crypts and the villi.

Characterisation of a putative poly(ADP-ribose)polymerase in the ascomycete *Podospora anserina*

Mathis Müller-Ohldach, Diana Brust, Andrea Hamann, Heinz D. Osiewacz

Institute of Molecular Biosciences, Department of Biosciences, Johann Wolfgang Goethe University, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany.
e-mail: a.hamann@em.uni-frankfurt.de

Podospora anserina is a filamentous ascomycete which is characterized by a defined lifespan. Wild-type strain s displays a lifespan of about 25 days. After this period of linear growth morphological and physiological changes occur leading to the development of a senescence syndrome and eventually to the death of the fungus. These changes are largely due to the accumulation of deficient mitochondria during aging. The key role of mitochondria in this degenerative process raises the question of whether or not an apoptotic machinery is involved in lifespan control. *In-silico* analysis of the genome sequence led to the identification of several putative apoptosis factors. One of those factors is a putative poly(ADP-ribose)polymerase (PARP). PARPs are a group of proteins linked to DNA repair, apoptosis and ageing. *In-silico* analysis showed that PaPARP has a high similarity to the human form of PARP-2. A c-terminal GFP fusion of PaPARP is, as expected, located in the nucleus. Numerous attempts to knock-out the gene by homologous recombination were to no avail, leading to the conclusion that the knock-out might be lethal. Verification of this assumption is under way by replacing *PaParp* in a transgenic strain carrying an ectopic *PaParp* integration. A future aim is to demonstrate the role of PaPARP in the apoptotic machinery of *P. anserina* by biochemically assessing its catalytic properties. There are already several mutants created overexpressing *PaParp* constitutionally. The lifespan as well as the resistance of these strains against genotoxic stresses are under investigation.

The protection of post-ischemic hearts by HO3538, a potent amiodarone analogue through inhibition of the mitochondrial apoptotic pathway

Arpad Szanto, Zita Bognar, Katalin Hanto, Aliz Szabo, Viktoria Nemeth, Antal Tapodi, Kalman Hideg, Gabor Varbiro, Ferenc Gallyas Jr., Balazs Sumegi

Deptartment of Biochemistry and Medical Chemistry, Department of Urology, Institute of Organic and Medicinal Chemistry, Faculty of Medicine, University of Pécs, Szigeti u. 12, H-7624 Pécs, Hungary.

HO3538 is a modified Amiodarone analogue. Amiodarone is a widely used and potent antiarrhythmic agent. Both amiodarone and its metabolite possess antiarrhythmic effect, and both compounds can contribute to toxic side effects.
Here, we compare the effect of HO3538, amiodarone and desethylamiodarone on mitochondrial energy metabolism in perfused hearts, and the mitochondrial functions (respiration, membrane potential, and permeability transition) in isolated mitochondria were also analyzed.
Amiodarone and HO3538, but not desethylamiodarone protects the mitochondrial energy metabolism in perfused heart during ischemia re-perfused. Amiodarone, when was present at low concentrations and predominantly in membrane bound form, protected heart and mitochondrial energy metabolism from ischemia-reperfusion-induced damages in Langendorff-perfused heart model. Up to the concentration of 10 mM, the drug considerably inhibited Ca^{2+}-induced permeability transition, while at higher concentrations it induced a cyclosporin A independent permeability transition of its own. At low concentrations, HO 3538 has the same effect as the amiodarone, by inhibiting the Ca^{2+}-induced mitochondrial swelling, whereas it dissipated the mitochondrial membrane potential ($\Delta\psi$), and prevented the ischemia-reperfusion-induced release of apoptosis- inducing factor (AIF). At higher concentrations, amiodarone but not HO3538 induced a cyclosporin A (CsA)-independent mitochondrial swelling.
In contrast to these, desethylamiodarone did not inhibit the Ca^{2+}-induced mitochondrial permeability transition, did not induce the collapse of $\Delta\psi$ in low concentrations, and did not prevent the nuclear translocation of AIF in perfused rat hearts, but it induced a CsA-independent mitochondrial swelling at higher concentration, like amiodarone.
In conclusion: amiodarone analogues, like HO3538, can possess cardio-protective effect by inhibiting the mitochondria apoptotic pathway: the permeability transition, the collapse of $\Delta\psi$, and the release of the pro-apototic proteins.

Identification of a link between Calmodulin kinase II and Jun-terminal kinase by mapping Ca2+-dependent apoptosis pathways

Aleksandra Mandic Havelka, Maria Hägg, Yun-Chun Li, Maria Berndtsson, Maria C. Shoshan and Stig Linder

Dept. of Oncology-Pathology, Cancer Center Karolinska, Karolinska Institute, S-171 76 Stockholm, Sweden.

We used a chemical genetics to characterize the importance of calcium signaling pathways for induction of apoptosis. A small library of 40 compounds that induce apoptosis in HCT116 colon cancer cells was first identified by screening the NCI Mechanistic Set. The majority of these pro-apoptotic compounds (65%) were found to be dependent on Ca2+ for efficient induction of caspase-cleavage activity. Ca2+-dependent apoptosis was found to primarily involve calpain and calmodulin. Inhibition of calmodulin kinase II (CaMKII) by KN-93 inhibited apoptosis, whereas inhibition of protein phosphatase 2B (calcineurin) by FK506 was less effective. Induction of CaMKII activity was observed by a number of compounds. Further studies suggested that Jun-N-terminal kinase (JNK) is an important mediator of apoptosis signaling down-stream of CaMKII. Our results suggest that a Ca2+/CaMKII pathway is responsible for JNK activation and apoptosis by various stimuli.

Noscapine Induce Apoptosis via Activation of Caspases-2,-3,-6,-8,-9 in Fas-independent manner in myeloblastic leukemic K562 cells

Nastaran Heidari Bahram Goliaei ,Parvaneh Rahimi Moghaddam and Massoud Mahmoudian .

Institute of Biochemistry and Biophysics, University of Tehran, Tehran ,Iran Razi Institute for Drug Research (RIDR), Department of Pharmacology, Iran University of Medical Sciences (IUMS), Tehran, Iran,e-mail:Heidari@ibb.ut.ac.ir

Monitoring apoptosis is becoming increasingly important in finding new chemoprevention drugs and their mechanism. Previously, the microtubule opium alkaloid noscapine was discovered as a microtubule destabilizing agent that arrests mammalian cells at mitosis and induces apoptosis. In spite of several reports on the efficacy of noscapine to induce apoptosis, there is no data on the mechanism of noscapine-induced apoptosis. We selected apoptosis-resistance, P53-null myelogenous leukemia k562 cells to monitor apoptosis and study of noscapine's mechanism. K562 cells showed delayed but effective response to noscapine treatment. We could monitor apoptosis by the DNA fragmentation, PARP cleavage and increasing activity of caspase-2,-3,-6,-8, and-9 with 20 µM noscapine after 24hr treatment. Using the peptide inhibitors of caspases help us to elucidate caspases cascade. We proved caspase-8 activity in a Fas-independent manner, downstream of caspase-9 activation. The increasing of Bax/Bcl-2 ratio in the first hours of apoptosis induction showed mitochondrial event involved in trigger of apoptosis. In conclusion, noscapine has potential to induced apoptosis in p53- null apoptosis- resistant K562 cells and can be a good candidate for preventive and therapeutic application for myeloid leukemia.

Real-Time Analysis of Initiator Caspases Activation and Activity during TRAIL induced Apoptosis in Human Cancer Cells

Hellwig C.T., Koehler B.F., Dussmann H., Prehn J.H.M., Rehm M.

Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin 2, Ireland, e-mail: mrehm@rcsi.ie

In recent years, fluorescence resonance energy transfer (FRET) probes (CFP linked to YFP via a short caspase specific amino acid sequence) provided us with valuable insight into the activation of effector caspases in single living cells. Here we describe the first reporter system that allows us to observe the activation process and the activity of initiator caspases-8 and -10 quantitatively and in real time. Importantly, the system is completely independent of any contribution of downstream caspases.

Following death receptor stimulation, caspases-8 and -10 activate the proapoptotic protein Bid by proteolytic cleavage. Truncated Bid (tBid) then triggers mitochondrial outer membrane permeabilisation (MOMP) and the release of cytochrome *c* (cyt-c) into the cytoplasm. Cyt-c release is accompanied by mitochondrial depolarisation and triggers the subsequent activation of caspases-9, -3 and -7. In a positive feedback, caspase-3 can activate additional procaspase-8/-10. Furthermore, caspases-3 and -8/-10 overlap in their substrate specificities.

We generated HeLa clones stably expressing a caspase-8/-10 optimized FRET probe (CFP-IETD-YFP). Caspase activation resulted in specific cleavage of the probe and control experiments showed that the FRET construct is fully functional in living cells.

To measure caspase-8/-10 activity without contribution of downstream caspases, we stably knocked down Bid expression. Signal progression was stopped at the level of caspase-8/-10 activation as mitochondrial membrane depolarisation could no longer be observed. Furthermore, these cells were highly resistant to death receptor ligand TRAIL but fully responsive to the Bid-independent stimulus staurosporine.

Caspases-8/-10 were activated within the first hour after addition of high doses of TRAIL (100 ng/ml). Following an activation phase of ~30min, a constant caspase activity could be monitored for several hours. In parental HeLa cells, effector caspases-3/-7 get activated rapidly and cleave a similar FRET substrate (CFP-DEVD-YFP) within 10-15 min, tightly coupled (minutes) with subsequent apoptotic morphological changes.

These results show that while effector caspase activation is a rapid event directly resulting in subsequent cell death, activation of caspases-8/-10 results in a low intracellular caspase activity that can persist for hours without causing immediate cell death.

This research was supported by a grant from Science Foundation Ireland to M Rehm.

Identification of small molecules that induce apoptosis in a Myc-dependent manner and inhibit Myc-driven transformation

Hao Mo, Marina Vita and Marie Henriksson

Department of Microbiology, Tumor and Cell Biology (MTC), Karolinska Institutet, Box 280, SE-171 77, Stockholm, Sweden

The Myc transcription factor is a key regulator of cell cycle progression, cell growth, apoptosis, differentiation and cellular transformation. Myc is a potent oncoprotein that is deregulated in a wide variety of human tumors and is an attractive target for novel cancer therapies. We have used a cellular screening approach to identify two low-molecular-weight compounds, Myc pathway response agents (MYRAs), that predominantly suppressed the growth of Myc-overepressing cells compared to cells with wild type Myc levels. Both MYRA-A and MYRA-B induce apoptosis in a Myc dependent manner and inhibit cellular transformation. However, their mechanism of action is different; MYRA-A inhibits Myc transactivation and interferes with DNA-binding of Myc family members but has no effect on the E-box binding protein USF. In contrast, MYRA-B induces Myc-dependent apoptosis without affecting Myc transactivation or Myc/Max DNA binding (Mo and Henriksson, 2006).

Furthermore, we have also identified another compound, F10, that induce apoptosis in a Myc-dependent manner and seems to act by targeting Myc for degradation. In addition, all three compounds were more efficient in inhibiting cell proliferation in cells with high compared to low MYCN expression.

We are now performing screens in cells with high MYCN levels using novel chemical libraries and have so far identified several compounds that show selectivity for cells with high MYCN expression. The characterization of these compounds will be presented and discussed.

In summary, our data show that cellular screening assays can be a powerful strategy for the identification of candidate substances that modulate the Myc pathway. These compounds can be useful tools for studying Myc function and may also be of therapeutic potential as leads for drug development.

Mo and Henriksson. Proc Natl. Acad. Sci. USA 103:16, 6344-6349 (2006)

Differential p53, Ca^{2+} and reactive oxygen responses in stress-induced apoptosis signaling by oxaliplatin and cisplatin

Emma Hernlund[1], Ozgur Kutuk[1,2], Elin Jerremalm[1], Claudia Burz[1,3], Juan Castro[1], Stig Linder[1], Theoharis Panaretakis[1] and Maria C. Shoshan[1]

[1] Cancer Center Karolinska, Department of Oncology and Pathology, Karolinska Institute and Hospital, S-171 76 Stockholm, Sweden
[2] present adress: Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA
[3] present adress: L'Université de Medicine et Pharmacie Iuliu Hatieganu, Cluj-Napoca, Roumania

Cisplatin (cDDP) and oxaliplatin (OXP) are both DNA-damaging agents but differ significantly in clinical use as well as in growth inhibition profiles (http://itbwork.nci.nih.gov/PublicServer/CompareServer). At low doses (<10 μM) cDDP but not OXP induced premature senescence. In the range of 1-5 μM, OXP was more efficient than cDDP in inducing apoptosis over 48 h, whereas the reversed was observed with ≥10 μM. For both drugs, ≥10 μM was required to induce acute, stress-induced apoptosis. The underlying apoptotic signaling involved JNK, but the two drugs differed with respect to involvement of Ca^{2+}, superoxide anion and p53. In contrast to cDDP, OXP-induced apoptosis did not require Ca^{2+}. Both drugs induced reactive oxygen species, but only cDDP-induced apoptosis required superoxide anion. Induction of p53 was greater and more rapid with OXP than with cDDP. p53-deficient as well as bax-deficient cells were more resistant to OXP than to cDDP. The greater p53-independance of cDDP was further evidenced in a higher proportion cells showing caspase-3 activation without concomitant p53 accumulation. We propose that although both drugs elicit DNA-damaging responses as well as acute stress signal-mediated apoptosis, the slower OXP-induced apoptosis depends more on DNA damage, whereas cDDP elicits additional, more rapidly acting stress signaling that contributes to its cytotoxicity.

Proteasome inhibitors efficiently reactivate TRAIL-induced apoptosis in malignant glioma

H. Hetschko, J. Masri, J. Weissenberger, D. Kögel

Department of Experimental Neurosurgery, Johann Wolfgang Goethe-University Clinics Frankfurt, Germany, e-mail: h.hetschko@med.uni-frankfurt.de

Glioblastoma multiforme (GBM) is characterized by a potent resistance against antineoplastic treatment. Although Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) seems to be a promising anti-cancer agent for various malignant diseases, many glioma cell lines are innately resistant to TRAIL. The purpose of this study was i) to evaluate the efficiency of combination therapies to reactivate TRAIL sensitivity in GBM and ii) to identify the molecular signalling events underlying this reactivation. Here we investigated the sensitivity of a panel of six human GBM cell lines to apoptosis induced by TRAIL [250 ng/ml], and TRAIL in combination with proteasome inhibitors (PIs) [2,5 µM MG132; 50 nM epoxomicin], or Bcl-2/Bcl-xL inhibitors [30 µM HA14-1, 30 µM BH3-I2´] and gamma-irradiation [20 Gy]. The cell lines evinced drastic differences in their sensitivity to these distinct apoptotic stimuli, with only two of them showing significant induction of cell death in response to TRAIL. The combinatory treatments revealed that apoptosis could be efficiently enhanced with the Bcl-2/Bcl-xL inhibitors BH3-I2´ and HA14-1 in three of the six cell lines. Gamma irradiation in combination with TRAIL potentiated cell death in only one of three cell lines. Interestingly, our data show that apoptosis was markedly enhanced by PIs in the TRAIL-sensitive cell lines U87 and U251, and could be potently reactivated in both TRAIL-resistant cell lines U343 and U373. The p53 status of the investigated GBM cell lines showed no correlation with the sensitivity to PIs, Bcl-2/Bcl-xL inhibitors or gamma irradiation. Knockdown of DR5 followed by combined treatment with MG132 and TRAIL resulted in a significant decrease of DR5 surface expression and cell death. In contrast, RNA interference (RNAi) against the putative DR5 upstream regulator and pro-apoptotic transcription factor GADD153/CHOP had no effect on the amount of cell death, suggesting that MG132 activated DR5 in a CHOP-independent fashion. Further studies employing JNK inhibitors and RNAi against c-Jun and DcR2 are currently being performed to delineate the critical upstream signals required for enhanced TRAIL sensitivity. In conclusion, combinatorial approaches based on TRAIL or agonistic TRAIL receptor antibodies in conjunction with PIs might be a promising strategy to reactivate apoptosis in therapy-resistant GBMs in the future.

Pro-apoptotic signaling induced by IFNa

Linn Hjortsberg[1], Theocharis Panaretakis[1], Ann-Charlotte Björklund[1], Bertrand Joseph[2], Katja Pokrovskaja[1], Dan Grandér[1]

[1]Department of Oncology and Pathology, Cancer Centre Karolinska, Karolinska Institutet and Karolinska University Hospital, S-171 76 Stockholm, Sweden.
E-mail: Linn.Hjortsberg@ki.se

Interferons (IFNs) are cytokines with pleiotropic effects on important cellular functions including inhibition of proliferation, induction of differentiation, modulation of the immune system and inhibition of angiogenesis. They are approved for the treatment of various conditions, mainly tumor- but also viral and neurological diseases. Despite their widespread use, the molecular mechanisms behind the clinical effects of IFNs are not fully elucidated. Several mechanisms have been suggested for the antineoplastic activities of IFNs, including indirect effects such as immune stimulation and inhibition of angiogenesis, but also direct effects on the tumor cells, including cell cycle regulation, differentiation and apoptosis. Most anticancer agents act by inducing apoptosis in the tumor cells. Since also IFNs induce cell death, apoptosis as the mechanism behind their clinical effects is an attractive and also likely thought. Therefore these studies were aimed at illuminating the mechanisms of programmed cell death in tumor cells in response to IFNα.

Several signaling pathways are activated following binding of IFNα to its receptor. The Jak-STAT pathway has been well studied; its activation give rise to altered expression of several hundred interferon stimulated genes, whose products carry out many of the effects of IFNα. However, IFNα also induces activation additional signaling cascades, including the phosphoinositide-3 kinase (PI3K) pathway.

We have shown that IFNα induces a caspase-dependent apoptosis, preceded by activation of the pro-apoptotic proteins Bak and Bax, loss of mitochondrial membrane potential and release of cytochrome C. The apoptotic process required functional PI3K/mTOR signaling, upstream of the mitochondria. Although abrogation of the PI3K/mTOR pathway potently blocked the ability of IFNα to induce apoptosis, it left the IFNα stimulated Jak-STAT signaling and gene expression changes intact. Also the protective effect against virus-induced cell death was unaffected, thus suggesting that IFNα-induced apoptosis relies mainly on cytoplasmic events. Thus we are now independently studying the importance of IFNα-induced STAT-mediated alterations of transcription and of cytoplasmic signaling in IFNα-stimulated cell death.

p30 – a new cleavage product at the CD95 Death-Inducing Signalling Complex (DISC)

Julia C. Hoffmann, Alexander Golks, Peter H. Krammer and Inna N. Lavrik

Division of Immunogenetics, Tumorimmunology Program, German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany, e-mail: julia.hoffmann@dkfz.de

Apoptosis can be triggered by a number of factors including UV or γ-irradiation, chemotherapeutic drugs and signalling from death receptors (DRs), such as CD95. Upon stimulation CD95 oligomerizes and the intracellular death-inducing signalling complex (DISC) is formed. Upon recruitment to the CD95 DISC procaspase-8 is autocatalytically activated by sequential cleavages at defined aspartate (Asp) residues and the resulting active heterotetramer $(p10)_2$ $(p18)_2$ is released into the cytosol. This processing has been described by a two-cleavage-step model.

In the process of procaspase-8 processing at the CD95 DISC a yet unknown cleavage product is generated, p30. The protein p30 comprises the two catalytic subunits and is generated upon CD95 stimulation by cleavage of procaspase-8 at Asp^{210} and can be further processed to form the active caspase-8 heterotetramer. Analysis of a stable cell line overexpressing p30 indicates that it has a functional role in CD95 signalling. Specific cell death after CD95 stimulation was significantly increased in the presence of p30, even though surface expression of CD95 and apoptosis induction by UV and Staurosporin were not altered. The identification of p30 as a new cleavage product of procaspase-8 processing upon CD95 stimulation provides new insights into the mechanism of procaspase-8 activation at the CD95 DISC. The further analysis of the production, the processing and the functional role of this protein will help to clarify the mechanism of procaspase-8 activation and CD95 signalling.

Apoptosis versus differentiation induced by interaction of butyrate and unsaturated fatty acids in normal and transformed colonic epithelial cells

Jirina Hofmanová, Alena Vaculová, Martina Hýždalová, Zuzana Koubková, Jaromíra Netíková, Alois Kozubík

Laboratory of Cytokinetics, Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic, e-mail: hofmanova@ibp.cz

Dynamic balance between proliferation, differentiation and apoptosis is necessary for healthy intestinal epithelium. This is regulated not only by endogenous factors like cytokines and hormones, but it is also significantly affected by exogenous dietary factors. Particularly dietary lipids appeared to be important modulators of behaviour of colon epithelial cells and their role in colon carcinogenesis is assumed. Among them, butyrate, the short -chain fatty acid produced by microbial fermentation of fibre, and essential polyunsaturated fatty acids (PUFAs) were shown to influence colonic cell kinetics. Suggesting mutual interaction of these agents, we investigated the response of human colonic cell lines derived from normal fetal tissue (FHC), colon adenocarcinoma (HT-29, HCT116) and/or lymph node metastasis (SW620) to combined treatment with sodium butyrate (NaBt, 3mM) and PUFAs (50 microM) of n-6 (arachidonic acid, AA) or n-3 series (docosahexaenoic acid, DHA). Flow cytometry, fluorescence microscopy and Western blotting were the main methodologies used. Depending on the level of cell transformation, the cells responded differently to the agents used both individually and in combination (NaBt+AA or DHA). We detected modulations of proliferation (cell number, cell cycle parameters), differentiation (alkaline phosphatase activity, carcinoembryonic antigen expression) and apoptotic parameters (subG0/G1 population, PARP cleavage, nuclear morphology – DAPI staining). In comparison with individual agents, combined NaBt and PUFA treatment increased apoptosis especially in FHC and SW620 cells, DHA being more effective than AA. On the other hand, the differentiation induced by NaBt was attenuated by PUFAs in well-differentiated FHC and HT-29 cells. Simultaneously with these changes, we showed that combination of NaBt with PUFAs caused changes of membrane lipid packing (merocyanine 540 fluorescence) and increased expression of fatty acid transporter protein (anti-FAT/CD36 monoclonal antibody), accumulation of lipid droplets in cytoplasm (Nile red fluorescence), and reactive oxygen species production (dihydrorhodamine-123 fluorescence). Changes of differentiation versus apoptosis were accompanied by dynamic changes of mitochondrial membrane potential, caspase-3 and -9 activities, and expression of regulatory Mcl-1 protein. We conclude that interaction of NaBt and PUFAs may cause significant changes in colonic cell lipids, influence oxidative metabolism and modulate colonic cell kinetics depending on the level of cell transformation.

Supported by grants No. 524/04/0895 GACR and No. 1QS500040507 IGA ASCR.

The effects of polycyclic aromatic hydrocarbons (PAHs) on apoptosis-related cell signalling may have implications for their mutagenic and carcinogenic potential

N. E. Landvik[1], X. Tekpli[2], A. Solhaug[1], D. Lagadic-Gossmann[2] and J. A. Holme[1]

[1]Division of Environmental Medicine, NIPH, P.O. Box 4404 Nydalen, N-0403 Oslo, Norway [2]Inserm U620, Université Rennes 1, Rennes, France, e-mail: jorn.holme@fhi.no

PAHs including nitro-PAHs in ambient air are considered to be contributing to the aetiology of human lung cancer. Studies indicate that several of the PAHs are found to cause mutations and tumors in animal models probably by the formation of reactive electrophilic metabolites that covalently bind to macromolecules including proteins and DNA.

In previous studies on Hepa1c1c7 cells, we have shown that some of the PAHs may trigger cell survival and inhibit pro-apoptotic signals. Here, we have examined the toxic effects of the nitro-PAHs 1-nitropyrene (1-NP), 1,3-dinitropyrene (1,3-DNP) and 1,8-dinitropyrene (1,8-DNP). The cell death induced seemed to be triggered by DNA-damage resulting in phosphorylation and nuclear translocation of the tumour suppressor gene p53. These effects lead to further activation and cleavage of enzymes central for receptor- and mitochondria-related apoptosis. Furthermore, we observed an increased phosphorylation of the stress related MAPKs p38 and JNK, which have been suggested to modulate the apoptotic response. The compounds also increased the level of certain cell survival signals including p-Akt.

Of special importance was the finding that the most mutagenic and carcinogenic compound tested, 1,8-DNP, induced less, if any, cell death, despite the fact that this compound seemed to give most DNA damage judged by increased phosphorylation of p53 and accumulation of cells in S-phase. Further immunocytochemical studies revealed that 1,8-DNP did not result in a translocation of the p53 protein into the nucleus. It is hypothesized that some genotoxic carcinogenic chemicals induce DNA damage that “normally” should have resulted in cell death, but that they simultaneously activate pathways inhibiting the triggering of the apoptotic process. One possible implication may be that more cells survive the DNA damage, but with an increased probability of having mutations and chromosomal aberrations. Such findings may help explaining why various types of DNA damage have different mutagenic potential, and contribute to the scientific basis on which a refinement of current classification of carcinogens and cancer risk assessments can be undertaken.

Elucidating the mechanism by which DT-IL3 kills Acute Myelogenous Leukemia cells

Henrick Horita and Andrew Thorburn

Department of Pharmacology, University of Colorado at Denver and Health Sciences Center, Aurora, Colorado, 80045, USA.

Only 20-30% of acute myelogenous leukemia (AML) patients enjoy long-term disease free survival with our current treatments. Thus, new, targeted therapies are needed for better treatment of AML. The fusion toxin protein DT-IL3 consists of the α and β subunit of the diphtheria toxin fused to an intereleukin-3 ligand (IL-3). Previous work has shown that DT-IL3 has selective toxicity towards cells with increased IL-3 receptors (IL-3R), which has been traditionally used as a marker of AML and this selective toxicity has provided a basis for ongoing clinical trials using DT-IL3 to treat AML. We wish to understand the mechanism by which DT-IL3 kills AML positive cells in order to better predict resistance mechanisms and to develop a rational basis for combination therapies. Previous work from our lab has shown that other AML targeted toxins kill by inducing caspase-dependent apoptosis. Cell death by DT-IL3 is independent of effector caspase 3 and 7 activation and caspase inhibitors provide no protection. DT-IL3 is not working through autophagic programmed cell death as we see no induction of LC-3II. Additionally, knocking down Beclin 1, an essential protein in autophagy, does not inhibit cell death. This left us with two questions: 1.Why do these cells not die by apoptosis? 2. How are these cells dying? To search for candidate proteins that may be involved in DT-IL3's mode of action we used MudPIT mass spectrometry. We identified several interesting proteins that were differentially regulated by the drug, including Nucleolin and Alix. Nucleolin, which was upregulated during DT-IL3 treatment, increases Bcl-2 protein levels by stabilizing its mRNA. We hypothesize that this increase in Bcl-2 could inhibit both apoptosis and autophagy. Alix, an inhibitor of paraptosis, decreased during DT-IL3 treatment. Thus, decreasing Alix may allow the cell to undergo paraptosis and we hypothesize that this is the mode of action by which DT-IL3 kills AML cells. Ongoing experiments are being conducted to further test these hypotheses.

Bcl-xAK, a novel Bcl-x splice product lacking a BH3 domain triggers apoptosis in human melanoma cells when applying a Tet-off adenoviral expression system

Amir M. Hossini, Bernd Gillissen, Lothar F. Fecker, Peter T. Daniel, Jürgen Eberle

Department of Dermatology and Allergy, Skin Cancer Center, University Medical Center Charité, Berlin, Germany,
Clinical and Molecular Oncology, University Medical Center Charité, Berlin, Germany

Proteins of the Bcl-2 family are key players in the control of apoptosis via the mitochondrial pathway and exert both proapoptotic and antiapoptotic functions. Most antiapoptotic proteins share four Bcl-2 homology domains (BH 1-4), whereas the proapoptotic proteins may lack some of these domains. However, all so far described proapoptotic Bcl-2 proteins carry a BH3 domain. The gene for Bcl-x gives rise to several alternative splice products resulting in proteins with distinct functions, e.g. the antiapoptotic Bcl-x_L with all four BH domains and the proapoptotic Bcl-x_S encompassing a BH3 and BH4. In a previous study, we demonstrated that overexpression of Bcl-x_S efficiently triggers apoptosis in human melanoma cells, whereas overexpression of Bcl-x_L resulted in inhibition of apoptosis. When screening for additional bcl-x-encoded proteins, we identified a novel splice product of 138 amino acids which we termed Bcl-x_{AK} (atypical killer). It carries a BH2 and BH4 as well as the transmembrane domain, but lacks a BH1 and BH3. Endogenous expression of Bcl-x_{AK} was found in melanoma and other tumor cell lines. To investigate the activated apoptosis pathways, a replication-deficient, tetracycline-regulatable (Tet-Off) adenoviral vector was constructed for overexpression of Bcl-x_{AK}. After infection and tetracycline withdrawal, apoptosis was efficiently induced in three human melanoma cell lines. In contrast to Bcl-x_S, Bcl-x_{AK} was found after exogenous overexpression in both mitochondrial and in cytosolic cell fractions. By these findings, a new class of Bcl-2 proteins is introduced which promotes apoptosis independent from the BH3 domain and which implies additional, new mechanisms for apoptosis regulation in melanoma cells.

RHAU, a stress-responding RNA helicase that represses transcription of DAPK

Fumiko Iwamoto, Sandra Pauli and Yoshikuni Nagamine

Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland, e-mail: fumiko@fmi.ch

RHAU is a DExH RNA helicase associated with AU-rich element (ARE) of mRNA. AREs are present in the 3'-UTR of many mRNA coding for early response gene products and confers instability on the mRNA. ARE-binding proteins are important gene regulators because they control ARE-mRNA stability in response to changing cellular environment. RHAU was identified in our laboratory as a novel ARE-associating protein by RNA-affinity chromatography. RHAU physically interacts with the polyadenylate ribonuclease PARN and the exosome, hence enhances deadenylation and decay of ARE-mRNA. Both *in vitro* and *in vivo* study showed that the ATPase activity of RHAU is required for the accelerated decay of ARE-mRNA. Thus we have shown that RHAU is the first mammalian RNA helicase involved in mRNA degradation.

To investigate biological roles of RHAU, we established stable HeLa cell lines expressing RHAU-shRNA in an inducible manner. In normal culture condition, viability and morphology of RHAU-downregulated cells were similar to control cells, whereas under stressed conditions of serum-starvation, RHAU-downregulated cells underwent caspase-dependent cell death significantly faster than the control cells. This apoptotic phenomenon of RHAU-downregulated cells could be explained by upregulation of proapoptotic factor, death-associated protein kinase (DAPK), in these cells. We found that RHAU transcriptionally regulated DAPK expression by nuclear run-on assay and luciferase reporter assay, suggesting that RHAU was a protein of dual functions, namely as a transcriptional regulator in the nucleus and an mRNA destabilizing factor in the cytoplasm. Co-immunoprecipitation analysis using anti-RHAU antibody identified a Bcl-2-interacting transcriptional repressor, Btf, as a RHAU-binding partner. We hypothesize that RHAU and Btf regulate each other or function together on the transcriptional repression of apoptosis-mediating genes in response to cellular stresses.

Terfenadine, an H1 histamine receptor antagonist, induces calcium dependent DNA-damage and apoptosis in human melanoma cells by acting on the cells apart from H1 histamine receptors.

Jangi SM, Ruiz-Larrea MB[a], Díaz-Pérez JL[b], Asumendi A, Gardeazabal J[b], Pérez-Yarza G, Díaz-Ramón JL[b] and Boyano MD.

Department of Cell Biology and Histology. Faculty of Medicine and Dentistry. University of the Basque Country. 48940 Leioa, Bizkaia, Spain.
[a]Department of Physiology. Faculty of Medicine and Dentistry, University of the Basque Country, Leioa E-48940, Bizcaya, Spain
[b]Service of Dermatology. Cruces Hospital. 48903 Barakaldo, Bizkaia, Spain

Histamine is a well known biogenic amine that has been described as a growth factor for various normal and malignant human cells. In previous work, we have found that H1 histamine antagonists induce apoptosis in several tumour cell lines (Jangi et al, 2004 and 2006). This apoptosis appears to be caspase-2 dependent and involves the mitochondrial pathway. In the present work we have investigated the mechanism and cell signalling pathways involved in the apoptosis induced by terfenadine, an H1 histamine antagonist. Since, it has been found that histamine modulates intracellular calcium level through H1 histamine receptors, we studied the effect of terfenadine on calcium level in the melanoma cells and, unsuspectedly we found that terfenadine sharply increased intracellular calcium level. This calcium rise is phospholipase C (PLC) dependent, as neomycin and U73122, two PLC inhibitors, were able to prevent intracellular calcium rise and the cell death induced by terfenadine. Treatment of melanoma cells with terfenadine induced DNA-damage, tested by comet assay. The DNA-damage was also preventable by PLC inhibitors and by BAPTA, an intracellular calcium chelator, indicating that DNA-damage induced by terfenadine is calcium dependent. Furthermore, we found that terfenadine arrests the cell cycle transiently at S- and G2-M- phases and increased the expression of p53 after one hour of the treatment. Finally, we found that histamine was unable to prevent terfenadine induced calcium rise and cell death totally. Furthermore, an inhibitor of the histidine decarboxylase, the only enzyme responsible for histamine synthesis in the cells, did not induce cell death to the same extent that induced by terfenadine. Moreover, histamine H1 receptor RNA interference was failed to induce cytotoxic effect and to prevent terfenadine induced cell death. We conclude that terfenadine induces calcium dependent DNA-damage and apoptosis in human melanoma cells that is not occur totally through its histamine antagonizing effects.

ACKNOWLEDGMENTS: This study was supported by grants from the University of the Basque Country (UPV 00075.327-14466/2002). Jangi SM, is from Hawler Medical University, Kurdistan-Iraq, he received a research fellowship from the Basque Government.

TRAIL – an exogenous and endogenous tumour growth factor

Agnieszka Toloczko[1], Eva Baader[1], Ahmed El-Gazzar[1], Harald Ehrhardt[1,2], Klaus-Michael Debatin[3], Irmela Jeremias[1,2]

[1]GSF – National Research Center for Environment and Health, Junior Research Group Apoptosis, Marchioninistrasse 25, 81377 Munich, Germany, e-mail: Irmela.Jeremias@GSF.de, [2]Dr. von Haunersches Kinderspital, Ludwig Maximilians Universität, Lindwurmstr. 4, 80337 Munich, Germany, [3]University Children's´ Hospital, Prittwitzstr. 43, 89075 Ulm, Germany

TRAIL (TNF-related, apoptosis-inducing ligand) is considered a potential future cytotoxic drug to treat cancer as TRAIL induces apoptosis in a wide variety of different tumour cells without toxicity in animal trials. The apoptosis signaling pathway activated by TRAIL includes formation of the receptor-proximal death-inducing signaling complex followed by direct or Bid/mitochondria-amplified activation of caspases.
We recently described for the first time that TRAIL exerted a second, opposite function: TRAIL induced survival and proliferation in tumour cells resistant against TRAIL-induced apoptosis (Oncogene 25, 3842-44, 2003). TRAIL, anti-DR4 or anti-DR5 induced apoptosis in tumour cells we called group A tumour cells and proliferation in group P tumour cells, while none had any effect on group 0 tumour cells. In group AP tumour cells, TRAIL induced proliferation in low concentrations and at early time points, while TRAIL induced apoptosis over time proving that both signaling pathways activated by TRAIL were functional within one single tumour cell. Both in cell lines as well as in primary cells of children with acute leukemia, more than 20 % of tumour cells belonged to group P (Cancer Res. 65, 7888-95, 2005). Important within the clinical context, TRAIL induced proliferation in group P cells even in the presence of cytotoxic drugs. When apoptosis defects proximal to the TRAIL-receptors like loss of Caspase-8 or dysfunction of FADD were introduced into group A tumour cells, TRAIL induced proliferation in these cells. In contrast, downstream apoptosis defects like overexpression of Bcl-2 or Bcl-xL or downregulation of Caspase-9 converted group A tumour cells into group 0 tumour cells. Thus, the decision between life and death signaling after stimulation with TRAIL was made close to the TRAIL receptors. Furthermore, spontaneous growth of group P tumour cells depended on the presence of endogenous TRAIL as downregulation of TRAIL expression using shRNA induced apoptosis in these cells.
Taken together, our data show that TRAIL induces both apoptosis and proliferation in tumour cells and that the determining switch between life and death upon stimulation with TRAIL lies at the level of receptor-proximal signaling.

CKIP-1 interacts with the intracellular domain of TNF and participates in the inflammatory response of macrophages

Kata Juhasz[1], Monika Debreczeny[1], Agnes Zvara[1], Agota Domonkos[1], Zsolt Balogi[1], Chie Kohchi[2], Erno Duda[3]

[1]Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Hungary
[2]Radioisotope center, Hiroshima University, Japan
[3]Albert Szent-Györgyi Medical Center, Szeged University, Szeged, Hungary

Engagement of the 26 kDa transmembrane form of TNF (tmTNF) by its receptor or agonist antibodies elicits a process in the TNF expressing cells, termed reverse signaling. To elucidate the molecular basis of this phenomenon proteins interacting with the intracellular domain of tmTNF were identified by yeast two hybrid technique. Casein kinase 2 interacting protein-1 (CKIP-1) revealed as a tmTNF interacting protein and the binding was confirmed by pull down experiments. CKIP-1 also interacts with a number of proteins, like casein kinase-2, actin-capping protein, ATM and c-Jun. The protein has plecstrin homology, cytoskeletal protein-, chromatin-, and Leu-zipper-binding domains. CKIP-1 shuttles between the plasma membrane and the nucleus and its role in cell signaling is cell-type specific. In fibrobalsts it represses AP-1 activity thereby inducing apoptosis. We demonstrated that mouse CKIP-1 is involved in the inflammatory response of murine macrophages. Its expression is regulated by LPS and its overexpression leads to the increased production of inflammatory cytokines in response to LPS challenge. CKIP-1 was found to transactivate the human TNF promoter and seems to cooperate with c-Jun in this process. The protein was also found to promote the activation of c-Jun and Elk-1, but did not influence NF-kappaB activity. Our data suggest that CKIP-1 might act as a signaling protein in pathways regulating cytokine expression and/or apoptosis in TNF reverse signaling during inflammation.

The pro-survival gene A1 is transcriptionally regulated by NFAT in mast cells

Mats Karlberg, Erik Ullerås, Gunnar Nilsson

Karolinska Institutet, Department of Medicine, Clinical immunology and Allergy unit, Stockholm, Sweden, mats.karlberg@ki.se

The pro-survival protein A1 has a crucial role in activation-induced mast cell survival. Upon activation via IgE receptor cross-linking, A1 is up-regulated resulting in increased survival, an effect which is not seen in A1 deficient mast cells. In macrophages, B- and T-cells, transcription of A1 is regulated by NFκB. In mast cells however, our data suggest an NFκB independent transcriptional regulation of A1.

Bone marrow derived mast cells from mice deficient in c-Rel and p50, two subunits of NFκB, still up-regulate A1 after IgE receptor cross-linking. Similarly, mast cells over expressing the NFκB super-repressor IκB-α, also up-regulates A1 after activation. Furthermore, reporter gene analysis of promoter sequences of A1, lacking the in B-cell described active NFκB binding site, still show an active promoter. All these data suggest a minor role for NFκB in transcription of A1 in mast cells.

Instead, we have data indicating that NFAT is the primary transcription factor for A1 in mast cells. Cyclosporine A, an inhibitor of calcineurin and NFAT, blocks the induced expression of A1 mRNA in mast cells upon IgE receptor aggregation, but not in monocytes activated by LPS. This has been confirmed both by reporter gene analysis and RT-PCR. Furthermore, a putative NFAT binding site in the promoter region of A1 show increased protein binding after IgE receptor cross-linking. Antibodies directed against NFAT confirmed identity of the binding protein in a band shift assay, either by quenching the protein-oligo binding, or by a band shift.

These results indicate differences in transcriptional regulation of genes in different cell types. In this study we show that the pro-survival gene A1 in mast cells, contradictory to other cell types, is not transcriptionally regulated by NFkB. Instead, our results suggest NFAT as the transcription factor regulating A1 in mast cells following IgE receptor cross-linking.

XIAP targeting sensitizes Hodgkin´s Lymphoma cells for cytolytic T cell attack

Hamid Kashkar[1,2] , Jens-Michael Seeger[1], Andreas Hombach[3], Anke Deggerich[1], Benjamin Yazdanpanah[1], Olaf Utermöhlen[1], Gerd Heimlich[4], Hinrich Abken[3], Martin Krönke[1,2]

[1]Institute for Medical Microbiology, Immunology and Hygiene; [2]Center for Molecular Medicine Cologne (CMMC);[3] Tumorgenetics, Clinic I Internal Medicine, University of Cologne, Cologne, Germany; and [4]the Laboratory of Signal Transduction, NIEHS, National Institutes of Health, Research Triangle Park, North Carolina.

The immunosurveillance of Hodgkin's Lymphoma (HL) by cytotoxic T lymphocytes (CTL) is insufficient and the clinical experience with adoptive transfer of CTLs is limited. We have previously reported that defects in mitochondrial apoptotic pathways and elevated XIAP-expression confer resistance to different apoptotic stimuli in HL cells. Here we aimed to develop molecular strategies to overcome the resistance of HL cells against CTL-mediated killing via granzyme B (grzB). In HL cells grzB-induced mitochondrial release of pro-apoptotic Smac is blocked, which results in complete abrogation of cytotoxicity mediated by CTLs. Cytosolic expression of recombinant mature Smac enhanced caspase activity induced by grzB and restored the apoptotic response of HL cells. Similarly, down-regulation of XIAP by RNA interference markedly enhanced the susceptibility of HL cells for CTL-mediated cytotoxicity. XIAP gene knock-down sensitized HL cells for killing by antigen-specific CTLs redirected by grafting with a chimeric anti-CD30scFv-CD3zeta immunoreceptor. The results suggest that XIAP targeting by Smac agonists or XIAP-siRNA can be used as a synergistic strategy for cellular immunotherapy of Hodgkin´s lymphoma.

Dynamic molecular regulation of phagocytosis in the apoptotic cells by macrophages

Klára Katona, Gyöngyike Májai, Ralph Rühl, Gábor Zahuczky and László Fésüs

Department of Biochemistry and Molecular Biology, Signaling and Apoptosis Research Group of the Hungarian Academy of Sciences, Research Center for Molecular Medicine, University of Debrecen, e-mail:katonak@indi.biochem.dote.hu

Phagocytosis plays an important role in the clearance of apoptotic cells in the human body. In spite of the many discoveries made so far in this field, the dynamics and molecular regulation of this process remains not well understood. We have observed that human monocyte-derived macrophages engulf increasing number of apoptotic neutrophils over time reaching saturation by 6 hours of co-incubation. The saturated state of these macrophages remains unchanged even after addition of a new batch of apoptotic neutrophils. During engulfment of neutrophils the total cholesterol content in macrophages is increased as compared to the unchallenged ones. Cholesterol pre-loading of macrophages significantly decreases their overall phagocytic capacity. Time-dependent gene expression studies in macrophages engulfing apoptotic neutrophils reflects the saturating dynamics of the engulfment process mainly in the early up-regulation of the mRNA level of well known phagocytic receptor genes (e.g. oxidized LDL receptor 1) and of molecules which opsonize apoptotic cells for phagocytosis (e.g thrombospondin). When the phagocytic process reaches a saturation state, these genes became down-regulated. Our data suggest that biochemical components of the engulfed apoptotic cells, particularly cholesterol, play an important role in the dynamics of the phagocytic process, thus, affecting the speed of the removal of dead cells and signaling a saturation state by a so far unknown mechanism.

Induction of apoptosis by plumbagin through ROS-mediated inhibition of topoisomerase II

Kawiak A[1], Piosik J[2], Stasilojc G[3], Gwizdek-Wisniewska A[2], Bigda J[3], Lojkowska E[1]

[1]Department of Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk & Medical University of Gdansk, Kladki 24, 80-822 Gdansk, Poland
[2]Department of Molecular and Cellular Biology, Intercollegiate Faculty of Biotechnology, University of Gdansk & Medical University of Gdansk, Kladki 24, 80-822 Gdansk, Poland
[3]Department of Medical Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk & Medical University of Gdansk, Debinki , 80-822 Gdansk, Poland

Plumbagin, a quinone present in plants from the *Droseraceae* and *Plumbaginaceae* families, has been proven to possess anti-tumour activity in *in vitro* and *in vivo* studies. Plumbagin has been reported to inhibit the activity of topoisomerase II and due to the presence of a quinone moiety, the generation of reactive oxygen species (ROS) has been implicated in the anti-tumour activity of this compound. Since ROS have been reported to selectively modify proteins and therefore regulate cellular signaling including apoptosis, the objective of this research was to clarify the role of ROS and topoisomerase II inhibition in the induction of apoptosis mediated by plumbagin.

Plumbagin inhibits the activity of topoisomerase II through the stabilization of the DNA-topoisomerase II cleavable complex, which leads to DNA strand breaks. As determined by the comet assay, plumbagin induced DNA cleavage in HL-60 cells, whereas in a cell line with reduced topoisomerase II activity - HL-60/MX2, the level of DNA damage was significantly decreased. The pre-treatment of cells with N-acetyl cysteine (NAC) attenuated plumbagin-induced DNA damage, pointing out to the involvement of ROS generation in cleavable complex formation. The level of intracellular ROS generated by plumbagin did not correlate with the formation of DNA strand breaks. The level of ROS reached a maximum after 40 min of incubation with plumbagin, and declined thereafter to reach basal values after 2h. However, the induction of DNA damage was visible after 1 h and increased in a time-dependant manner. Moreover, in HL-60/MX2 cells ROS were generated at a similar rate, whereas a significant reduction in the level of DNA damage was detected in this cell line. This suggests that plumbagin-induced ROS does not directly damage DNA but requires the involvement of topoisomerase II. Furthermore, experiments carried out using light spectroscopy indicated no direct interactions between DNA and plumbagin. The induction of apoptosis was significantly delayed in HL-60/MX2 cells indicating the involvement of topoisomerase II inhibition in plumbagin-mediated apoptosis. Thus, the experimental data obtained so far strongly suggest ROS-mediated inhibition of topoisomerase II as an important mechanism contributing to the anti-tumour properties of plumbagin.

Caspase-1 and TRIM16/EBBP: Two key regulators of inflammation and interleukin-1 secretion in keratinocytes

Martin Keller, Andreas Rüegg, Laurence Feldmeyer, Sabine Werner and Hans-Dietmar Beer

Institute of Cell Biology, ETH Zurich, Zurich, Switzerland,
e-mail: martin.keller@cell.biol.ethz.ch

The two variants of interleukin (IL)-1, IL-1α and -β, are key players in the regulation of local and systemic pro-inflammatory processes. They have similar biological activities and are expressed as precursors. IL-1α is active in its pro-form, whereas biologically active IL-1β is generated through cleavage by caspase-1. IL-1β is mainly secreted by activated macrophages, but human keratinocytes can also release large amounts of active IL-1β and proIL-1α after UV irradiation. Both proteins are not secreted via the endoplasmic reticulum/Golgi pathway, but by as yet poorly characterized mechanisms. Processing of proIL-1β is dependent on caspase-1, which in turn is activated by the inflammasome. We found that TRIM16/EBBP, a member of the growing family of TRIM proteins with various cellular functions, is a novel enhancer of IL-1α and -β secretion. This was demonstrated in cells overexpressing TRIM16 and in UVB-irradiated human keratinocytes with an siRNA-mediated TRIM16 knock-down. We showed that endogenous TRIM16 binds to IL-1β as well as to components of the inflammasome and that the binding correlates with IL-1β secretion. This may explain the regulatory effect of TRIM16 on the secretion of IL-1β, but not on IL-1α, because the latter is not a substrate of caspase-1. Nevertheless, macrophages of caspase-1-deficient mice show reduced IL-1α release, but the reason for this deficit is as yet unknown. We found that IL-1α and -β release is also caspase-1-dependent in human keratinocytes. Additionally, we performed binding studies and secretion experiments using caspase-1 with a mutated active site. These results provide insight into the mechanisms underlying the caspase-1-dependent release of IL-1.
Taken together, we identified TRIM16 as a novel regulator of IL-1 release. Furthermore, we showed a general effect of caspase-1 on IL-1 secretion, which is dependent on caspase-1 activity. Finally, our results suggest a similar mechanism for inflammasome activation and IL-1 release in keratinocytes as previously shown for macrophages. This suggests that keratinocytes are directly implicated in the onset of inflammation and that TRIM16 and caspase-1 are promising targets for the treatment of inflammatory skin diseases.

Structure-function analysis caspase-1/CARD-mediated NF-kB activation

Kristof Kersse, Mohamed Lamkanfi, Sigrid Cornelis, Tom Vanden Berghe and Peter Vandenabeele

Molecular Signalling and Cell Death Unit, Department of Molecular Biomedical Research (http://www.dmb.rug.ac.be), VIB, Ghent University, technologiepark 927, B-9052 Zwijnaarde, Belgium, e-mail: peter.vandenabeele@dmbr.Ugent.be

Caspase-1 contributes through two independent pathways to an inflammatory response. On the one hand it mediates the maturation of pro-interleukin (IL)-1β and pro-IL-18 through its proteolytic activity, while on the other the N-terminal caspase recruitment domain (CARD) is necessary and sufficient for the activation of NF-κB. The latter is dependent on the interaction with RIP2, a CARD-containing serine/threonine kinase involved in multiple NF-κB activating pathways. COP/Pseudo-ICE, INCA and ICEBERG are three CARD-only proteins that share a high degree of identity with the prodomain of caspase-1. All three proteins bind with caspase-1 and prevent the maturation of pro-IL-1β and pro-IL-18. However, in contrast to INCA and ICEBERG, only COP interacts with RIP2 and gives rise to NF-κB activation. Hence, we can divide these homologous CARD-proteins in two groups, the NF-κB activators (caspase-1 CARD and COP) and the non-activators (INCA and ICEBERG). Potentially critical amino acids on the surface of the caspase-1 CARD for the interaction with RIP2 and the subsequent NF-κB activation were selected by comparing the sequences and the three-dimensional models of these two groups. Mutation of these differential amino acids in caspase-1 CARD resulted in the identification of two amino acids that are absolutely crucial for the activation of NF-κB as pointmutation completely abrogated the NF-κB inducing capacity of caspase-1 CARD. Furthermore, reverting these mutations in INCA partially restored INCA-mediated NF-κB activation, underlining the importance of these amino acids. However, the other caspase-1 function is not affected by this mutation as the maturation of pro-IL-1β can still occur. In this way we can dissect both caspase-1 functions opening the possibility to study their contributions separately.

Regulation of FBP1 expression and changes in glucose metabolism are crucial for an appropriate response to DNA damage and aging in *S.cerevisiae*

Ana Kitanovic and Stefan Wölfl

Institute for Pharmacy and Molecular Biotechnology, Ruperto-Carola University of Heidelberg, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany, e-mail: Ana.Kitanovic@urz.uni-heidelberg.de

All eukaryotic cells are constantly exposed to exogenous or endogenous agents that damage DNA. Occurring over various time spans or as the consequence of repeated exposures, the accumulation of mutations may lead to increased genetic instability, elevation of intracellular levels of reactive oxygen species (ROS) and facilitate tumor promotion and progression. It was proved earlier that DNA damaged or chronologically aged yeast cells die exhibiting typical markers of apoptosis.

Investigating the transcriptional response of *S.cereveisiae* to low doses of the alkylating agent methylmethane sulfonate (MMS) we observed that metabolic adaptation and pre-induction of environmental stress response (ESR) program, as a consequence of nutrient deprivation, helps cells to cope better with the impact of genotoxic agents applied later. Deletion of the key enzyme of gluconeogenesis, fructose-1,6-bisphosphatase (*FBP1*), resulted in lower sensitivity to MMS and reduced ROS production, leading to better recovery of the mutant after a long-term MMS treatment. Moreover, delayed production of ROS in the mutant cells was also observed in the first fifteen days of aging culture. Reintroduction of *FBP1* in the knockout strain restored the wild-type phenotype while overexpression enhanced MMS-sensitivity of yeast cells, significantly increased the activity of the *RNR2* promoter and shortened life-span, but did not have any influence on ROS accumulation. In the direct competition *Dfbp1* mutant cells were more viable only in the first 3 days of aged MMS treated culture. In the non-treated culture wild-type cells were constantly dominant. When we tried to re-grow 15 days old cells we observed that cells which overexpress *FBP1* are able to adapt better to a new environment containing fresh medium. Interestingly, MMS diminished enzymatic activity of this protein what was not followed by the reduction in protein level.

Taken together, our results clearly indicate that *FBP1* influences the connection between DNA damage, aging and subsequently apoptosis program either through direct signalling or an intricate adaptation in energy metabolism. In conclusion, the tight regulation of *FBP1* expression and age-associated changes in glucose metabolism are not only crucial for the control of gluconeogenesis but also for an appropriate response to aging and DNA damage.

Mechanisms involved in apoptosis of HT-29 cells after 5-LOX pathway modulation followed by photodynamic therapy with hypericin

Ján Kleban[1], Jaromír Mikeš[1], Viktor Horváth[2], Veronika Sacková[1], Jirina Hofmanová[2], Alois Kozubík[2], Peter Fedorocko[1]

[1]Institute of Biology and Ecology, Faculty of Sciences, P. J. Šafárik University, Moyzesova 11, 040 01 Košice, Slovakia, e-mail: jankleban@yahoo.com
[2]Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Kralovopolska135, 612 65 Czech Republic

It may be hypothesized that the lipoxygenase (LOX) metabolic pathway plays an important role in photodynamic therapy (PDT) of malignant tumours, and modification of this pathway may result in administration of lower doses of photodynamic active agents accompanied by reduced side effects. We discovered that pre-treatment of HT-29 cells with specific 5-lipoxygenase inhibitor MK-886 followed by photodynamic therapy with hypericin induced cell cycle arrest and an increase of apoptosis (Kleban et al., Journal of Photochemistry and Photobiology B: Biology, 2006, in press). More detailed study of signaling pathways leading to increase of apoptosis and massive changes of cell cycle was conducted.

Expression of key apoptotic signal molecules (caspase-3, -8, -9; PARP; Mcl-1; Bax) using Western blotting, caspase activity, reactive oxygen species production (ROS) using flowcytometry (dihydrorhodamine123; hydroethidine and 2´,7´- dichlorodihydrofluorescein diacetate staining) and survival of cells using clonogenicity test were analysed.

Only minimal changes in expression of caspases in cells treated with nontoxic concentrations of both agents alone or their mutual combination were observed. However, their activity (caspase-3, -8, -9) was increased in according to their relevant position in apoptotic signal pathway. Treatment with nontoxic concentration of MK-886 had minimal influence on ROS production as compared to control cells. In contrast, hypericin alone exponentially increased the level of ROS (approximately two fold), but no additional effect of MK-886 pre-treatment was detected. Influence of both treatment models (in presence or absence of PDT) on the production of various types of oxygen radicals will be presented. Clonogenicity test displayed disruption of colony formation after mutual combination of both agents as compared to MK-886 alone and PDT alone.

In conclusion, our study indicates that increase of apoptosis is transducted preferentially through mitochondrial pathway (partitipation of ROS and caspase-9).

This work was supported by Science and Technology Assistance Agency under the contract No. APVT-20-003704 and by grant agency VEGA under the contract No.1/2329/05.

Key words: photocytotoxicity, hypericin, MK-886, HT-29, apoptosis signalling.

A novel activity of Bak at the endoplasmic reticulum.

Martina Klee and Felipe X. Pimentel-Muiños

Centro de Investigación del Cáncer, Universidad de Salamanca-CSIC, Salamanca, 37007 Spain

Proapoptotic Bcl-2 family members Bak and Bax play a redundant, but essential role in multiple death pathways. At the mitochondrial outer membrane both proteins trigger apoptosis through the release of apoptogenic factors which subsequently activate downstream effector caspases. In addition to their presence at the mitochondria, Bak and Bax are also localized to the endoplasmic reticulum where they control apoptosis by regulating calcium homeostasis. In this subcellular compartment, both proteins were also found to modulate the unfolded protein response through a direct interaction with IRE1alpha. However, evidence in the literature suggests that this functional redundancy may not always apply, opening the possibility for specific functions carried out by only one of these effectors.

To search for specific activities of Bak we took advantage of findings in previous reports where the overexpression of Bak and Bax was shown to trigger spontaneous signaling. Our results show that coexpression of Bak and Bcl-X(L) provokes extensive swelling and vacuolization of reticular cisternae. A Bak version lacking the BH3 domain suffices to induce this phenotype, and reticular targeting of this mutant retains the activity. Expression of upstream BH3-only activators in similar conditions recapitulates ER swelling and vacuolization if ryanodine receptor calcium channel activity is inhibited. Experiments with Bak and Bax-deficient mouse embryonic fibroblasts show that endogenous Bak mediates the effect, whereas Bax is mainly irrelevant. These results reveal a novel role of reticular Bak in regulating structure and volume of this organelle. Because this activity is absent in Bax, it constitutes one of the first examples of functional divergence between the two multidomain homologues.

Novel proteins interacting with death receptor-6 (DR6 / TNFRsf21)

Martin Klíma, Jitka Zájedová, Ladislav Andera

Laboratory of Cell Signaling and Apoptosis, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, e-mail: klima@leuko.biomed.cas.cz

Death receptor-6 is a death domain containing receptor of the TNFR (tumor necrosis factor-receptor) superfamily that apparently participates in the regulation of proliferation and differentiation of T- and B-lymphocytes. Overexpression of DR6 in HeLa or HEK293 cells leads to apoptosis, and/or to activation of NF-κB and stress kinases of the JNK/SAPK family. DR6-knockout (DR6-/-) mice have expanded T-cell populations, increased secretion of IL-2 and IL-4 in response to mitogens, and grafted DR6-/- HSC induce more severe graft-versus-host disease. DR6-/- B cells show increased proliferation in response to anti-IgM, anti-CD40 or LPS. DR6 ligand was not, in contrast to majority of other TNFR receptors, yet identified.

The first step in deciphering the DR6 signaling is the identification of individual components of the DR6 membrane signaling complex. We employed the yeast-two-hybrid screening of several cDNA libraries for proteins interacting with the intracellular part of DR6. We have identified several specific interactors and at the present we proceed with the characterization of their role in DR6 signaling. One of them is a phox-homology domain containing protein sorting nexin-3 (SNX3). DR6 and SNX3 directly interact both in yeast (Y2H system) and in human cells (coimmunoprecipitation), and after cotransfection into mammalian cells they colocalize in vesicular endosome-like structures. Thus, SNX3 may play a role in membrane trafficking and recycling of DR6.

Ectopic expression of another DR6-interacting protein, a calcium-binding domain containing protein copine-7 (CPNE7), inhibits JNK activation induced by DR6 overexpression. We currently analyze if CPNE7 could be a downstream effector of signaling from the activated DR6.

Chemokine receptors CXCR4 and CCR7 mediate inhibition of anoikis in cancer cells

Marina Kochetkova and Shaun R. McColl

School of Molecular and Biomedical Sciences, Adelaide University, Adelaide, Australia

Specific molecular and cellular mechanisms utilised by tumour cells to invade and colonise organs distant from the primary site remain largely undiscovered. Understanding of these mechanisms will eventually lead to the treatment of the metastatic tumours causing 90% of cancer related deaths. It has been shown that matrix-independent survival of metastatic cells during dissemination through blood and/or lymph passageways is an important stage in the invasion cascade and thus anoikis resistance may significantly contribute to malignant spread by allowing epithelial cancer cells to survive in the absence of attachment in the circulation or in hostile stromal environment at distant sites.

Recent studies have suggested that activation of a number of chemokine receptors on cancer cells might contribute to the invasive phenotype of cancer cells in a chemokine ligand/receptor-dependent, organ-specific manner. We have investigated a specific role for chemokine receptor stimulation in promoting cancer cells survival and have found that CXCL12 and CCL19 chemokines, ligands for the CXCR4 and CCR7 receptors respectively, were able to inhibit anoikis specifically in metastatic breast and colon tumour cells via distinct novel molecular pathways. The specificity of the chemokine receptor inhibition of anoikis was confirmed using shRNA mediated knockdown and treatment with Gi specific inhibitor Pertussis toxin. In addition we demonstrate that the inhibition of anoikis by chemokines in invasive tumour cells is mediated by the phosphatidylinositol 3-kinase (PI3K) dependent regulation of the BH3 only protein Bmf and the pro-apoptotic protein Bax.

In summary, our findings support novel, previously undescribed chemokine receptor function of the inhibition of anoikis in highly invasive cancer cells which may represent one of the mechanisms used by malignant cells to survive in the hostile environment in the circulation or at distant sites of metastasis. The results also, for the first time highlight potential pathological role for the chemokine family members in metastatic cancer disease as survival factors.

ER stress-induced apoptosis is independent of the death receptor pathway but sub-lethal ER stress sensitizes cells to death receptor stimuli by up-regulating DR5

Barbara F. Koehler[1,2] , Donat Koegel[2] and Jochen H. M. Prehn[1]

Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin 2, Ireland[1] and Experimental Neurosurgery in the Center for Neurology, Johann Wolfgang Goethe-University Clinics Frankfurt, Germany[2].

Prolonged endoplasmic reticulum (ER) stress, resulting from accumulation of misfolded proteins in the ER lumen, induces caspase activation and apoptosis. Recently we have described that transcriptional induction of the Bcl-2 homology 3 (Bh3)-only protein PUMA and activation of the mitochondrial pathway of apoptosis is central to ER stress-induced apoptosis. Emerging evidence suggests that the activation of the death receptor pathway via TRAIL (tumor necrosis factor-related apoptosis inducing-ligand) may also have a key role in this process. TRAIL binding to either death receptors (DR) 4 or 5 results in caspase activation and cleavage of the BH3-only protein Bid to a truncated form capable of engaging the mitochondrial apoptotic pathway.

In order to delineate the requirement of death receptor signaling during ER stress induced apoptosis we utilized HeLa cells stably transfected with either Bid-specific or control siRNA-vectors. Impairment of Bid expression rendered cells highly resistant to TRAIL-mediated apoptosis. However, ER stress-induced apoptosis was independent of Bid expression as treatment with the ER stressor tunicamycin provoked similar levels of apoptosis in both knock down and control cells. Similarly, use of death receptor blocking peptides had no effect on the levels of apoptosis. Given that DR5 has previously been reported to be up-regulated following ER stress we hypothesized that low levels of ER stress may synergistically sensitize cells to TRAIL. Indeed, caspase activation, PARP cleavage and apoptosis were significantly increased following TRAIL treatment in tunicamycin pre-treated cells, an effect which was abrogated by Bid knock down. Interestingly, protein synthesis was required since preincubation with cycloheximide abolished the synergy. Real-time qPCR and western blotting revealed that tunicamycin did not alter DR4 expression but increased DR5 expression on both mRNA and protein levels. Moreover, use of a DR5-blocking peptide completely abolished the sensitizing effects of tunicamycin to TRAIL-induced apoptosis.

Our data suggest that ER stress-mediated apoptosis proceeds independently of the death receptor pathway but low doses of tunicamycin can have significant effects in sensitizing cells to TRAIL-mediated apoptosis via up-regulation of DR5.

Supported by grants from Science Foundation Ireland and Deutsche Forschungsgemeinschaft to JHMP.

K_{ATP} channel openers uncouple oxidative phosphorylation of cardiac mitochondria *in situ*

Dalia M. Kopustinskiene[1,2] and Adolfas Toleikis[2]

[1]*Department of Biochemistry, Kaunas University of Medicine, Kaunas, Lithuania*
[2]*Laboratory of Biochemistry, Institute for Biomedical Research of Kaunas University of Medicine, Kaunas, Lithuania. Email: daliammk@mail.lt*

K_{ATP} channel openers protect ischemic-reperfused myocardium by mimicking ischemic preconditioning, however, the protection mechanisms have not been fully clarified yet. Since the skinned fibers technique gives an opportunity to investigate an entire population of mitochondria in their native milieu, in this study we have investigated the effects of K_{ATP} channel openers diazoxide and pinacidil on the respiration rate of rat heart mitochondria *in situ*, oxidizing pyruvate and malate (6+6 mM). Respiration rates were recorded by the means of Clark-type oxygen electrode in the physiological salt solution (37°C). Our results showed that both diazoxide and pinacidil (60-1250 μM) in a concentration-dependent manner increased pyruvate-malate supported State 2 respiration rate of skinned cardiac fibers by 15-120%. Moreover, diazoxide did not affect, whereas pinacidil (60-1250 μM) decreased the State 3 respiration rate of skinned cardiac fibers by 4-27%. Thus, in contrast to diazoxide, pinacidil not only uncouples pyruvate and malate oxidizing mitochondria in skinned cardiac fibers, but also inhibits the respiratory chain, whereas diazoxide has only uncoupling action. Since moderate mitochondrial uncoupling prevents excessive ROS generation and Ca^{2+} overload, our results support the hypothesis that uncoupling might be responsible for the cardioprotection by K_{ATP} channel openers.

Apoptosis induced by iron deprivation in tumor cells

Jan Kovár[1,2], Michal Koc[1], Jaroslav Truksa[1], Marie Ehrlichová[1] and Zuzana Nadová[1]

[1]Department of Cell Signalling and Apoptosis, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic, e-mail: kovar@biomed.cas.cz
[2]Department of Cell and Molecular Biology, 3rd Faculty of Medicine, Charles University, Prague, Czech Republic, e-mail: jan.kovar@lf3.cuni.cz

Iron deprivation specifically induces apoptosis in some cultured tumor cells while other cells are resistant. We studied the mechanisms involved in apoptosis induction by iron deprivation in tumor cells. To discriminate between changes coupled only with iron deprivation and changes involved in the induction of apoptosis by iron deprivation, we compared sensitive 38C13 cells (mouse B lymphoma) and resistant EL4 cells (mouse T lymphoma). Iron deprivation was achieved by incubation in a defined iron-free medium. We detected caspase-3 activation as well as caspase-9 activation in sensitive cells under iron deprivation. Iron deprivation led to the release of cytochrome c from mitochondria in sensitive cells but it did not affect the cytosolic localization of Apaf-1. The mitochondrial membrane potential ($\Delta\psi_m$) was dissipated in sensitive cells due to iron deprivation, however, reactive oxygen species (ROS) production was not significantly affected. The antiapoptotic Bcl-2 protein was associated with mitochondria and the association did not change under iron deprivation. However, we detected translocation of the proapoptotic Bax protein from the cytosol to mitochondria in sensitive cells under iron deprivation. Taken together, we suggest that iron deprivation induces apoptosis in 38C13 cells via mitochondrial changes concerning proapoptotic Bax translocation to mitochondria, collapse of the mitochondrial membrane potential, release of cytochrome c from mitochondria, and activation of caspase-9 and caspase-3. Events upstream of Bax translocation during apoptosis induction by iron deprivation remain to be elucidated. However, the p53 pathway is not involved here as we demonstrated previously. We also compared the mechanisms involved in apoptosis induction by iron deprivation in mouse 38C13 cells with the mechanisms involved in sensitive human Raji cells (Burkitt lymphoma). Caspase-3 and caspase-9 were also activated in Raji cells under iron deprivation. However, cytochrome c release from mitochondria was not detected in Raji cells. Similarly, mitochondrial membrane potential ($\Delta\psi_m$) was not dissipated and Bax translocation was not detected in Raji cells under iron deprivation. Caspase-6 and caspase-7 were not activated. We conclude that together with “regular” pathway of caspase-3 activation by iron deprivation in 38C13 cells there is another pathway of caspase-3 activation by iron deprivation in Raji cells which is independent of cytochrome c release.

Macrophages use different internalization mechanisms to clear apoptotic and necrotic cells without activation of NF-kB transcription factor

Dmitri V. Krysko[1], Geertrui Denecker[2], Katharina D'Herde,[1,*] and Peter Vandenabeele[2,*]
[*]These authors share senior author ship

[1]Department of Human Anatomy, Embryology, Histology and Medical Physics, and [2]Molecular Signaling and Cell Death Unit, Department for Molecular Biomedical Research, VIB, Ghent University, Ghent, Belgium. Dmitri.Krysko@ugent.be

The present study characterized two different internalization mechanisms used by macrophages to engulf apoptotic and necrotic cells. Our *in vitro* phagocytosis assay used a mouse macrophage cell line, and murine L929sAhFas cells that are induced to die in a necrotic way by TNFR1 and heat shock or in an apoptotic way by Fas stimulation. SEM revealed that apoptotic bodies were taken up by macrophages with formation of tight fitting phagosomes, similar to the "zipper"-like mechanism of phagocytosis, whereas necrotic cells were internalized by a macropinocytotic mechanism involving formation of multiple ruffles directed towards necrotic debris. Two macropinocytosis markers (lucifer yellow and horseradish peroxidase) were excluded from the phagosomes containing apoptotic bodies, but they were present inside the macropinosomes containing necrotic material. In addition, in order to address the controversial issue of cell specificity we have used in our *in vitro* phagocytosis assay another cell type as target cell. For this purpose we applied a well- characterized model system for apoptotic or necrotic cell death, being Jurkat E cells stimulated with anti-Fas or FADD-deficient Jurkat cells stimulated with hTNF and we could confirm the data obtained for L929sAhFas cells. Addition of wortmannin (50 nM and 100 nM), an inhibitor of phosphatidylinositol 3` -kinase (PI3K), reduced by 50% the uptake of apoptotic bodies by macrophages, while the engulfment of necrotic cells remained unaffected, suggesting a differential involvement of PI3K. Induction of a pro-inflammatory response in macrophages by necrotic cells is still controversial and therefore we investigated whether following the uptake of apoptotic and necrotic cells the activity of NF-κB transcription factor (a key regulator of genes involved in immune and inflammatory reactions) would be modulated. To this end we generated a lentiviral vector containing a NF-κB-dependent luciferase reporter gene and transduced Mf4/4 with this vector. This approach revealed that neither apoptotic nor necrotic cells induce NF-κB activation in macrophages upon their uptake. In conclusion the present study demonstrates (1) that apoptotic and necrotic cells were internalized differently by macrophages and (2) that uptake of neither apoptotic nor necrotic L929 cells modulates the NF-κB activity in macrophages.

Large-conductance potassium channel activators in neuroprotection

Bogusz Kulawiak, Marta Glab, Adam Szewczyk

Laboratory of intracellular ion channels, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland

Potassium channels have been identified in the inner-mitochondrial membrane. ATP-regulated potassium channel (mitoK_{ATP} channel) was described in liver, heart and brain mitochondria. Recently, a large-conductance Ca^{2+}-activated potassium (BK_{Ca}) channel was described in heart, glioma and brain mitochondria.
The activity of BK channels is modulated by various pharmacological substances such as potassium channel openers: benzimidazolone derivative NS1619 and indol derivatives CGS7181 and CGS7184. The BK_{Ca}-type channel is blocked by neurotoxins, peptides charybdotoxin and iberiotoxin, and paxilline, alkaloid produced by *Penicilium paxilli*.
Potassium channels are believed to play a crucial role in cytoprotection. Activation of mito-K_{ATP} channel with use of potassium channel openers (diazoxide) can lead to cardioprotection and neuroprotection. Similarly, activation of mitochondrial BK_{Ca} channel by NS1619 can lead to cardioprotection.
The aim of our experiments was to test the influence of BK_{Ca} channel openers NS1619, CGS7181 and CGS7184 on neuronal cell viability. In our research we used HT-22 mouse hippocampal cell line. HT-22 cells lack glutamate ionotropic receptors. It is known that treatment of HT-22 cells with glutamate leads to oxidative stress and in consequence to cell death. Cell survival was measured after 18h with two methods: MTT reduction and LDH release. Here, we show that treatment of HT-22 cells with low concentration (3μM) of CGS7184 and CGS7181 in the presence of 3mM glutamate increases cell survival in comparison to cells treated with glutamate alone. These effects were not observed when NS1619 was used. Protective effects of CGS7184 and CGS7181 were not reversed by addition of BK_{Ca} channel inhibitors: charybdotoxin, iberiotoxin and paxilline. Additionally level of reactive oxygen species was monitored. Glutamate alone significantly increased ROS level in HT-22 cells. Interestingly in the presence of CGS7184 and glutamate ROS level was similar.
Additionally we found that high concentration of CGS7181 and CGS7184 induce death of HT22 cells. Incubation of HT-22 with 30μM CGS7184 for 18 hours caused LDH release. Moreover, 3μM CGS7184 induced apoptosis of HT-22 observed after long time incubation. After 36h incubation with 3μM CGS7184 we observed formation of sub-G1 fraction. Toxic effects of CGS7184 were not inhibited by caspase inhibitor z-vad-fmk, calpain inhibitor VI and cyclosporine A.

Death or Survival? Differential effects of NF-kappaB on apoptosis induced by DNA-damaging agents.

*E. Strozyk, **D. Kulms

**Department of Dermatology, University of Muenster, D-48149 Muenster;*
***Department of Cell Biology and Immunology, University of Stuttgart, D-70569 Stuttgart*

Recently much effort is made to selectively kill tumor cells via induction or enhancement of apoptosis. Combination therapies with death ligands and chemotherapeutic drugs, as well as selective inhibition of upstream activators of NFκB are two prominent approaches. Activation of NFκB is generally accepted to be associated with induction of antiapoptotic pathways, provoking tumor maintenance. Correspondingly, IL-1 mediated NFκB activation was previously shown to suppress death ligand-induced apoptosis. In contrast, UVB-mediated apoptosis was shown to be significantly enhanced due to NFκB-dependent repression of antiapoptotic genes and concurrent release of TNFα, which additionally activated TNF-R1-driven apoptosis in an autocrine fashion. These findings implied UVB-induced DNA damage to be critically involved in changing the properties of NFκB. To determine whether NFκB dependent enhancement of apoptosis is a general occasion in response to DNA damaging agents used in cancer therapy we investigated the influence of IL-1 mediated NFκB activation on apoptosis induced by the cytostatic drugs Cisplatin, Doxorubicin, and Etoposide, by γ-radiation, UVB and UVA.
Whereas UVB, UVA and Cisplatin induce base modifications (pyrimidine dimers, bulky purine adducts) the topoisomerase inhibitors Doxorubicin and Etoposide as well as γ-radiation mainly induce DNA strand breaks. We could observe an enhancement of apoptosis upon UVB-radiation and Cisplatin treatment and to a lesser extend upon UVA-radiation, always coinciding with TNFα release. Apoptosis induced by Doxorubicin, Etoposide and γ-radiation remained unaffected by IL-1 mediated NFκB activation although TNFα transcription was conducted. TNFα protein was found to be trapped within the cytoplasm most likely due to inhibition of PKCδ, the enzyme that usually facilitates export of TNFα to the plasma membrane.
Taken together the present study indicates that NFκB may be converted from an antiapoptotic into a proapoptotic mediator in a DNA damage-dependent manner. We show that NFκB-mediated enhancement of apoptosis is critically dependent on the type of DNA lesion induced and is always associated with TNFα release. Since the observed phenomenon is restricted to certain types of DNA lesions, these findings should carefully be taken into account when developing new anti cancer strategies.

Caspase-4 is involved in apoptosis induced by endoplasmic reticulum (ER) stressors and polyglutamine aggregates

Umayal Lakshmanan[1], Takashi Momoi[2] and Alan G Porter[1]

[1]*Cell Death and Human Disease Lab, Institute of Molecular and Cell Biology, 61 Biopolis Drive (Proteos) Singapore 138673* [2] *Division of Development and Differentiation, National Institute of Neuroscience, Kodaira, Tokyo 187-8502, Japan email: mcbagp@imcb.a-star.edu.sg*

Caspase-4 is a human caspase without an obvious corresponding mouse homologue. Based on sequence homology and gene positioning in the cluster of caspases located on human chromosome 11 compared to mouse chromosome 9, caspase-4 might be a functional homologue of mouse caspase-12 (human caspase-12 seems to be non-functional because of a frame-shift mutation). Just like caspase-12, we found that caspase-4 is processed exclusively in response to endoplasmic reticulum (ER) stress. Using a neuroblastoma cell line stably expressing short hairpin (shRNA) against caspase-4, we show that caspase-4 is not required for cell death induced by many stimuli, but contributes only to endoplasmic reticulum stress induced by tunicamycin, thapsigargin, Brefeldin A and the calcium ionophore A23187. Moreover, caspase-4 is localized predominantly in the ER. An expansion of polyglutamine tracts is an underlying mutational mechanism of several neurodegenerative diseases; and aggregates or deposits of polyglutamine [poly(Q)] protein are a prominent pathological characteristic of most polyglutamine diseases. Evidence suggests that an expanded poly(Q) tract exists in an abnormal conformation, and this mis-folded protein might trigger an 'unfolded protein response' in the classical ER stress pathway. Here we show that poly$(Q)_{72}$ aggregates induce ER stress. Caspase-4 is also cleaved in the presence of poly$(Q)_{72}$ aggregates, and polyglutamine-induced apoptosis is significantly reduced in the clones stably expressing shRNA against caspase-4. Poly(Q) may therefore manifest its pathology in neurodegenerative diseases through ER stress, and hence we speculate that caspase-4 may have a role in the pathogenesis of eight well-known disorders caused by expansion of an unstable CAG triplet (SBMA, Huntington's, DRPLA, and SCA-1,-2, -3,- 6, and - 7).

Metacaspases and Bax Inhibitor-1 as regulators of programmed cell death in plants

Eric Lam and Naohide Watanabe

Biotech Center, Rutgers The State University of New Jersey, 59 Dudley Road, New Brunswick, 0890, USA, e-mail: Lam@aesop.rutgers.edu

In spite of the prevalence of programmed cell death (PCD) in plant development and stress responses, molecular description of the key executioners and details of the pathway involved remain elusive. Several studies have provided evidence for the participation of plant caspase-like proteases (CLPs) during the activation of PCD including hypersensitive response (HR) cell death. However, to date no homologue of animal caspases has been identified in plants. Recent discovery that revealed the legumain VPE (vacuolar processing enzyme) as a CLP with YVADase activity which can mediate PCD during the HR suggested the existence of conserved cell death-related protease activities that can be provided by structurally divergent enzyme molecules. Metacaspases, a family of cysteine proteases found in plants, fungi and protozoa, were identified to have significant tertiary structural homologies to animal caspases. We and others have now shown that this class of cysteine proteases does not have CLP activity *in vitro* and instead, they prefer target sites containing basic amino acid residues at the P1 position. However, evidence in yeast and plant systems suggests that metacaspases may mediate the activation of PCD by reactive oxygen or developmental cues via stimulation of downstream CLPs that remain to be identified. In order to determine whether and how plant metacaspases could be involved in PCD, we have been characterizing insertional mutants in some of the 9 genes that encode metacaspases in the model plant *Arabidopsis thaliana*. Some of these results will be presented in this meeting. Aside from metacaspases, the conserved protein Bax Inhibitor-1 (BI-1) has been shown to act as a PCD repressor in all eukaryotes tested so far, including yeasts. We have recently demonstrated that BI-1 loss-of-function mutants in *Arabidopsis thaliana* display accelerated death phenotypes upon activation by mycotoxin or abiotic stress that can induce PCD in plants. Using this genetic approach, we are examining whether BI-1 action in plant PCD may also involve the endoplasmic recticulum as has been suggested for animal PCD.

Inhibition of caspases shifts propionibacterial SCFA-induced cell death from apoptosis to autophagy

Annaïg Lan[1,2], Catherine Brenner[3], Dominique Lagadic-Gossmann[4] & Gwénaël Jan[1]

[1] UMR 1253 INRA–Agrocampus Sciences et Technologie du Lait et de l'œuf, 65 rue de Saint Brieuc, 35042 Rennes cedex, France, [2] Laboratoires Standa, 68 rue Kaskoreff, 14050 Caen cedex 4, France, [3] CNRS UMR 8159, LGBC, Université de Versailles, 45 avenue des Etats-Unis, 78035 Versailles, France, [4] INSERM U620, Université Rennes1, Faculté de Pharmacie, 2 avenue du Pr Léon Bernard, 35043 Rennes cedex, France. annaig.lan@rennes.inra.fr

Colorectal cancer constitutes a major concern in developed countries and is linked to environmental factors such as lifestyle and particularly diet. Therefore, a growing interest focuses on probiotics as cancer-preventing agents. To identify new probiotics, we investigated the potential of the food-grade propionibacteria, to kill cancer cells *in vitro*. In a previous report, we have shown that a dairy species, *Propionibacterium freudenreichii,* can kill two human adenocarcinoma cell lines. The short chain fatty acids (SCFA), propionate and acetate, which are the major end-products of *P. freudenreichii*, were shown to be responsible for this lethal process.

In this report, we have specified the molecular mechanisms leading to SCFA-induced cell death. We have shown that propionibacterial SCFA triggered apoptosis via the mitochondrial pathway. Indeed, SCFA induced mitochondrial alterations like increase of reactive oxygen species generation and decrease of mitochondrial transmembrane potential. These events led to release of cytochrome c, and redistribution of Bax, Smac and AIF and processing of initiator and effector caspases. In order to investigate the role of caspases in this lethal process, we used the pan-caspase inhibitor (zVADfmk). We showed that zVADfmk did not prevent SCFA-induced death of HT-29 cells. However, in the presence of the pan-caspase inhibitor, typical signs of autophagy were evidenced. Thus, inhibition of caspases shifted colorectal cancer cell death from apoptosis to autophagy under propionibacterial SCFA treatment.

Dairy propionibacteria and their metabolites could exert a local action on cancerous cells in the colon and thus constitute a promising food supplement to anticancer drugs for the prevention and/or the prophylaxis of digestive cancers. In this context, understanding molecular mechanisms of different cell death pathways induction by propionibacterial SCFA could constitute a useful tool in cancer treatment modality. Moreover, activating or inhibiting precisely target molecules that mediate cell death pathways, could permit the use of chemotherapeutic agents more effective and less toxic in association with propionibacteria.

The mammalian circadian clock gene *Per2* modulates cell death in response to oxidative stress

Sonja Langmesser, Maria Chiara Magnone, April Bezdek, Tiziano Tallone, Sandro Rusconi and Urs Albrecht

Department of Medicine, Division of Biochemistry, University of Fribourg, Rue du Musée 5, 1700 Fribourg, Switzerland

The selective advantage of the ability to keep track of time and to predict daily recurring changes in environmental conditions has lead to the development of circadian clocks. These clocks play an important role in the regulation of numerous physiological processes.
In mice, one of the core oscillator components is encoded by the *mPer2* gene. We found that mouse embryonic fibroblasts (MEFs) isolated from *mPer2*^{Brdm1} mice showed enhanced survival after treatment with the ROS-inducing agent paraquat as compared to wild-type MEFs. The same elevated survival was seen after treatment with various other ROS-inducing agents but not after treatment with H_2O_2, which induces necrotic cell death rather than apoptosis. In synchronized cultures of MEFs, cytotoxicity oscillated in a circadian manner, with better survival of *mPer2*^{Brdm1} MEFs at every time point analyzed.
Infection of MEFs with adenoviruses carrying expression constructs for mPer2 restored survival of *mPer2*^{Brdm1} MEFs to wild-type levels whereas infection with viruses carrying GFP did not. The elevated survival thus appears to be a direct effect of the lack of functional mPer2.
Analysis of the activities of aconitase and superoxide dismutase, two enzymes involved in intracellular ROS defense, as well as of the number of mitochondria by fluorescence microscopy did not reveal any differences between wild-type and *mPer2*^{Brdm1} MEFs. These results indicate that enhanced survival of *mPer2*^{Brdm1} MEFs is not caused by alterations in ROS defense mechanisms or in mitochondrial number.
By performing cDNA microarray analysis, we found an increase in the expression of anti-apoptotic *bcl2* in *mPer2*^{Brdm1} MEFs. A massive upregulation of basal *bcl2* expression could be confirmed in liver tissue of *mPer2*^{Brdm1} mice by Northern blot. Additionally, semiquantitative PCR analysis showed that, although *bcl2* expression was reduced in MEFs of both genotypes after paraquat treatment, levels were still significantly higher in *mPer2*^{Brdm1} MEFs.
Taken together, we present evidence for the involvement of the murine clock gene *mPer2* in the cellular response to oxidative stress, possibly by controlling expression levels of *bcl2*. These data hint at a role of the clock in the regulation of apoptosis, which would make it yet another factor to control this highly complex process.

DC-developed from CD34+ cells used as vaccine for possible anticancer therapy

Paula Lazarova[1'2], Gunnar Kvalheim[1], Krassimir Metodiev[3], Jahn Nesland[1]

1Norwegian Radium Hospital, Inst. Cancer Research, Oslo, Norway
2District University Hospital "St. Anna", Varna, Bulgaria
3Medical University, Varna, Bulgaria

The most powerful antigen-presenting cells, specialized to initiate and regulate the immune response, are the dendritic cells (DC). Their clinical use as cellular adjuvants in vaccination strategies is well-known. Based on some published papers on the matter, in addition to our previous studies, it is obvious that enriched CD34+ can be expanded ex-vivo to become DC; the serum-free culture procedure provide better conditions for production of DC-cells. We have found that derived in such way, those DC work and act more efficiently as antigen-presenting cells than other DC derived from blood monocytes.
Our present experimental study aims to establish ex-vivo culture conditions for expansion and preparation of DC for clinical use and to test the optimal immunologic properties of these cells: the authors try to optimize serum-free culture conditions for expansion of CD34+ derived DC and to evaluate phenotypically by flowcytometry and immunohistochemistry maturated DC.
Materials and methods: a) cell sources - leukapheresis harvest samples are obtained from cancer patients undergoing peripheral blood stem cell mobilization. The clinical models are cases with leucemia and prostate cancer. The mean purity of 19 samples is 89%. b) media - Dulbecco's Modified Eagle Medium supplemented with 10% FCS; alternatively - serum-free CellGro/SCGM (CellGenix) supplemented with 25% Albumin. c) DC cultures - CD34+ enriched cells in culture bags (CellGenix). d) Recombinant human cytokines and growth factors - GM-CSF, TNF-alpha, IL-4, flt-3, SCF. e) Monoclonal antibodies - FITC- or PE-labeled maBs against CD1a, CD14, CD33, CD34, CD40, CD80, CD83, CD86, HLA-DR.
Our results show that the serum-free culture conditions with appropriate combination of cytokines allow to generate large amounts of DC from CD34+ progenitors. The differentiation of CD34+ cells into mature DC is analyzed flowcytometrically. The DC phenotyping confirms a high expression of CD86, CD83, CD40, CD80 and HLA-DR, whereas CD14 and CD33 are less actively expressed. The overall evaluation of the achieved results allow the authors to continue with the next steps (examination of stimulatory capacity and immunologic properties of ex-vivo cultured DC, by T-stimulation, transfection and ability of cultured DC to induce T-cell proliferation in MLR), which will give a green light for clinical application.
The results are encouraging for future successful application of such vaccine therapy on cancer patients.

Overexpression of Epstein-Barr virus latent membrane protein 1 (LMP1) in EBV Latency III B-cells induces caspase 8 mediated apoptosis: role of CD95.

Le Clorennec Christophe[1], Ouk Tan-Sothéa, Jayat-Vignolles Chantal, Youlyouz-Marfak Ibtissam, Adriaenssens Eric[2,4], Coll Jean[2], Bornkamm Georg W[3], Feuillard Jean[1].

[1]Centre National de la Recherche Scientifique, UMR CNRS 6101, Faculté de Médecine de Limoges, Université de Limoges, CHU Dupuytren, Laboratoire d'Hématologie, 2 Rue du Docteur Marcland, 87025 Limoges Cedex, France
[2]CNRS UMR 8527, Institut de Biologie de Lille, BP 447, 59021 Lille Cedex, France.
[3]Institute of Clinical Molecular Biology and Tumor Genetics,1 GSF-National Research Center for Environment Health, D-81377 Munich, Germany.
[4]INSERM ERI8, Université de Lille I, 59655 Villeneuve d'Ascq.

Epstein-Barr virus (EBV) contributes to oncogenesis by transforming normal B lymphocytes into lymphoblastoid cell lines (LCLs) in latency III. LMP1 has been described as the major transforming protein of EBV latency III program. In different cellular type, LMP1 could, by diverting TNF-R family signaling pathway, induce apoptosis.Thus, recent studies show that LMP1 overexpression would be toxic for cell. This process of positive regulation of apoptosis by LMP1 is not well-known. In precedent studies, we demonstrated that LMP1 is responsible of CD95 regulation via principally NF-kappaB and secondary STAT1 and p53, in EBV-latency III B-cells.This work aims to study the regulation by LMP1 of cellular pathways implied in the apoptotic response in EBV latency III B-cells lymphocyte immortalized.
To understand the toxic effect of LMP1 in LCLs, we cloned wild type LMP1 into a novel double inducible episomal vector, pRT-1, who expressed the gene of interest under control of a bidirectional doxycycline regulatable promoter that allowing simultaneous expression of truncated NGF receptor, used as a surrogate marker of inducibility. These plasmids were stably transfected either into LCLs. Induction of the cDNA of interest was performed with doxycycline for 24h to 120h. Positive cells for NGFR were purified using magnetic beads.
In order to describe the process of apoptosis by LMP1 in LCLs, we tested effect of LMP1 overexpression. We observed markedly increased CD95 expression in cell surface detected by FACS and confocal microscopy and rapidly CD95 aggregation, DISC formation and CD95 internalization were observed. Then, caspase 8 activity was detected and causing activation of caspase 9 and caspase 3. Finally, late stages of apoptosis were observed thereafter, like cleavage of PARP, as well as DNA's fragmentation. We performed immunoprecipitation experiments of CD95 and caspase 8 and we demonstrated that caspase 8 activity was due exclusively to formation of CD95 DISC. Consequently, we demonstrated, in EBV Latency III B-cells, that CD95 aggregation and apoptosis was dependent of LMP1 overexpression, via LMP1 signalling. Finally, overexpression of LMP1 potentiates CD95 mediated apoptosis.
Therefore, our results suggest that LMP1 overexpression potentiates EBV-latency III immortalised B-cells to CD95 mediated apoptosis.

Endoplasmic reticulum stress induces calcium-dependent permeability transition, mitochondrial outer membrane permeabilization and apoptosis

Christophe Lemaire, Aurélien Deniaud, Ossama Sharaf El Dein, Evelyne Maillier and Catherine Brenner

LGBC, Université de Versailles/St Quentin, CNRS UMR 8159, Versailles, France,

Mitochondrial calcium (Ca^{2+}) accumulation plays a stimulatory role on mitochondrial functions, and above a certain threshold, Ca^{2+} can also transmit and amplify an apoptotic signal. Here, we characterized the capacity of physiological stimuli (e.g. histamine, IP_3) and endoplasmic reticulum (ER) stress agents (e.g. A23187, thapsigargin, tunicamycin, brefeldin A) to release Ca^{2+} from ER stores, induce mitochondrial Ca^{2+} accumulation, and trigger cell death in several human cancer cell lines. Thus, only sustained Ca^{2+} accumulation in response to some ER stress agents into the mitochondrial matrix induces the full hallmarks of mitochondrial pro-apoptotic alterations (permeability transition, electrochemical potential dissipation, matrix swelling and Bax/cytochrome c/AIF relocalisation), while rapid and time-limited physiological Ca^{2+} accumulation failed to affect cell viability. The specificity of the process was addressed with pharmacological inhibitors, which target VDAC (DIDS, NADH), IP_3R (2-APB), the permeability transition pore (CsA, BA), Ca^{2+} uniport (Ruthenium Red, CCCP), Ca^{2+} (BAPTA-AM), caspases (z-VAD), and protein synthesis (CHX). Moreover, we designed a novel cell-free system of purified organelles, which allowed the identification of IP_3R, VDAC and/or an unknown channel, Ca^{2+} uniport and the permeability transition pore as key proteins in healthy and tumoral organelles, and reveals that the whole process can occur independently of any other component in less than one hour in vitro.

GSH depletion, protein S-glutathionylation and mitochondrial transmembrane potential hyperpolarization are early events in initiation of cell death induced by a mixture of isothiazolinones in HL60 cells.

Alessandra Leonini[1], Anna Ettorre[1], Simona Frosali[2], Paolo Di Simplicio[2] and Anna Di Stefano[1]

1 Department of Molecular Biology, University of Siena, Via Fiorentina 1, 53100 Siena, Italy
2 Department of Neuroscience , University of Siena, Via A.Moro, 53100 Siena, Italy

We recently described that brief exposure of HL60 cells to a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one (CMI) and 2-methyl-4-isothiazolin-3-one (MI) induces apoptosis at low concentrations (0.001- 0.01%) and necrosis at higher concentrations (0.05- 0.1%). In this study, we show that glutathione (GSH) depletion, reactive oxygen species generation, hyperpolarization of mitochondrial transmembrane potential ($\Delta\Psi m$) and formation of protein-GSH mixed disulphides (S-glutathionylation) are early molecular events that precede the induction of cell death by CMI/MI. When the cells exhibit common signs of apoptosis, they show activation of caspase-9, reduction of $\Delta\Psi m$ and, more importantly, decreased protein S-glutathionylation. In contrast, necrosis is associated with severe mitochondrial damage and maximal protein S-glutathionylation. CMI/MI-induced cytotoxicity is also accompanied by decreased activity of GSH-related enzymes. Pre-incubation with L-buthionine-(S,R)-sulfoximine (BSO) clearly switches the mode of cell death from apoptosis to necrosis at 0.01% CMI/MI. Collectively, these results demonstrate that CMI/MI alters the redox status of HL60 cells, and the extent and kinetics of GSH depletion and S-glutathionylation appear to determine whether cells undergo apoptosis or necrosis. We hypothesize that S-glutathionylation of certain thiol groups accompanied by GSH depletion plays a critical role in the molecular mechanism of CMI/MI cytotoxicity.

VDAC and Bak recombinant proteoliposomes induce caspase 9 activation in cancer cell lines

Lavinia Liguori, Bruno Marques, Ana Villegas-Mendez, Romy Rothe and Jean-Luc Lenormand

HumProTher laboratory, Laboratoire d'Enzymologie – GREPI, University Joseph Fourier, CHU de Grenoble; BP217 ; 38043 Grenoble Cedex 9. France

Delivery by nanoparticles and lipid vesicles of functional therapeutic proteins into targeted living cells is one of the most promising strategies for cancer treatment. These technologies are especially efficient for drugs and soluble proteins. The use of these systems in the delivery of membrane proteins directly into cells has not been tested because methods for producing membrane proteins and functional proteoliposomes are still difficult to carry out. Here we report the effect of bio-active proteoliposomes containing pro-apoptotic VDAC and Bak synthesized with an optimized cell-free expression system. Recombinant VDAC and Bak proteins are directly integrated into the lipid bilayer of natural liposomes and the lipid environment stabilize the proteins and support the right folding. The Voltage-dependent anion channel (VDAC) has been shown to play an essential role in apoptosis in mammalian cells by regulating the cytochrome c release from the mitochondria. This effect is mediated by the Bcl-2 family members such as Bax, Bak, Bid and Bik which modulate mitochondrial membrane permeability upon activation. Bak and Bax can directly bind to VDAC and regulate its activity. Treatment of different cancer cell lines with proteoliposomes containing Bak, VDAC or both proteins in combination, induce an early cleavage of caspase 9, release of cytochrome c and activation of an apoptotic pathway that remains to be still elucidated. Delivery of VDAC and Bak is targeted to mitochondria membranes as revealed by immunofluorescence microscopy and cell fractionation. The importance of these interactions in the mitochondrial apoptosis regulation, elects these two transmembrane proteins (VDAC and Bak) as excellent candidate for protein therapy.

Modulation of Bcl2 expression by gemcitabine and nimesulide in pancreatic tumor cell lines

Longoni Biancamaria, Giovannetti Anna, Bertacca Laura, Piras Antonio, Navarra Teresa and Mosca Franco

Department of Oncology, Transplantation and Advanced Technologies in Medicine, University of Pisa

Gemcitabine (Gtb) is an inhibitor of ribonucleotide reductase and DNA synthesis and is an effective antitumoral agent used for the treatment of pancreas cancer. The majority of human primary pancreatic carcinoma overexpress the prostaglandin endoperoxide synthase Cox-2 (inducible form) in contrast to the benign pancreatic tumor. Cox-2 activity produces prostaglandin E2 and induces the production of angiogenic factors, which can favour tumorigenesis through neoangiogenesis.
The present study analysed the effects of gemcitabine and nimesulide (Nms), a selective cox-2 inhibitor, on the expression of the anti-apoptotic Bcl-2 protein in two pancreatic tumor cell lines BxPc3 and MiaPaCa, characterized by high and low cox-2 expression respectively. We defined 2 pharmacological doses, for each drug, able to induce a nontoxic stimuli. We assayed the mitochondrial activity of 30x10^4 living cell treating with different concentractions (Nms 15-30-60-120 µM and Gtb 15-30-60-120 nM) for 24-48-72h. The cytotoxicity test shows that Bxpc3 are more sensitive than MiaPaca to both drugs and the lowest toxicity effect corresponds to Nms 30µM and Gtb 15nM. Cell lines were also exposed to parallel treatments for 24,48,72h: Gtb 15nM; Nms 30µM, Gtb 15nM+Nms 30 µM. Total proteins were extracted and the Bcl-2 expression level was assayed by westhern-blot.
BXPC3 showed: at 24h an increase of Bcl-2 upon Gtb and Gtb+Nms treatment, while Nms alone did not modify the Bcl-2 level; at 48h Nms induced an increase of Bcl-2 higher than that induced by Gtb or Gtb+Nms; at 72h a similar high level of Bcl-2 was observed for each treatment.
The Bcl-2 levels in Miapaca-2 at 24h and 48h were increased only upon Gtb+Nms treatment. On the contrary at 72h showed a decrease in Bcl-2 level upon each treatment.
The increase of the antiapoptotic Bcl-2 protein upon chronic treatment of BxPC3 and Miapaca2 with gemcitabine 15nM may be part of a resistance mechanism of cancer cell to this chemotherapeutic agent. The decrease of Bcl-2 observed in MiaPaca (low cox2 expression) upon Nms 30µM may be explained by inhibition of antiapoptotic cox-2-dependent pathway. High Cox-2 expression in BxPc3 may be responsible for the lack of inhibition of the antiapoptotic pathway by nimesulide.

Fenretinide-induced apoptosis via endoplasmic reticulum stress- New targets for melanoma therapy?

Penny Lovat[1+2], Marco Corazzari[3], Jane Armstrong[2], Andrew Harbottle[1], Gian Maria Fimia[3], Mark Birch-Machin[1], Christopher Redfern[2] and Mauro Piacentini[3]

Dermatological Sciences[1] and Northern Institute for Cancer Research[2] School of Clinical Laboratory Sciences[2], University of Newcastle, Newcastle Upon Tyne, NE2 4HH, UK and INMI-IRCCS Lazzaro Spallanzani[3], Rome, 00149 Italy.

Fenretinide, a synthetic derivative of retinoic acid, induces apoptosis of neuroectodermal tumour cells including melanoma. The signalling mechanism of fenretinide-induced apoptosis is mediated by ceramide accumulation with subsequent metabolism to the diasialoganglioside GD3, resulting in oxidative stress, ROS generation through increased 12-lipoxygenase activity, and induction of the DNA damage-inducible transcription factor GADD 153. GADD 153 induction is also a hallmark of apoptosis induced by endoplasmic reticulum (ER) stress. Since ER stress-induced apoptosis is a potential target pathway for therapy of melanoma, resistant to apoptosis mediated by death receptor ligation or DNA damage, we have tested the hypothesis that fenretinide-induced apoptosis of melanoma results from ER stress.

In SK MEL 110 and A375 melanoma cells, fenretinide induced phosphorylation of the translation initiation factor EIF2a, and splicing of Xbp I; these are both key markers of the unfolded protein response to ER stress. Several ER stress response genes were also induced including GADD153, Grp78, calnexin and calreticulin. We have also found that ERp57 and ERdj5, two genes forming part of the protein folding machinery of the ER, are induced in response to fenretinide. siRNA-mediated knockdown of these two genes increased the susceptibility of melanoma cells to fenretinide-induced apoptosis. These results suggest therefore, that targeting cellular mechanisms protecting against ER stress in combination with ER stress-inducing agents such as fenretinide may define a novel and more-effective therapeutic strategy for melamoma.

Selective elimination of mitochondria from living cell induced by inhibitors of bioenergetic functions.

K.G. Lyamzaev, O. Yu. Pletjushkina, B.V., Chernyak

A.N. Belozersky Institute, Moscow State University, 119992 Moscow, Russia
E-mail: Lyamzaev@mail.ru

Mitochondria in various cell types form dynamic reticulum which was suggested to play a role of cable-like energy transporting system of the cell. In agreement with this idea fragmentation of the mitochondrial reticulum, observed under various stressful conditions.
We have studied the dynamics of mitochondrial in human carcinoma cells HeLa. It was shown that inhibitors of respiration (piericidin, antimycin, myxothiazol), ATP synthase (oligomycin, aurovertin) and uncouplers (such as DNP and FCCP) did not cause any loss in viability of these cells during a 48 h inhibition process. The mitochondrial inhibitors in the presence of glucose induced significant changes in mitochondrial structure independent of ATP depletion or apoptotic events. In the following experiment we have took the advantage of this model for investigation of the long-term consequences of mitochondrial fission. The most rapid fission of mitochondria was induced by combined treatment with respiratory inhibitors and uncouplers simultaneously. At the final steps of the treatment with uncouplers in combination with the respiratory in the fragmentation gathered near the nucleus and formed several aggregates. When HeLa cells were treated for 72h with FCCP or DNP in combination with antimycine or myxothiazole, a fraction of the cells (60-70 %) died by apoptosis but the rest of population was viable, without any signs of apoptosis (normal nucleus, normal cytoskeleton, no annexin V stainig). The total amount of mitochondrial material decreased significantly. Cytochrome c was not released from mitochondria until the very last steps while the release of the other mitochondrial proteins could not be excluded. We did not observed co-localization of mitochondrial clusters with autophagosomes. Probably mechanism of mitoptosis in HeLa cells during treatment with mitochondrial inhibitors is closer to the mechanism of elimination of organelles during the lens and erythroid differentiation, which is independent from major components of autophagic machinery. It could be suggested that this mechanism provide cells with selective advantages due to low content of pro-apoptotic mitochondrial proteins and elimination of the major source of ROS production and probably underlies the low mitochondria content observed in some tumors in vivo.

Nucleophosmin regulates Bax-mediated apoptosis.

David C. MacDonald, Jui-Lee A. Birse-Archbold, John Sharkey & Lorraine E. Kerr.

Astellas CNS Research, The University of Edinburgh, The Chancellor's Building, 49 Little France Crescent, Edinburgh EH16 4SB, UK, email: D.C.Macdonald@sms.ed.ac.uk

Apoptosis is a fundamental biological process regulated by the balance of pro- and anti-apoptotic members of the Bcl-2 family. Disturbances to this balance are linked to many diseases ranging from cancer to inflammation and from Alzheimer's disease to stroke. Activity of Bax, the prototypical apoptosis-inducing member, is pivotal to cell survival. When activated, Bax changes structure, migrates to the mitochondria and initiates a cascade of events resulting in cell death. However the precise molecular mechanisms whereby Bax becomes activated and translocates to the mitochondria to induce apoptosis, remain poorly understood. We have identified nucleophosmin (B23, numatrin) as a novel Bax-binding protein and have investigated the role of nucleophosmin in regulating apoptosis using a human neuroblastoma cell line (SH-SY5Y) treated with the intrinsic apoptotic pathway inducer, staurosporine (STS). Following induction of apoptosis (500nM STS), cytochrome c was progressively released from mitochondria into the cytosol and reaching a plateau between 4-5hrs. Similarly the levels of cleaved caspase-3 in the cytosol steadily increased. Although total nucleophosmin expression levels did not change over the 5 hour time course of STS treatment as assessed by Western blotting, confocal microscopy revealed that its subcellular distribution altered. Nucleophosmin was confined to the nucleolus of control cells but was distributed throughout the cytoplasm of apoptotic cells at 2 and 4 hours of staurosporine treatment. In contrast, Bax translocation to the mitochondria was not evident until 4 hours. Knockdown of nucleophosmin protein expression by approximately 50% using shRNA attenuated the progression of the intrinsic pathway, as measured by mitochondrial cytochrome c release and pro-caspase 3 cleavage. These data demonstrate that nucleophosmin is a novel regulator of Bax-mediated apoptosis and opens up new areas for therapeutic intervention.

The cytomegalovirus mitochondrial inhibitor of apoptosis (vMIA) is required for virus replication and cross-species infection

Claudia Mack, Igor Jurak, Stefanie Hölzer and Wolfram Brune

Division of Viral Infections, Robert Koch Institute, Nordufer 20, 13353 Berlin, Germany, e-mail: gehrkec@rki.de

Human cytomegalovirus (HCMV) encodes two well-characterized inhibitors of apoptosis by its open reading frames UL36 and UL37 exon 1. The viral inhibitor of caspase-8 activation (vICA) protects cells from external, death receptor-mediated stimuli. The vICA gene is dispensable for HCMV replication and is inactive in the most commonly used laboratory strain AD169 due to an adventitious mutation. By contrast, the viral mitochondrial inhibitor of apoptosis (vMIA) is essential for replication of the HCMV strain AD169. Cells infected with a deletion mutant of the vMIA gene undergo apoptosis involving activation of caspase-9, caspase-3 and PARP. Caspase activation and apoptosis are inhibited when viral DNA replication is blocked, suggesting that viral DNA replication triggers apoptosis.
To test whether the requirement of vMIA depends on the presence or absence of a functional vICA, we constructed a vMIA deletion mutant of the HCMV strain FIX, which possesses a functional vICA gene. This mutant virus could replicate only on cells that overexpress bcl-XL or an analogous viral protein. Replacement of the vMIA gene in the viral genome by the bcl-2 family genes bcl-2, bcl-XL, E1B-19k, or KSbcl-2 resulted in replication-competent mutant viruses. This indicated that the requirement of vMIA for inhibition of virus-induced apoptosis is independent of vICA, and that cellular and viral bcl-2 family proteins can substitute for vMIA. Moreover, we showed that infection of human cells with the murine or the rat cytomegalovirus triggered apoptosis. Expression of vMIA or a bcl-2 family protein prevented apoptosis and facilitated replication of the rodent cytomegaloviruses in human cells. These results indicate that induction of apoptosis limits cross-species infection of rodent cytomegaloviruses, and that these viruses do not encode a vMIA homolog that is functional in human cells.

Nutlin-3, a hdm2 antagonist, suppresses cell proliferation in human p53 wild-type glioblastoma cell lines.

Federica Mennillo, Roberta Magrini, Luisa Massai, Giovanni Gaviraghi, Georg C. Terstappen, Annette Bakker.

Siena Biotech S.p.A., Siena, Italy, e-mail: rmagrini@sienabiotech.it

Nutlin-3, a Roche compound identified as a selective and potent hdm2-p53 inhibitor, is a small molecule that binds hdm2 and renders it inaccessible to p53 thus preventing p53 from degradation. Moreover, it has been demonstrated to induce the arrest of tumour proliferation in vitro and in vivo employing different tumour model systems. Glioblastoma multiforme, the most aggressive astrocytic form of gliomas, is known to show aberrations in the p53 pathway in a high percentage of cases and inactivating p53 mutations and amplifications of hdm2 were found to be mutually exclusive. A therapeutic strategy targeting glioblastoma cells with amplified or overexpressed hdm2 to undergo apoptosis or stop proliferation might be feasible.
Employing a panel of glioblastoma cell lines differing in their p53 status we show that in p53 wt cell lines (expressing hdm2 to various levels) the p53 pathway is reactivated by nutlin-3 treatment as demonstrated by p53 protein induction and transcriptional upregulation of different p53 target genes such as p21, mdm2 and PUMA. The phenotypic result is that p53 wt cells are inhibited in their proliferation to a greater extent than p53 mut cell lines. In a p53 wt background, this inhibition of proliferation is due to cell cycle arrest in G2/M phase and not to caspase-driven apoptosis since caspase 3 was not activated. Taken together, our data suggest that in glioblastoma cells another form of cellular death might play a role in the response to nutlin-3.

PPARgamma dependent regulation of human macrophages in phagocytosis of apoptotic cells

Gyöngyike Majai, Zsolt Sarang, Krisztián Csomós, Gábor Zahuczky, László Fésüs

Department of Biochemistry and Molecular Biology, Signalling and Apoptosis Research Group of the Hungarian Academy of Sciences, Research Center for Molecular Medicine, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary, e-mail: fesus@indi.dote.hu

Macrophages acquire their capacity for efficient phagocytosis of apoptotic cells during their differentiation from monocytes. The peroxisome proliferator activated receptor gamma (PPARγ) is highly up-regulated during this maturation program. We report that addition of PPARγ antagonist during differentiation of human monocytes to macrophages significantly reduces the capacity of macrophages to engulf apoptotic cells. The antagonist acted in a dose-dependent manner, had to be present during the differentiation process and could also prevent the previously observed augmentation of phagocytosis by glucocorticoids. Synthetic PPARγ agonist could not increase phagocytosis capacity. Blocking activation of PPARγ did not prevent differentiation of macrophages but down-regulated the expression of pro-phagocytic genes such as CD36, transglutaminase 2 and pentraxin 3. Inhibition of PPARγ dependent gene expression did not block the anti-inflammatory effect of apoptotic neutrophils or synthetic glucocorticoid but significantly decreased production of IL-10 induced by LPS. Our results suggest that during differentiation of macrophages natural ligands of PPARγ are formed regulating both expression of genes responsible for effective clearance of apoptotic cells and macrophage-mediated inflammatory processes.

Old Yellow Enzymes; new contrasting roles for old enzymes in oxidative stress and programmed cell death in yeast

Osama Odat, Samer Matta, Sotirios C. Kampranis and Antonios M. Makris

The Old Yellow Enzyme (OYE) group of FMN-oxidoreductases has received extensive attention over the years from biochemists, for their catalytic properties. However, until very recently there was no information about their biological function.

The OYE2 gene caught our attention when its C-terminal fragment was found to restore sensitivity to Bax lethality, and the proper mitochondrial-GFP targeting in a mutant yeast strain. Its highly related homologue OYE3 had previously been implicated in the Bax phenotype. Several other FMN-oxidoreductases (yAIF, yAMID) have also been shown to participate in apoptotic processes in yeast.

Overexpression of OYE2, but not OYE3, lowered the endogenous levels of ROS, and effectively removed excess ROS generated by various prooxidant insults. Logarithmically growing yeast overexpressing oye2p, when treated with H_2O_2, recovered much faster than control cells, whereas cells overexpressing oye3p failed to recover. The results showed a strong antioxidant capacity of the oye2 protein, enhancing survival, and an opposing role for oye3, antagonising cell recovery. The full length OYE2 was also able to suppress Bax lethality in wild type cells.

Deletion of OYE2 but not OYE3 rendered cells more sensitive to Bax lethality and H_2O_2 induced programmed cell death, further confirming its antioxidant role.

Surprisingly, *Doye2 oye3* double knockout cells became highly resistant to H_2O_2-induced PCD, and under certain conditions exhibited reduced chronological aging. The cells had lowered levels of endogenous or subsequent to H_2O_2 treatment ROS, compared to the wild type parental cells. In the DKO cells, respiration was upregulated, as evidenced by their ability to grow in glycerol containing media, which can explain the reduced ROS and the extension of their lifespan. Additionally, these data suggest that OYE3 exerts its antagonistic function via OYE2.

In contrast to *Doye2 oye3* cells, double deletions of OYE2 with interacting protein partners or antioxidant genes exacerbated their H_2O_2 sensitivity. In the case of *Doye2 glr1* (glutathione reductase) very high levels of ROS were detectable, compared to the single mutant counterparts. This led to extensive spontaneous cell death which was caused by actin cytoskeleton abnormalities and failure of the daughter cells to receive properly a nucleus. Defects in the partition of other organelles were also evident. The oye2 protein had recently been shown to affect actin polymerization. Our data link the function of oye2p with regulation of ROS levels, cytoskeletal rearrangements and the control of cell death and division.

Standardized extract of Ashwaghandha of induce apoptosis in HL60 cells through ROS generation and stimulate Th1-response in mice.

Fayaz Malik, Jaswant Singh and G N Qazi

Division of Pharmacology, Regional Research Laboratory (Council of Scientific and Industrial Research), Canal road Jammu Tawi, India 180001

Balb/c mice were orally administrated extract (SE) daily for 15 days, immunized and challenged with complete Freund's adjuvant (FA) on day 1 and incomplete FA and 7, respectively. SE stimulated Th1 immune response and macrophage function reaching peak value with 25mg dose. SE stimulated significantly both IgG2a and IgG2b with no effect on IgG1 in blood sera of mice. Flow cytometric analysis of lymphocyte surface markers of T cells (CD3+, CD4+, CD8+) and B cells (CD19+) indicated predominant enhanced proliferation and differentiation of T and B cells.

SE dose dependently modulate the CD80, CD86, CD11a, CD11b on splenocytes isolated from mice. SE activated LPS stimulated peritoneal macrophage functions in vitro in terms of enhanced secretion of nitrite, IL-12 and TNF-a. In contrast IL-10 remained unchanged.

SE dose dependently inhibited cell proliferation of HL 60 cells after 48h, analyzed by MTT reduction assay IC50=15µg/ml

SE induced apoptosis in HL60 cells was analyzed by annexin -V and PI staining of cells further analyzed by FACS.SE induce dose dependent ROS generation in HL60 cells, ROS was estimated FACS after cells were stained with DCFHDA after 4h treatment. SE induce the dose dependent Mitochondrial membrane potential loss in HL60 cells after 4 h treatment.SE induce the expression of Caspase3/9 in HL60 cells.

The studies Showed that AGB supports predominantly Th1 immunity with increase in macrophage functions and immunoglobulins secretion. AGB also induce apoptotic cell death in HL-60 cells in vitro via ROS generation suggesting that standardized extract of no toxicological consequences might find useful applications against immune suppressed diseases.

MDM4 function: a proapoptotic activity for a negative regulator of p53

Mancini F, Giglio S, Prodosmo A, Rinaldo C, Soddu S, Sacchi A, Pontecorvi A[2], Moretti F[1].

Lab. Molecular Oncogenesis, Regina Elena Cancer Institute, Rome, [1]Neurobiology and Molecular Medicine Inst., National Council of Research, Rome, [2]Inst. Medical Pathology, Catholic University, Rome, Italy

MDM4 (MDMX) is the closest analogue to MDM2, the well known inhibitor of the tumor suppressor gene p53. Biochemical and genetic studies have indicated that under normal growth conditions, MDM4 may act as a negative regulator of p53 levels and/or activity. On the other hand, overexpressed MDM4 stabilizes p53 and, under some circumstances, it positively regulates p53 activity. These data leave open the question of the biological role of MDM4 in human cells and particularly of enhanced MDM4 levels as it has been reported in some human tumors.
The present report shows that following DNA damage (UV or genotoxic agents), overexpressed MDM4 specifically switches p53 function toward the apoptotic response. This switch is mediated by the ability of MDM4 to activate two different p53 apoptotic pathways: the transcription-dependent and -independent ones. Indeed, MDM4 enhances phosphorylation of proapoptotic p53 phospho-site (Ser-46) and induces transcriptional activation of proapoptotic genes (AIP-1, Noxa) but not of the growth-arrest mediator p21/Waf1. Moreover, MDM4 stabilizes preferentially cytoplasmic fraction of p53 causing its relocalization to the mitochondria with consequent cytochrome C release. The MDM4 p53-binding and Ring Finger domains are both required to induce such effects. In agreement with these data, siRNA for MDM4 in tumor cell lines significantly decreases the apoptotic rate following genotoxic stress while increases growth arrest phenotype.
This study helps to clarify the oncogenic role of MDM4 and provides potential useful information for those therapeutic strategies aimed at reactivating p53 oncosuppressive function.

Investigating the role of PIDD in cell death and survival

C. Manzl[1], V. Labi[1], J. Tschopp[2] and A. Villunger[1]

[1]*Division of Developmental Immunology, Innsbruck Medical University, Innsbruck, Austria*
[2]*Department of Biochemistry, University of Lausanne, Lausanne, Switzerland*

The p53-induced protein with a death domain (PIDD) has been identified as a gene induced in response to p53 activation in a wide variety of cells and tissues. PIDD is involved in the activation of caspase-2 by forming a tri-molecular complex via the adapter protein RAIDD, called the "PIDDosome". Alternatively, PIDD can recruit the serin/threonine kinase RIP1 and the IKK complex component NEMO, leading to DNA damage-induced NF-κB activation. These facts suggest that PIDD is activating two pathways simultaneously in a competitive manner and additional signals may be required to tilt the balance towards death or survival.
To address the physiological relevance of PIDD, we have generated *pidd*$^{-/-}$ mice by homologous recombination. Due to a *lacZ* insertion in-frame with the PIDD open reading frame we set off to analyze expression of PIDD mRNA expression during embryonic development and in the adult organism as well as in response to DNA damage induced by whole-body ionizing radiation. Preliminary results suggest that PIDD-deficiency is compatible with normal embryonic development since mice are born at the expected Mendelian ratio and histological assessment revealed no gross abnormalities in 8-12 week old adult mice.
Cell death induction was monitored in isolated thymoctes as well as mature T and B cells derived from wt and PIDD-deficient mice. We observed that primary *pidd*$^{-/-}$ lymphocytes responded similar to wt cells in response to a broad range of cell death inducing agents and DNA-damaging drugs. If anything, we observed a sensitization of PIDD deficient cells to cell stress that was most pronounced in response to heat shock. Although preliminary, our data suggest that the dominant function of PIDD in vivo may be the activation of NF-kB signalling and survival, whereas its pro-apoptotic function in response to genotixic stress appears to be redundant with the Apaf-1/caspase-9/caspase-3 "apoptotsome" pathway of caspase activation.

Reactive oxygen species and PKC delta: a crosstalk aimed at triggering apoptosis of MYCN amplified neuroblastoma cells

Barbara Marengo*, Chiara De Ciucis, Vito Pistoia*, Damiano Cottalasso, Maria Adelaide Pronzato, Umberto Maria Marinari, Cinzia Domenicotti.

Department of Experimental Medicine, General Pathology Section, Genoa University, Italy
** G. Gaslini Institute, Genoa, Italy*
e-mail: cdomeni@medicina.unige.it

Reactive oxygen species (ROS) are a by-product of biological processes with high chemical reactivity and are essential for cell biology and physiology; in fact they play a critical role in differentiation, proliferation and apoptosis acting as “second messengers” in the activation of signaling proteins. Redox changes of molecules able to regulate the balance between the production of oxidant species and the decrease in antioxidant defences, might be the cause of differentiation, aging and neoplastic transformation. Recent studies have demonstrated that alterations of cell redox state might be associated to tumoral phenotype as consequence of apoptotic dysregulation and enhancement of cell proliferation. Previously, we have demonstrated that L-buthionine-S,R-sulfoximine (BSO) treatment of human neuroblastoma (NB) cells, induced apoptosis by stimulating protein kinase C (PKC) delta activation. BSO is a glutathione (GSH)-depleting agent commonly used in NB clinical treatment, and exploits this biological peculiarity to induce cell death.
Here, we show that MYCN amplified NB cells are more sensitive to BSO than non-amplified cells; their death which is brought about by calpains and caspases is inhibited by antioxidant vitamin C, by DPI, a NADPH oxidase inhibitor and by rottlerin, a PKC-delta inhibitor. BSO-induced oxidative stress causes DNA damage, reduces bax/bcl-2 heterodimerization and leads to a stable interaction between PKC-epsilon and bax. Our findings demonstrate that ROS activate PKC-delta, which is involved in NADPH oxidase activation and in the overproduction of ROS. Intracellular oxidation is a crucial step in the conformational changes in pro- and anti-apoptotic proteins that drive MYCN-amplified neuroblastoma lines to cell death. Then, the opportunity to modulate intracellular redox state and key molecular intermediates such as PKC isoforms might be useful in activating the mitochondrial pathway of cell death in MYCN- amplified NB cells, thereby sensitising this tumour to therapeutic approaches. *(Grants from PRIN n° 2004063943_001 and n° 2004068552_002, Italian Ministry of Health and Compagnia S. Paolo)*

Endoplasmic Reticulum Stress and Apoptosis in the Liver of Scorbutic Guinea Pigs

Éva Margittai, Gábor Bánhegyi, Gábor Nagy, József Mandl, and Miklós Csala

Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, POB 260, 1444 Budapest, Hungary, margeva@freemail.hu

Previous studies indicated the role of ascorbate in oxidative protein folding in the endoplasmic reticulum (ER). The protein or glutathione thiol groups are more oxidized (to disulfide bonds) in the ER lumen than in the cytosol. The way in which the luminal thiol oxidizing environment is generated and maintained has not been elucidated. Some protein components of the thiol oxidizing electron transfer chain were identified, e.g., Ero1p and PDI, but some links are missing. The addition of ascorbate to hepatic microsomes in vitro leads to enzymatic ascorbate consumption accompanied by an enhanced oxidation of protein thiol groups. On the basis of these observations, ascorbate deficiency (scurvy) can be expected to impair protein maturation in the ER. The defective protein folding, in turn, activates a signaling network called the unfolded protein response (UPR). UPR includes upregulation of ER chaperones (e.g., GRP78, GRP94) and foldases (e.g., PDI, ERP72). When these mechanisms do not ameliorate the stress situation, apoptosis is initiated in higher eukaryotic organisms, presumably to eliminate unhealthy cells. Insufficient ascorbate intake causes scurvy in certain species. Beyond its known functions, it has been suggested that ascorbate participates in oxidative protein folding in the ER. Because redox imbalance in this organelle might cause ER stress and apoptosis, we hypothesized that this might contribute to the pathology of scurvy.

Guinea pigs were divided into 7 groups: the control group was fed a commercial guinea pig food containing 0.1 g/100 g ascorbate for 4 wk, 5 groups consumed an ascorbate-free food for 0, 1, 2, 3, or 4 wk and 1 group was fed this scorbutic diet for 2 wk and then the commercial food plus 1 g/L ascorbate in drinking water for 2 wk. TBARS generation and the expression of some ER chaperones and foldases were determined in hepatic microsomes. The apoptotic index was assessed in histological sections.

Although ascorbate, measured by HPLC, was undetectable in the livers of the guinea pigs after they had consumed the scorbutic diet for 2 wk, the microsomal TBARS level was elevated relative to the initial value only at wk 4. Western blot revealed the induction of GRP78, GRP94, and protein disulfide isomerase at wk 3 and 4. Apoptosis was greater than in the control, beginning at wk 3. None of the alterations occurred in the groups fed the commercial guinea pig food or ascorbate-free food followed by ascorbate supplementation. Therefore, persistent ascorbate deficiency leads to ER stress, unfolded protein response, and apoptosis in the liver, suggesting that insufficient protein processing participates in the pathology of scurvy.

Menadione-induced apoptosis is affected by low frequency 50 Hz magnetic field in murine fibroblast cells.

Ari Markkanen, Jukka Juutilainen, and Jonne Naarala.

University of Kuopio, Department of Environmental Sciences, P.O. Box 1627, FI-70211 Kuopio, Finland.

The extremely low frequency (ELF) magnetic fields (MF) has been classified as "possible carcinogenic to humans" by the International Agency for Research on Cancer (IARC 2002). This classification is mainly based on epidemiological studies, especially evidence for an association between residential ELF MF exposure and childhood leukaemia. Our previous *in vitro* findings indicate that 50 Hz MF exposure modifies cell cycle of UVB-irradiated yeast cells (Markkanen et al., Bioelectromagnetics,22:345-350,2001). According to those findings, MF exposure may slow down recovery of yeast cells from growth delay caused by UV damage. Our *in vivo* findings also suggest that 50 Hz MFs modify apoptosis induced by solar-simulated UV radiation in mouse skin (Kumlin et al., Radiat Environ Biophys,41:155-158,2002).

In the present study we co-exposed murine fibroblast cell line (L929) to 50 Hz MF (intensity of 100 or 300 µT) and menadione (2-methylnaphthalene-1,4-dione, MQ; 150 µM) or UVB radiation (dose 160 J/m^2). The samples were stained with propidium iodide (PI) and analysed by Becton Dickinson FACSCalibur cytometer and Cell Quest program. The apoptotic cells were defined as sub G1 events.

The amount of apoptotic cells were significantly decreased ($p<0.001$) when cells were first exposed 24 hours to MF, then to MQ for 1 hour and finally another 24 hours to MF compared to MQ alone exposed cells. In the UVB-irradiated cells that were exposed in the same kind of protocol no differences were seen. Moreover, when cells were first exposed to MQ and then 24 or 48 hours to MF the amount of apoptosis were same as merely MQ exposed cells. The MQ is known to accelerate the production of free radicals in mitochondria and also produce different radicals than UVB. By these results it seems that MF may affect directly electron transport chain and MF exposure given before MQ may enhance the effect of genotoxic agent by sensitizing the cells. These findings are potentially important to reveal the cellular effects of MF.

Selective clearance of macrophages in atherosclerotic plaques via drug-induced nonapoptotic cell death

Wim Martinet,[1] Stefan Verheye,[2] Arnold G Herman[1] and Guido RY De Meyer[1]

Division of Pharmacology, University of Antwerp, Wilrijk, Belgium; Antwerp Cardiovascular Institute Middelheim, Antwerp, Belgium, e-mail: wim.martinet@ua.ac.be

Macrophages play a key role in atherosclerotic plaque destabilization and rupture whereas smooth muscle cells (SMCs) contribute to plaque stability. Selective removal of macrophages from plaques via pharmacological therapy could therefore represent a promising approach to stabilize 'vulnerable', rupture-prone lesions. Yet, how macrophages can be eliminated from plaques without influencing other cell types, in particular SMCs, is unknown. In this study, we could demonstrate that the pancaspase inhibitor z-VAD-fmk induces nonapoptotic cell death in macrophages but not in SMCs. Cell death was characterized by bulk degradation of long-lived proteins, LC3 processing and cytoplasmic vacuolization, which are all markers of autophagy. However, also necrosis occurred and the number of necrotic cells rapidly increased during z-VAD-fmk treatment. Further evidence indicated that the expression level of receptor interacting protein 1 (RIP1) mediates the sensitivity to z-VAD-fmk. However, upon z-VAD-fmk treatment, macrophages overexpressed and secreted several chemokines and cytokines, including TNFα. The combination of z-VAD-fmk and TNFα but not TNFα alone, induced SMCs necrosis via a mechanism that required RIP1 expression. These results suggest that z-VAD-fmk, despite its selective cell death inducing capacity, would be detrimental for the stability of atherosclerotic plaques due to enlargement of the necrotic core, stimulation of inflammatory responses and indirect induction of SMC death. We therefore examined an alternative strategy to selectively induce macrophage cell death in atherosclerosis. Stents eluting the mTOR inhibitor and rapamycin derivative everolimus, implanted in atherosclerotic arteries of cholesterol-fed rabbits, led to a marked reduction in macrophage content without altering the amount of SMCs. In vitro studies showed that everolimus induced inhibition of translation both in macrophages and SMCs. Moreover, everolimus triggered autophagic cell death, but only in macrophages. Autophagic macrophage cell death was confirmed by transmission electron microscopy both in cultured cells and in explanted atherosclerotic segments derived from collar-treated rabbit carotids. Massive macrophage-specific initiation of cell death was also obtained with cycloheximide, suggesting that inhibition of protein translation leads to selective macrophage cell death. However, cycloheximide did not induce autophagy but apoptosis. All together, stent-based delivery of everolimus but not z-VAD-fmk represents a promising approach to selectively clear macrophages in atherosclerotic plaques by autophagy.

Cell death in the primary enamel knot – mitochondrial pathways

Eva Matalova, Jana Setkova, Ivan Misek, Paul Sharpe* and Abigail Tucker*

Laboratory of Animal Embryology, Academy of Sciences, Brno, Czech Republic
**Department of Craniofacial Development, King´s College, London, UK*
matalova@iach.cz

The primary enamel knot (PEK) of developing molar teeth is an important signalling centre during transition from the cap stage to the bell stage, critical for forming the future tooth cusps. Cell population of PEK consists from not dividing cells, expressing lots of signalling molecules and is finally eliminated my massive apoptosis. PEK apoptosis may represent a passive process as well as act actively in tooth shape formation and size determination. Mechanisms of dental apoptosis have not been known yet.

Knock-out mice (embryonic day: 15.5 – bell shape of the first molar tooth germ) were exploited to investigate dental apoptosis in absence of Apaf-1, caspase-9 and caspase-3, crucial molecules of mitochondrial apoptosis pathways. Additionally, proliferation, marker gene expression (Shh, Fgf-4) and tooth germ morphology were evaluated using immunohistochemistry (PCNA staining), *in situ* hybridization and histology. Morphological criteria and TUNEL test were applied to detect apoptotic bodies and DNA breaks in PEK cells.

In the wild type molar tooth germs, apoptotic bodies and TUNEL positive cells were observed in PEK, in the stalk, so called enamel navel, and in the epithelial cell layer facing the oral cavity. In the Apaf-1 and caspase-9 deficient mice, the tooth shape corresponded to the wild type, however, no apoptosis was found in PEK, navel, stalk or surrounding tissues. In caspase 3 -/- /129X1/SvJ, the location of the first molar tooth germs was shifted posteriorly in the upper jaw. In contrast, in the caspase-3 -/-/B57BL/6, altered morphology of the first molar tooth germs was found in both jaws. In particular, the concave region where the PEK would have originated appeared to be strongly disorganised. No apoptotic bodies were found in the mutant tooth germs and TUNEL labelling showed negative or very weak staining. Location of *Shh* mRNA in homozygous mutants was found to be expanded into the inner dental epithelium compared to the wild type. *Fgf-4* expression was found to be normal. All mutant mice showed the same PCNA staining pattern as the wild type mice.

Absence of Apaf-1, caspase-9 and caspase-3, respectively, prevents apoptosis in PEK. Further signalling networks and possible compensatory mechanisms are under study.

Supported by the Grant Agency of the Czech Academy of Sciences (B500450503).

Genotype-dependent priming to self- and xeno-cannibalism in heterozygous and homozygous lymphoblasts from patients with Huntington disease

Paola Matarrese°, Walter Malorni°, Elisabetta Mormone°^, Antonella Tinari*, Milena Cannella§, Vittorio Maglione§, Maria Grazia Farrace^, Luigi Frati, Mauro Piacentini^, and Ferdinando Squitieri§

(°) Departments of Drug Research and Evaluation and (*) Technology and Health, Istituto Superiore di Sanita', Rome, Italy; (^) Department of Biology, University of Rome "Tor Vergata", Rome, Italy; (§) Neurogenetic Unit, IRCCS Neuromed, Pozzilli (IS), Italy

Huntington's Disease (HD) is a neurodegenerative diseases caused by the expansion of a trinucleotide repeat in IT15 gene that results in an exceedingly high number of contiguous glutamine residues in the translated protein huntingtin. HD is usually reported as a genetic disease that in heterozygous patients presents a variable expansion of trinucleotide repeats. However, when homozygosis occurs, the disease appears as more severe and is associated with high CAG repeats numbers. Patients homozygous and heterozygous for the HD mutation carrying the same CAG expansion length, despite a similar age at the onset, disease progresses more rapidly in homozygot.
Although the primary site of cell toxicity is the nucleus, recent studies in animal models highlighted a direct mitochondrial toxicity that may specifically contribute to the occurrence of the clinical phenotype.
In the present work we studied the mitochondrial function and cell death pathway(s) in heterozygous and homozygous immortalized cell lines from patients with Huntington disease. Heterozygosis was characterized by specific alterations of mitochondrial membrane potential, a constitutive hyperpolarization state of mitochondria, and was correlated with an increased susceptibility to apoptosis. Lymphoblasts from homozygous patients were instead characterized by a significant percentage of cells displaying autophagic vacuoles. These cells also demonstrate a striking attitude toward a significant cannibalic activity. Considering the pathogenetic role of cell death in HD, the present work provides new useful insights regarding the role of mitochondrial dysfunction, i.e. hyperpolarization, in hijacking i) HD heterozygous cells towards apoptosis and ii) HD homozygous cells towards a peculiar phenotype characterized by both self- and xeno-cannibalism. These events can however be viewed as an ultimate attempt to survive more that a way to die. The present work underline the possibility that HD-associated mitochondrial defects could be tentatively bypassed by the cells by activating cellular "phagic" activities, including the so called "mitophagy" and "cannibalism", that only finally lead to cell death.
Altogether these data seem to indicate that mitochondrial dysfunction occurring in HD cells may potently bolster the scavenging of these organelles by autophagy. This powerful "phagic" potential could represent an ultimate attempt to survive or, finally, an unwanted way to die.

Hsp90 inhibitor radicicol changes the mode of cisplatin-induced cell death.

Mateva R**,** Grancharov K., Detcheva R., Gorneva G.

Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria, mateva@obzor.bio21.bas.bg

The induction of apoptosis is thought to be one of the main mechanisms of the antitumor action of many cancer therapeutics including cisplatin. The increased resistance of tumor cells to cisplatin is often correlated with appearance of mutations in a number of proteins connected to apoptotic machinery. On the other hand, tumor cells are characterized by a very high content (up to tenfold than in normal cells) of Hsp90 which acts as molecular chaperone. Among its client proteins are key enzymes of apoptosis, cells cycle regulation, DNA-repair as well as oncoproteins of signal transduction pathways. It has been shown that the Hsp90 specific inhibitor, the macrocyclic lactone radicicol, causes depletion of mutant p53, Raf-1, ErbB2 and Akt pools. This compound increases specifically sensitivity to cisplatin in cisplatin-resistant, mismatch repair-deficient cells. It is known that mismatch-repair mechanism plays an important role in realization of the antitumor activity of cisplatin.

The role of Hsp90 in the increased tumor resistance to chemotherapy is not fully understood. Our results showed that kinetics of induction of Hsp90 upon cisplatin treatment of murine leukaemia cells strictly correlated with their high tolerance toward this agent. We found that combined treatment of F4N leukemia cells with relatively low concentrations of cisplatin and radicicol results in synergetic cytotoxic action. The analysis of cell death mode showed that cisplatin alone induces apoptosis with typical morphologic sings. Radicicol alone did not induce apoptosis at concentrations much higher that those used in the combination. Upon combined treatment, the morphological analysis showed a great amount of binuclear and multinuclear cells, as well as an increased number of mitotic cells. We conclude that the combined treatment of F4N cells with radicicol and cisplatin results not in increase of apoptosis, but rather in cell death through mitotic catastrophe.

This work was supported by a grant No L1412 of the National Fund for Scientific Research at the Bulgarian Ministry of Education and Science.

Effects of oxazaphosphorines on DNA degradation in leukaemic cells

Mazur L., Niziol A., Opydo M., Dabrowski Z. and *Niemeyer U.

*Laboratory of Experimental Haematology, Department of Animal Physiology, Institute of Zoology, Jagiellonian University, Cracow, Poland, e-mail: maz@zuk.iz.uj.edu.pl *NIOMECH, University of Bielefeld, Germany*

Oxazaphosphorines are a class of alkylating agents widely used in chemotherapy. D-19575 (glufosfamide, ß-D-glucosyl-isophosphoramide mustard), D-18864 (4-hydro-peroxy-cyclophosphamide) and D-17272 (mafosfamide cyclohexylamine salt) are new generation oxazaphosphorines. DNA is known to be a main target of action of alkylating drugs on cells. DNA cleavage is also considered to be one of the biochemical hallmarks of programmed cell death. The aim of the present study was to assess and compare the effects of D-19575, D-18864 and D-17272 on induction of DNA degradation in neoplastic haematopoietic cells undergoing programmed cell death - apoptosis, necrosis and/or mitotic catastrophy. The experiments were carried out *in vitro* on human promyelocytic leukaemic HL-60 cells. The research was conducted using flow cytometry as well as light and fluorescence microscopy methods. Two approaches were used to detect and identify leukaemic cells expressing DNA cleavage and chromatin degradation. The first approach relied on the *in situ* labeling of DNA strand breaks with fluorescein-conjugated deoxynucleotides in the enzymatic reaction catalyzed by exogenous terminal deoxynucleotidyl transferase. APO-BrdU assay, TUNEL technique, was used to identify cells with DNA fragmentation. The second one based on morphological analysis of HL-60 cells after staining with DAPI - the DNA specific fluorochrome and sulforhodamine 101. After application of three oxazaphosphorines, different patterns of changes in the frequency of cells expressing DNA strand breaks, the frequency of apoptotic, necrotic and mitotic cells and in the total number of HL-60 cells, were determined. The greatest DNA degradation was observed following D-18864 administration, intermediate after D-17272 treatment and the least one when D-19575 was applied. Temporary alterations in the frequency of cells expressing DNA fragmentation and the number of cells undergoing different modes of cell death were dependent on the oxazaphosphorine agent given, its dose, the parameter tested and the time intervals after the compound application.

Supported by Polish Research Projects BW/33/2005 and DS/IZ/2006

Identification of a Serum Factor Required for Augmented Phagocytosis of Apoptotic Cells

Aisleen McColl, Adriano Rossi, Chris Haslett, Ian Dransfield

MRC Centre for Inflammation Research, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, EH16 4TJ. Scotland. Email: s0571550@sms.ed.ac.uk

Inflammation is normally a beneficial response to infection and injury. Neutrophils are the first leukocyte to migrate into the inflamed site. During self-limiting inflammation, neutrophils undergo constitutive apoptosis, a form of programmed cell death important for immune cell homeostasis. Neutrophils undergoing apoptosis display 'eat me' signals, such as exposure of phosphatidylserine, that distinguish them from viable neutrophils. This allows their selective recognition, uptake and degradation by phagocytes via receptors such as the putative phosphatidylserine receptor, avb3/CD36 thrombospondin receptors and scavenger receptors. The rapid removal of intact apoptotic neutrophils in this way protects tissues from the noxious contents and does not illicit proinflammatory secretory responses by the phagocyte, promoting resolution of inflammation. Impaired clearance of apoptotic neutrophils has been suggested to contribute to the pathogenesis of many persistent inflammatory diseases, such as systemic lupus erythematosus (SLE). The modulation of phagocyte clearance of apoptotic leukocytes represents a potential therapeutic target for the control of inflammatory diseases. Anti-inflammatory glucocorticoids, such as dexamethasone (DEX), have been shown to enhance nonphlogistic phagocytosis of apoptotic leukocytes by macrophages. DEX-treated monocyte-derived macrophages (mj) displayed enhanced phagocytosis of human neutrophils cultured for 20hr in the presence of serum, confirming previous observations. This effect appeared to be reversible by washing the neutrophils with PBS without cations, and recoverable by adding back serum during the phagocytosis assay. DEX-treated mj showed a defect in phagocytosis of apoptotic neutrophils cultured under serum-free conditions that is recovered by adding back serum. This observation implies there is a serum factor required for the enhanced phagocytosis of apoptotic neutrophils by DEX-treated mj. Preliminary data suggests that this serum component is not CRP, IgG, a platelet factor, C3b/iC3b and that the phagocytosis is not b2-integrin mediated. Studies are underway to identify the serum factor and subsequently to elucidate the receptor mechanism employed by DEX-treated mj to engulf apoptotic neutrophils.

Role of the transcription factor Prep1 in apoptosis

Nicola Micali, Luis Fernandez, Francesco Blasi and Massimo Crippa

Laboratory of Molecular Genetics, Di.Bi.T. - S. Raffaele Scientific Insititute, Milano, Italy

TALE proteins, including the MEINOX and PBC subfamilies, are homeodomain transcription factors. Prep1, belonging to the MEINOX family, heterodimerizes with Pbx proteins, in a DNA-independent manner. The complex is translocated to the cell nucleus, where it acts as a transcriptional regulator.
In a previous work, the functional role of Prep1 during early zebrafish development was investigated using a morpholino antisense oligonucleotides strategy. Prep1 morphants showed massive apoptotic cell death, particularly in the hindbrain and spinal chord. In this study, we have investigated the role played by Prep1 in the apoptotic behaviour of mammalian cells, using MEFs (Mouse Embryo Fibroblasts) cells obtained from WT and Prep1 hypomorphic mice (Prep1i/i). We observed that Prep1i/i MEFs were more sensitive to apoptosis after UV induction. The apoptotic phenotype of Prep1i/i MEFs was supported by a strong increase of active Caspase 9 and active Caspase 3 and by the altered expression level of several pro- and antiapoptotic proteins (BAX, BCL-XL BCL2, p53). Moreover, using different apoptotic stimuli than UV light, such as Etoposide, which causes p53-mediated apoptosis, we observed a dramatic increase in apoptosis of Prep1i/i MEFs in comparison to WT MEFs, whereas TNF-alpha treatment, which causes p53-independent apoptosis, the observed increase was marginal. These results suggest that the lack of Prep1 affects specific apoptotic pathways and could contribute to understand the biological role of Prep1 and its involvment in programmed cell death.

The pro-inflammatory cytokine TNFa critically regulates macrophage clearance of apoptotic neutrophils.

Sylwia Michlewska, Deborah A. Sawatzky, Ian L. Megson, Ian Dransfield and Adriano G. Rossi

MRC Centre for Inflammation Research, The Queen's Medical Research Institute, University of Edinburgh Medical School, 47 Little France Crescent, Edinburgh, Scotland, UK; e-mail: s.michlewska@sms.ed.ac.uk

A defect in the resolution of inflammation likely contributes to the development of chronic inflammatory diseases such as rheumatoid arthritis, atherosclerosis or autoimmune disorders. The pro-inflammatory cytokine, TNFα, is believed to play a fundamental role in many inflammatory diseases and anti-TNFα therapy has been successfully used for the treatment of certain inflammatory disorders. There is emerging evidence indicating that apoptosis of inflammatory cells and their subsequent non-inflammatory clearance by macrophages are key mechanisms involved in the resolution of inflammatory responses. Although TNFα has been demonstrated to influence rates of inflammatory cell apoptosis, little is known about the effects of TNFα on macrophage phagocytosis of apoptotic neutrophils. To address this question, we treated human monocytes for 6 days with TNFα or bacterial-derived lipopolysaccharide (LPS) and assessed the ability of the differentiated macrophages to ingest apoptotic neutrophils. Both LPS and TNFα treatment specifically inhibited macrophage clearance of apoptotic neutrophils whereas phagocytosis of human IgG opsonised erythrocytes was unaffected. The effects were concentration- and time-dependent and, importantly, reversed by soluble TNF receptor fusion protein providing convincing evidence that LPS mediated inhibition of phagocytosis are likely due to TNFα production. Furthermore, analysis of supernatants from LPS-treated macrophages revealed that the degree of inhibition of phagocytosis is positively correlated with the concentration of TNFα. We also demonstrated that dexamethasone, a synthetic glucocorticoid, reversed the inhibitory effects of both LPS and TNFα on clearance of apoptotic neutrophils. Analysis of culture supernatants indicated that dexamethasone inhibited LPS-induced production of TNFα. These results suggest that TNFα at sites of inflammation may lead to reduced clearance of apoptotic neutrophils, thereby exacerbating the inflammatory response and possibly leading to further tissue damage. These findings may also explain, in part, the clinical success of anti- TNFα antibodies or soluble TNF receptors for the treatment of inflammatory diseases.

DNA damage checkpoint control programs interfere with p14^{ARF} induced apoptosis

Ana Milojkovic , Dilek Güner, Philipp G. Hemmati , Bernd Gillissen, Jana Wendt, Clarissa von Haefen, Peter T. Daniel

Clinical and Molecular Oncology, University Medical Center Charité, Campus Berlin-Buch and Max Delbrück Center for Molecular Medicine, Germany

P14^{ARF} is a tumor suppressor gene product being encoded by the human INK4A gene locus. This protein has been shown to play a unique role in the induction of apoptosis and the regulation of cell cycle and senescence following oncogenic stress. To dissect cell death signaling by p14^{ARF}, we used MCF-7 breast carcinoma cells which have lost the key executioner caspase-3 and MCF-7 cells that were stably re-transfected with procaspase-3. Cell cycle arrest in G1 phase was induced by p14^{ARF} using an adenoviral vector system in both mock and caspase-3 transfectans. Checkpoint abrogation by the ATM/ATR kinase inhibitor caffeine was accompanied by apoptotic cell death strictly depending on caspase-3. Thus, p14^{ARF} triggers a permanent G1-arrest which can be abrogated by caffeine leading to caspase-3 dependent apoptosis. p14^{ARF} induced upregulation of p21$^{CIP/WAF-1}$ and caused activation of the cell cycle program without triggering apoptosis. Furthermore, investigations showed that caffeine induces decrease of p21$^{CIP/WAF-1}$ protein levels and leads to activation of the cell cycle progression and caspase-3 dependent apoptosis. This indicates that loss of the CDK inhibitor p21$^{CIP/WAF-1}$ facilitates p14^{ARF} induced apoptosis. Apoptosis was executed via the mitochondrial pathway as evidenced by the breakdown of the mitochondrial membrane potential, cytochrome c release and caspase activation. These data demonstrate that cell cycle arrest programs interfere with p14^{ARF}-induced apoptosis. Aberrant re-entry into the cell division cycle results in massive apoptosis that proceeds via a caspase-3 dependent pathway.

A potential role for Cdk5 in apoptotic events following stroke in man

Nicholas Mitsios[1], Roberta Pennucci[1], Coral Sanfeliu[2], Jerzy Krupinski[3], John Gaffney[1], Pat Kumar[1], Shant Kumar[4] and Mark Slevin[1]

[1]School of Biology, Chemistry & Health Science, Manchester Metropolitan University, UK [2]Department de Farmacologia i Toxicologia, IIBB, Barcelona, Spain [3]Servicio de Neurologia, Hospital Universitari de Bellvitge, Barcelona, Spain [4]Department of Pathology, University of Manchester, Manchester, UK

Neuronal cell death after brain ischaemia may be associated with activation of cyclin-dependent kinases (Cdk). Cdk5 is expressed in neurons and growing evidence suggests that it can promote neuronal death. However, although other neurological diseases have been studied, little has been published on Cdk5 expression after stroke in man. In this study, RT-PCR, western blotting and immunohistochemistry/immunofluorescence have been employed to study Cdk5 expression in human post-mortem stroke tissue, and in human cerebral cortical foetal neurons (HFN) and human brain microvascular endothelial cells (HBMEC) exposed to oxygen-glucose deficiency (OGD) and reperfusion. Since Cdk5 activation results both from binding to its regulatory subunit p35 and from phosphorylation, expression of both p35 and phosphorylated Cdk5 (p-Cdk5) was investigated. An increase in the number of neurons and microvessels stained for Cdk5, p-Cdk5 and p35 occurred in the infarct and penumbra of many patients after stroke. Staining became irregular and clumped in the cytoplasm and nuclear translocation occurred. Association of Cdk5 with apoptosis after stroke was found, as Cdk5 colocalized with TUNEL positive neurons from penumbra and p-Cdk5 was found in the nucleus of active caspase-3 stained cells. Similar upregulation in HFN subjected to OGD was seen. These results have provided new evidence for a potential role of Cdk5 in apoptotic events associated with response to ischaemic injury.

A role for the DED domain –containing DEDD in cell-cycle and cell-size regulation

Toru Miyazaki* & Satoko Arai

Div. of Molecular Biomedicine for Pathogenesis, CDBIM, Faculty of Medicine, The University of Tokyo.7-3-1, Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan.

Accumulating evidence has shown that many molecules, including some cyclin-dependent kinases (Cdks) and cyclins, as well as the death-effector domain (DED) containing FADD, function for both apoptosis and cell cycle. In order to investigate the physiological function of the DED-containing DEDD, a possible pro-apoptotic nuclear protein, we have generated DEDD-deficient (DEDD-/-) mice. Although fibroblast cells from DEDD-/- embryos exhibited normal responses against various apoptosis-inducing stimuli, they revealed significant reduction in rRNA and protein levels, and thus, in cell volume. In consequence, DEDD-/- mice showed decreased body and organ sizes relative to DEDD+/+ mice. In addition, proliferative rates were accelerated in DEDD-/- cells, due to a specific decrease in duration of the G2/M stage during cell-cycle. Collectively, DEDD extends the length of G2/M, and increases rRNA and protein synthesis, which overall appears to support sufficient cell growth.

Loss of HtrA2 results in increased mitochondrial stress, enhanced ROS production and perturbation of calcium homeostasis

Nicoleta Moisoi, Valentina Fedele, Kristina Klupsch*, Wang Hao, Julian Downward*, Luis Miguel Martins

Cell Death Regulation Laboratory, MRC Toxicology Unit, Leicester, UK
e-mail: nm105@le.ac.uk
**Signal Transduction Laboratory, Cancer Research UK London Research Institute, UK*

HtrA2 is a mitochondrial serine protease, which is released into the cytoplasm upon apoptotic stimulation contributing to cell death both through IAP binding and consequent caspase activation and through its own proteolytic activity. However, *in vivo* studies suggest that HtrA2 is essential for cell survival rather than cell death. HtrA2 KO mice fail to gain weight approximately two weeks after birth and display a progressive neurological phenotype with parkinsonian features [1]. In humans, two loss-of-function mutations in HtrA2 have been implicated in Parkinson disease [2]. Thus, loss of HtrA2 proteolytic activity appears to impair normal cell function leading to neuro-degeneration and cell death.

We aim to understand how HtrA2 loss of function contributes to cell death using the HtrA2 KO mouse model. Our working hypothesis is that HtrA2 may be involved in a protective stress response pathway similar to its bacterial homolog Deg S.

We studied the effect of a number of drugs known to affect mitochondrial function and to induce parkinsonism, on reactive oxygen species (ROS) accumulation in cells derived from KO and WT HtrA2 mice. We show that treatment with 6-OHDA or H_2O_2 led to cellular accumulation of ROS, more specifically of mitochondrial superoxide. to a greater degree in cells lacking HtrA2. Increased oxidative stress may impair various cell functions including Ca^{2+} homeostasis and contribute to the induction of stress pathways. We have evidence that both MEFs and primary neurons derived from HtrA2 KO animals display altered Ca^{2+} homeostasis; the main feature being reduced Ca^{2+} availability in ER stores. We also identified stress pathways that are activated to a higher degree in cells lacking HtrA2 following different stimuli. We conclude that loss of HtrA2 sensitizes cells to stress stimulation leading to increased ROS production and deregulated calcium signaling. This supports the hypothesis that HtrA2 is involved in a protective response to stress.

[1] Martins, L. M. *et al.* 'Neuroprotective role of the Reaper-related serine protease HtrA2/Omi revealed by targeted deletion in mice' Mol Cell Biol. 2004 Nov;24(22):9848-62.
[2] Strauss K.L *et al.* 'Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease' Hum Mol Genet. 2005 Aug 1;14(15):2099-111

Bcl-2 and Bcl-XL are indispensable for the late phase of mast cell development

Christine Möller[1], Mats Karlberg[1], Magnus Åbrink[2], Keiichi I. Nakayama[3], Noboru Motoyama[4] and Gunnar Nilsson[1]

[1]Department of Medicine, Karolinska Institutet, Stockholm, Sweden, [2]Department of Molecular Biosciences, Swedish University of Agricultural Sciences, Uppsala, Sweden, [3]Division of Cell Biology, Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, Kyushu University and [4]Department of Geriatric Research, National Institute for Longevity Sciences, National Center for Geriatrics and Gerontology, Obu, Aichi, Japan.
e-mail: Christine.Moller@ki.se

In this study we wanted to determine the importance of Bcl-2 and Bcl-XL for mast cell development and survival. Bcl-2 and Bcl-XL are anti-apoptotic Bcl-2 family members that play a major role in regulating cell survival by suppressing cell death related to a variety of apoptotic stimuli. However, the direct involvement of Bcl-2 and Bcl-XL in dictating mast cell survival has not been defined. To determine the importance of Bcl-2 and Bcl-XL for mast cell development and survival *bcl-x*$^{-/-}$ and *bcl-2*$^{-/-}$ mouse embryonic stem cells were maintained in medium supplemented with either IL-3 or IL-3 in combination with stem cell factor (SCF) to favor mast cell development. Deficiency in either *bcl-x* or *bcl-2* totally inhibited the development of embryonic stem cell-derived mast cells (ESMCs) when IL-3 alone was used as a mast cell growth factor. Intriguingly, when IL-3 was used in combination with SCF, the ESMCs developed normally the first two weeks but thereafter the cell numbers dropped drastically. ESMCs lacking *bcl-x* or *bcl-2* exhibited strong expression of A1, another pro-survival Bcl-2 family member. Thus, for the first time we provide direct evidence that both *bcl-x* and *bcl-2* are indispensable for mast cell development.

Defect of Ubiquitin/Proteasome-ERAD Stimulates Autophagic Pathway and ER Stress-mediated Cell Death Pathway.

Eriko Fujita, Yoriko Kouroku, Akiko Soyama, and Takashi Momoi

Divisions of Development and Differentiation, National Institute of Neuroscience, 4-1-1 Ogawahigashi-machi, Kodaira, Tokyo 187-8502, Japan

Endoplasmic reticulum (ER) quality control system extracts, retrotranslocates, and degrades the aberrant proteins by ubiquitin/proteasome, ER-associated degradation system (ERAD), but if not sufficiently degrades, it finally activates ER stress-mediated cell death pathway. We have shown that expanded polyglutamine 72 repeat (polyQ72) aggregates, which inhibit ubiquitin/ proteasome activity, induce the ER stress-mediated cell death and autophgy formation. In the present study, we examined the relationship between ER stress-mediated cell death and autophagy caused by inhibition of ubiquitin/proteasome activity. Rapamycin, a stimulator of autophagy, inhibited the polyQ72-induced cell death with caspase-12 activation. PolyQ72 stimulated Atg5-Atg12-Atg16 complex-dependent LC3 conversion from LC3-I to -II, which plays a key role in autophagy. The eIF2a A/A mutation, a knock-in to replace a phosphorylatable Ser51 with Ala51, and dominant negative PERK inhibited polyQ72-induced LC3 conversion. Furthermore, Atg5 deficiency as well as the eIF2a A/A mutation increased the number of cells showing polyQ72 aggregates and polyQ72-induced caspase-12 activation. Dysferlin, type II membrane protein with calcium-dependent membrane fusion domain and causative gene of LGMD2B/MM, was localized at ER/Golgi and substrate for ubiquitin/proteasome ERAD. The dysferlin was associated with Sec61a, a component of retrotranslocon for the extraction of misfolded proteins from the ER to the cytosol. LLL, inhibitor of proteasome, induced ubiquitination of dysferlin and their aggregation in the ER. LLL and overexpression of dysferlin induced ER stress and finally apoptotic cell death. During these process, LLL and dysferlin aggregates induced LC3 conversion from LC3-I to –II. Furthermore, inhibition of lysosomal enzyme and knockdown of the autophagy pathway stimulated the dysferlin aggregation in the ER. Excess dysferlin is degraded by autophagy/lysosome pathway when ubiquitin/proteasome ERAD system is defected. Thus, autophagy/lysosome is an alternative ERAD degradation system.

Could Fatty Acids act as surrogate Free Radical Scavengers?

Christos Moraitis and Brendan P. G. Curran

School of Biological and Chemical Sciences, Queen Mary College, University of London, Mile End Road, London E1 4NS, UK

Previous work from this laboratory has demonstrated that HS-sensitivity is tightly correlated with the level of cellular Unsaturated Fatty Acids (UFAs) in the yeast Saccharomyces cerevisiae. Anaerobically-grown cells in particular, which contain Polyunsaturated Fatty Acids (PUFAs) were found to be considerably less sensitive to heat-shock than aerobically-grown cells which do not contain any PUFAs. More recently, we have shown that the sensitivity of the yeast HSR is strongly associated with the accumulation of Reactive Oxygen Species (ROS). Here, over a range of heat shock temperatures, we reveal that when comparing anaerobically-grown cells with different cellular lipid profiles, elevated levels of Lipid Hydroperoxides (LOOHs), as determined by the Thiobarbituric Acid Reactive Substances (TBARS) assay, are associated with a decrease in the induction of an HS-reporter gene in both the presence and absence of H_2O_2. In addition, cells that accumulate a higher level of LOOHs are also more resistant to H_2O_2-induced oxidative death. More profoundly, when lipid peroxidation is inhibited by ascorbic acid, anaerobically-grown cells become less thermotolerant and more sensitive to heat-shock, irrespective of their lipid profile. In contrast to anaerobically-grown cells, aerobically-grown fermenting cells are found to be considerably more sensitive to heat-shock and less thermotolerant in both the presence and absence of H_2O_2. Overall, they also accumulate a lower level of LOOHs whereas, the inhibition of lipid peroxidation in these cells is accompanied by enhanced thermotolerance and decreased HS-sensitivity. A comparison of anaerobically-grown cells with different lipid profiles also reveals that the HS-induced increase in LOOH levels can be inversely related to the HS-induced increase in the overall level of ROS, as determined by an intracellular probe, which in turn, can be directly correlated to the level of expression from the HS-reporter gene. We therefore conclude that in anaerobically grown cells, lipid peroxidation may confer a cytoprotective effect, which is accompanied by a reduction in the overall level of ROS and a decrease in HS-sensitivity.

Keywords: Lipid Hydroperoxides; Polyunsaturated Fatty Acids; Reactive Oxygen Species; Heat Shock Response; Intrinsic Thermotolerance; Cytoprotective Effect; Saccharomyces cerevisiae.

Role of protein phosphatase 4 in the regulation of apoptosis and cell cycle

Mirna Mourtada-Maarabouni and Gwyn T. Williams

Institute for Science and Technology in Medicine, Keele University, Keele, Staffs, ST5 5BG, UK, e-mail: bia19@biol.keele.ac.uk

The identities of some apoptosis-controlling molecules have already been established and further components are being identified through sequence similarities and physical interactions with known components. In contrast, functional expression cloning offers a complementary approach to the identification of critical genes which does not rely on such prior assumptions and allows direct targeting of the molecules involved in the crucial commitment stages of the apoptosis. This is feasible as this strategy exploits the gene's cell survival-promoting activity itself to allow its isolation. The application of this strategy to the isolation of cDNAs conferring resistance to dexamethasone and γ-irradiation identified protein phosphatase 4 (PP4) as a potential apoptosis regulatory gene in mouse T-lymphocytes. The importance of this phosphatise is further indicated by conservation between mouse and human (all 307 amino acids identical). Overexpression of the catalytic subunit of PP4 (PP4C) in a number of human cell lines resulted in cell cycle (G1) arrest and an increase in apoptosis. In addition, siRNA mediated silencing of PP4C resulted in an increase in cell proliferation and apoptosis inhibition. Interestingly, proteomic analysis revealed that modulation of PP4 expression affected the phosphorylation state of a number of proteins implicated in apoptosis and cell cycle. These observations suggest that PP4 plays an important role in the regulation of both apoptosis and cell proliferation.

This work was financed by a grant from Wellcome Trust

A fraction of cytochrome c is retained in mitochondria until advanced stages of apoptosis

Mussche S, Pyfferoen L and D'Herde K

Dept of Anatomy, Embryology, Histology and Medical Physics, Ghent University, Godshuizenlaan 4, 9000 Ghent, Belgium.e-mail: s.mussche@UGent.be

Release of cytochrome c from mitochondria is considerd to be a complete and rapid event (Goldstein et al., 2000). However, several investigators including ourselves have found a more gradual cytochrome c release and thus heterogeneity of mitochondrial response. In granulosa *explants* we previously observed two populations of mitochondria in apoptotic cells: respiring condensed mitochondria still containing cytochrome c and a second population of orthodox mitochondria which had released their cytochrome c (D'Herde et al. 2000). We localised cytochrome c in mitochondria using a enzymehistochemical method based on the oxidation and precipitation of diaminobenzidine (DAB) by cytochrome c in the copresence of cytochrome oxidase The same technique was used in the present study in which we looked at cytochrome c release in primary granulosa cell cultures.. Apoptosis was induced by serum-free culture and ceramide-8 and evaluated 5h later by DAPI-staining. Microscopical observations showed the presence of DAB-positive – this is, cytochrome c containing - mitochondria in apoptotic cells containing condensed and fragmented nuclei. This finding was confirmed by electron microscopy (EM). The specificity of the staining was checked by performing incubations in the presence of KCN as an inhibitor of cytochrome oxidase.

Detailed EM-analysis revealed that all mitochondria of pre- and early apoptotic cells (as seen by atypical condensation of the chromatin) contained cytochrome c. However, two subgroups could be distinguished: mitochondria with cytochrome c staining in the intermembrane and intracristal space and a second group of mitochondria showing DAB-staining in the mitochondrial matrix as well. Apoptotic cells, with the classical chromatin condensation pattern and fragmentation of the nucleus, showed again DAB-staining in all mitochondria but the amount of cytochrome c staining and of mitochondria/cell was decreased and there was no staining in the mitochondrial matrix. In these cells the mitochondria often were clustered.

These results of granulosa single cell cultures differ in some aspects from the findings in granulosa explants. In the granulosa cell cultures no negative mitochondria were found. However, in these 2 different granulosa models there is no complete cytochrome c release during apoptosis.

Goldstein et al. (2000) *Nature Cell Biol.* **2**:156-162
D'Herde et al. (2000) *CDD* **7**:331-337

Dramatic increase of cardiomyocytes necrosis in blockade of programme pathways of cell death

Vasyl S. Nagibin, Victor E. Dosenko, Lesya V. Tumanovska, Alexei A. Moibenko

Department of Experimental Cardiology, Bogomoletz Institute of Physiology, 01024, Kiev, Ukraine, e-mail: nvasya@ukr.net

The possibility of triggering between apoptosis and autophagy (or apoptosis and oncosis) and some hierarchy of cell death pathways have been proposed recently. The main direction in cytoprotection now is preferential prevention of apoptosis or autophagy and decreasing of these types of cell death in different pathologies is interpreted as positive. However, we showed that inhibition of programmed cell death can initiate necrosis.

Primary cultures of rat neonatal cardiomyocytes underwent 30 minutes of anoxia followed by 60 minutes of reoxygenation. We used the specific proteasome inhibitor clasto-lactacystin beta-lactone (10 microM) to induce programmed cell death. We also used N-3-methyladenine (100 mM) to prevent autophagic cell death, and caspase inhibitor N-acetyl-Asp-Glu-Val-Asp-al (DEVD) (100 microM) to prevent apoptotic cell death during anoxia-reoxygenation or clasto-lactacystin beta-lactone application. The percentage of living, necrotic, and apoptotic cells were determined by staining with Hoechst 33342 and propidium iodide. Autophagy was demonstrated by staining vascular structures in vivo by monodansyl cadaverine.

The inhibition of autophagy with N-3-methyladenine during anoxia-reoxygenation caused an increase in the necrotic cell number (1.9 times comparing to anoxia-reoxygenation, $P<0.001$) and the live cell population decrease (by 5.5 %, $P=0.03$). Moreover, simultaneous inhibition of both autophagy and apoptosis (N-3-methyladenine and caspase-3 inhibitor application) in anoxia-reoxygenation led to dramatic increase of necrotic cardiomyocytes number (4.5-fold, $P<0.001$) and a concomitant decrease in the living cell number (by 9 %). Application of DEVD during anoxia-reoxygenation also caused an increase in the necrotic cell number (2.8 times comparing to anoxia-reoxygenation, $P<0.001$). The inhibition of autophagy with N-3-methyladenine during application of clasto-lactacystin beta-lactone caused an increase in the necrotic cell number (2 times comparing to clasto-lactacystin beta-lactone alone, $P=0.01$). Simultaneous application of N-3-methyladenine and caspase-3 inhibitor during proteasome activity inhibition led to significantly increase of necrotic cardiomyocytes number (13.4 times, $P<0.001$) and a concomitant decrease in the living cells number (1.5 times, $P<0.001$). Naturally, N-3-methyladenine and DEVD completely prevented autophagic and apoptotic cell death correspondingly in all cases. Obtained data indicate that selective blockade of programmed cell death pathways (both apoptosis and autophagic cell death) has a negative effect for whole cell population and such cytoprotection strategy is deadlock.

Hepatoprotection by Cyclosporin A in Experimental Hepatitis. Sorting Desensitization of the Mitochondrial Permeability Transition Pore from Immunosuppression

Luca Nicolosi[1], Elena Palma[1], Maria Eugenia Soriano[1], Francesca Finetti[2], Grégoire Vuagniaux[3], Jean-Maurice Dumont[3], Cosima T. Baldari[2] and Paolo Bernardi[1]

From the [1]Department of Biomedical Sciences and CNR Institute of Neuroscience, University of Padova, Italy; the [2]Department of Evolutionary Biology, University of Siena, Italy; and [3]Debiopharm, Lausanne, Switzerland.

We studied the mitochondrial, cellular and hepatoprotective effects of Cyclosporin A (CsA) and DEBIO-025, a CsA derivative where Sar in position 3 and MeLeu in position 4 have been substituted by D-MeAla and EtVal, respectively. At variance from CsA, DEBIO-025 did not prevent nuclear translocation of a Nuclear Factor of Activated T Cells-GFP fusion protein, nor did it inhibit activation of purified mouse T cells, while it was more potent than CsA at desensitizing the mitochondrial permeability transition pore (PTP) to Ca^{2+} both *in vitro* and *ex vivo*. We have compared the effects of CsA and DEBIO-025 in fulminant hepatitis induced in the outbred CD1 mouse strain (i) by injection of lipopolysaccharide of *E. Coli* (LPS) plus D-Galactosamine (D-GalN), a treatment that sensitizes the liver to the proapoptotic effects of TNFalpha; and (ii) by injection with the Jo2 antibody, a treatment that causes hepatic damage by direct stimulation of the Fas receptor. We found comparable levels of hepatoprotection (as assessed by caspase 3 cleavage, release of aminotransferases and animal survival) with CsA and DEBIO-025 after treatment with LPS + DGalN but not with the Jo2 antibody. These results help define the hepatocyte death pathways where the PTP plays a critical role *in vivo*; allow a clear-cut separation of the effects of cyclophilin ligands from calcineurin inhibition; and suggest that DEBIO-025 may be a safe and useful tool for the treatment of TNFalpha-dependent acute hepatitis.

Differences in the anticarcinogenic mechanisms of bexarotene and N-(4-hydroxyphenyl)retinamide in Cutaneous T-cell lymphoma

Nieto-Rementeria N.[1], Apraiz A.[1], Pérez-Yarza G.[1], Boyano MD.[1], Díaz-Pérez JL.[2], Ratón JA.[2], Izu R.[2], Asumendi A.[1]

Department of Cell Biology and Histology. University of the Basque Country.48940-Leioa, Spain[1]. Dermatology Department. Cruces Hospital.48903-Barakaldo, Spain[2] e-mail: aintzane.asumendi@lg.ehu.es

Cutaneous T-cell lymphoma represents a heterogeneous group of skin-homing T cell lymphocytes. No curative treatment exists. Therefore, there is a great need for the development of novel therapies. LGD1069 (bexarotene) is a synthetic nuclear retinoid X receptor (RXR)- selective retinoid approved by the FDA for the treatment of cutaneous T cell lymphoma (CTCL). The ability of RXRs to form heterodimers with various receptor partners important in cellular function indicates the biological activities of bexarotene are diverse. Ligands have a profound influence on nuclear receptor dimerization and affect the activation of some genes or others. However, remission rate of bexarotene treatment in CTCL varies from one patient to another. N-(4-hydroxyphenyl)retinamide (4HPR) is a low-toxicity synthetic-derivative of retinoic acid, which has shown great potential as antineoplasic agent on several lymphoblastic leukaemia cell lines.

We compared the anticarcinogenic effects of 4HPR and bexarotene on Hut-78, HH and MJ, well-established CTCL cell lines. Both 4HPR and bexarotene induced a moderate loss of viability in CTCL cell lines after 4 days of incubation. Annexine V assays demonstrated that treated cells underwent apoptosis. Apoptosis rate was higher in 4HPR than bexarotene treated cells. Clonogenic survival assays on methylcellulose after 15 days of culture exhibited similar results as those obtained for 4HPR cytotoxicity; however, self-renewal ability on methylcellulose was strongly inhibited after bexarotene treatment, which seems to be, at least partially, the result of cell cycle arrest. That is why, we further investigated changes in cell cycle related protein expression: p53, p21, cyclin B1, cyclin D1, cdc2(p34), pAkt.

In conclusion, both bexarotene and 4HPR reduced proliferation rate of CTCL cell lines in a time dependent manner and while bexarotene is associated with the inhibition of cell cycle arrest, 4HPR induces mainly mitochondrial apoptotic-pathway.

Pro-apoptotic activity of the naturally occurring compound, 13-Hydroxy-15-oxozoapatlin.

P. Nigro, E. Bloise, F. Dal Piaz, N. De Tommasi and M.A. Belisario.

Biochemistry Laboratory, Department of Pharmacological Sciences, University of Salerno, Fisciano (SA), Italy, e-mail: panigro@unisa.it

13-Hydroxy-15-oxozoapatlin *(*Oxo), from *Parinari sprucei* bark, was originally identified as a compound that inhibited the proliferation of human cancer cell lines and caused cell cycle arrest in G_2/M (Lee et al., *Chem. Biol. Interact.*, 99, 193-204, 1996). Here we show that Oxo induced apoptosis in Molt 4 and U937 cells, two leukemia-derived cell lines, in a dose- and time-dependent manner. Apoptosis induction was confirmed by the increase of hypodiploid cell number, caspase 3-activation and phosphatidylserine externalization. In addition, we demonstrated that Oxo induced mitochondrial depolarization and cytochrome c release. Interesting, we found that at doses of Oxo = 10 µM, the onset of apoptosis was preceded and/or concomitant to cell cycle arrest in G2/M, whereas at higher doses (25 µM) of Oxo cells did not arrest in G2/M before apoptosis commitment. The pro-apototic activity of Oxo appeared to be strictly dependent on the presence of an alpha, beta-unsaturated carbonyl, as the Oxo analogue, PS72, lacking this functional group (Braca et al., Planta Med.,70, 540-550, 2004), was not cytotoxic. As the alpha,beta-unsaturated carbonyl might serve as a Michael acceptor reacting with intracellular sulphydryls, we evaluated the effect of Oxo on intracellular thiols (protein -SH and GSH). We found that Oxo reduced dose-dependently the levels of intracellular thiols. Alkylation appeared to be the main mechanism of oxo-mediated –SH depletion at short incubation times. On the contrary, GSH oxidation became the predominant effect observed in 24h cell cultures. As GSH system is one of the intracellular thiol-reducing factors to antagonize the excessive oxidation, we hypothesized that Oxo induced apoptosis through a redox–dependent mechanism. This hypothesis was further supported by the following experimental evidences: i) Oxo increased up to 3.4 fold the levels of intracellular reactive oxygen species in cell exposed for 1 hr to different doses of the compound; ii) the presence of antioxidants such as desferoxamine and thiourea strongly reduced the extent of Oxo-induced apoptosis; iii) cell pre-treatment with N-acetyl cysteine ablated almost completely the pro-apototic activity of Oxo.
Further studies are in progress on apoptosis mediator proteins, bearing the –SH group, as potential target of Oxo.

The phycotoxin okadaic acid induces apoptotic death via oxidative stress in cultured glial cells.

Amaya Ferrero-Gutiérrez[1,3], Anabel Pérez-Gómez[1,3], Antonello Novelli[2,3], Maria Teresa Fernández-Sánchez[1,3].

[1]Dept. Biochemistry and Molecular Biology, [2]Dept. Psychology, and [3]University Institute for Biotechnology of Asturias, University of Oviedo, 33006 Oviedo, Spain, e-mail: anovelli@uniovi.es

Okadaic acid (OKA) is a phycotoxin commonly contaminating shellfish seafood, that potently inhibits protein phosphatase 2A (PP2A). OKA induces apoptotic degeneration of cultured neurons, and we investigated whether it could also affect glial cells. Exposure of cultured glial cells from the rat cerebellum to increasing concentrations of OKA resulted in glial degeneration and death, with 80% of cell death at 50 nM OKA. after 8 h. The time course of glial degeneration was characterized by progressive shrinking of the cell body and significant morphological changes at earlier time than in neurons. OKA glial toxicity was associated with DNA laddering in agarose gel electrophoresis, chromatin condensation and fragmentation and activation of caspase-3. All these molecular events are compatible with the activation of an apoptotic process and occurred in cultured neurons as well. However, differently from neurons, OKA toxicity in glial cells was potentiated by treatment with protein synthesis inhibitors such as cycloheximide or actinomycin D. Glial cells treatment with toxic concentrations of OKA promoted oxygen free radical formation, as detected by confocal laser microscopy, and significantly reduced both catalase and glutathion peroxidase activity,
as well as reduced glutathion cellular levels. The presence of antioxidants such as α-tocoferol, in the growth media during exposure to OKA, partially reduced both oxidative stress and toxicity. The effect of OKA treatment on the phosphorylation levels of several proteins in neurons and glial cells will be discussed.

Identification of a Second Transactivation Domain in the Carboxyl Terminus of p73: Cell, Promoter and Process Specificity

U. Nyman, B. Zhivotovsky, and B. Joseph.

Division of Toxicology and Neurotoxicology, Institute of Environmental Medicine (http://www.ki.se/imm), Karolinska Institutet, Sweden, e-mail: Ulrika.Nyman@imm.ki.se.

The p53 family member protein, p73, is encoded by the gene P73. This gene gives rise to many different isoforms due to alternative promoters and C-terminal splicing. Importantly, these various isoforms have different effects on cellular processes like cell death, cell cycle and differentiation. The full length isoforms, TAp73, have for quite some time been regarded as pro-apoptotic molecules, while the shorter ΔNp73, lacking the transactivation (TA) domain has been regarded as anti-apoptotic ones, being able to counteract the effect of the full length p73 as well as p53. However, we have previously shown that in a cell-type specific manner, the full length TAp73α can inhibit apoptosis whereas TAp73β promotes it. We also showed the TA domain of p73 to be important for p73's pro-apoptotic activity, but not for its anti-apoptotic actions. In line with these studies we have performed experiments showing that full length TAp73 can be active in transcription, despite a point mutation, which inactivates the TA domain due to conformational change. This TA-independent effect seems to be both cell-type and promoter specific. Indeed, both p73α and p73β isoforms, having a mutated TA domain, were able to activate transcription of the cell cycle regulatory genes MDM2, P21 and CYCLIN G, whereas being inactive on the promoter of the pro-apoptotic gene BAX in small cell lung carcinoma H82 cells. Hence, the TA domain seems to be crucial for activating genes involved in apoptosis, whereas the putative second TA domain is important in activation of cell cycle regulatory genes. Taking advantage of two point mutations situated in the C-terminus of p73, known to occur in tumors of the lung and neuroblastoma, we found these mutants still being active on promoters of cell cycle regulatory genes, but to a somewhat lesser extent than the full length p73. These results indicate that whereas the TA domain is important in the activation of pro-apoptotic genes, the second TA domain might, in a cell-type specific manner, be essential in activation of certain genes significant for cell cycle regulation, giving a deeper insight on the complex involvement of p73 in proliferation and apoptosis, and its structure-function properties.

Investigating the role of serine proteases in leukaemic cell death

A.R. O Connell[1], B.W Lee[2], C. Stenson-Cox[1§]

[1]National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland. [2]Immunochemistry Technologies LLC., 9401 James Avenue South Suite 155, Bloomington, MN 55431, USA.

Staurosporine causes a rapid induction of apoptosis in virtually all mammalian cell types. It has also been shown to activate features of cell death under caspase-inhibiting conditions. The aim of this work was to investigate the proteolytic mechanisms responsible for cell death in this case. First we demonstrated that the broad-spectrum caspase inhibitor z-VAD.FMK, does not affect staurosporine-induced apoptotic morphology, nuclear condensation or DNA fragmentation in HL-60 cells, despite its cell permeability and prevention of caspase-3 processing and activity.

Enzymatic assays were employed to assess apoptosis-associated proteolytic activities and large-scale inhibitor studies, including fluorescently labelled inhibitors were used to deduce specificity; regulation and positioning of serine proteases activated during cell death in HL-60 cells.

In this way we show that inhibitors of serine proteases delay cell death in one such system. Furthermore through profiling of proteolytic activation, we demonstrate for the first time that staurosporine activates a chymotrypsin-like serine protease mediated cell death program in HL-60 cells, independently of, but in parallel to caspase activation. Features of serine protease mediated cell death include cell shrinkage and apoptotic morphology, regulation of caspase-3, altered nuclear morphology, generation of an endonuclease and DNA degradation. We also reveal a staurosporine-induced activation of a p16 chymotrypsin-like protein during apoptosis.

In conclusion, we have characterised for the first time, a caspase independent serine protease mediated cell death program in HL-60 cells. This work contributes directly to the growing knowledge of caspase-independent cell death pathways and has implications for apoptosis-based cancer therapies. We are currently investigating the intracellular localisation of the staurosporine-induced 16kDa protease and chymotrypsin-like proteolytic activity, as well as the role of the relatively z.VAD.fmk resistant caspases, -2 and -8 in serine protease mediated events in HL-60 cells.

Investigating the tumorsuppressor potential of the BH3-only proteins Bmf and Bad in *c*-myc induced lymphomagenesis.

Anna Olsson and Andreas Villunger

Division of Developmental Immunology, Innsbruck Medical University, Innsbruck, Austria

Impaired apoptosis has been recognized as a hallmark of tumor development. Deregulation of various members of the Bcl-2 family in cancers has been described. Among them, loss of the BH3-only protein Bim or shRNA-mediated knock-down of *puma* have been shown to accelerate lymphoma development in mice.
Loss of the BH3-only protein Bmf in mice induces polyclonal B cell hyperplasia but not tumorigenesis. The observed accumulation of B lymphocytes is most likely caused by a decreased sensitivity of Bmf-deficient B cells to cytokine withdrawal and/or B cell receptor ligation. Mice deficient for another BH3-only protein, Bad, have normal lymphocyte development but aged mice were reported to develop diffuse large B-cell lymphomas, with an incidence of about 20%.To explore the tumorsuppressor potential of these two BH3-only proteins in B cell lymphomagenesis mice deficient for Bmf or Bad were inter-crossed with transgenic mice expressing the *c-myc* oncogene under control of the *Eμ* heavy chain enhancer. These animals usually develop a bulky pre-B or B cell lymphomas with almost normal white blood counts, within a few months of life.
In contrast to data published using Bim-deficient mice our data from $bmf^{+/-}$ or $bad^{+/-}$ $E\mu\text{-}myc^{+}$ lymphomas so far reveal no significant acceleration of c-Myc-mediated lymphomagenesis. Haploinsufficiency for *bad*, however, was found to promote leukemia development, as previously been reported for loss of bim. This effect was not seen in animals lacking one allele of *bmf*. To explore the role of BH3-only proteins in the response to chemotherapy primary lymphoma cells derived from *Eμ-myc* mice and from *Eμ-myc* mice heterozygous for *bmf*, *bad* or *bim* were subjected to a range of anticancer drugs *in vitro* in order to correlate drug-resistance with the loss of individual BH3-only proteins. However, haploinsufficiency does not confer protection to any of the anti-cancer agents tested.

Effects of oxazaphosphorines on DNA damage in the erythropoietic system

Mazur L., Niziol A., Opydo M., Radowska A. and *Niemeyer U.

*Laboratory of Experimental Haematology, Department of Animal Physiology, Institute of Zoology, Jagiellonian University, Cracow, Poland, e-mail: maz@zuk.iz.uj.edu.pl *NIOMECH, University of Bielefeld, Germany*

Chemotherapeutic agents act on neoplastic cells and damage also normal cells, especially cell populations that have rapid cell turnover. The cytotoxicy of oxazaphosphorines, a class of alkylating drugs, is known to be mainly dependent on DNA alkylating properties. However, available information about possible effects on DNA degradation in normal cells of the haematopoietic system by the new generation alkylating drugs is scarce. Thus, the aim of the study was to show and compare the extend to which three oxazaphosphorine compounds D-17272 (mafosfamide cyclohexylamine salt), D-18864 (4-hydro-peroxy-cyclophosphamide) and D-19575 (glufosfamide, ß-D-glucosyl-isophosphoramide mustard) can affect DNA degradation in erythroblasts and cell death induction in erythrocytes as well as changes in erythroblast proliferation. The experiments were carried out *in vivo* on adult male mice. The research was conducted using light, fluorescence and confocal microscopy methods. In the case of erythropoiesis, the outcome of DNA damage occurring in erythroblasts after cytotoxic agent(s) application is micronuclei formation which can be observed in erythrocytes. It is accepted that erythrocytes containing one micronucleus or micronuclei in the cytoplasm die by 'delayed apoptosis'. The number of polychromatic erythrocytes is an indicator of cell proliferation rate. Temporary changes in the frequency of micronucleated polychromatic erythrocytes among immature polychromatic erythrocytes, the frequency of micronucleated normochromatic erythrocytes among mature normochromatic erythrocytes as well as the number of polychromatic erythrocytes among polychromatic and normochromatic erythrocytes, were determined in bone marrow, peripheral blood and spleen. The patterns of temporary alterations in the frequency of micronucleated erythrocytes and the number of polychromatic erythrocytes observed in the mouse bone marrow, peripheral blood and spleen, were dependent on the chemotherapeutic agent given and the time intervals after the drug application. DNA damage caused by the oxazaphosphorines resulted in triggering 'delayed apoptosis' of erythrocytes and changes in erythroblast proliferation.

Supported by Polish Research Projects BW/33/2005 and DS/IZ/2006

Assembly of pro-apoptotic Bcl-2 family proteins in the yeast outer mitochondrial membrane

Martin Ott, Erik Norberg, Boris Zhivotovsky and Sten Orrenius

Division of Toxicology, Institute for Environmental Medicine, Karolinska Institute, Stockholm, Sweden

Mitochondrial outer membrane permeabilization is considered a key event during the early phase of the apoptotic process and involves members of the Bcl-2 family of proteins. The Bcl-2 family consists of more than 30 proteins, which can be divided into three subgroups: Bcl-2-like survival factors, Bax-like death factors, and BH3-only death factors. The interaction of the caspase-8-cleaved form of the BH3-only death factor Bid(tBid) with Bax results in the insertion of Bax into the outer mitochondrial membrane. Subsequently, Bax assembles into oligomeric structures that promote the release of mitochondrial intermembrane space proteins into the cytosol, where they can participate in the control of the apoptosis machinery.
We are employing a reconstituted system of recombinant proteins and mitochondria isolated from different yeast mutants to clarify the targeting specificity and the assembly process of the pro-apoptotic Bax-pores in the mitochondrial outer membrane. Yeast does not have members of the Bcl-2 family. However, a combination of recombinant tBid and Bax causes cytochrome c release from isolated yeast mitochondria, which is similar to mammalian mitochondria. Using mitochondria isolated from a yeast mutant lacking cardiolipin we found that tBid/Bax-induced cytochrome c release was indistinguishable from that observed with wildtype mitochondria. This indicates that cardiolipin is not required to target tBid/Bax to the outer mitochondrial membrane.

Role of lipid rafts in intrinsic versus extrinsic apoptosis signaling in B lymphoma cells: implications for cancer treatment

Astrid Ottosson, Yu Sun and Bengt Fadeel

Division of Biochemical Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden;
e-mail: astrid.ottosson.439@student.ki.se

Lipid microenvironments of specific constitution in the plasma membrane known as lipid rafts are involved in cellular signaling and have been shown to play an important role in Fas death receptor signaling. Furthermore, lipid rafts have been suggested to play a role in chemoresistance in certain Burkitt lymphoma (BL) cell lines. Hence, our group has shown that the Raji cell line fails to undergo intrinsic (mitochondria-dependent) apoptosis in response to the anti-cancer agent etoposide due to lipid raft sequestration of apoptotic protease-activating factor-1 (APAF-1) (Sun et al., Blood, 2005). APAF-1 is a component of a protein complex termed the apoptosome and needed for caspase-3 activation and cell death. We now show that the chemosensitive Jurkat T cell line and the chemoresistant Raji B cell line both express the Fas death receptor and are sensitive to Fas-mediated (extrinsic) caspase-3 activation. Furthermore, our data suggest that Fas signaling can be impaired by adding the lipid raft disrupting agents, ß-methylcyclodextrin (ß-MCD) and nystatin. Chemoresistance due to defective apoptosis of cancer cells is a major problem in the treatment of cancer patients. Based on our findings, it is possible that the effect of conventional anti-cancer treatment could be amplified using lipid raft disruptors, but at the same time, this adjuvant form of treatment could also hamper Fas-dependent immune killing of cancer cells.

Novel approaches to evaluate the alteration in membrane traffic induced by FasL signalling.

Sihem Ouasti and Mauro Degli Esposti

Faculty of Life Sciences, The University of Manchester, Stopford Building, Oxford Road, M13 9PT Manchester, UK. E-mail: sihemouasti@manchester.ac.uk.

Death receptor ligation promotes caspase activation and engagement of mitochondria and other organelles. We have outlined a novel sequence of cellular events connecting surface death receptors with internal organelles at the junction of caspase activation.
Soon after ligation, Fas (CD95/Apo1) enhances endocytosis with consequent alteration in membrane traffic that produces cytological rearrangement of organelles that appear to mix with each other.
We have used several approaches to highlight and verify Fas-induced alteration of membrane traffic. In particular, we have implemented a novel technique using an animal lectin, *Helix pomatia* agglutinin (HPA). HPA has a high affinity with GalNAc residues of membranes proteins residing in early secretory organelles (ER and the *Cis*-Golgi), and also present at the surface of undifferentiated T-cell lines like Jurkat.
In live cells, surface binding of HPA is followed by constitutive endocytosis, which is greatly enhanced by FasL treatment of Jurkat cells. So, surface staining with HPA becomes a very effective way to visualize endocytic event both qualitatively, especially at the cell surface, and quantitatively by monitoring membrane traffic.
We have then developed a dual HPA assay that combined evaluation of endocytosis in live cells with subsequent labelling of internal secretory organelles in permeabilised cells.
Briefly, this dual HPA staining consists of incubating red labelled HPA in live cells. After fixation and permeabilisation, cells are then treated with green labelled HPA as in conventional static staining of the Golgi.
This procedure allows the visualisation of the global changes of the traffic of the same membrane elements that occur after FasL treatment. External HPA was maximally internalised after 30 min, while internal membranes labelled with green HPA disperse away from the Golgi cluster, spreading towards the cell periphery and surface.
When using the pan-caspase inhibitor z-VAD, HPA internalization appears to be unchanged, whereas the dispersal and spread of internal HPA-labelled membranes is clearly inhibited. Hence, activation of apical caspases is essential for the centrifugal dispersal of endomembranes and enhanced exocytosis that follow early stimulation of Fas.

Mathematical Modeling of Apoptotic Caspase Activation

[1]Pace V, [1]Bellizzi D, [2]Carotenuto L, [1]De Benedictis G

[1] Department of Cell Biology, University of Calabria. Italy
[2] Department of Electronics, Informatics and System Science, University of Calabria. Italy

Mathematical modeling is a powerful tool to elucidate complex signal transduction networks. We developed a quantitative mathematical model of the apoptotic caspase activation. The model consists of a set of 1st order nonlinear ordinary differential equations which describe, according to the mass action law, the relationships between the concentrations of some molecular species (states of dynamical system) involved in caspase activation. For any physically meaningful set of parameters, the model has an equilibrium state characterized by the absence of activated caspases. We investigated the stability of this equilibrium states, and provided some theoretical original results. By extensive numerical searches in the parameter space we obtained a complete picture of three different patterns of equilibrium states, for which we propose a putative biological meaning. Moreover, we simulated the time evolution of the concentrations of relevant chemicals by solving the system differential equations for several parameter values and initial states, thus trying to mimic the quantitative cell response to external stimuli.
To check the biological reliability of our model, we are comparing *in silico* data with data of *in vitro* experiments carried out in synchronized 143B.TK$^-$ cells. After treatment with recombinant Apo2L/TRAIL as stress factor, we measure caspase 3 and caspase 8 activity (bioluminescence assays) as a function of the variable parameters that have a pivotal role in our model (time and concentrations).
At our knowledge, this is the first time that experimental data in cell culture are used to check the biological reliability of a mathematical model describing apoptotic caspase activation.

Molecular mechanisms of resistance of human colorectal COLO 205 cancer cells to extrinsic apoptosis

Beata Pajak, Arkadiusz Orzechowski

Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Nowoursynowska 159, 02-776 Warsaw, Poland, e-mail: bepaj@wp.pl

The resistance of transformed colon epithelial cells to immune system-mediated extrinsic apoptosis leads to grow and development of colon cancer. In the past, several tactics were shown how colon adenocarcinomas avoid cell deletion and remain viable. In particular, colorectal cancer cells resist death ligands-induced apoptosis by expressing antiapoptotic protein FLIP. By direct interaction with FADD, FLIP inhibits the death signal descending from death receptors in COLO 205 cells. Regardless of the presence of active membrane receptors, colorectal cancer cells resist interferons-mediated cell death, either. In our studies, we assumed that IFN-R and TNF-R1 receptors compete for STAT-1α kinase. Firstly, it was shown that STAT-1α kinase is bound to TRADD protein in TNF-R1 signalosome, and that the amount of sequestered STAT-1α is directly proportional to the TNF-R1 ligand. Secondly, we observed that STAT-1α kinase bound to TRADD was phosphorylated at Y701 residue (P-STAT-1α) and that the amount of P-STAT-1α ligated to TNF-R1 signalosome was diminished after IFN-α pretreatment. It was not the case when COLO 205 cells were pretreated with IFN-γ. Thirdly, to our surprise IFN-α stimulated cell survival, rather than to promote COLO 205 cell death. The latter was accompanied by NF-κB activation, known to promote antiapoptosis. We suggest, that since IFN-α but not IFN-γ pretreatment released TNF-R1 signalosome from P-STAT-1α-dependent blockade of antiapoptosis it is most likely that IFN-Rα-dependent upstream kinase for STAT-1-α renders colon adenocarcinoma COLO 205 cells less susceptible to TNF-α-induced apoptosis.

Methods: immunoprecipitation, western blotting, immunocytochemistry, electron microscopy

Mitochondria as therapeutic target for the treatment of fungal infections. Implications for the effector function of the immune system.

Pardo, Julian[1], Urban, Christin[2], Müllbacher, Arno[3], Wallich, Reinhard[4], Borner, Christoph[2], Simon, Markus M[1]

[1]Metschnikoff Laboratory, Max-Planck-Institut for Immunbiology, D-79108 Freiburg, Germany, [2]Institute of Molecular Medicine and Cell Research, Center for Biochemistry and Molecular Research, Freiburg, Germany, [3]John Curtin School of Medical Research, Australian National University, Canberra, Australia, [4]Institute for Immunology, University Clinic Heidelberg, Heidelberg, Germany

Induction of cell death via apoptosis has been adapted by both, vertebrates and parasites during their co-evolution as one strategy for mutual defense and survival. The immune system of vertebrates employs apoptotic mechanisms to combat intracellular parasites such as viruses and tumours. The two principal cytolytic effector lymphocyte populations, natural killer (NK) and cytotoxic T cells (CTL) execute their function primarily via the granule exocytosis pathway mediated by perforin and the two granzymes (gzm) A and B.. By using *ex vivo* derived CTL from mice deficient in either gzmA and/or gzmB we have demonstrated, that gzmA and gzmB independently are able to induce different pro-apoptotic processes, like oligonucleosomal DNA fragmentation, mitochondrial depolarization, ROS production and PS exposure at the plasma membrane by different mechanism. Recent results from our lab also suggest that pro-apoptotic members of the Bcl-2 family (Bid, Bak or Bax) are not necessary for the final cell death of target cells induced by CTL, as shown by clonogeneic survival assays, although can modulate some processes. Pathogens have also evolved mechanisms to induce apoptosis of host immune cells as a mean of survival strategy. One case in point is the opportunistic human pathogen *Aspergillus fumigatus*. It is known that one of its secondary metabolite, gliotoxin (GT), has immunosuppressive properties as a result of induction of apoptosis in relevant target cells. By analysing the molecular mechanism(s) of GT-induced apoptosis we have confirmed previously findings that GT triggers apoptosis via mitochondrial depolarization and ROS production. Furthermore, we have shown the molecular mechanism involved in this process: Gliotoxin-induced mitochondrial perturbation is induced by Bak conformational change, independent of caspases and/or the pro-apoptotic protein Bid, and associated with cytochrome c, AIF release and cell death.
Thus the differences in the apoptotic pathways activated by the immune system on the one hand and *A. fumigatus* on the other may open new avenues for therapeutic treatment of the pathogen without interfering with the immune defense.

Antirheumatic biologic agents induce leukocyte-dependent apoptosis of synovial fibroblasts.

Pattacini L.[1], Casali B.[1], Nicoli D.[1], Farnetti E.[1], Boiardi L.[2], Salvarani C[2].

Molecular Biology Unit[1] and Rheumatology Division[2], Ospedale Santa Maria Nuova (http://www.asmn.re.it),Reggio Emilia, Italy.
E-mail: laura.pattacini@asmn.re.it

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease of unknown aetiology characterized by hyperplasia of synovial cells and inflammatory cell infiltration. Synovial macrophages secrete tumor necrosis factor α (TNF-α), which induces the production of pro-inflammatory cytokines, such as IL-6. Treatment with TNF-α antagonists, the antibodies anti-TNF-α infliximab and adalimumab or the recombinant TNF-α receptor etanercept, has been shown to improve disease symptoms. In clinical trials, improvement of the disease activity has been achieved by inhibiting IL-6 by means of the humanized anti-IL-6 receptor tocillizumab. Nevertheless, the precise mechanism of action of these drugs has not been fully understood.
In our study, we investigated the pro-apoptotic effects of these four biologic drugs on synovial fibroblasts (SF) obtained from three osteoarthritis (OA) and three RA patients. SF were cultured alone, with peripheral blood mononuclear cells (PBMC), or with the adherent fraction isolated from PBMC, then exposed to either 10µg/ml infliximab, 10µg/ml adalimumab, 10µg/ml etanercept or 25µg/ml tocillizumab for 7 days. Apoptosis induction was determined by light microscopy and quantization of nucleosome release through ELISA. ERK, bax and active caspase 3 were detected by western blot.
We observed that only when the entire PBMC population was co-cultured in direct contact with SF, apoptosis of RA SF, but not of OA SF, was induced by the four drugs with a different extent in the three analyzed samples. In fact, one sample resulted resistant to infliximab, adalimumab and tocillizumab but sensitive to etanercept, with a 1.7 fold increase in apoptosis induction, one responded only to the two anti-TNF-α antibodies (1.3 fold increase) and one showed a comparable response for all the tested drugs, with apoptosis increases of 1.3 folds when treated with infliximab, adalimumab and tocillizumab and a 1.7 folds when exposed to etanercept. This response was associated with an increase in MAPKs and Bax levels as well as of the active form of caspase 3.
These findings suggest that the efficacy of the biologic drugs in use for RA treatment could be due, at least in part, to leukocyte-dependent apoptosis induction of SF.

A model inducible system to examine the toxic effects of a-synuclein on human neuronal cells

Maria Pavlaki, Kostas Vekrellis, Evangelia Emmanouilidou, Matina Maniati, Leonidas Stefanis

Division of Basic Neurosciences, Foundation for Biomedical Research of the Academy of Athens, Athens, Greece

a-synuclein is thought to be central in the pathogenesis of Parkinson's Disease (PD). Dominant point mutations or multiplications of the α-synuclein gene are sufficient to cause familial PD and α-synuclein deposition is a major hallmark of the sporadic disease. The manner in which aberrant α-synuclein causes neuronal degeneration is unclear, but it is thought that the propensity of α-synuclein to form oligomers and eventually aggregates and inclusions and to affect proteasomal function may be causal. We have generated tightly controlled tetracycline-regulated human neuroblastoma SH-SY5Y cell lines expressing either wild type or A53T mutant α-synuclein, or beta-galactosidase, as a control. Five to 7 days following concurrent induction of wild type or mutant α-synuclein expression and neuronal differentiation with retinoic acid, we have observed marked morphological abnormalities, including neurite retraction and vacuolization. In contrast, no such phenotype was observed in cells expressing beta-galactosidase or in cells in which α-synuclein was maintained in an uninduced state, due to the continuous presence of doxycycline. No inclusions or frank protein aggregation were observed by immunocytochemistry, but oligomeric forms of α-synuclein were apparent. Preliminary results, based on the incorporation of the lipophilic, lysosomotropic dye monodansylcadaverine within cells, indicate that there is an increase in autophagic vacuoles in the cells expressing α-synuclein, consistent with their appearance at the light microscope level. Cell death, as quantified by the incorporation of the dye Ethidium Homodimer, was increased in cells expressing α-synuclein. Cell death occurred at a relatively low level, but was continuous over the period of 5-9 days. No processed caspase 3 was detected by Western immunoblotting. In cells expressing wild type α-synuclein enzymatic proteasomal activity was normal at 5 and 6 days following induction, when neuronal degeneration was already apparent. Our results suggest that α-synuclein can lead to degeneration of human neuronally differentiated cells in a manner that is independent of inclusion formation and proteasomal dysfunction. The cell death that occurs due to the accumulation of α-synuclein appears to be autophagic and non-apoptotic. Further studies will examine the molecular and biochemical underpinnings of neuronal degeneration in this model.

Sphingosine kinase: apoptosis regulating enzyme and chemotherapy target in prostate cancer.

Pchejetski D.[1], Doumerc N.[2], Golzio M.[1], Rischmann P.[2], Kohama T.[3], Tessie J.[1], Malavaud B.[2], Cuvillier O.[1]

[1] Institute of pharmacology and structural biology, Toulouse, France [2] Department of Urology, Rangueil Hospital, Toulouse, France [3] Sankyo Ltd., Tokyo, Japan

Recently we showed that inhibition of a known oncogenic sphingosine kinase (SK) is linked to chemo-sensitivity of prostate cancer cells to various drugs (Pchejetski et al., Cancer Res., 2005). Currently we tested whether SK can serve as a potential target for sensitization of chemoresistant prostate cancer cells.

Pharmacological SK inhibition with novel, highly specific inhibitor (SKI) induced cell death in PC-3 cells in a dose-dependent manner. Cell death, triggered by SKI had apoptotic features, as confirmed by fluorescent microscopy and flow cytometry, and is mediated by caspase-8, -7 and -3 activation, subsequently followed by PARP cleavage. Similar effect an be achieved using siRNA to SK1 whereas enforced expression of sphingosine kinase rendered cells resistant to these treatments. Previously we found that chemoresistance of PC-3 cells is linked to the absence of SK inhibition. Here we report that pretreatment of PC-3 cells with small, non-toxic doses of SKI or with siRNA to SK overcomes chemoresistance of PC-3 cells.

SKI appears to have no general toxicity in vivo. Mice orthotopically engrafted with PC-3/GFP fluorescent cells were treated weekly with either SKI, camptothecin and both SKI and camptothecin together. SKI alone induced small tumor diminishment, however twice potentiated the effect of camptothecin. SKI treatment also reduced on 35% the number of micrometastases, assessed by GFP fluorescence.

To conclude, these results demonstrate a novel, potent SK inhibitor as efficient proapoptotic agent in prostate cancer treatment. Moreover, pharmacological sphingosine kinase inhibition can sensitize PC cells to chemotherapy both in vitro and in vivo proving the critical role of this enzyme in cell survival, and outlining its potential as a target for chemotherapy.

Gene and protein expression changes in human brain following acute ischemic stroke: identification of potential novel mediators of apoptosis

Roberta Pennucci[1], Nicholas Mitsios[1], Coral Sanfeliu[2], Jerzy Krupinski[3], John Gaffney[1], Pat Kumar[1], Shant Kumar[4] and Mark Slevin[1]

[1]School of Biology, Chemistry & Health Science, Manchester Metropolitan University, Manchester, UK, e-mail: r.pennucci@mmu.ac.uk
[2]Departament de Farmacologia i Toxicologia, Institut d'Investigacions Biomediques (IIBB), Barcelona, Spain
[3]Servicio de Neurologia, Hospital Universitario de Bellvitge, Barcelona, Spain
[4]Department of Pathology, University of Manchester, Manchester, UK

Both pro-apoptotic and survival-promoting mechanisms are triggered by ischemic injury. A better control over suppression of apoptosis and enhancement of neuroprotective mechanisms would be a major benefit towards stroke recovery. Previous work from our group using cDNA microarrays, for the first time directly on human brain tissue from stroke patients, identified novel genes deregulated following cerebral ischemia. In particular, normal cellular Prion Protein (PrP^c), Matrix Metalloproteinase 11 (MMP11) and Mixed Lineage Kinase 3 (Mlk3) were shown to be up-regulated in patients who survived for 2-37 days after stroke. RT-PCR, Western blotting and immunohistochemistry were used to confirm the above results. Findings from several research groups have suggested a pro-apoptotic role for Mlk3 and an anti-apoptotic role for PrP^c and MMP11. Therefore, we used *in vitro* studies to elucidate the involvement of these proteins in cell survival/death pathways triggered by ischemia. Primary human foetal neurons (HFN) and human brain microvessel endothelial cells (HBMEC) were exposed to oxygen-glucose deprivation (OGD) and reperfusion and the expression of Mlk3, PrP^c and MMP11 was analysed. The preliminary results provide new evidence for a potential involvement of these molecules in cellular responses to ischemic injury. Further studies will determine whether increased expression of these proteins is protective or detrimental to neurons and blood vessels.

Comparison of detection methods of cell death and cell viability after exposure to Doxorubicin, Actinomycin D and Mitomycin C *in vitro*.

Piia Penttinen[1,2], Jukka Pelkonen[2,3], Maija-Riitta Hirvonen[1]

1. Department of Environmental Health, National Public Health Institute, Kuopio, Finland. 2. University of Kuopio, Kuopio, Finland. 3. Kuopio University Hospital, Kuopio, Finland.

Dying cells are going through a complex network of pathways resulting into either apoptotic or necrotic cell death. Cellular events underlying the cytotoxicity evoked by cytostatic compounds produced by gram positive bacteria Streptomycetes were investigated in this study. Mouse (RAW264.7) macrophages were exposed to Doxorubicin, Actinomycin D and Mitomycin C in dose-dependent manner. Cell death and cell viability were detected after 24 hours exposure by using flow cytometric methods including ApoAlert assay for mitochondria membrane depolarization, DNA content analysis, PI (propidium iodide) exclusion test of fresh cells and live gate analysis. In addition, functional mitochondria were assessed by the MTT-test. All the studied compounds slightly irritated the mitochondria of macrophages assessed as a collapse of mitochondria membrane potential. In flow cytometric analysis Actinomycin D seemed to be the most cytotoxic, PI exclusion test and DNA content analysis indicate that 40% of cells were dead after exposure to the highest dose of Actinomycin D (0.1µM). In addition, Actinomycin D exposure caused the most significant decrease in the cell viability as detected by the MTT-test (35% of the control), whereas the viability was 70% based on the flow cytometric live gate analysis. The results were the opposite after Doxorubicin and Mitomycin C exposure; nearly 30% of cells were dead according to PI exclusion test, but DNA content analysis indicate only 4% and 8% of dead cells (Doxorubicin 1µM and Mitomycin C 0.8µM, respectively). After Doxorubicin exposure the cell viability detected by the live gate analysis was as low as 20%, while the MTT test indicates that 60% of cells were alive. Furthermore, the MTT-test did not indicate any decrease in the cell viability after Mitomycin C exposure although the viability was only 50% based on the live gate analysis. The present results demonstrate that different mechanisms of cell death can be activated by these agents although they belong to the same group of chemicals with cytostatic activity. Since detection of the induced cytotoxicity can be strongly dependent on the methods used, a variety of different assays are needed for in-depth understanding of the pathways of cell death caused by an agent, and for proper evaluation of its toxicity.

p25a induces a-synuclein dependent cell death in a cellular model for neurodegenerative disorders.

Christine L. Petersen and Poul Henning Jensen

Institute of Medical Biochemistry, University of Aarhus, Denmark, e-mail: clp@biokemi.au.dk

The neurodegenerative disorders, Parkinson's disease (PD), multiple system atrophy (MSA), and dementia with Lewy Bodies (DLB) belong to the group of α-synucleinopathies. Their unifying hallmark is the presence of intracellular inclusions containing aggregates of α-synuclein (Asyn). The inclusions comprise neuronal Lewy bodies in PD and DLB and glial cytoplasmic inclusions in MSA. The mechanisms underlying Asyn aggregation are not clear but are likely to involve an altered expression of pro-aggregatory factors.

We have identified the brain-specific protein, p25 α, as an accelerator of Asyn aggregation suggesting a role for p25 α in the neurodegenerative process. The cellular expression of p25 α is abnormal in degenerating neurons and oligodendrocytes in PD and MSA where it co-localizes with Asyn in Lewy bodies and glial cytoplasmic inclusions.

Here we have investigated the role of p25 α on cellular toxicity in an oligodendrocyte cell line stably expressing human wild-type Asyn. These cells display cellular degeneration when transiently transfected with p25 α as demonstrated by shrinkage of the cytosol, perinuclear localization of the microtubules, and nuclear condensation. Cell death is partially blocked by Congo red (an inhibitor of aggregation) and an anti-aggregatory peptide directed against Asyn. Furthermore, the p25 α-mediated cell death is significantly reduced by inhibitors of caspase-3 and -8 but not by a caspase-9 inhibitor. Our results indicate that the p25 α-mediated Asyn-dependent cell death is caused by the pro-aggregatory activity of p25α. Furthermore, the caspase 8/-3 cascade seems to be activated in the apoptotic process. Based on our results, we suggest that p25α contributes to the cytotoxicity underlying several α-synucleinopathies.

Understanding the molecular and cellular mechanisms whereby Asyn exerts ts neurotoxicity may help to develop novel neuroprotective strategies for diseases caused by Asyn aggregation.

Reduction of the cardiolipin per se do not mimic the effects on cell death associated with CL remodelling in Barth syndrome lymphoblasts

Petit P.X.[1], F. Gonzalvez [1,2] , S.-Y. Choi[3], F. M.A. Frohman[3] and E. Gottlieb[2].

[1]Institut Cochin, CNRS 8104, 24 Rue du Faubourg Saint-Jacques, Paris, France ; Beatson Institute, [2]Cancer Research UK, Glasgow, Scotland ; [3]Academic Medical Center , Laboratory Genetic Metabolic Diseases, Amsterdam, Nederland

Cardiolipin (CL) is a unique phospholipid with dimeric structure, carrying four acyl groups and two positive charges and usually known for anchoring respiratory chains proteins, or «gluing» the respiratory complex together and also attaching cytochrome *c* to the outer surface of the inner mitochondrial membrane, as come in recent years to be recognize as playing a key role in the cascade of events that takes place when apoptotic cell death is induced through the mitochondrial pathway. CL level drop in the initial phase of apoptosis via oxidative processes and generates free cytochrome *c* into the intermembrane space. So, this line of argument suggest that decreased level of CL should be pro-apoptotic because high level of freely releasable cytochrome *c*.

The present work address studies of the mammalian CLS gene, the role of cardiolipin in cardiolipin defiencent HeLa cells and its impact in cell death signal transduction through the mitochondrial pathway. We describe here cloning of the human gene for CLS and analysis of its knockdown on cell death progression. We find that decreased level of CL accelerates death and constitutes a switch between apoptosis and necrosis.

At the contrary, the Barth syndrome lymphoblastoid cells are characterized by a reducted concentration of CL associated with changes in their fatty acid composition. We have shown that defect in CL metabolism disturb mitochondrial structure and energetics and confer resistance to Fas induced apoptosis.

Based on these results, we are now characterising the link between mitochondrial function and structure, CL and apoptosis.

Supported by the AFM (Association Française contre les Myopathies) Grant n° n°11557 to PXP and CNRS

In vitro and in vivo evidence of ER stress and ER-mediated cell death during exposure to nephrotoxic drugs.

Mathieu Peyrou & A.E. Cribb

Laboratory of Pharmacogenetics, University of Prince Edward Island, Charlottetown, Canada. E-mail: acribb@upei.ca

Renal toxicity is a frequent adverse drug reaction encountered with otherwise useful clinical drugs. Although the mechanisms of renal toxicity are incompletely understood, evidence is accumulating that the endoplasmic reticulum (ER) is sometimes involved during renal cell damage. Studies in cells from other tissues (brain, liver) have revealed the dual role of the Unfolded Protein Response (UPR) triggered by ER-stress: it can help cells cope with stress by upregulation of protein chaperones and translation attenuation, but can also elicit ER-mediated cell death through pathways which involve specific proteases (caspase 12, calpain…) associated with markers of ER stress (GRP78 and GRP94 upregulation, XBP-1 processing…). Our major goals were to determine if the ER is a common target for nephrotoxic drugs and if the associated ER stress and UPR play a significant role during cell death. In a first set of *in vitro* experiments, we studied the effects of a mild ER stress triggered by known ER-stress inducers (tunicamycin and oxidized DTT) on the toxicity of clinically relevant nephrotoxic drugs in four renal cell lines (LLC-PK1, NRK-52E, MDCK, HEK-293). We demonstrated an overall protective effect associated with upregulation of ER protein chaperones GRP78 and GRP94, although some discrepancy appeared with certain drugs. In a second set of *in vivo* experiments, Spague-Dawley rats were injected with nephrotoxic doses of 3 clinically-relevant compounds: gentamicin, cisplatin and p-aminophenol, a metabolite of acetaminophen (paracetamol). ER stress markers were consistently observed after administration, indicating a disruption of ER homeostasis. Finally, a third *in vivo* experiment was conducted to document ER-mediated cell death after p-aminophenol administration. In addition to ER-stress markers, caspase-12 and calpain activation was monitored by immunoblotting and specific activity assays at 6h and 24h post-administration. Cleavage and reduced activity of calpain was observed at 24h, as well as caspase-12 activation and upregulation. This study suggests a significant involvement of the ER during renal toxicity and illustrates its multiple roles: a potential target (ER stress), a site of compensatory mechanisms (UPR), and an active player during cell death (ER-mediated cell death).

Reactive oxygen species affects structural and functional unity of mitochondrial network

Olga Pletjushkina, Konstantin Lyamzaev, Ekaterina Popova, Olga Nepryakhina, Olga Ivanova, Lidia Domnina, Boris Chernyak

A. N. Belozersky Institute, Moscow State University, 119992 Moscow, Russia
E-mail: pletjush@genebee.msu.ru

Fragmentation of the mitochondrial network (reticulum), following gathering of mitochondria in the perinuclear area and clusterization are induced by oxidative stress. Inhibitors of the respiratory chain (piericidin and myxothiazol) cause fragmentation of mitochondria in HeLa cells and fibroblasts without any signs of apoptosis. The mitochondria-targeted antioxidant (MitoQ) inhibits fragmentation indicating that ROS produced in mitochondrial matrix are responsible for this effect. Hydrogen peroxide also induces fragmentation of mitochondria and this effect is stimulated by the inhibitors of respiration in agreement with hyperproduction of reactive oxygen species (ROS) detected in the cells using dichlorodihydrofluorescein diacetate (DCF-DA). MitoQ suppresses hyperproduction ROS and fragmentation of mitochondria caused by hydrogen peroxide alone or in combination with the inhibitors of respiration confirming the major role of endogenous mitochondrial ROS production in structural changes of mitochondrial network. In untreated cells, the mitochondrial reticulum consists of numerous electrically-independent fragments. These fragments are identified after local photodamage of mitochondria, which causes immediate depolarization of electrically connected units. Prolonged treatment with MitoQ resulted in drastic increase in size of these fragments. These data indicate that in resting cell structural and functional dynamics of mitochondria could be regulated by mitochondrial ROS.

Apoptosis induction in pig macrophages by African swine fever virus (ASFV) correlates to virulence of the agent

R. Portugal[1], A. Leitão[2] and C.L.V. Martins[1]

[1]Infectious Diseases Laboratory, CIISA, Faculdade de Medicina Veterinária, Lisbon, Portugal;
[2]IICT, CIISA, Faculdade de Medicina Veterinária, Lisbon Portugal Insert your affiliation

Different viruses are known to modulate apoptosis in order viral replication is accomplished in the host. African swine fever virus (ASFV), the sole member of the *Asfarviridade* family is a large dsDNA virus that replicates on pig macrophages. Modulation of macrophage apoptosis by isolates with different virulence and its implications in pathogenesis are still poorly understood. ASFV possesses homologues to *bcl-2* and *iap* genes (5-HL and 4-CL respectively). These genes are effective when expressed in mammalian cell lines, but their role in the macrophage remains unknown. With the aim of studying apoptosis development in porcine macrophages after infection with highly and low virulent ASFV isolates, blood derived pig macrophage cultures were either mock infected, infected with the highly virulent ASFV/L60 or with the low virulent ASFV/NH/P68 at similar multiplicity of infection of 3 (MOI3). At 7h (ongoing replication, early infection phase) and at 18h (accomplished replication, late infection phase) post infection (PI), levels of apoptosis were determined by quantification of DNA internucleosomic fragmentation, caspase-3 activity and nuclei morphology visualization. Our results showed induction of higher levels of apoptosis by the low virulent ASFV/NH/P68 at both early and late phases of infection when compared to mock and ASFV/L60 infected macrophages. Study on the expression of viral genes 5-HL and 4-CL at 1.5, 4, 8, 12 and 16h PI through quantitative PCR showed a marked increase in expression along infection time with both viral isolates. However, immediately at very early infection (1.5h PI) a higher expression for both genes was obtained in ASFV/L60 infected macrophages in comparison to NH/P68. Interestingly 5-HL expression was lower than 4-CL at all time points in both viral infections.
Our studies show that highly virulent ASFV/L60 is able to repress apoptosis more effectively in the host cell than ASFV/NH/P68, which may contribute for a better understanding on the pathogenesis of ASFV infection.

Acknowledgments: Supported by Project POCTI/CVT/59122/2004 (FCT, Portugal) and grant SFRH/BD/10576/2002 (FCT, Portugal).

Caspase-3 activation in the neuronal cell line NG108-15 during differentiation and staurosporine induced cell death.

Bruna Pucci[1], Francesca Bertani[1], Valeria Colafrancesco[2], Marco Tafani[1,2]and Matteo A. Russo[2].

[1]Department of Cellular and Molecular Pathology, IRCCS San Raffaele Pisana, Rome Italy, [2]Department of Experimental Medicine and Pathology, La Sapienza University, Rome, Italy,

The hybridoma cell line NG108-15 differentiated in neurons previous treatment with 1mM sodium butirrate. The differentiated cells showed the characteristic morphology and structure of neuronal cells, besides they expressed the neuronal molecular marker (MAP-1). To test the hypothesis that differentiated neurons die by apoptosis with a different kinetic respect the undifferentiated cells we treated sodium butirrate treated and untreated cell with 100 nM
Staurosporine (STS). MTT assay and Hoechst staining indicated that 85% of undifferentiated NG108-15 died after 6h of STS treatment. Only 5% of differentiated cells died after the same treatment. 70% of sodium butirrate differentiated cells died only after 24h of treatment. Caspase-3 assay and caspase-3 western blot showed that caspase-3 resulted active after 3h of STS treatment in both differentiated and undifferentiated cells. Surprising differentiated NG108-15 cells showed caspase-3 activation in absence of STS treatment. PARP was cleaved with the same kinetic, after 3 h of STS treatment, in both differentiated and undifferentiated cells. In conclusion, differentiated neuronal cells NG108-15 were characterized by caspase-3 activity but there was no cell death during their differentiation. Moreover, after treatment with STS they died with a slow kinetic respect the undifferentiated cells despite the presence of active caspase-3. We hypothesized a role of caspase-3 in NG108-15 differentiation and the existence of a block of caspase-3 activity in the apoptotic pathway. Since neuronal degenerative diseases are characterized by late activation of apoptotic mechanisms we believe that this differentiation model can help us in understanding the molecular pathways that govern cell death in neurodegenerative diseases. Such knowledge will allow us to interfere with the degrading action of the apoptotic machinery in order to avoid the structural damage and the lost of neuronal function.

Glucocorticoids and Control of Cell Growth: Role of GILZ

Riccardi Carlo, Massetti Michela, Marchetti Cristina, Di Virgilio Rosa, Ayroldi Emira.

Section of Pharmacology Toxicology and Chemotherapy; Dept of Clinical and Experimental Medicine; Via del Giochetto, 06122 Perugia, Italy, e-mail: riccardi@unipg.it

Glucocorticoids (GCs) are able to modulate cell death and proliferation. These effects are consequent to genomic and non-genomic signals activated by GCs interaction with glucocorticoid receptor (GR). However, mRNA and/or protein synthesis inhibition counters GCs-induced apoptosis as well anti-proliferative activity thus indicating that gene transcription is required. GILZ (glucocorticoid induced leucine zipper) is a protein rapidly induced by GCs treatment and its induction may represent one of the mechanisms contributing to GCs activated cell growth inhibition. In fact, GILZ is able to induce apoptosis in different cells, including T lymphocytes, and also inhibits cell proliferation consequent to T cell activation. While GCs-induced apoptosis correlates with genomic and non-genomic mechanisms, inhibition of cell proliferation is due to GILZ interaction with MAPKs, a transduction pathway involved in cell growth control. GILZ//MAPKs binding results in cell growth inhibition in vitro and in vivo.
GCs modulate cell apoptosis and proliferation through a wide number of molecular mechanisms, such for example GR/NF-kB interaction. Our results indicate that induction of GILZ expression may be another molecular mechanisms contributing to GCs effects.

Life and death of a neutrophil: The role of NF-kB dependent and independent survival proteins in neutrophil apoptosis.

Nicola A. Riley, Christopher Haslett and Adriano G. Rossi

MRC Centre for Inflammation Research, Queen's Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh, EH16 4TJ, Scotland, UK, e-mail: n.a.riley@sms.ed.ac.uk

Neutrophils make up the highest proportion of the leukocytes in the circulation (approximately 50 - 70%) defending the body against bacterial and fungal infections. Failure of granulocytes to appropriately enter apoptosis at a suitable rate or, alternatively, a defect in phagocytic clearance of the apoptotic granulocytes can lead to the inflammatory response being disrupted. In such instances this could prevent effective resolution of inflammation resulting in development or promotion of various inflammatory disorders. As the survival pathways that determine the fate of the cell remain shrouded in some mystery we aim to elucidate the key survival proteins in the neutrophil. Through the use of various cytokines and inflammatory mediators that affect neutrophil longevity at inflammatory loci data has been obtained that implicates an important role for the anti-apoptotic proteins Mcl-1 and X-IAP. For example, we have demonstrated that TNF-a has the capacity to decrease levels of X-IAP in neutrophils at early time points. Another focal point of our research is the important role played by the transcription factor NF-κB in the regulation of neutrophil apoptosis. It has been previously shown in our laboratory that NF-κB is activated by several important inflammatory agents (e.g. LPS and TNF-a) resulting in the production of anti-apoptotic proteins. Through use of the NF-κB inhibitors it has been demonstrated that the anti-apoptotic effects of LPS and TNF-a can be over-ridden by inhibition of NF-κB and work is currently being undertaken to shed further light on the mechanisms of this pathway with particular regard to the anti-apoptotic proteins involved. Therefore we believe that unravelling the survival pathways within neutrophils could provide invaluable information with the potential to form the basis for novel therapeutic agents which have the ability to increase selective granulocyte apoptosis thereby aiding resolution.

Is Apoptosis a Cause or Effect in Myocardial Reperfusion Injury and Donor Heart Preservation?

Robicsek, Francis, Masters, Thomas N, Schaper Jutta, Freude Bernd, Fokin Alexander A. and Robicsek, Steven.

Carolinas Heart Institute, Dept. of Cardiovascular and Thoracic Surgery and Heineman Medical Research Laboratories, Carolinas Medical Center, Charlotte, NC. USA. Max-Planck-Institute, Dept. of Experimental Cardiology, Bad Nauheim, Germany. College of Medicine, University of Florida at Gainesville, Gainesville, FL, USA. E-mail: robicsek@ufl.edu

The length of blood flow interruption to the heart as a result of cardiopulmonary bypass, coronary artery occlusion, or during donor heart preservation limits the myocardial viability once flow is restored. Multiple mechanisms have been described to substantiate numerous cellular changes that result during the lack of blood flow and also during the restoration of blood flow. Included in these cellular changes is apoptotic mechanisms implicated as a direct cause of myocardial injury commonly occurring after surgical or cardiological restoration of impaired myocardial blood supply. Its role in reperfusion injury and/or myocardial preservation was the purpose of the present experiments. This study used a heterotopic transplanted working cervical donor heart preparation in a canine model. Inducing asystolic ischemia of various time-periods both protected (hypothermia with microperfusion) and unprotected (normothermic ischemia) represented the ischemic challenge to the heart. Function of the transplanted heart was monitored during reperfusion from the host animal by functional parameters and tissue changes including apoptosis and necrosis determined with electron microscopy, HPLC, western blot analyses and immuno-histochemistry for lamin B and activated caspase-3.

Apoptosis was initiated during ischemia as seen by the reduction in lamin B and completed during reperfusion since apoptosis requires ATP. Irreversible damage in the heart resulted from apoptosis with no necrosis evident after 18-24 hours of hypothermic preservation. Using cyclosporine A (apoptotic inhibitor) during the preservation phase, the viability of the heart was extended from 10-12 hours to 18-24 hours. In conclusion, myocardial reperfusion injury is, at least in part, a result of apoptosis and measures to prevent apoptotic initiation with an apoptotic inhibitor should be started during the ischemic period for acute myocardial ischemia or for donor heart preservation.

Ursodeoxycholic acid modulates hepatocyte apoptosis through nuclear steroid receptors

S. Solá,[1,2] R.E. Castro,[1] J.D. Amaral,[1] R.M. Ramalho,[1] P. Borralho,[1] M.M. Aranha,[1] B.T. Kren,[2] C.J. Steer,[2] C.M.P. Rodrigues[1]

[1]Centro de Patogénese Molecular, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal; and [2]Department of Medicine, University of Minnesota Medical School, Minneapolis, MN, USA

Ursodeoxycholic acid (UDCA), an endogenous bile acid, is widely used in the treatment of liver disorders. It acts as a potent inhibitor of the classical mitochondrial pathway of apoptosis, in part, by directly stabilizing membranes. Furthermore, as a cholesterol derived molecule, UDCA interacts with nuclear steroid receptors (NSR), such as the glucocorticoid receptor (GR), suggesting that it may also modulate gene expression. In these studies, we investigated whether regulation of upstream mitochondrial events by UDCA prevents hepatocyte apoptosis. In addition, we explored the role of NSR in the antiapoptotic function of UDCA. UDCA inhibited TGF-beta1-induced apoptosis of primary rat hepatocytes, by modulating the expression of E2F-1, Mdm-2, p53 and Bcl-2 family members, in a caspase-independent manner. Moreover, UDCA specifically prevented induction of p53 and Bax by overexpression of E2F-1 and p53, respectively. We also demonstrated that UDCA modulates the E2F-1/Mdm-2/p53 apoptotic pathway in hepatocytes through a NSR-dependent mechanism. In fact, pretreatment with UDCA upregulated NSR expression. UDCA was further shown to promote GR/hsp90 dissociation, thus inducing subsequent NSR translocation. However, when the C-terminal region of GR was deleted, UDCA no longer induced GR/hsp90 dissociation and GR nuclear translocation nor protected against apoptosis. Surprisingly, the bile acid does not require NSR transactivation for preventing apoptosis in hepatocytes. Finally, UDCA appeared diffuse in the cytosol but aggregated in the nucleus of hepatocytes. The nuclear trafficking of UDCA occurred through a NSR-dependent mechanism. In conclusion, this work revealed additional pathways for the antiapoptotic function of UDCA, demonstrating that the bile acid regulates the expression of specific targets upstream of mitochondrial commitment. Further, UDCA interacts with a specific region of NSR to translocate into the hepatocyte nuclei and inhibit apoptosis-related genes. (Supported by FCT, Lisbon, Portugal)

Localization of Active Caspase-6 in the Kinetochores Influences the Timing of Mitosis Progression

Salvador Rodriguez-Nieto and Boris Zhivotovsky

Institute of Environmental Medicine. Division of Toxicology and Neurotoxicology. Karolinska Institute. Sweden. e-mail: Salvador.Rodriguez-Nieto@ki.se

Accumulating evidences suggest the involvement of caspases in processes beyond apoptosis. Thus, alternative functions of caspases are related to the regulation of cell cycle progression and cell differentiation. Previously, it has been shown that the executioner caspase-6 can participate in cell cycle entry of quiescent T cells and its inhibition blocked this process. Here, we report further clues on the localization and the role of caspase-6 in cell cycle progression. We show that processed caspase-6 is localized in prometaphasic kinetochores of HCT116 cells undergoing mitosis. Using low doses of the microtubule depolymerizing drug nocodazole, we were able to synchronize the cell population in G2/M-prometaphase and analyze the function of caspase-6. In cells arrested in prometaphase with nocodazole caspase-6 strongly localized to the kinetochores and co-immunoprecipitated with the kinetochore proteins, CENP-E and CENP-F. VEIDase, was found to be the major caspase activity detectable in arrested cells, while DEVDase activity was not evident. Using a model of G2/M block/release with nocodazole, the effects of the selective caspase-6 inhibitor z-VEID-fmk or siRNA on the timing of mitosis was monitored. Inhibition of caspase-6 activity delayed cell cycle re-entry upon nocodazole block/release, while the caspase-3/-7-like inhibitor, z-DEVD-fmk, did not influence this process. Our results suggest that, although active caspase-6 is not essential for cell cycle progression, its inhibition strongly influences the timing of mitosis.

PEA-15/PED is phosphorylated in response to glucose withdrawal and protects glioblastoma cells from glucose deprivation-induced cell death

Anika Eckert, Barbara Böck, Tobias Haas, Jaromir Sykora, Kerstin Schultze, Christel Herold-Mende, Otmar Wiestler, Henning Walczak, Wilfried Roth

German Cancer Research Center (DKFZ),Im Neuenheimer Feld 280, Heidelberg, Germany; email: W.Roth@dkfz.de

PEA-15/PED (Phosphoprotein enriched in astrocytes 15 kD/ Phosphoprotein enriched in diabetes) is a death effector domain-containing protein which is involved in the regulation of apoptotic cell death, insulin resistance in diabetes, and MAP kinase signaling. Since PEA-15 is highly expressed in cells of glial origin, we studied the role of PEA-15 in human malignant glioma. Immunohistochemical analysis of PEA-15 expression shows strong immunoreactivity in glioblastoma multiforme. Interestingly, immunohistochemistry with an antibody specific for phosphorylated PEA-15 (S116) predominantely results in staining of the cells in perinecrotic areas in glioblastomas, suggesting that PEA-15 is phosphorylated in response to cellular stress. *Ex vivo* isolated human glioblastoma cells exhibit high levels of S116-phosphorylated PEA-15. Glucose deprivation of glioblastoma cells specifically induces phosphorylation of PEA-15 at S116. Incubation of the cells with BIS-VIII, a protein kinase C inhibitor, or KN-93, a calcium/calmodulin-dependent protein kinase inhibitor, abolishes phosphorylation of PEA-15 upon glucose withdrawal. Overexpression of PEA-15 induces marked resistance against glucose deprivation-induced cell death, which is accompanied by a decreased activation of Caspase 3. Downregulation of endogenous PEA-15 by transfection of specific siRNA oligonucleotides results in sensitization to glucose withdrawal-mediated cell death and Caspase 3 cleavage. The PEA-15-dependent cellular resistance to glucose deprivation is accompanied by an increased phosphorylation of ERK1/2, and treatment with an ERK1/2 inhibitor abrogates the PEA-15-mediated increase in resistance to glucose withdrawal. Our findings suggest that PEA-15 increases the resistance of glioblastoma cells in poor microenvironments with a lack of nutrients, such as in perinecrotic areas, which possibly further hampers the efficacy of chemo- and radiotherapy.

Cybrids with mtDNA mutations causing Leber's hereditary optic neuropathy are sensitized to apoptotic death induced by a mitochondrial oxidative stress

Anna Maria Ghelli, Claudia Zanna, *Valerio Carelli, §Andrea Martinuzzi and Michela Rugolo

*Dip. di Biologia Evol. Sperim. and *Dip. di Scienze Neurologiche, Università di Bologna;*
§IRCCS "E.Medea", Conegliano V., Italy (michela.rugolo@unibo.it)

Reduced activity in mitochondrial complex I is associated with a wide spectrum of neurodegenerative diseases, possibly through stimulation of mitochondrial production of ROS. Reduced complex I activity due to mitochondrial DNA point mutations is found in many cases of Leber's Hereditary Optic Neuropathy (LHON), which is characterized by a selective death of retinal ganglion cells. The transmitochondrial cytoplasmic hybrids or cybrids, bearing one of the three most frequent LHON mutations at nucleotide positions 11778/ND4, 3460/ND1 and 14484/ND6, have been widely used to model LHON pathophysiology at the cellular level. This cell system is generated by the repopulation with patient-derived mutant mitochondria of an immortalized cell line, previously devoid of its original mtDNA. It was shown that LHON cybrids were more prone to undergo Fas- and metabolic stress-induced apoptosis. In this study, we have investigated whether cybrids with LHON mutations are also susceptible to an oxidative insult. To this purpose, LHON cybrids have been exposed to two different treatments: incubation with the pro-oxidant tert-butyl hydroperoxide (t-BH) to induce an exogenous oxidative stress and incubation with the complex I inhibitor rotenone, to selectively increase superoxide production within mitochondria. After both treatments, LHON cybrids underwent an apoptotic type of cell death. However, the oxidative stress induced by t-BH similarly affected control and LHON cybrids and the parental cell line 143B.TK-, suggesting that its effect was independent of the mtDNA mutations. Conversely, rotenone induced a significant loss of viability in LHON cybrids, but not in control cybrids or in the parental 143B.TK- cell line. It seems therefore that inhibition of complex I specifically affect cybrids with mtDNA LHON mutations through a mitochondrial oxidative damage. Different antiapoptotic and antioxidant compounds have been tested to identify those able to counteract the loss of cell viability induced by rotenone. From these results, a model will be proposed to explain LHON cybrids predisposition to cell death.

Supported by grants from Telethon, MIUR and Ministero della Salute.

Tumor Necrosis Factor-induced cell death is mediated via the Receptor for Advanced Glycation End products.

Stefanie Salliau[1¶], Franky Van Herreweghe1[¶], Anneleen Cottyn[¶], Mathias Laga[¶], Winnok De Vos[‡], Patrick Van Oostveldt[‡], Joël Vandekerckhove[¶] and Katia Vancompernolle[¶].

[¶]*Department of Medical Protein Research, Molecular and Metabolic Signaling Unit, Faculty of Medicine and Health Sciences, Ghent University and Flanders Interuniversity Institute for Biotechnology (VIB), Albert Baertsoenkaai 3, B-9000 Ghent, Belgium and* [‡]*Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium; Email: katia.vancompernolle@UGent.be*
[1] *These authors contributed equally to this work.*

Engagement of TNF receptor 1 by TNFa in the fibrosarcoma cell line L929 activates NF-κB and a caspase-independent cell death pathway that requires the generation of oxidative stress in the mitochondria. We have recently shown that TNF induces a substantial increase in the cytotoxic glycolytic metabolite methylglyoxal, which, together with the phosphorylation of glyoxalase I, leads to the formation of specific methylglyoxal-derived Advanced Glycation End products (AGEs). Here we report that TNF-induced cell death, but not TNF-induced NF-κB activation, is mediated through the Receptor for AGEs (RAGE), a multi-ligand cell surface receptor implicated in a variety of diseases. Within 5 to 10 min, TNF induces a nucleocytoplasmic translocation of full-length RAGE. When cell death proceeds, TNF induces a considerable reduction in the amount of FL-RAGE and its higher molecular weight complexes, which is accompanied by the generation of a truncated form of RAGE in the cytosol. Overexpression of the extracellular ligand binding domain of RAGE (sRAGE) or inhibition of a secretory pathway by Brefeldin A strongly inhibits TNF-induced cell death as well as the TNF-induced nucleocytoplasmic translocation of FL-RAGE and the subsequent reduction of FL-RAGE and its higher molecular weight complexes, but does not affect the TNF-induced NF-κB activation. We propose that TNF induces a ligand-dependent dislocation of full-length RAGE from the endoplasmatic reticulum into the cytosol. There, FL-RAGE can have two fates: either it can be translocated to the nucleus, or it can be degraded or processed in the cytosol via a yet unknown mechanism.

Calpains are involved in the activation of both AIF and caspase-12 that mediate apoptosis in retinal degeneration

Daniela Sanges[1], Roberta Tammaro [2] and Valeria Marigo[1,2]

[1] *Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy*
[2] *Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy*

Programmed cell death has a crucial role in the pathogenesis and the course of a wide spectrum of neurodegenerative diseases including Retinitis Pigmentosa (RP). This form of retinal degeneration, that form the largest cause of blindness in the developed world, results from the photoreceptor cell death. However the molecular mechanisms underlying apoptosis during retinal degeneration are still elusive and this hampers the development of a cure for this blinding disease. An autosomal recessive form of RP, that naturally occurs in the *rd1* mouse model, is caused by a mutation in the β catalytic subunit of the phosphodiesterase gene (*PDE6B*). Lack of PDE activity controlling calcium influx through cGMP-gated channels, lead to the increase in intracellular calcium that has been linked with apoptosis in this animal model.
In this study we characterized *in vitro* differentiated retinal stem cells in which spontaneous cell death is caused by lack of Pde6b activity. We show that two apoptotic pathways, one from the mitochondrion and one from the endoplasmic reticulum, are co-activated during the degenerative process in this *in vitro* model of photoreceptor degeneration. We found that *in vitro* and *in vivo* both AIF and caspase-12 move to the nucleus in the same apoptotic photoreceptors and that their co-activation is dependent on intracellular calcium. As seen by treatment with pan-caspase inhibitor and calpain inhibitors, the apoptotic mechanism is not mediated by executor caspases but by calpains. Knock-down experiments defined that AIF plays a key role in the apoptotic death, while caspase-12 has only a reinforcing effect.
Most interestingly, by blocking calpain activity *in vitro* and *in vivo*, we abolish apoptosis and activation of AIF and caspase-12 in the degenerating retina. This study provides the first link between two caspase independent apoptotic pathways involving mitochondrion and endoplasmic reticulum in a degenerating neuron.

Tissue transglutaminase promotes alternative cell death of erythrocytes

Zsolt Sarang, Andras Madi and Zsuzsa Szondy

Department of Biochemistry and Molecular Biology, University of Debrecen, Hungary

Mature erythrocytes can undergo a rapid self-destruction process sharing several features of apoptosis, including cell shrinkage and phosphatidylserine (PS) externalization. During this regulated form of programmed cell death intracellular Ca2+ concentration increases and activates the Ca2+ regulated enzymes μ-calpain and tissue transglutaminase (TG2). By crosslinking several membrane skeletal proteins TG2 has been shown to be responsible for the Ca2+ -induced irreversible shape change and permanent loss of membrane deformability in dying erythrocytes. Using TG2-/- erythrocytes we demonstrate that in the absence of TG2 the uptake of cells by macrophages and the externalization of PS upon calcium ionophore treatment are delayed without any alterations in the cell surface distribution of PS as compared to wild-type cells. On the other hand, following hyperosmotic shock or oxidative stress, signals known to elicit cell shrinkage and annexin-V binding, we found no delay in the PS exposure in TG2-/- cells. By performing two dimensional gel electrophoresis and subsequent mass spectrometric analysis using samples from calcium ionophore treated, biotinylated monodansylcadaverine loaded erythrocytes, we have identified calpain small subunit 1 (CSS1) as a new TG2 substrate. Western blot analysis also revealed that the calpain large and small subunits are crosslinked by TG2. To determine the effect of TG2-dependent crosslinking on calpains, the calpain activity was determined in both TG2+/+ and -/- erythrocytes before and after calcium ionophore treatment using the fluorescent substrate N-succinyl-Leu-Tyr-7-amido-4-methylcoumarin and by monitoring the degradation of the calpain substrate protein spectrin. We found that the calcium ionophore-treated TG2+/+ erythrocytes displayed the highest calpain activity. Our data suggest that TG2 promotes the rate of calcium ionophore-induced cell death by regulating calpain activity.

Supported by the Hungarian Research Fund (OTKA T049445) and by the Hungarian Ministry of Health (ETT 100/2003).

State of proteins B23/nucleophosmin and UBF in HeLa cells during apoptosis induced by alpha-TNF/emetine

E. Sautkina, N. Potapenko, N. Vladimirova

Department of Structural Biochemistry, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry Russian Academy of Sciences, Moscow, Russia, e-mail: vla@ibch.ru,

The structural states of two major nucleolar proteins, UBF and B23/nucleophosmin (both monomeric and oligomeric forms) were analyzed in samples containing 25, 45, and 100% of cells with apoptotic nuclei. It was shown by immunoblotting that TNF-α-induced apoptosis of HeLa cells was associated with proteolysis of UBF and production of a 76-kD fragment, the content of which increased in correlation with the fraction of apoptotically changed cells. The N- and C-terminal amino acid sequences of UBF and its 76-kD fragment were characterized, and the site of the apoptosis-induced specific proteolysis was identified. Unlike UBF, nucleophosmin was not cleaved during the course of apoptosis in TNF-treated HeLa cells and its content was not changed even in cells with fully fragmented nuclei. However in apoptotic cells that reached terminal stages of apoptosis the ratio of monomeric forms to oligomeric forms of the nucleophosmin was different from that of the control cells. Moreover, the apoptosis-specific forms of the B23 protein were detected. This study was supported by the Russian Foundation for Basic Research (project 06-04-48388)

Search by virtual screening techniques for XIAP inhibitors which target BIR3 domain.

Lilianna Schyschka[1], Nicole Barth[1], Caroline Bliem[2],Hermann Stuppner[2] and Angelika M. Vollmar[1]

[1]Department of Pharmacy, Center of Drug Research, University of Munich, Munich, Germany
[2]Department of Pharmacognosy, Institute of Pharmacy, University of Innsbruck, Innsbruck, Austria

IAPs (inhibitor of apoptosis proteins) play a pivotal role in regulation of apoptosis, cell cycling, and ubiquitylation. XIAP is the most potent member of eight mammals IAPs, which inhibited caspase -9, -3 and -7 activities. A number of cancer cells overexpress XIAP, what make them resistant to multidrug chemotherapy. The effect of XIAP on apoptosis can be antagonized for example by the mitochondrial proteins SMAC/DIABLO and Omi/HtrA2 as well as regulated by calpains or the proteasome. Through BIR3 domain XIAP binds to active caspase-9, and therefore blocks caspases cascade at initiator level. For that reason it would be of high interest to identify specific inhibitors which interact with this domain. Through virtual screening of commercial databases various compounds, both synthetic and of natural origin, were identitied as potential XIAP inhibitors. The respective compounds were tested in wild type and XIAP overexpressing human leukemia Jurkat T cells for induction of apoptosis either by themselves or in combination with low dose of etoposide. Some of the compounds in fact are able to sensitize for etoposide-induced apoptosis, and may have the potential to overcome the apoptosis resistant effect of XIAP.

Cis-platinum treatment of subconfluent NIH/3T3 cultures induces a form of autophagic cell death in polyploid cells

Alessandra Spano, Gianni Monaco, Sergio Barni* and Luigi Sciola

*Department of Physiological Biochemical and Cellular Sciences, University of Sassari; *Department of Animal Biology, University of Pavia, Italy. e-mail: sciola@uniss.it*

In this work, we have analysed the effects induced by different concentrations (50, 75, 100 μM) of the cytostatic drug cis-platinum (cDDP) in the NIH/3T3 cells. Cultures of this mouse fibroblast line, in conditions of cell subconfluence, showed the appearance of polyploid cells with DNA content values ranged between 6c and 24c. The results obtained after cDDP treatment demonstrated that concentrations of 50 and 75 μM induced a cytostatic effect, while 100 μM concentration surprisingly showed a lower antiproliferative action. All treatment conditions caused cell detachment with the appearance of classical apoptosis, which incidence appeared to be drug concentration-dependent. Morphological analysis of still adherent cells demonstrated the presence of patterns of alternative cell degeneration, especially in polyploid cells, with strong alterations at both nuclear and cytoplasmic levels. In particular, there were events of micronucleation and intense phenomena of multilobulation and furrows of the nucleus that preceded the formation of nuclear fragments with heterogeneous size; these nuclear changes appeared to be correlated with the actin cytoskeleton reorganization at the perinuclear area. The ultrastructural and immunofluorescence analyses at cytoplasmic level showed the appearance of intermediate filament bundles and the persistence of microtubular network. Transmission electron microscopy also showed the presence of autophagocytotic phenomena accompanied by an increase of secondary lysosomes, residual bodies and myelin figures, indicative of intense autodegradation of endomembranes and organelles. In the complex, these cellular aspects indicated events of autophagic apoptosis in which, as known, autophagosome formation seemed to be dependent on microfilaments and intermediate filaments, while the successive fusion with lysosomes seemed to be dependent on microtubules. Furthermore, the persistence of cytoskeletal components, at least up to advanced stages of degenerative phenomena, indicated the lack, at cytosol level, of the proteolytic activity expressed during the typical apoptosis. Our data permitted to conclude that the same pharmacological treatment was able to induce different mechanisms of cell degeneration related to cytokinetic activity, cell size and ploidy. More actively proliferating cells (DNA content 2c – 4c) died throughout typical apoptosis, while polyploid cells degenerated by means of mechanisms of alternative cell death, partly referable to autophagic apoptosis.

Nucleolus disassembly in mitosis and apoptosis: dynamic redistribution of phosphorylated-c-Myc, fibrillarin and Ki-67

Cristiana Soldani [1], Maria Grazia Bottone[1,2], Carlo Pellicciari[1] and Anna Ivana Scovassi[2]

1Dipartimento di Biologia Animale, University of Pavia, and 2Istituto di Genetica Molecolare del CNR, Pavia, Italy, e-mail: scovassi@igm.cnr.it

During apoptosis, the complex structure of nucleolus is irreversibly dismantled; in particular, the dense-fibrillar (DFC) and granular (GC) components segregate and may form, in late apoptosis, heterogeneous aggregates with other RNP-containing structures which migrate to the cytoplasm. We investigated in HeLa cells the rearrangement of three representative nucleolar proteins, i.e. fibrillarin (that is a marker of the nucleolar DFC), and Ki-67 and phosphorylated c-Myc (which are both involved in cell proliferation control). The analysis was carried out by conventional and confocal microscopy after fluorescence immunolabeling in order to elucidate whether these nucleolar proteins are differently redistributed and/or degraded during apoptosis. We also followed their dynamic redistribution during mitosis, when the nucleolus is reversibly disassembled with a temporaneous relocation of its components to different subcellular regions. We observed that in interphase control cells, fibrillarin and P-c-Myc always colocalize in the nucleolus, whereas Ki-67 does not, consistent with its cycle-related redistribution in interphase; during mitosis, P-c-Myc, Ki-67 and fibrillarin redistribute differently and never colocalize, until the completion of cytodieresis. In early apoptotic nuclei these nucleolar proteins undergo segregation, and may migrate to the cytoplasm with the final formation of apoptotic blebs containing different nucleolar proteins. This evidence confirms that the apoptotic bodies may be variable in size and content, and include heterogeneous aggregates of proteins and/or nucleic acids of nuclear origin. Proteolysis is not a constant feature for nucleolar proteins, during apoptosis. Indeed, we found that Ki-67 is cleaved during apoptosis, being immunodetectable only in early apoptotic cells; on the contrary, undegraded fibrillarin and P-c-Myc move into the cytoplasm and are found in apoptotic blebs, where they often do not colocalize. In conclusion, during apoptosis the nucleolar proteins, P-c-Myc, fibrillarin and Ki-67 do have distinct fates being either cleaved (Ki-67) or extruded into the cytoplasm with a different kinetics in an ordered, non chaotic program.

Antisense treatment against anti-apoptotic genes induces apoptosis and acts as chemoadjuvant in head and neck cancer.

Himani Sharma and Neeta Singh

Department of Biochemistry, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029.INDIA.

We have earlier reported that the inhibition of apoptosis in head and neck squamous cell carcinomas (HNSCC) is because of upregulated expression of Bcl-2, Bcl-XL and Survivin. Hence, we addressed the question whether antisense approach towards these inhibitors of apoptosis could restore the apoptosis in HNSCC. Further, we wanted to see whether chemotherapeutic efficacy of Cisplatin could be enhanced by using this drug in combination with antisense oligonucleotides in human laryngeal carcinoma HeP2 and cells. The effect of these antisense oligonucleotides was examined on the mRNA expression by RT-PCR and on protein expression by Western blotting. Apoptosis was measured by flowcytometry, TUNEL assay and caspase-3 activity assay. Treatment of HeP2 cells with 400nM antisense oligonucleotides against Bcl-2, Bcl-XL and Survivin for 48 hrs decreased their expression both at the mRNA as well as at the protein level, resulting in the induction of apoptosis. Treatment of HeP2 cells with these antisense oligonucleotides augmented Cisplatin induced apoptosis. Our findings emphasize the importance of Bcl-2, Bcl-XL and Survivin as survival factors in HNSCC cells. Antisense treatment against these survival factors in combination with lower doses of chemotherapy offers potential as a less toxic chemoadjuvant therapy.

Molecular events associated with a-Lipoic acid-induced Apoptosis of Hepatoma cells

Gabriella Simbula, Laura Sanna, Marta A. Kowalik, Manuela Deidda, Amedeo Columbano, Giovanna M. Ledda-Columbano, Monica Pibiri.

Department of Toxicology, Unit of Oncology and Molecular Pathology, University of Cagliari, Italy, email:gsimbula@unica.it

Lipoic acid is a thiol containing antioxidant widely used for the treatment of a variety of diseases, including liver cirrosis, heavy metal poisoining, and free-radical related diseases, such as diabetic polyneuropathy. In addition to its recognized protective effect against oxidative stress, lipoic acid has been shown to induce apoptosis in leukaemic and colon cancer cells. However, little is known about the apoptotic effect of LA in hepatoma cells. Herein, we investigated i) whether lipoic acid induces apoptosis in two different hepatoma cell lines, FaO and HepG2, and ii) the molecular events underlying its apoptotic effect. The results showed that lipoic acid inhibits the growth of both FaO and HepG2 cells as indicated by reduction in cell number, reduced expression of cyclin A and increased levels of the cyclin/CDKs inhibitors p27 and p21. Cell cycle arrest was accompanied by a decreased cell viability and DNA laddering pattern indicative of apoptosis. Lipoic acid-induced apoptosis was associated with ROS generation, p53 activation, increased expression of Bax, release of cytochrome–c from mitochondria to the cytoplasm, activation of caspases 3, 6 and 9 and of the anti-apoptotic protein survivin. Moreover, α-lipoic acid treatment was accompanied by activation of pro-apoptotic signaling (i.e JNK), and inhibition of antiapoptotic signaling (i.e. PKB/Akt) pathways. In conclusion, this study provides evidence that lipoic acid induces apoptosis in both FaO and HepG2 hepatoma cell lines and suggests that it may prove useful in liver cancer therapy.

Mast cells induce cell death via secreted granzyme B

Simon, Markus M[1], Wallich, Reinhard[2], Ebnet, Klaus[3], Iden, Sandra[3], Martin, Praxedis[1], Ekiciler, Aynur[1], Müllbacher, Arno[4], Huber, Michael[5], Pardo, Julian[1]

[1]Metschnikoff Laboratory, Max-Planck-Institut for Immunbiology, D-79108 Freiburg, Germany, [2]Institute for Immunology, University Clinic Heidelberg, Heidelberg, Germany, [3]Institute of Cell Biology, ZMBE, University of Muenster, D-48149 Muenster, [4]John Curtin School of Medical Research, Australian National University, Canberra, Australia, [5]Molecular Immunology, Biology III, University Freiburg & Max-Planck-Institut for Immunbiology, Freiburg.

The proteases, granzyme (gzm) A and gzmB have been mainly linked to NK cells and cytotoxic T lymphocytes (CTL) and their lytic machinery so far. Together with perforin (perf) both gzms are released through exocytosis of NK/CTL secretory granule contents into a synapsis formed between the killer cell and its target cell. After their access to the target cell cytosol they initiate alternative, but strictly perf-dependent, proteolytic pathways leading to apoptosis (1). However, in addition to these intracellular activities, gzmA and gzmB may also function extracellularly. Evidence suggest that both gzms cleave components of extracellular matrices and basal laminae, such as fibronectin, vitronectin, collagens and laminin and that these processes may result in cell detachment of susceptible targets and, indirectly, in their delayed death, termed anoikis (2-5).
Here we present evidence that differentiated mouse bone marrow derived mast cells (BMMC) express granzyme (gzm) B, but not gzmA or perforin. GzmB is associated with cytoplasmic granules of BMMC and secreted after Fcε-receptor mediated activation. BMMC from wild type but not gzmB-deficient mice cause cell death in susceptible adherent target cells indicating that the perforin-independent cytotoxicity of BMMC is executed by gzmB. Together with the finding that gzmB also induces a disorganization of endothelial cell-cell contacts the data suggest that activated mast cells contribute, via secreted gzmB, to anoikis of susceptible perivascular target cells, increased vascular permeability, leukocyte extravasation and subsequent inflammatory processes, including atherosclerosis and rheumatoid arthritis.

1-Pardo, J., et al. 2004, J Cell Biol. 167:457-68.
2-Choy, J.C., et al. 2004, Arterioscler Thromb Vasc Biol. 24:2245-50.
3- Buzza, M.S., et al. 2005, J Biol Chem. 280:23549-58.
4- Simon, M.M., M.D. et al. 1991, Immunology. 73:117-9.
5- Simon, M.M. et al. 1988, Biol Chem Hoppe Seyler. 369 Suppl:107-12.

Mitoptosis, elimination of mitochondria

V.P. Skulachev

Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119992, Russia. E-mail skulach@belozersky.msu.ru

A concept has been put forward (V Skulachev, IUBMB Life 2000; 49: 365-373) that such complex biological systems as mitochondria, cells and organisms are equipped with mechanisms of self-elimination which are actuated when the system in question appears to be unwanted for system(s) of higher positions in biological hierarchy ("Samurai principle"). Some examples of operation of this principle in malfunctioning (ROS-overproducing or ATP-hydrolyzing) mitochondria will be presented.

(1) ROS overproduction results in (i) oxidative modification of an inner mitochondrial membrane (IMM) protein (most probably, oxidation of some SH-groups in the ATP/ADP antiporter), (ii) permeability transition pore opening, an event entailing collapse of electric potential difference across IMM and (iii) cessation of electrophoretic stage of import by mitochondria of precursor of mitochondrial proteins. Moreover, ROS inactivate aconitase which functions not only as the initial enzyme of the Krebs cycle but also as a mitochondrial DNA-stabilizing protein (like histones for nuclear DNA). As a result, mitochondria perish (mitoptosis), the mitochondrial remains being consumed be autophagosomes (mitophagia).

(2) Treatment of mitochondria with an uncoupler and a respiratory chain inhibitor converts the organelles from ATP producers to ATP consumers. We have found that in some cells possessing active glycolysis, such a treatment initiates (i) decomposition of extended mitochondria to small spherical mitochondria ("the tread-grain transition"), (ii) their movement to the nucleus and concentration in certain place near the nuclear membrane, (iii) formation of a very large, single-membrane surrounded mitochondrial cluster ("mitoptotic body"), (iv) exocytosis of this body from the cell which survives for certain time without mitochondria, using glycolysis an the only energy source. Possible involvement of the type two mitoptosis in elimination of mitochondria during maturation of erythrocytes and lens fiber cells is considered.

Amazing journey down the tube – enterocyte turnover along the gastro-intestinal tract in young rats

Paulina Slazak, Michal M. Godlewski, Agnieszka Piastowska, Mikolaj A. Gralak

Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Nowoursynowska 159, 02-776 Warsaw, Poland

This preliminary study aimed to evaluate the kinetics of cell turnover in the intestinal mucosa of young rats for further evaluation of diet-supplemented biologically active substances on enterocyte turnover. The process of remodeling of intestinal mucosa is associated with changes in the ratio of proliferation (mitosis) to programmed cell death (apoptosis and autophagy) of enterocyte populations. This study is the first description of the dynamics of mitosis, apoptosis and autophagy along the entire gut. In the study 8 week-old healthy rats from control group were used (n=10). Mitosis was evaluated on the basis of Ki67 expression, apoptosis and autophagy – on the expression of active caspase 3 and MAP I LC-3, respectively. Evaluated indexes for duodenum, prox-jejunum, mid-jejunum, distal-jejunum and ileum were: 7.4%, 13.6%, 11.1%, 10.3% and 8.7% for mitosis, 28.2%, 25.6%, 12.1%, 20.6%, 12.3% for apoptosis and 1.7%, 2.2%, 1.5%, 1.5%, 1.1% for autophagy. Interesting feature was observed in duodenum. Half of the rats exhibited high apoptosis index (32.0 – 43.6%) whereas other half low (9.8 – 16.5%). No significant changes in growth rate or health between these rats were observed. This observation agrees with other authors describing variations in the enzyme activity and morphology of duodenum of young animals. This phenomenon could be due to extreme conditions an variations in pH caused by gastric digesta content appearing in duodenum.

The effect of leptin and ghrelin on survivin expression in the small intestinal mucosa of neonate piglets

Monika Slupecka and Jaroslaw Wolinski

The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jablonna, Poland

The aim of the present study was to investigate the effect of leptin and ghrelin on survivin expression in the small intestinal mucosa of neonate piglets. Survivin is a member of the inhibitor of apoptosis gene family that is expressed during development and in human cancer *in vivo*. Its expression was also found in normal: human endometrium, human colonic mucosa and gastric mucosa. Leptin and ghrelin are hormones that are involved in the small intestinal development in neonatal piglets. It has been shown that leptin enhances, while ghrelin delays growth and maturation of the small intestinal mucosa. These hormones were found in substantial amounts in sow colostrum and milk.

Four groups of piglets were studied: sow reared (SR), fed with artificial milk (C7), fed with milk formula plus leptin (10µg/kg b. wt.) administration (L10) and fed with milk formula plus ghrelin (7,5 µg/kg b. wt.) administration. Staining for survivin was observed in all epithelial cells in groups: C7, ZG, L10 and confirmed by Western blot analysis. Survivin was expressed in both nuclei and cytoplasm of epithelial cells. Interestingly, there was no survivin expression in group of piglets fed first with colostrum and than with sow's milk (SR).

Present study claims that kind of feeding influence on the survivin expression in the small intestinal mucosa and the role of leptin and ghrelin supplementation require further studies.

Survival signaling by C-RAF: mitochondrial ROS and Ca2+ are critical targets

Julija Smigelskaite[1], Andrey V. Kuznetsov[1], Christine Doblander[1], Manickam Janakiraman[1], Martin Hermann[2], Martin Wurm[2], Robert Sucher[1], and Jakob Troppmair[1]

[1]Daniel Swarovski Research Laboratory, and [2]KMT Laboratory, Department of General- and Transplant Surgery, Innsbruck Medical University, Innsbruck, Austria

Gene ablation and overexpression studies demonstrated the antiapoptotic activity of RAF. Survival signaling by RAF includes inactivation of pro-apoptotic (MST2, ASK1, BAD) as well as transcriptional upregulation of antiapoptotic proteins and the activation of additional survival pathways, which maintain mitochondrial integrity and thereby assure continued energy production and cell survival. These diverse safeguard mechanisms allow cells to cope with varying apoptotic stimuli. Currently we do not know whether there is a common intermediate in cell death signaling, which initiates the collapse of mitochondrial membrane integrity and the resulting cell death. In the study presented here, we provide evidence that reactive oxygen species (ROS) may fulfill such a role and demonstrate that they are critical determinants of RAF- suppressible cell death induction. Growth factor removal from the IL-3 dependent promyeloid cell line 32D results in the production of ROS, which is not observed in cells protected by either IL-3 or oncogenic C-RAF (vRAF). Antioxidants not only decreased intracellular ROS levels but at the same time delayed the onset of apoptotic cell death caused by IL-3 withdrawal or treatment with staurosporine (STS) or the direct-acting oxidative stress-inducing agent *tert* butyl hydroperoxide (*t*-BHP). Our experiments demonstrate that cell death under conditions of enhanced ROS production goes along with increased mitochondrial Ca^{2+} levels in control but not in vRAF expressing 32D cells. Preventing mitochondrial Ca^{2+} overload after IL-3 deprivation delayed apoptotic cell death. Taken together, our data show that survival control by C-RAF impinges on controlling cellular ROS production, which otherwise perturbs mitochondrial Ca^{2+} homeostasis. A possible link between RAF signaling and the mitochondrial events observed here will be presented.

p21 blocks irradiation-induced apoptosis downstream of mitochondria by inhibition of cyclin-dependent kinase-mediated caspase-9 activation

Dennis Sohn, Frank Essmann, Klaus Schulze-Osthoff and Reiner U. Jänicke

Institute of Molecular Medicine, University of Düsseldorf, Universitätsstrasse 1, 40225 Düsseldorf, Germany, e-mail: Dennis.Sohn@uni-duesseldorf.de

The role of the cyclin-dependent kinase (CDK) inhibitor p21 as a mediator of p53-induced growth arrest is well established. In addition, recent data provide strong evidence for new emerging functions of p21 including a role as a modulator of apoptosis. The mechanisms, however, by which p21 interferes with the death machinery, especially following ionizing radiation (IR), are largely unknown. Here we report that IR induced caspase-9 and –3 activation and subsequent apoptosis only in p21-deficient colon carcinoma cells, whereas similar treated wild-type cells were permanently arrested in the G2/M phase, correlating with the induction of cellular senescence. Interestingly, activation of the mitochondrial pathway including caspase-2 processing, depolarization of the outer mitochondrial membrane, and cytochrome c release was achieved by IR in both cell lines, indicating that p21 inhibits an event downstream of mitochondria, but preceding caspase-9 activation. IR-induced p21 protein expression was restricted to the nucleus and no evidence for a mitochondrial or cytoplasmic association was found. In addition, p21 did neither interact with caspase-3 or -9, suggesting that these events are not required for the observed protection. Consistent with this assumption, we found that CDK inhibitors potently abrogated IR-induced caspase processing and activation without affecting mitochondrial events. Also in vitro caspase activation assays yielded higher caspase-3 activities in extracts of irradiated p21-deficient cells compared to extracts of similar treated wild-type cells. Thus, our results strongly indicate that p21 protects cells from IR-induced apoptosis by suppression of CDK activity that appears to be required for activation of the caspase cascade downstream of the mitochondria.

Changes in the organization of the Golgi apparatus and the endoplasmic reticulum during apoptosis

C. Soldani[1], M.G.Bottone[1,2], C. Alpini[3], A. Fraschini[1] and C. Pellicciari[1]

[1]Dipartimento di Biologia Animale, University of Pavia; [2]Istituto di Genetica Molecolare (Sezione di Istochimica e Citometria) del CNR; [3]Laboratorio di Analisi Biochimico-Cliniche, IRCCS San Matteo, Piazzale Golgi 1, Pavia, Italy, e-mail: soldani@unipv.it

In interphase cells, the Golgi apparatus (GA) exists as stacks of cisternae organized as a perinuclear lace-like reticulum. During the cell cycle, GA as well as all other membranous organelles must grow before undergoing division into a multitude of tubulo-vesicular clusters and partitioning between the two daughter cells, at mitosis. It is worth considering that GA vesicles and cistermae are often found in association with the microtubule cytoskeleton, both in interphase and mitosis. A disassembling of GA was also observed to occur during apoptosis when all the cytoplasmic organelles undergo significant changes in their distribution and structure, essentially due to the concerted action of activated caspases. In this study, we aimed at describing the changes in the organization of endoplasmic reticulum (ER) and GA during apoptosis, in relation to the rearrangement of the microtubular cytoskeleton. HeLa cells have been induced to apoptosis in response to different stimuli, i.e. drugs which arrest dividing cells by causing DNA damage or by targeting products of proliferation-related genes. Conventional and confocal fluorescence microscopy and electron microscopy have been used to investigate the reorganization of cytoplasmic organelles after specific cytochemical techniques. We found that the ER and GA disperse during apoptosis, without massive degradation, as much as it occurs during their mitotic fragmentation before cytodieresis. Interestingly enough, this reorganization during apoptosis is paralleled by restructuring of the microtubular cytoskeleton: this confirms the strict structural relationship between ER or GA and the microtubules, and is fully consistent with the evidence that a reversible fragmentation and dispersal of GA and ER may be elicited by the drug-induced depolymerization of microtubules. The evidence that apoptosis may be induced by agents (such as the photosensitizing agent, Rose bengal acetate) which directly damage ER and GA membranes suggest that these organelles my act as stress sensors in the activation of the caspase cascade.

Vap-1/SSAO-mediated Methylamine Oxidation Induces Apoptosis on Vascular A7r5 and Huvec Cell Types.

Solé M[1], Hernández M[1], Boada M[2,3] and Unzeta M[1]

[1]*Institut de Neurociències. Departament de Bioquímica i Biologia Molecular, Facultat de Medicina, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Espanya.*
[2]*Servei de Neurologia, Hospital G. U. Vall d'Hebron . Barcelona, Espanya.*
[3]*Fundació ACE. Institut Català de Neurociències Aplicades. Barcelona, Espanya.*

Semicarbazide sensitive amine oxidase (SSAO), is a multifunctional enzyme (*O'Sullivan J. et al., 2004*) showing different biological roles depending on the tissue were it's expressed. SSAO is present in almost all mammalian tissues (*Andres N. et al., 2001*), specially those highly vascularized. It metabolizes primary amines (*Lyles GA., 1996*) generating hydogen peroxide (H_2O_2), ammonia (NH_3) and the corresponding aldehyde as final toxic products able to contribute to cellular oxidative stress. SSAO shows also an insulinomimetic effect in adipocytes (*Enrique-Tarancón G. et al., 1998*), and it behaves as a vascular adhesion protein (VAP-1) in endothelial cells. However, its physiological function is still far from clear. SSAO/VAP-1 is expressed in the cells in a membrane-bound form and is also present as a soluble enzyme in plasma; in this concern, it has been postulated (*Abella A, et al., 2004*) that the soluble form comes from the membrane-bound one by a shedding process metalloprotease-dependent. We have described that SSAO/VAP-1 is overexpressed in human cerebrovascular tissue from Cerebral Amyloid Angiopathy linked to Alzheimer's Disease (CAA-AD) (*Ferrer I. et al., 2002*) and also that the plasma SSAO activity increases significantly with the severity of Alzheimer's Disease (*del Mar Hernandez M. et al., 2005*). We have also reported that the soluble form of SSAO/VAP-1 catalysis induces apoptosis in vascular smooth muscle cells (*Hernandez M. et al., 2006*). The aim of this work has been to study the cytotoxic effect of the methylamine oxidation by the transmembrane form of this enzyme. Because of the SSAO/VAP-1 expression fenotype is lost in cultured cells, A7r5 (smooth muscle cells from rat aorta) and HUVEC (human umbilical vein endothelial cells), were stably transfected with vector pcDNA 3.1 containing hVAP-1/SSAO. Cells were treated with different concentrations of Methylamine (1-3mM), the physiological SSAO substrate. A time-dependent and dose-response cytotoxic effect was observed. The activation of caspase-3 corroborates that apoptosis is involved in this cell death. This results allow us to suggest that in pathological conditions such as CAA-AD, in which SSAO is overexpressed (*Ferrer I. et al., 2002*), this enzyme through its catalytic action could contribute to the vascular tissue damage observed in CAA-AD.

ATM deficiency triggers resistance to Fas-induced apoptosis in lymphoid cells

Maria Giovanna di Bari# (1, 2), Venturina Stagni# (1, 2,), Ivano Condò (2), Roberto Testi (2), Yaniv Lerenthal (3) Enrico Cundari (4) and Daniela Barilà (1, 2, 5)*

(1) Dulbecco Telethon Institute, Department of Experimental Medicine and Biochemical Sciences, University of Rome "Tor Vergata", Via Montpellier, 1 00133 Rome, Italy.
(2) Laboratory of Immunology and Signal Transduction, Department of Experimental Medicine and Biochemical Sciences, University of Rome "Tor Vergata", Via Montpellier,1 00133 Rome, Italy.
(3) The David and Inez Myers Laboratory for Genetic Research, Department of Human Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel.
(4) Istituto di Biologia e Patologia Molecolari Consiglio Nazionale delle Ricerche, 00185 Rome, Italy.
(5) Fondazione Santa Lucia, 00179, Rome, Italy

Ataxia Telangiectasia (AT) is a rare cancer predisposing genetic disease, caused by the lack of functional ATM kinase, which is a principle actor of the DSB DNA-damage response. AT patients show a broad and diverse phenotype which in part is seen in an increased rate of lymphoma and leukemia development. Fas-induced apoptosis plays a fundamental role in the homeostasis of the immune system and its deficiency has been associated to autoimmunity and to lymphoma development.
We therefore investigated the role of ATM kinase in Fas-induced apoptosis. Using a lymphoid cell line we could show that Fas-receptor stimulation triggers the activation of an ATM-mediated response similar to the typical DNA damage-induced ATM activation. This implied the phosphorylation of ATM at Ser1981 and of its downstream substrates p53, Chk2 and H2AX. According to previous reports, ATM was cleaved by caspases and, interestingly, only caspase-cleaved ATM was found phosphorylated at Ser1981. Immunofluorescence experiments showed that ATM phosphorylation at Ser1981 occurs only in those cells exhibiting a clear apoptotic morphology, characterized by condensed nuclei. This observation, together with the finding that p53, Chk2 and H2AX phosphorylation is completely dependent on caspase activity, suggests a link between Fas-dependent ATM activation and apoptotic DNA fragmentation. In this system, ATM seems to play a pro-apoptotic role since ATM deficient cells were resistant to Fas-induced apoptosis. This is consistent with the finding that AT cells up-regulate FLIP-S protein, a well known inhibitor of Fas-induced apoptosis. Reconstitution of ATM activity was sufficient to decrease FLIP levels and to restore Fas-sensitivity. Overall, these data suggest a novel molecular mechanism through which ATM deficiency promotes lymphoma development.

Pyk2 involvement in membrane depolarization-promoted cerebellar granule neuron survival.

Strappazzon Flavie*, Torch Sakina*, Trioulier Yaël #, Blot Béatrice*, Sadoul Rémy*, Verna Jean-Marc*.

**Institut National de la Santé et de la Recherche Médicale (INSERM), Laboratoire Neurodégénérescence et Plasticité, Equipe Mixte INSERM 0108, and University Joseph Fourier, Grenoble I, F-38043 Grenoble, France.*
 # URA CEA-CNRS 2210, Service Hospitalier Frédéric Joliot, DRM, DSV, CEA, 4 place du Général Leclerc, F-91401 Orsay cedex, France.

The 110-kDa nonreceptor tyrosine kinase Pyk2 is a member of the focal adhesion kinase (FAK) family. Upon stimulation, Pyk2 undergoes trans-autophosphorylation at Tyr^{402}, inducing its activation. A variety of extracellular stimuli activate Pyk2, including stress signals and stimuli that elevate cytoplasmic free Ca^{2+} concentration and/or activate protein kinase C. Chronic membrane depolarization greatly enhances the survival of many types of neurons in culture. In particular, cerebellar granule neurons (CGN) can survive *in vitro* in high potassium (25 mM KCl) concentration in the absence of serum or growth factors but die when shifted to physiological K^+ concentration (5 mM KCl). We investigated whether Pyk2 is involved in CGN survival induced by depolarization. We show that CGN grown in depolarizing K25 medium exhibit detectable Pyk2 activity, which depends on membrane depolarization-induced Ca^{2+} influx. In addition, we demonstrate that Pyk2 phosphorylation requires Calmoduline (CaM) activity as W13, a CaM antagonist, abolishes depolarisation-induced Pyk2 phosphorylation. We also provide findings showing that a 70% increase in Tyr^{402}-phosphorylated Pyk2 levels occurs following induction of apoptosis by potassium depletion. This early and transient enhancement of Pyk2 activity is likely triggered by Ca^{2+} release from intracellular stores as it is suppressed by chelation of cytosolic Ca^{2+} with BAPTA-AM. Finally, we demonstrate that silencing Pyk2 activity by expressing a dominant-negative Pyk2 mutant leads to the extensive death of CGN grown in 25 mM KCl-containing medium; only 40% of the mutant Pyk2-expressing neurons were alive 24h after transfection when compared to control. By contrast, the overexpression of wild-type Pyk2 blocks CGN apoptosis induced by K^+ lowering, increasing CGN survival by 70 % 24h after medium shift when compared to control. Overall, the present findings demonstrate for the first time the role of Pyk2 in mediating the Ca^{2+}, CaM-dependent survival effect of membrane depolarization on CGN.

Apoptosis of photoreceptors and second order neurons in animal models of Retinitis Pigmentosa.

Enrica Strettoi, Francesca Mazzoni, Elena Novelli, Eva Terzibasi, Chiara Rossi

Istituto di Neuroscienze del CNR, Area della Ricerca, Via Giuseppe Moruzzi 1, 56100 Pisa, Italy

Retinitis pigmentosa (RP) comprises a group of inherited retinal dystrophies causing progressive photoreceptor degeneration and blindness. In typical RP, a primary genetic defect is found in rod photoreceptors, which die progressively by an apoptotic mechanism. At later times, cones are also lost.
We used well known animal models of RP to investigate a) photoreceptor apoptosis; 2) the secondary degeneration of neurons postsynaptic to photoreceptors. These cells represent the biological platform to attempt restoring vision in RP by means of electronic prostheses or cellular transplantation and are therefore very important for perspective treatment of RP. Rd10 and rd1 mutant mice were used. Using various morphological techniques, we found that photoreceptor degeneration in the rd10 retina peaks between 20 and 30 days. Ethidium staining showed nuclear condensation (pycnosis) in degenerating photoreceptors, which could also be labeled with Fluoro Jade histochemistry and TUNEL staining, indicative of neuronal damage and DNA fragmentation. Antibodies against the activated form of the pro-apoptotic enzyme Caspase 3 also showed a specific pattern of staining of photoreceptors, particularly around P30.
The morphology and distribution of second order neurons remained normal up to P30, when most rods have degnerated. Later, rod bipolar cells exhibited progressive dendritic atrophy and loss of postsynaptic receptors, until a cellular loss of about 20% was detected around 3 months. Horizontal cells followed a similar regressive pattern. Since dendritic atrophy resemble typical neuronal responses to trophic factor deprivation, we developed a strain of mice, expressing both the rd1 mutation and overexpressing bcl-2 in the inner retina. Bcl-2 is know to confer cells the ability to resist axotomy, trophic factor deprivation and ischemia. Double mutant mice exhibited all the morphological abnormalities described in rd1 mice, including dendritic atrophy. Yet, the secondary degeneration of second-order neurons was prevented by bcl-2 overexpression, showing its apoptotic nature.
Thus, photoreceptor degeneration in rd mutant mice is apoptotic. Secondary loss of inner retinal cells is probably also apoptotic, and can be prevented by bcl-2 overexpression. This notion is important in view of the possibility to prevent vision loss in RP by means of antiapoptotic agents.

Internalization is essential for the anti-apoptotic effects of exogenous thymosin beta-4 on human corneal epithelial cells

Su, Yeu, Ho, Jennifer H., Lee, Oscar K.

Department of Medical Research & Education, Veterans General Hospital-Taipei, Taiwan, R.O.C.

Thymosin β-4 ($T\beta_4$) is a widely distributed peptide consisting of 43 amino acids that has pleiotropic effects on different types of cells. In addition to preventing the death of cardiomyocytes induced by ischemia, exogenous $T\beta_4$ has been shown to inhibit the apoptosis of human corneal epithelial cells triggered by ethanol. In the present study, we found that the susceptibility of the immortalized human corneal epithelial (HCE-T) cells to FasL and H_2O_2 was decreased by pretreating them with the recombinant $T\beta_4$ produced by *E. coli*. Moreover, FasL-mediated activation of caspases-8 and -3 as well as H_2O_2-triggered stimulation of caspases-9 and -3 in these cells was also abolished by $T\beta_4$ pretreatment. Interestingly, internalization of this G-actin sequestering peptide into HCE-T cells was demonstrated by immuno-fluorescence staining. Unexpectedly, when cells were incubated with cytochalasin D, an actin depolymerization reagent, prior to the addition of exogenous $T\beta_4$, not only was the internalization but also the cell death protection of this G-actin binding protein abrogated. To our best knowledge, this is the first report to show that internalization is essential for the anti-apoptotic activity of exogenous Tβ4 on human corneal epithelial cells.

**The caspase-generated fragments of PKR cooperate to activate full length PKR
and inhibit translation**

Vanessa Suin[1], Xiao-Ming Wang[2], Ann Meeus[3], Xavier Saelens[3], Peter Vandenabeele[3] and Michael Kalai[1]

[1]Laboratory of Cellular Microbiology, Molecular Microbiology Unit and [2]Laboratory of Enzymology, Pasteur Institute, Rue Engeland 642, B-1180 Brussels, Belgium and [3]Molecular Signalling and Cell Death Unit, Department for Molecular Biomedical Research, VIB and Ghent University, Technologiepark 927, B-9052 Zwijnaarde, Belgium.

The dsRNA-activated protein kinase PKR inhibits translation via phosphorylation of translation initiation factor 2-alpha (eIF2-a). We showed that in apoptosis protein synthesis is inhibited and that caspases cleave PKR, separating the kinase domain (KD) from the N-terminal domain (ND). Here, we compared the fates of PKR and eIF2-a in apoptosis and necrosis induced by dsRNA. Phosphorylation of PKR and eIF2-a occurred in apoptosis, was caspase dependent and coincided with appearance of caspase mediated PKR fragments. DsRNA-induced necrosis did not induce PKR and eIF2-a phosphorylation or inhibition of translation. We demonstrate that both ND and KD interact with full length PKR. KD leads to PKR and eIF2- a phosphorylation and inhibits protein translation, while ND does not. Co-expression of ND and KD promotes their interaction with PKR, PKR and eIF2-a phosphorylation and suppresses protein translation better than KD alone. These findings suggest a caspase dependent mode of activation of PKR in which the KD fragment can bind and activate intact PKR. Interaction of ND with PKR is suggested to facilitate the binding and activation of PKR by KD.

Nitric oxide as a mediator in regulation of hypoxia inducible factor-1alpha accumulation under normoxic conditions and low oxygen availability

Vadim V. Sumbayev[1,2,3], Inna M. Yasinska[2]

[1]University of Aarhus, Denmark, [2]Mechnikov Odessa University, Ukraine, [3]University of Kaiserslautern, Germany
e-mail: sumbayev@yahoo.co.uk

Hypoxia-inducible factor-1 alpha (HIF-1α), a regulatory subunit of HIF-1 transcription complex that upregulates genes involved in angiogenesis and glycolysis as well as serves as an essential regulator of inflammatory and innate immune responses. In particular, it induces the expression of glycolytic enzymes providing the energy for inflammatory reactions as well as CD18, which supports adhesion of neutrophils on the injured sites. In addition, HIF-1α protein plays critical role in apoptotic signal transduction by mediating p53 stabilization. All these processes are regulated by cytokines and other signaling proteins, which induce expression of nitric oxide synthase (NOS) and respectively production of NO and other reactive nitrogen species (RNS). These species were found to regulate HIF-1α accumulation under normal/low oxygen availability. We have studied the mechanisms of RNS-dependent regulation of HIF-1α accumulation. We have found that RNS mediate accumulation of HIF-1α under normoxic conditions and S-nitrosate the accumulated protein. S-nitrosation of Cys800 of HIF-1α protein supports its interaction with p300/CBP and enhances HIF-1 transcriptional activity. On the other hand, nitric oxide inhibits HIF-1α accumulation under low oxygen conditions via formation of peroxynitrite, which leads to damage of mitochondria and their respiratory chain followed by the release of 2-oxoglutarate and iron (the components needed to activate HIF-1α proline hydroxylases – PHDs) into cytosol. We suggested that peroxynitrite serves as an alternative donor of oxygen in HIF-1α PHD reactions. Taken together our data suggest that RNS are the mediators in regulation of HIF-1α protein accumulation.

Murine cytomegalovirus m41 protein protects from apoptosis induced by PKC inhibitors

Maren Syta and Wolfram Brune

Division of Viral Infections, Robert Koch Institute, Nordufer 20, 13353 Berlin, Germany, e-mail: SytaM@rki.de

Cytomegaloviruses are slow replicating, large DNA viruses of the herpesvirus family. Especially the slow replicating viruses are prone to abortive infection due to cell death prior to virus egress. To prevent the premature apoptosis of infected cells, cytomegaloviruses evolved several genes with antiapototic function.
Previously, we have shown that the murine cytomegalovirus (MCMV) protein m41 prevents premature apoptosis of infected cells. The protein localized predominantly to the cis-Golgi compartment, but is also found in the nuclear membrane. However, the molecular mechanism by which the m41 protein inhibits apoptosis has remained unknown. In the present work we further characterized the function of m41. An MCMV mutant with a deleted m41 ORF (MCMVΔm41) was used to analyze infected cells for their sensitivity against various apoptotic stimuli. Cells infected with the Δm41 mutant were protected against TNFa-, but not against staurosporin-induced cell death, whereas wildtype MCMV-infected cells were protected against both stimuli. Staurosporine is an inhibitor of protein kinase C (PKC) and other protein kinases, and a well-established inducer of apoptosis. However, the mechanism of staurosporine-induced apoptosis is largely unknown. To narrow down the possible pathway by which m41 interferes with staurosporine-mediated cell death, we tested a selective PKC (alpha, beta and epsilon) inhibitor, Ro-32-0432. Like staurosporine, this drug induced apoptosis in cells infected with Δm41 and in uninfected control cells, but not in cells infected with wildtype MCMV, suggesting a role of PKC for the function of m41. This suggestion is hardened by the observation that cells stably expressing m41 are protected from staurosporine induced apoptosis.
The family of PKCs consist of several isozymes. Some isozymes have a proapoptotic function whereas others are antiapoptotic. PKC-alpha and –beta belong to the antiapoptotic group of PKCs and are known to phosphorylate Bcl-2, which is required for its antiapoptotic function. Thus, we hypothesize that the m41 gene product inhibits apoptosis by compensating for a lack of phosphorylated Bcl-2.

TRAIL induces tumour cell apoptosis exclusively through DR4 or DR5

Eva Szegezdi, Devalingam Mahalingam and Afshin Samali

Department of Biochemistry and National Centre for Biomedical Engineering Science, National University of Ireland, Galway

Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the TNF death ligand family. It is best known for its ability to induce apoptosis selectively in cancer cells but not in untransformed cells. The mechanism that protects untransformed cells from TRAIL-induced death, although was widely examined, still not understood. TRAIL is recognized by four distinct membrane-bound receptors: DR4/TRAIL-R1, DR5/TRAIL-R2, DcR1/TRAIL-R3 and DcR2/TRAIL-R4. Of the four receptors, only DR4 and DR5 contain a death domain, which is required for induction of apoptosis. DcR1 and DcR2 do not have a functional death domain and thus they are thought to act as decoy receptors. Most cells in the human body express all four receptors; however neither their expression level nor their ratio correlates with TRAIL-sensitivity, suggesting that intracellular mechanisms control TRAIL-responsiveness. The aim of this study was to examine the intracellular determinants of TRAIL-sensitivity. Study of a panel of cancer cell lines confirmed that TRAIL receptor expression on the cell surface cannot predict sensitivity, and presence of high DR4/DR5:DcR1/DcR2 ratio is not sufficient to induce TRAIL-mediated cell death. Furthermore, study of the DR4- and DR5-mediated cell death pathways individually by using receptor-selective TRAIL variants and agonistic antibodies revealed that the two death-inducing receptors contribute to TRAIL-induced cell death in different proportions. In Colo205 colon carcinoma cells, activation of the DR5-pathway by DR5-selective TRAIL mutants induced 72±8% cell death, while activation of the DR4-pathway could not induce more than 16±6% cell death. Furthermore, preincubation with a DR4-specific neutralizing antibody could only prevent up to 12±6% of TRAIL-induced cell death, while the DR5-specific neutralizing antibody could prevent 66±9% of TRAIL-induced cell death. Similar pattern, but opposing contribution of the two receptors was identified in ML-1 acute myeloid leukemia cells. Our studies point out, that so far unexplored processes regulate the activity of the two death-inducing receptors and TRAIL responsiveness, which is likely to be independent of the decoy receptors.

Adenosine A_{2A} Receptor-Mediated Cell Death of Mouse Thymocytes Involves Adenylate Cyclase, Bim and is negatively regulated by Nur77

Ildikó Kiss [1*], Henriett Oskolás [1*], Réka Tóth[1], Philippe Bouillet[2], Katalin Tóth[1], András Fülöp[1], Beáta Scholtz [3], Catherine A. Ledent[4], László Fésüs[1] and Zsuzsa Szondy[1]

[1]Department of Biochemistry and Molecular Biology, Signaling and Apoptosis Research Group, Hungarian Academy of Sciences, Research Center of Molecular Medicine and 3Clinical Genomic Center,University of Debrecen, Debrecen, Hungary ,H-4012, ,[2]Walter Elisa Hall Institute of Medical Research, PO Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia [4]IRIBHM, Université Libre de Bruxelles, Brussels, B-1050 Belgium

Adenosine is generated in the microenvironment of emerging thymocytes through normal mechanisms of lymphocyte selection. In a normal thymus most of the adenosine is catabolized by adenosine deaminase, however, in an environment where up to 95% of the cells undergo programmed cell death, a sufficient amount of it is accumulated to trigger cell surface adenosine receptors. Here we show that accumulated adenosine can induce apoptosis in mouse immature thymocytes mostly via adenosine A_{2A} receptors. The signaling pathway is coupled to adenylate cyclase activation, induction of the Nur77 transcription factor, Nur77-dependent genes, such as Fas ligand and TRAIL, and the proapoptotic BH3-only protein Bim. We analyzed several knock out and transgenic mouse lines and found that adenosine–induced killing of mouse thymocytes required Bim, occurred independently of “death receptor” signaling, and was inhibited by Bcl-2 and Nur77. Collectively our data demonstrate that adenosine-induced cell death involves signaling pathways originally found in negative selection of thymocytes, and suggests a determining role of Bim and a regulatory role for Nur77.

Supported by OTKA OTKA T049445

Proliferation and apoptosis in relation to treatment response in pituitary adenomas

Cristiana Tanase*, E. Codorean*, V.Ciubotaru**, I. Ogrezean**, E. Raducan*, C. Ardeleanu*

* *"Victor Babes" National Institute of Pathology, Bucharest, Romania*
** *"D. Bagdasar" Hospital, Neurosurgery Dept., Bucharest, Romania*

The relationship between proliferation and apoptosis plays an important role in predicting the behaviour of pituitary tumors and might be a target of anti-tumor therapy.
AIM: to estimate the influence of specific chemotherapy (somatostatin analogs and dopamine agonist) on proliferative activity and apoptosis and the in pituitary adenomas.
Methods. Were investigated 40 paraffin-embedded pituitary tumors: 24 invasive adenomas – 11 treated (9 with octreotide and 2 with bromocriptine). Immunohistochemical analysis was performed for Ki67 (proliferation) and Bax, Bcl-2 expression (apoptosis); apoptotic index (AI) was determined by the in situ end-labelling technique (TUNEL).
Results. The mean Ki-67 (labelling index – LI) in both treated and untreated patients was 3,68%. We noticed a positive correlation between the proliferative activity and the tumoral invasiveness (Ki67 LI: 4.82±0.4% in invasive and 1.25±0.3% in non-invasive adenoma; P< 0,01). Patients treated showed a lower Ki67 LI (2,9%±0,72) than untreated controls (4,5%±0,57; P<0,05). Expression of Bax and Bcl2 was found in 80% respectively 50% of tumors and was moderate or low in most of the adenomas with Bax/Bcl-2 > 1 in treated pituitary adenomas. Apoptosis was found in 60% of tumors, higher in some of the invasive ones, with a positive correlation between the proliferation rates and apoptotic indices (AI: 4.54±1.22 in total versus 5.42±1.46 in invazive pituitary adenomas). Also, some differences were recorded in treated and untreated adenomas (AI: 5.36%±1.62 versus 4,60%±1.92). There was a positive correlation between the Ki67 LI and the apoptotic index.
Conclusions. The assessment of proliferation, and apoptosis might be used to evaluate the susceptibility of adenomas to therapy.

Structure-based design of non-peptidic small molecular inhibitors of caspase-3

Sei-ichi Tanuma, Jun-ichi Sakai, Ryoko Takasawa and Atsushi Yoshimori

Department of Biochemistry, Faculty of Pharmaceutical Sciences, and Genome & Drug Research Center, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan, e-mail: tanuma@rs.noda.tus.ac.jp

Caspases are known to play critical roles during the execution of apoptosis. In pathological conditions, such as ischemic brain injury and amyotrophic lateral sclerosis (ALS), main apoptotic caspases, particularly caspases-3 and -9 are activated and cause excessive neuronal cell death. Hence, the inhibitors of caspases have been expected to be beneficial pharmaceuticals for neurodegeneration. Although many peptide inhibitors of caspases have been reported and commercially available, they have intrinsic limitations for therapeutic use because of their poor cell-permeability, in vivo stability, and bioavailability. Here, we describe the computational development of non-peptidic small molecular inhibitors of caspase-3.

Previously, using our computational program for designing of optimized binding peptides of the target domains (Hot Spots) on target proteins, named Amino acid Positional Fitness (APF) method, we designed a novel caspase-3 specific peptide inhibitor, Ac-DNLD-CHO. Thus, we performed computational design of small molecular inhibitors of caspase-3 on the binding mode of DNLD peptide. By our optimized peptide mimetic screening method, we discovered a novel caspase-3 specific non-peptidic small molecule inhibitor, named CS4566. This inhibits caspase-3 in vitro with IC50 value of 14 ƒÊM. These results suggest that this novel class of small molecular inhibitor of caspase-3 is a promising lead compound for creating non-peptidic small morecular inhibitors and pharmaceuticals for caspase-mediated diseases.

Non-apoptotic programmed cell death induced by a copper(II) complex in human cancer cells

Saverio Tardito[§], Ovidio Bussolati[§], Luciano Marchiò[#], Maurizio Lanfranchi[#], Renata Franchi-Gazzola[§]

[§]Unit of General and Clinical Pathology, Department of Experimental Medicine, [#]Department of General and Inorganic, Analytical, and Physical Chemistry, University of Parma, Italy, e-mail: saverio.tardito@nemo.unipr.it

A_0, a novel Cu(II) thioxotriazole complex, produces severe cytotoxic effects in 8 of 25 human solid tumor cell lines tested with a potency comparable to that exhibited by cisplatin while no cytotoxic effect was observed in normal human dermal fibroblasts. In the cell panel tested, sensitivities to A_0 and cisplatin were unrelated, pointing to different mechanisms of action of the two drugs. In particular, both a parental, cisplatin-sensitive ovarian cancer cell line and its cisplatin resistant subline were comparably affected by the copper complex. In all A_0-sensitive cell lines, the complex induced a characteristic array of changes, hallmarked by the formation of vacuoles negative for proteins, lipids, extracellular fluid. The death process has been characterized in the most sensitive line, human fibrosarcoma HT1080 cells. No evidence of nuclear fragmentation or caspase-3 activation was detected in cells treated with A_0 which, rather, inhibited cisplatin-stimulated caspase-3 activity. Membrane functional integrity, assessed with calcein and propidium iodide, was spared until the late stages of the death process induced by the copper complex exluding an oncotic death. Vacuoles were negative for the autophagy marker monodansylcadaverine and their formation was not blocked by 3-methyl-adenine, an inhibitor of autophagic processes. Ultrastructural analysis indicated that A_0 caused the appearance of many electronlight cytoplasmic vesicles, possibly related to the endoplasmic reticulum, which progressively enlarged and coalesced to form large vacuolar structures that eventually filled the cytoplasm. The mechanism of action of A_0 was not based on his capability to generate extra- or intra-cellular ROS but, rather, seemed strictly related to the amount of copper taken up by cells. It is concluded that A_0 triggers a non-apoptotic, type 3B programmed cell death (Clarke PG (1990) Anat Embryol (Berl) 181: 195-213), characterized by an extensive cytoplasmic vacuolization. This peculiar cytotoxicity pattern may render A_0 of particular interest for inducing death of apoptosis-resistant cancer cells.

High Antitumor Activity of Novel Glycosylated Indolocarbazole Is Determined by Strong Intercalation Into DNA

Victor V.Tatarskiy Jr., Dmitry N. Kaluzhny, Anna K.Shchyolkina, Filipp S.Bondarev, Irina L. Plikhtyak, Tatyana D.Miniker, Stalina Ya. Mel'nik, Zoya S. Smirnova, Olga F.Borisova and Alexander A. Shtil

N.N.Blokhin Cancer Center, Moscow, Russia; Engelhardt Institute of Molecular Biology, Moscow, Russia.

Chemical modifications of indolocarbazoles are aimed at obtaining clinically applicable derivatives of this perspective class. We synthesized a series of indolocarbazoles with various carbohydrate substituents. The compounds were tested in the National Cancer Institute panel of 60 human tumor cell lines and in *in vivo* hollow fiber assays. One of the most potent agents selected in this screening is the water soluble 12-(α-*L*-arabinopyranosyl)indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione) (**1**). Compound **1** contains *L*-arabinose residue linked to the pharmacophore via nitrogen atom in position 12 of indolocarbazole. Treatment of mice with transplanted colon carcinoma or leukemia resulted in the complete cure of animals or significant tumor regression at tolerable concentrations of **1**. Search for mechanisms of antitumor effects revealed that **1** potently inhibited cyclin dependent kinase (CDK) 1 *in vitro* (IC_{50}=45 nM); the molecular docking showed that **1** fits well into the ATP binding pocket of CDKs 2, 4 and glycogen synthase kinase 3. However, nanomolar concentrations of **1** were insufficient for cell kill, implying that the target(s) other than the above enzymes is critical for cytotoxicity. Indeed, the submicromolar concentrations of **1** were required for apoptotic death of solid and hematopoietic tumor cells. Compound **1** formed two types of intercalating complexes with double stranded DNA. Scatchard plots showed that type 1 complexes (formed at 0.5-1 μM of **1**) had remarkably strong binding constant (K_1=6x10^6 M^{-1}, one intercalated molecule per 8 nucleotides). The type 2 complexes (detectable with 2-5 μM of **1**) were weaker but more frequent (K_2=1.6x10^5 M^{-1}, one intercalation event per 2 nucleotides). The preferential sites of intercalation were GC pairs. Dichroism studies and *in silico* analysis provided a model for interaction of **1** with the duplex. Importantly, it is the amounts of **1** that formed type 1 complexes that were necessary for DNA replication block, activation of p53 transcription, G_2 arrest and death of HCT116 human colon carcinoma cells. Thus, although **1** interacted with several targets in a dose dependent manner, its strong intercalation into DNA is a key prerequisite for anticancer efficacy.

P53-independent apoptosis induced by peroxynitrite in Jaws II bone marrow cells is inhibited by Bcl2

Emma Taylor, Adriano Rossi and John Harlan

[1]MRC Centre for Inflammation Research, Queen's Medical Research Institute, University of Edinburgh, Scotland, UK and [2] Division of Hematology, Research and Training Building, Harborview Medical Center, University of Washington, Seattle, WA, USA. Email etaylor1@staffmail.ed.ac.uk

Nitric oxide (NO) and related species such as peroxynitrite ($ONOO^-$) promote apoptosis in many cell types, particularly at high concentrations that stress the cell. In some cell types, this may dependent on the function of the tumour suppressor, p53, which becomes active during cellular stress and DNA damage, and acts to arrest the cell cycle and promote apoptosis. Jaws II is a GM-CSF-dependent bone marrow cell line derived from the p53-null mouse, and was used to determine whether the peroxynitrite-donating compound, GEA 3162, can induce caspase activation and apoptosis despite the absence of functional p53. Bcl2 is an anti-apoptotic protein that promotes mitochondrial stability, and overexpression in Jaws II cells was used to determine its effect on $ONOO^-$ induced apoptosis.

Apoptosis is a caspase dependent process, therefore total caspase activation was measured in wild-type (Jaws II-GFP) and Bcl2 overexpressing (Jaws II-Bcl2) cells in the absence and presence of GEA 3162 (30 – 100 μM). After 4 hours incubation, there was a concentration-dependent increase in caspase activation in Jaws II-GFP cells, which was blocked by co-incubation with zVAD, demonstrating that increased fluorescence was indeed due to enhanced caspase activity. In contrast, caspase activity in Bcl2-overexpressing cells was unaffected by exposure to GEA 3162. Analysis of specific caspases showed that caspases 3 and 2 were strongly activated with a weaker activation of caspases 8 and 9.

Mitochondrial permeability was also measured to determine the potential apoptotic pathway through which peroxynitrite-induced apoptosis proceeds in Jaws II-GFP cells. A concentration-dependent increase in permeability occurred in the presence of GEA 3162, which was reduced but not abolished by Bcl2 overexpression. A role for p38 activation in the apoptotic pathway was also observed using the p38 MAP kinase inhibitor, SB203580 (10-50 μM), alongside a potential small role for JNK, using the inhibitor, SP600125 (10-50 μM).

In summary, GEA 3162 induces p53-independent apoptosis in the Jaws II bone marrow cell line, which proceeds via p38 activation, mitochondrial permeabilisation and activation of caspases 3 and 2 in particular. Overexpression of the anti-apoptotic protein, Bcl2, blocks caspase activation and mitochondrial changes.

Sensory Cell Death in the Inner Ear

Ruth R Taylor and Andrew Forge

Centre for Auditory Research, The Ear Institute, University College London
332 Gray's Inn Road, London. WC1X 8EE email:Ruth.r.taylor@ucl.ac.uk

Death of the sensory 'hair' cells in the inner ear through noise, ototoxic drugs and ageing is the major cause of acquired sensorineural deafness. Aminoglycosides are well known to promote hair cell death in the inner ear of all vertebrates. To investigate mechanisms of hair cell death both the newt, a urodele amphibian well known for regeneration, and mice, in which hair cell death in the cochlea (auditory organ) results in no recovery but structural reorganisation, were used as models.
In cultured explants of inner ear from newt, directly exposed to gentamicin, TEM and fluorescence microscopy showed hair cell death occurred by apoptosis. Cell bodies were retained within the epithelium and supporting cells were found to contain apoptotic cell bodies. Labelling with antibody to activated caspase3 demonstrated that activated caspase3 was involved in the hair cell death mechanism. There was no evidence for macrophage recruitment.
In mammals, the outer hair cells at the base of the cochlea are more vulnerable to cisplatin or aminoglycosides and ageing than those at the apex, whilst inner hair cells are more resistant. Through studies using isolated outer hair cells and strips of the entire organ of Corti, this has been shown to be an intrinsic difference in sensitivity to damage. Our aim is to identify the proteins involved in the pathways of cell death in order to determine whether the mechanism of inner hair cell death differs from that of outer hair cells. For initial investigations, we have developed a single dosing regime using kanamycin and bumetanide to produce a loss of hair cells in the organ of Corti of mice. At 7 days post-treatment all outer hair cells have disappeared but inner hair cells remain. At the later timepoint of 14 days, many inner hair cells have been lost. This model enables us to determine whether both types of hair cells die via programmed cell death pathway mediated by caspases or by other means. As with the newt, macrophage recruitment is not seen around the time of hair cell death in mature or early postnatal mouse organ of Corti.

Regulation of BARD1 subcellular localization and apoptotic function

Varsha Tembe, Beric Henderson

Westmead Millennium Institute, University of Sydney at Westmead Hospital, Westmead NSW 2145, Australia

BARD1 (BRCA1-associated Ring Domain 1) is a major cellular binding partner of BRCA1. BARD1 is an important regulator of the tumor suppressor function of BRCA1, and evidence that the BARD1 gene is mutated in a subset of breast/ovarian cancers implicates BARD1 itself can act as a tumour suppressor. BARD1 is known to regulate BRCA1 stability, localization and function. BARD1 and BRCA1 form a functional heterodimer, through the binding of their ring finger domains. This interaction is thought to stabilize both proteins, as the respective monomers are unstable. The BRCA1/BARD1 heterodimer has ubiquitin ligase activity that targets proteins involved in cell cycle regulation and DNA repair for degradation. BARD1 can function independently of BRCA1 in inducing apoptosis.

The apoptotic function of BARD1 is dependent on functional p53, but independent of, and inhibited by, BRCA1. BARD1 is up-regulated in response to genotoxic stress, and overexpression of ectopic BARD1 leads to apoptosis. Our lab recently showed that BARD1 is regulated by nuclear-cytoplasmic shuttling, and that nuclear export leading to increased cytoplasmic localization of BARD1 is associated with apoptosis. Mutation of the nuclear export signal, but not the nuclear localization signal, of BARD1 reduced its apoptotic activity. In contrast to a previous report, introduction of the cancer mutation Q564H did not abolish BARD1-dependent apoptosis. We have subsequently mapped the apoptotic regions to the C-terminus of human BARD1, including a sequence corresponding to the Ankyrin repeat protein interaction domain. The implications for regulation of apoptotic activity will be discussed.

Bone microenvironment-related growth factors and zoledronic acid modulate differentially the PTHrP expression in PC-3 prostate cancer cells.

Roxane Tenta, Antigone Sourla, Michael Koutsilieris.

Department of Experimental Physiology, Medical School, University of Athens, Greece.
e-mail: roxane@med.uoa.gr

PC-3 is an androgen insensitive human prostate cancer cell line, widely used for the study of prostate cancer.
Parathyroid hormone related peptide (PTHrP) mediates a wide range of local paracrine/autocrine and intracrine functions in various tissues under physiological and pathological conditions. The consequences of the intracrine mode of action of PTHrP may modulate processes of vital importance to the prostate cancer cell such as the inhibition of apoptosis, thereby suggesting that PTHrP can act as survival factor on prostate cancer cells. Bone microenvironment-related growth factors, such as insulin-like growth factor 1 (IGF-1), transforming growth factor beta 1 (TGFß1), basic fibroblast growth factor (bFGF) and interleukin 6 (IL-6), act as survival factors on metastatic prostate cancer cells, thereby inhibiting chemotherapy apoptosis, in vitro.
Bisphosphonates were shown to inhibit the proliferation and to induce apoptosis of certain prostate cancer cell lines, in vitro and zoledronic acid (zometa), has been approved for the treatment of skeletal complications in patients with hormone refractory prostate cancer.
The goal of the present study was to evaluate whether bone survival factors and zoledronic acid affect PTHrP and PTH.1R (PTH/PTHrP receptor) expression in PC-3 prostate cancer cells.
The actions of zoledronic acid (50 μM, 100 μM) and of bone survival factors (25ng/ml) on PTHrP expression in PC-3 prostate cancer cells were analysed by semi-quantitative rt-PCR, Real-Time PCR, immunocytochemistry and immunofluorescence.
Our data suggest that (i) IGF-1, TGFß1, bFGF and IL-6 induced PTHrP mRNA expression (ii) zoledronic acid suppressed PTHrP mRNA expression, whereas IGF-1, TGFß1, bFGF, IL-6 and zoledronic acid didn't affect PTH.1R mRNA expression in PC-3 cells.
In this study we show that the growth factors which are present in the bone-microenvironment increase, whereas zoledronic acid suppresses PTHrP mRNA expression in prostate cancer cells. Therefore, PTHrP may mediate, at least in part, the growth factors-induced inhibition of apoptosis of prostate cancer cells. The overall actions of PTHrP remain to be clarified in order to fully understand its contribution in metastatic cancer disease and in order to design specific therapies against the tumor metastasis microenvironment.

Role of tissue transglutaminase in the process of phagocytosis of apoptotic cells

Beáta Tóth[1], Daniel Aeschlimann[2], László Fésüs[1], Zsuzsa Szondy[1]

[1]Department of Biochemistry and Molecular Biology, University of Debrecen, Hungary
[2]Matrix Biology and Tissue Repair Research Unit, Dental School, Cardiff University, UK

The rapid and efficient phagocytosis of apoptotic cells plays a crucial role in preventing secondary necrosis, inflammation as well as in tissue remodeling and regulating immune responses. However, the molecular details of engulfment are just beginning to be elucidated.
In this clearance process the professional phagocytes, so called macrophages, play a central role. Engulfment of dying cells initiates cytoskeletal reorganization in macrophages and these events are evolutionally highly conserved and well regulated. Members of the Rho-family GTPases regulate cytoskeletal rearrangements underlying cell shape changes in migration and phagocytosis. Tissue transglutaminase is a GTP binding protein, and it also has transamidation function. Previous work from our laboratory has shown that TG2-/- mice has a defect in the phagocytosis of apoptotic cells and on long term autoimmunity is developed.
Here we show that phagocytosis of TG2-/- macrophages is also defective under *in vitro* condition and this is related to altered cytoskeletal reorganization and have a defect in the signaling pathway that leads to rac activation. In the present study we have investigated the mechanism of the observed deficiency in the phagocytosis of apoptotic cell. We have focused on the signaling events upstream Rac and identification of what function of TG2 plays role in the clearance of apoptotic cells. A series of adenoviruses have been generated to transduce mouse TG2, crosslinking deficient TG2, GTP-binding deficient TG2 or double defective enzyme. So far we could recover the phagocytosis deficiency in TG2-/- peritoneal macrophages by infecting them with adenovirus whose genoms code wild type TG2 gene. Using adenoviral gene transfer, rescue experiments with TG2-/- cells were carried out to identify whether TG2 is required in intracellular cell signalling directly controlling cytoskeletal rearrangements or in cell-cell communication.

Supported by: OTKA T049445, ETT 100/2003 and QLK3-CT-2002-02017

Ligation of RARa enhances glucocorticoid-induced apoptosis of T cells by facilitating glucocorticoid receptor mediated transcription

Katalin Tóth, Júlia Buslig, László Fésüs and Zsuzsa Szondy

Department of Biochemistry and Molecular Biology, Signaling and Apoptosis Research Group, Hungarian Academy of Sciences, Research Center of Molecular Medicine, University of Debrecen, Debrecen, Hungary, e-mail: tkati@indi.dote.hu

Glucocorticoid-induced apoptosis of thymocytes is one of the first recognized forms of programmed cell death. It was shown to require gene activation induced by the glucocorticoid receptor translocated into the nucleus following ligand binding. Here we report that retinoic acids, physiological ligands for the nuclear retinoid receptors, enhance glucocorticoid-induced death of mouse thymocytes both *in vitro* and *in vivo*. The effect is mediated via retinoic acid receptor (RAR) alpha, and occurs when both RARs and RXRs are ligated by natural retinoic acids. Using coimmunoprecipitation technique and mammalian two-hybrid system we demonstrate that the ligated RARα directly interacts with the ligated glucocorticoid receptor. This interaction results in an enhanced transcriptional activity of the glucocorticoid receptor. Our data demonstrate a new type of interaction between these two nuclear receptors in the regulation of thymocyte cell death.

Supported by Hungarian National Research Fund (OTKA T049445, TS44798), Hungarian Ministry of Health (ETT 100/2003),

Magnesium: A New Player in the Apoptotic Signalling Cascade?

Valentina Trapani[*], Giovanna Farruggia[+], Patrizio Sale[§], Stefano Iotti[++], Luca Prodi[+++], Alma Boninsegna[*], Matteo Simonacci[*], Paul B. Savage[#], and Federica Wolf[*]

[*]*Istituto di Patologia Generale, Università Cattolica del Sacro Cuore, Roma*
Dipartimento di Biochimica[+], Medicina Clinica[++] e Chimica[+++], Università di Bologna
[§]*Dipartimento di Medicina Sperimentale e Patologia, Università "La Sapienza", Roma*
[#]*Brigham Young University, Provo, Utah, USA*

The participation of magnesium (Mg) in key events for proliferation and differentiation suggests a possible role for this cation in apoptosis as well. While the important role of intracellular Ca^{2+} in apoptosis is generally accepted, so far the involvement of Mg has largely been ignored, although it has been reported that an increase in intracellular Mg occurs as an early event in apoptosis in different cell lines. It is now established that mitochondria are primary actors in triggering programmed cell death, but only recently these organelles have been identified as intracellular Mg stores[1]. Therefore, we set to investigate whether and how this cation might be a control element in life vs death decisions.
We took advantage of a new potent tool for Mg detection: DCHQ is a novel fluorescent indicator with unequalled properties of sensitivity and specificity[2]. Confocal imaging enabled the study of real-time distribution and movements of Mg in live cells, in stationary and dynamic conditions. Treatment with arachidonic acid (AA), implicated in the TNFα apoptotic signalling, caused a rapid and substantial increase in the intracellular Mg, which could be partially inhibited by cyclosporin A (CsA), suggesting an involvement of the permeability transition pore. Concomitantly, AA also caused a dose and time-dependent increase in the percentage of apoptotic cells, as revealed by Annexin V staining. Based on these preliminary results, we tried to determine the temporal and causal sequence leading to cell death, by analysing the effect of AA in terms of mitochondrial membrane depolarisation, cytochrome c release, caspase activation, and by using specific mitochondrial inhibitors.
In conclusion, by employing an innovative live cell imaging approach, we demonstrated that a transient increase in intracellular magnesium occurs prior to typical apoptotic events. The comparison between the response of normal epithelial cells to that of apoptosis-resistant tumour cells will allow to assess whether this Mg rise is to be considered a messenger signal necessary to trigger downstream events, or rather an associated phenomenon, whose significance should nevertheless be cautiously evaluated, in view, for example, of the known role of Mg as an essential cofactor for endonucleases.

[1]Kubota *et al* (2005) *Biochim Biophys Acta* 1744, 19-28
[2]Farruggia *et al* (2006) *J Am Chem Soc* 128, 344-350

RelA/NF-kB subunit binds the *bax* gene promoter and inhibits p73-induced *bax* transcription in CD28-costimulated T lymphocytes

Roberta Cianfrocca[1], Marzia Soligo[1], Valeria Marzano[1], Michela Muscolini[1], Barbara Marinari[2], Massimo Levrero[3], Antonio Costanzo[2] and Loretta Tuosto[1]

[1] Dept. Cell. and Develop. Biol. University "La Sapienza", [2] Dept. Dermatol. University "Tor Vergata", [3] Fondazione Andrea Cesalpino, Policlinico Umberto I, University "La Sapienza", Rome, Italy email: loretta.tuosto@uniroma1.it

The balance between anti-apoptotic and pro-apoptotic proteins of the Bcl-2 family is critical in determining the cell fate in response to death stimuli. P53 family of transcription factors generally regulates the expression of pro-apoptotic genes, such as *bax*. By contrast, NF-κB subunits are involved in the transcription of anti-apoptotic Bcl-2 members. The survival of T lymphocytes in response to T cell receptor (TCR) triggering has been recently connected to co-stimulation and NF-κB-mediated inhibition of TCR-induced p73 expression. The mechanisms by which both p73 induces apoptosis in T cells and NF-κB interferes with the activity of p73, remain still unknown. Here we show that CD28 co-stimulatory molecule protects T cells from irradiation-induced apoptosis by both up-regulating *bcl-x*$_L$ and inhibiting *bax* gene expressions. We found that p73, but not p53, binds to and *trans*-activates the *bax* gene promoter in irradiated T cells. The activation of RelA/NF-κB subunit in CD28 co-stimulated T cells suppresses *bax* gene transcription by interfering with the recruitment of both p73 and RNA polymerase II to the *bax* gene promoter. The data obtained from both *ex vivo* and *in vitro* analyses suggest that CD28 suppresses p73-induced *bax* gene transcription by mediating CBP/p300 sequestration as well as the binding of RelA containing repressor complexes to the κB consensus regions in the *bax* gene promoter. These findings identify RelA/NF-κB as a critical regulator of T cell survival through its ability to regulate both pro- and anti-apoptotic Bcl-2 family members.

The influence of EHV-1 infection on cytoskeleton in vitro

Agnieszka Turowska, Michal M. Godlewski, Anna Chmielewska, Anna Tucholska, Marcin W. Banbura.

Department of Preclinical Sciences, Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Ciszewskiego 8, 02-786 Warsaw, Poland

Consequences of equine herpesvirus type 1 (EHV-1) infection on cellular structure, in particular its effect on the cytoskeleton, are not clear as yet. Actin filaments play an important role during EHV-1 infection of cells derived from the specific host (ED, equine dermal cell line). In my studies distortion and decomposition pattern of cytoskeleton have been observed in infected cell cultures. Interestingly, F-actin in heterogenous cell line Vero (green monkey kidney) was not affected by EHV-1 infection. In contrast to actin filaments, there was some increase in a–tubulin polimerization in Vero cell line, what was evaluated by confocal microscopy and IOD technique. Unexpectedly, we have also observed structures specific for cell death, which may be caused by viral infection.
The aim of my doctoral thesis is to investigate changes in actin and tubulin structures in different cell lines (ED and Vero), infected with different strains of EHV-1 and to explain the nature and the mechanism of this cell death.

Balance between pro-survival and death signals – important determinant of colon cancer cell sensitivity to TRAIL-induced apoptosis

Alena Vaculová, Jirina Hofmanová, Alois Kozubík

Laboratory of Cytokinetics, Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, 612 65 Brno, Czech Republic

The tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of TNF family, is particularly interesting for its unique properties to induce cancer cell death (including colon) while sparing most normal cells. This implies its use as a promising anti-cancer agent [1, 2]. However, besides its potential to kill selectively the tumour cells, the physiological role of TRAIL is more complex. The ability of TRAIL to promote survival and proliferation of non-cancer cells has been reported [3]. Furthermore, in certain tumour cells, simultaneous or consecutive TRAIL-induced activation of apoptotic and pro-survival pathways was demonstrated [4]. The balance between these pathways is therefore very important in determining the cell fate.

The aim of this study was to highlight the importance of the balance between MAPK/ERK or PI3K/Akt and TRAIL signalling pathways in regulation of apoptosis of colon cancer cells and to study the mechanisms involved. Specific inhibitors of the MEK/ERK or PI3K/Akt pathways enhanced significantly the TRAIL-induced apoptosis of HT-29 human colon adenocarcinoma cells. The modulation of the apoptotic process was associated with potentiation of caspase-8 activity and significant changes at the level of mitochondria, such as decrease of mitochondrial membrane potential, and particularly changes of Mcl-1 protein level. We showed a different regulation of the level of this antiapoptotic protein under MEK/ERK and PI3K/Akt pathway inhibition. These changes were accompanied by significant modulation of GSK3β and ERK1/2 activity.

Our results contribute to the elucidation of mechanisms involved in the interaction between TRAIL-induced apoptotic pathways and pro-survival pathways, which is very beneficial to predict the effectiveness of TRAIL in cancer therapy.

Supported by the Grant Agency of the Czech Republic No. 524/04/0895 and IGA AS CR No. 1QS500040507.

References:
1. Walczak, H. et al., Nat Med. 5, 157-63, 1999.
2. MacFarlane, M., Toxicol Lett. 139, 89-97, 2003.
3. Secchiero, P., et al., Circulation. 107, 2250-6, 2003.
4. Baader, E., et al., Cancer Res. 65, 7888-95, 2005.

Caspase-2 in ovarian cancer cells

Helin Vakifahmetoglu, Magnus Olsson, Sten Orrenius and Boris Zhivotovsky

Division of Toxicology, Institute of Environmental Medicine, Karolinska Institutet 171 77 Stockholm, Sweden. email: Helin.vakifahmetoglu@imm.ki.se

During mammalian fetal development ovarian germ cells produced through clonal expansion undergo massive apoptosis. Using the “rate of cell death analysis” in tissues, it was shown that caspase-2 is an important mediator of germ cell death in the fetal ovary to control the germ cell pool of the female. In addition, studies on caspase-2 null mice showed an increase in number of primordial follicles in the postnatal ovary. These findings, combined with the fact that oocytes lacking caspase-2 are resistant to apoptosis following exposure to chemotherapeutic drugs, led us to hypothesize that caspase-2 might play an important role in treatment of ovarian cancer.

Here, we investigated the role of caspase-2 in DNA damage-induced apoptosis in different human epithelial ovarian carcinoma (EOC) cells. The experiments were performed using Cao-V-4 and Sk-o-V-3 cell lines and different apoptotic features were analysed.

The results demonstrated a differential response of these cells to cisplatin-induced apoptosis and caspase-2 activation. Within 48 hours after treatment, Cao-V-4 cells showed a time-dependent caspase-2 processing and activation followed by a pronounced cytochrome c release and oligonucleosomal DNA fragmentation. In contrast, almost 95 % of Sk-o-V-3 cells were resistant to cisplatin-induced apoptosis during the same time period. Further investigations revealed that this resistance depends on a delay of caspase-2 activation and dephosphorylation of caspase-2 by DRB, a specific PKCK2a inhibitor, is able to sensitize EOC cells to cisplatin by activating caspase-2.

The detailed mechanism underlying the difference in the sensitivity to cisplatin-induced apoptosis and the delayed caspase-2 activation were investigated. Thus far, our data suggest that non-activation and/or a delay of caspase-2 activation may be linked with the resistance to cisplatin-induced apoptosis and consequently to chemotherapy in some ovarian cancer cells.

Single-cell image analysis of cellular localization and colocalization of endonuclease G, AIF and AMID in living cells

Miroslav Varecha, Jana Amrichová, Michal Zimmermann, Vladimír Ulman, Pavel Matula, Petr Matula, and Michal Kozubek

Centre for Biomedical Image Analysis, Faculty of Informatics, Masaryk University, Brno, Czech Republic, email: mvara@fi.muni.cz

We studied the cellular localization and colocalization of proteins AIF, endonuclease G and AMID, which were previously found to be involved in caspase-independent apoptosis. We designed mammalian expression vectors, which carry genes encoding the proteins of interest fused to the fluorescent proteins to study cellular localization and colocalization of these proteins. We predicted the cellular localization of the proteins of interest using software, which analyses the aminoacid sequence of the protein with various algorithms. We also designed and applied a novel method of single-cell image analysis of the translocation of the fluorescent proteins into the nucleus. Using the listed methods, we managed to confirm by software prediction the localization of AIF and endoG to mitochondria. We confirmed experimentally the colocalization of AIF and endoG in the mitochondria of living cells using cotransfection of U-2 OS cells with mammalian expression vectors coding fusions proteins AIF-HcRed-tandem and endoG-EYFP. We also observed and confirmed their translocation from mitochondria to the nucleus during the staurosporine-induced apoptosis by time-lapse microscopy of living cells. Using same methods for protein AMID indicates, that AMID is not localised exclusively on the outer mitochondrial membrane, neither dissolved in the cytoplasm, as it was observed and published. Overexpression of fusion protein AMID-HcRed-tandem didn't have lethal effect on cells or mitochondria as it was also previously described. According to all our predicted and experimental results, AMID is localised to the cytoplasm, but more likely bound to the lipids of the cellular membranes. We did not observe its role in the staurosporine-induced apoptosis, because AMID wasn't transferred into the nucleus as was endoG and AIF and therefore it could not directly participate in the chromatin degradation process. The possible role of AMID in apoptosis was not observed and our results contribute to the comprehension of localization and function of AMID, AIF, and endoG proteins.

This work was supported by the Grant Agency of the Czech Republic (grant numbers 202/04/0907, 204/03/D031, and 204/05/P090) and by The Ministry of Education, Youth and Sports of the Czech Republic (project number MSM0021622419).

Caspase-3 and Caspase-3s roles in etoposide-induced apoptosis.

Végran F[1,2], Boidot R[1,2], Hamman A[2], Sequeira-Legrand A[2], Solary E[2] et Lizard-Nacol S[1].

[1]Laboratoire de Génétique Moléculaire – Centre GF Leclerc - 1, rue du Pr MARION – 21 079 DIJON, France. E-mail : fvegran@club-internet.fr
[2]INSERM U517 – Faculté de Médecine – 7, Bld Jeanne d'Arc – 21 000 DIJON, France.

Several studies have shown that apoptosis disregulation is highly involved in carcinogenesis and chemoresistance. Caspase-3 is one of the most important protein of apoptosis. A new variant, called Caspase-3s and stemmed from alternative splicing of exon 6 was identified. This variant has lost its catalytic site, suggesting a lose of apoptotic function.
In order to characterize each transcript role, the full-length cDNAs were stably transfected in MCF-7 (caspases-3 deficient), and in HBL100 (caspases-3s proficient) breast tumor cell lines. A transient co transfection of both variants was also performed in MCF-7. Apoptosis was induced by etoposide treatment in both cell lines (with 35 and 1.7µM, respectively), and was further measured by flow cytometry.
In untreated cells, transfection with each transcript has no effect on apoptosis rate (8 and 10% respectively for both cell lines).
When MCF-7 was treated with etoposide, 11% of apoptosis was observed which increased by the presence of Caspase-3 or Caspase-3s (35% and 22%, respectively). A co transfection was then performed in MCF-7 to observe the effect of the presence of both transcripts. The results showed, 7% of apoptosis before treatment and 10% after treatment in MCF-7 co transfected cells.
HBL100 treatment induced 34% of apoptosis in wild type cells, 46% in Caspase-3 transfected cells and only 14% in Caspase-3s one's.
Thus, expression of Caspase-3 or Caspase-3s in MCF-7 cells, increased etoposide-induced apoptosis but when both were co-transfected cell death was inhibited. This observation was confirmed when both transcripts were co expressed in HBL100. These results suggest that the pro apoptotic activity was higher with Caspase-3 than Caspase-3s and that each transcript has its own role with different activity when they are co-expressed.

Glucocorticoids and NF-kB inhibition: glucocorticoid-induced leucine zipper (GILZ)/NF-kB interaction

Enrico Velardi, Valerio Donato, Michela Massetti, Rosa Di Virgilio, Cristina Marchetti, Stefano Bruscoli and Carlo Riccardi

Section of Pharmacology Toxicology and Chemotherapy; Dept of Clinical and Experimental Medicine; Via del Giochetto, 06122 Perugia, Italy

Glucocorticoids (GCs) induce apoptosis through differents molecular mechanisms including glucocorticoid receptor (GR)-mediated NF-kB inhibition. We performed experiments to evaluate the possible role of GILZ in GR-mediated NF-kB inhibition. GILZ (Glucocorticoid-Induced Leucine Zipper) is a 137 aa protein expressed in different tissues including lymphoid and myeloid cells, and is rapidly induced by GCs treatment, known as potent anti-inflammatory and immunomodulatory molecule. GILZ effects on immune system relate in part to the inhibition of NF-kB activity. We have previously shown that GILZ, independent from I-kB, inhibits NF-kB activation, nuclear translocation and DNA binding by a direct protein-to-protein interaction with NF-kB.
GILZ is characterized by a leucine zipper (aa 76-97) domain, an -NH2 terminal domain (aa 1-75) and a -COOH terminal PER region (aa 98-137) rich in proline and glutamic acid residues. In the present study we used a number of mutants with the aim to define the GILZ molecular domains responsible for GILZ/p65NF-kB interaction. Results, obtained by *in vitro* and *in vivo* co-immunoprecipitation and by *in vivo* transcription experiments, using an NF-kB-sensitive reporter gene, indicate that GILZ homo-dimerization, through the leucine zipper domain, and part of the PER domain (aa 98-127) are necessary for GILZ interaction with NF-kB and inhibition of its transcriptional activity. NF-kB is involved in immune system development and its activation is associated to important pathological conditions including inflammatory/autoimmune diseases. Results here reported identify the molecular characteristics of GILZ for binding to and inhibition of NF-kB.
Defining the molecular domains of GILZ involved in NF-kB binding and inhibition is necessary for future studies aimed to design new therapeutic approaches able to mimic or antagonize GILZ/NF-kB interaction, thus modulating apoptosis.

Caspase-mediated cleavage of 5-lipoxygenase during apoptosis in T lymphoma cells

Maria Rita Rippo, Federica Villanova, Simona Moretti, Barbara Tomassini, Alfonso Catalano and Antonio Procopio

Dept. Of Molecular Pathology and Innovative Therapies, Polytechnic University of Marche, Ancona, Italy

5-lipoxygenase (5-LOX) is a tightly regulated enzyme involved in the production of leukotrienes from arachidonic acid and promotes cancer cell proliferation and survival. In our laboratory it was previously demonstrated that 5-LO expression and activity are induced by genotoxic agents in a p53-independent manner and antagonized p53- or genotoxic drug-induced apoptosis in a variety of cancer cells. Furthermore, it has been recently demonstrated that it is processed and inactivated by caspase-6 (giving a 62kDa fragment) in high 5-LO-expressing B cells after splitting. Nevertheless, we have found several hypothetical cleavage sites for caspases which have not been tested. Here we show that 5-LO is processed during apoptotic cell death of HuT-78 and Jurkat T lymphoma cell lines. In these cells 5-LO protein expression increases during time in culture and the levels are comparable with those of 5-LO positive cells, such as PC3. 5-LO is processed under Fas-, UV- and doxorubicin-induced cell death, giving a main and common fragment of 40kDa, which is inhibited by the pan-caspase inhibitor zVAD-fmk. The sub-cellular fractionation shows that the 40kDa 5-LO fragment is localized into the nucleus. In vitro cleavage assay shows that human recombinant 5-LO is cleaved by caspase-3. To unravel the precise cleavage site, several Asp to Ala mutants, corresponding to the hypothetical cleavage sites, have been prepared to be transfected in 5-LO non-expressing T cells. The role of the 5-LO fragment in apoptosis and the caspases responsible of the cleavage are currently under investigation.

Inhibition of apoptosis by induction of hypoxia-inducible factor-1. Cell survival in the face of injury.

Margret C.M. Vissers, Prachee Gokhalé, Mary Morrison.

Free Radical Research Group, Pathology Dept, Christchurch School of Medicine and Health Sciences, University of Otago, Christchurch, New Zealand.
email: margret.vissers@chmeds.ac.nz

Hypoxia-inducible factor (HIF)-1 is a transcription factor that controls cell responses to a low oxygen environment, although more recently a range of cellular conditions such as iron deficiency, ascorbate deprivation, or increased glucose metabolites have been shown to result in its up-regulation. HIF-1 is a heterodimer and its activity is controlled by hydroxylation of proline and asparagine residues by hydroxylases enzymes that require O_2, Fe^{2+}, 2-oxoglutarate and ascorbate. Deprivation of any of these substrates prevents the hydroxylation reactions and results in stabilisation of the HIF-1a and active gene transcription. HIF-1 controls many genes involved in the cell's response to hypoxia, including angiogenesis, erythropoeisis and glycolysis. Target genes also include those involved with cell proliferation, survival and apoptosis. The induction of pro-survival and pro-apoptotic genes by hypoxia can vary between cell types, and represents something of a paradox in the pro-survival vs pro-apoptotic pathways.

We have previously found that exposure of cultured endothelial cells to an enduring oxidant stress can inhibit apoptosis initiated by serum withdrawal. The effective oxidants used were amino acid chloramines, formed as a consequence of myeloperoxidase activity. These are relatively stable oxidants that are capable of crossing cell membranes and reacting almost exclusively with intracellular thiols. We have now found that exposure to chloramines can inhibit apoptosis in Jurkat and HL60 tumour cells treated with chemotherapy agents, and that the cells apparently survive. Treatment of the cells with chloramines resulted in up-regulation of HIF-1a, and this correlates with inhibition of apoptosis. If HIF-1a up-regulation is prevented, then apoptosis proceeds. These results demonstrate a novel oxidant-driven effect on apoptosis and HIF-1a signaling pathways that has implications for the outcome of inflammatory cells and tumour survival and progression. Understanding the interplay between these pathways will be important in determining the role of HIF-1 and oxidative stress in regulating cell death or survival.

The cell cycle inhibitor p57^{Kip2} promotes apoptotic mitochondrial changes and cell death.

P. Vlachos, U. Nyman, N. Hajji, T. Perlmann, and B. Joseph.

Division of Toxicology and Neurotoxicology, Institute of Environmental Medicine (http://www.imm.ki.se/), Karolinska Institute, Sweden, e-mail: Pinelopi.Vlachos@imm.ki.se.

The p57^{Kip2} gene, together with p21^{Cip1} and p27^{Kip}, belongs to the Cip/Kip family of cyclin-dependent kinase inhibitors, and has been suggested to be a tumor suppressor gene, being inactivated in various types of human cancers. p57 displays a more restricted pattern of expression than the other Cip/Kip family members. Only p57 has been shown to play vital roles during embryogenesis that other cell cycle inhibitors cannot compensate for. p57 null-mutant mice exhibit severe developmental defects. Many of the defects described are not directly linked to increased cell proliferation. In addition, p57 has been shown to influence cell differentiation of several cell types by mechanisms that have remained unknown, implying that p57 may have a broader scope of cellular action than only being a cell cycle inhibitor. p57 is structurally the most complex member of the Cip/Kip family and may be particularly important in processes that are beyond its prescribed role in cell proliferation. However, little is known concerning p57^{Kip2} possible interplay with the apoptotic cell death machinery and its possible implication for cancer. Here, we report that selective p57^{Kip2} expression sensitize HeLa cells to cell death induced by apoptotic agents, such as cisplatin, etoposide and staurosporine, via a mechanism which does not require p57^{Kip2}-mediated inhibition of cyclin-dependent kinases. Moreover, our studies reveal that p57^{Kip2} primarily promotes the mitochondrial apoptotic pathway, favoring loss of mitochondrial transmembrane potential, and consequent release of cytochrome-*c* into cytosol, caspase-9 and caspase-3 activation. In accordance, we found that Bcl-2 overexpression or VDAC inhibition were able to inhibit p57^{Kip2}'s cell death promoting effect. Thus, in addition to its established function in control of proliferation, these results reveal a mechanism whereby p57^{Kip2} influences the mitochondrial apoptotic cell death pathway in cancer cells.

Targeting XIAP overcomes apoptosis resistance of pancreatic carcinoma cells and suppresses tumor growth and invasion *in vivo*

Meike Vogler, Marjana Jovanovic, Katharina Dürr, Klaus-Michael Debatin, Simone Fulda

University Children's Hospital, Ulm, Germany

Pancreatic cancer is one of the leading causes of cancer-related death due to its resistance towards conventional therapies. A better understanding of the molecular mechanisms underlying apoptosis resistance of pancreatic cancer is therefore crucial to improve cancer therapy. Here, we identify X-linked inhibitor of apoptosis (XIAP) as a key determinant of apoptosis resistance of pancreatic carcinoma cells. XIAP was expressed at high levels in primary pancreatic carcinoma samples and the majority of pancreatic carcinoma cell lines. Stable downregulation of XIAP by RNA interference significantly reduced viability and enhanced TRAIL-induced apoptosis of pancreatic carcinoma cells. Importantly, knockdown of XIAP also strongly inhibited clonogenicity of pancreatic cancer cells treated with TRAIL indicating that XIAP promotes clonogenic survival. Also, downregulation of XIAP significantly increased CD95- or gamma-irradiation-induced apoptosis, whereas it had no effect on 5-fluorouracil, etoposide or gemcitabine-induced apoptosis. Analysis of apoptosis signaling pathways revealed that knockdown of XIAP resulted in enhanced cleavage and enzymatic activity of caspase-3, -9, -2 and -8. Interestingly, downregulation of XIAP also led to enhanced drop of mitochondrial membrane potential and increased cytochrome c release after stimulation with TRAIL, indicating that XIAP also functions upstream of mitochondria in TRAIL-induced apoptosis. In support of this notion, inhibition of caspase-3 completely inhibited drop of mitochondrial membrane potential in TRAIL-treated pancreatic carcinoma cells, in which XIAP was knocked down. Most importantly, knockdown of XIAP profoundly inhibited tumor growth and invasion of pancreatic carcinoma cells *in vivo.* Similar to XIAP knock down by RNAi, inhibition of XIAP by small molecule antagonists sensitized pancreatic cancer cells to TRAIL-, CD95- or gamma-irradiation-induced apoptosis. By demonstrating that targeting IAPs significantly enhanced death receptor or gamma-irradiation-induced apoptosis and also suppressed tumor growth and invasion of pancreatic carcinoma cells *in vivo*, our findings indicate that targeting IAPs represents a novel, promising strategy to overcome apoptosis resistance of pancreatic cancer, which has important clinical implications.

TRAIL Increases Caspase Activity and Apoptosis in Primary Human Hepatocytes

Xandra Volkmann[1], Michael P. Manns[1], Klaus Schulze-Osthoff[2], and Heike Bantel[1]

[1]Dept. of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, [2]Institute of Molecular Medicine, University of Duesseldorf, Duesseldorf, Germany

TRAIL has been implicated to exert its cytotoxicity *in vivo* only in transformed and infected cells without causing apoptosis in healthy tissue. However, warning concerns recently arose when human hepatocytes were shown to be TRAIL sensitive. Re-evaluation of these results suggested that the hepatotoxicity depends on the version of recombinant TRAIL.
In this study, we analyzed the toxicity of different TRAIL-versions compared to Flag-tagged CD95L in primary human hepatocytes 16 hours after isolation using a luminometric enzyme assay as well as by western blot analysis for detection of caspase-3 activity and by hypodiploid DNA staining for detection of apoptosis. Compared to untreated primary human hepatocytes, 50 or 100 ng/ml of polyhistidine-tagged TRAIL or untagged TRAIL as well as of agonistic TRAIL-R1 and TRAIL-R2 antibodies (1 ug/ml) significantly increased caspase activity in a dose-dependent manner. Whereas polyhistidine-tagged TRAIL and untagged TRAIL induced significant ($p<0.01$) lower caspase activity compared to Flag-tagged CD95L (100 ng/ml), no significant differences in hepatotoxicity was observed between agonistic TRAIL-R1 or TRAIL-R2 antibodies and Flag-tagged CD95L. To analyze whether TRAIL-induced increased caspase activation in primary human hepatocytes is due to isolation-mediated oxidative stress at day 1, we cultured hepatocytes over 4 days and compared caspase activity following treatment with Flag-tagged CD95L (100 ng/ml) or untagged TRAIL (50 ng/ml) for 6 hours at days 1, 2, 3 and 4. Compared to the respective untreated control, Flag-tagged CD95L- as well as untagged-TRAIL-induced caspase activation increased with the time of culturing, indicating that TRAIL-induced caspase activation is not an artefact due to the isolation procedure. To analyze whether CD95L- and TRAIL-induced caspase activation results in apoptosis, we simultaneously measured caspase activity and apoptosis by luminometric enzyme assay and hypodiploid DNA staining. Similary to caspase activation, the strongest apoptosis rate was observed after treatment of hepatocytes with Flag-tagged CD95L or agonistic TRAIL-R antibody, whereas treatment with polyhistidine-tagged TRAIL or untagged TRAIL was associated with lower apoptosis rates. In summary, these results implicate that TRAIL is toxic in primary human hepatocytes. There is a strong need for better *in vitro* / *in vivo* models to analyze potential side effects and hepatotoxicity of TRAIL.

Synthetic phospholipid analogues induce apoptotic cell death through death ligand independent formation and activation of FADD/caspase-8 DISC complexes

Clarissa von Haefen, Jana Wendt, Claus Belka, Silke Radetzki, Werner Reutter, Kerstin Danker, Peter T. Daniel

Department of Hematology, Oncology and Tumor Immunology, University Medical Center Charité, Campus Buch, Humboldt University, Berlin-Buch
Department of Radiation Oncology, University of Tübingen
Department of Biochemistry and Molecular Biology, University Medical Center Charité, Campus Benjamin Franklin, Berlin

Ino-PAF, a novel glycosidated synthetic analogue of platelet-activating factor (PAF), shows an impressive anti-proliferative and apoptosis inducing activity. Ino-PAF and other phospholipid analogues are incorporated into cellular membranes where they exert their cytotoxic activity.Consequently, their effects are independent of the proliferative state of the target cell. Here, we show that cell death induction occurs via regulated induction of apoptosis through the death receptor pathway that is, however, independent of ligand triggered death receptor activation. Cell death induced by Ino-PAF and a glucosidated derivate, Glc-PAF, was dependent on FADD and caspase-8 and could be inhibited upon overexpression of a dominant negative FADD (FADD-DN) or crmA. Apoptosis proceeded, however, in the absence of CD95 death ligand expression and receptor binding and resulted in low rates of ligand independent formation of FADD/Caspase-8 death inducing signaling complexes (DISC). Execution of cell death was inhibited upon ectopic expression of Bcl-2. This interfered with breakdown of the mitochondrial membrane potential, release of cytochrome c and activation of caspases-9 and -3. Thus, Ino-PAF and Glc-PAF trigger a type-II-like but ligand independent death inducing signaling machinery that relies on the extrinsic death pathway. This is of significant interest as the death receptor pathway often remains active in cancer and therefore provides an interesting and powerful tool for the development of anticancer drugs.

Involvement of full-length RasGAP in the anti-apoptotic response induced by its cleavage fragments.

Joël Walicki, Jiang-Yan Yang, Christian Widmann

Institute of Cellular Biology and Morphology, Lausanne University, Switzerland

We have recently identified RasGAP, a Ras-specific GTPase-activating protein, as a caspase substrate. RasGAP is cleaved at position 455 at low caspase activity generating an amino-terminal fragment (fragment-N) and a carboxy-terminal fragment (fragment-C). Surprisingly, fragment-N generates a potent survival signal that is at odds with the usual pro-apoptotic function of cleaved caspase substrates. Fragment N protects cells by activating the Ras-PI3K-Akt pathway. Here we have addressed the question as to whether fragment N could induce anti-apoptotic response independently of its parental RasGAP molecule. Our results show that expression of fragment N in mouse embryonic fibroblast lacking RasGAP did not result in Akt activation. Full-length RasGAP is therefore required for fragment N to induce a survival signal. It appears however that this requirement does not involve a direct interaction of RasGAP with its cleavage fragment as these two molecules didn't bind to each other in co-immunoprecipitation experiments. Our results indicate that fragment N modulates the function of its parental molecule to generate a protective signal in cells having mildly activated their caspases.

Stress activation protein kinases: key signalling molecules as therapeutic targets for hair cell death in the injured inner ear

J. Wang, J. Ruel, S. Ladrech, C. Domec and J.L. Puel

INSERM - UMR 583 and Université de Montpellier 1: Physiopathologie et thérapie des déficits sensoriels et moteurs, Institut des Neurosciences de Montpellier, Hôpital Saint Eloi, 80, rue Augustin Fliche, 34295 Montpellier, France.

Hearing loss can be caused by a variety of insults, including acoustic trauma and exposure to ototoxins, which principally effect the viability of sensory hair cells via the mitogen activated protein kinase (MAPK) cell death signaling pathway that incorporates c-Jun N-terminal kinase (JNK).

We evaluated the otoprotective efficacy of D-JNKI-1, a cell permeable peptide that blocks the MAPK/JNK signal pathway. The experimental studies included organ cultures of neonatal mouse cochlea exposed to an ototoxic drug and, cochleae of adult guinea pigs that were exposed to acoustic trauma. Results obtained from the organ of Corti explants demonstrated that the MAPK/JNK signal pathway is associated with injury and that blocking of this signal pathway prevented hair cell death initiated by this ototoxin. Results from *in vivo* studies showed that apoptosis is the predominant mode of cell death following acoustic trauma. Analysis of the events occurring after acoustic trauma demonstrates that JNK/SAPK activates a mitochondrial cell death pathway (i.e. activation of Bax, release of cytochrome c, activation of procaspases and cleavage of fodrin). The direct application of D-JNKI-1 into the scala tympani of the guinea pig cochlea prevented acoustic trauma-induced permanent hearing loss in a dose dependent manner.

These results indicate that the MAPK/JNK signal pathway is involved in both ototoxicity and acoustic trauma induced hair cell loss and permanent hearing loss. Blocking this signal pathway with D-JNKI-1 is of potential therapeutic value for long-term protection of both the morphological integrity and physiological function of the organ of Corti during times of oxidative stress.

Sensitization of Hepatocytes by Methyltransferase-Inhibitor 5-Azacytidine against Cytokine-mediated Cell Death

Weiland T, Wendel A, Künstle G

Chair of Biochemical Pharmacology, University of Konstanz, Germany
e-mail: Timo.Weiland@uni-konstanz.de

Aims: 5-Azacytidine (5-aza-CR) was developed as an antineoplastic agent long before its inhibitory activity on DNA methyltransferases and thus its ability to reverse aberrant gene silencing became evident. Today, 5-aza-CR is the first DNA hypomethylating agent approved for treatment of myelodysplastic syndromes and represents a promising drug for epigenetic cancer therapy. However, 5-aza-CR is contraindicated for patients with pre-existing liver diseases due to adverse hepatotoxic effects for yet unknown reasons. This study was initiated in order to identify the mechanisms that underlie 5-aza-CR-associated hepatotoxicity. Results: In a time- and concentration-dependent manner, hepatocytes, i.e. primary murine and human hepatocytes and HepG2 cells, became highly sensitive towards death receptor-mediated cell death (induced either by CD95L, TRAIL or TNF) when pre-treated with sub-toxic concentrations of 5-aza-CR. 5-aza-CR-enabled hepatocyte cell death was characterized by classical hallmarks of apoptosis such as membrane blebbing, chromatin condensation and exposure of phosphatidyl serine on the outer membrane. Moreover, in presence of 5-aza-CR activation of downstream caspases was markedly increased when the extrinsic pathway of apoptosis was activated by heterologous and transient expression of caspase-8. The latter finding was further substantiated by using a cell-free model of the extrinsic apoptosis pathway. Addition of recombinant caspase-8 into cytosolic extracts derived from 5-aza-CR-pretreated cells resulted in a significant higher activation of downstream caspases when compared to cytosolic extracts derived from non-pretreated cells. In contrast, direct activation of the intrinsic pathway of apoptosis by UV-radiation or staurosporine did not result in a higher rate of apoptosis. Conclusions: This study reveals that 5-aza-CR sensitizes hepatocytes against death receptor agonists. As a mechanistic rationale, we provide evidence for a preferential exacerbation of the extrinsic death signaling pathway. In summary, our findings offer a reasonable explanation for the adverse hepatotoxic properties of 5-aza-CR observed in patients with pre-existing liver disorders.

A novel Bcl-2-like inhibitor of apoptosis is encoded by the parapoxvirus, orf virus

Dana Westphal[1], Ellena M. Whelan[1], Stephen B. Fleming[1], Merilyn H. Hibma[1], Elizabeth C. Ledgerwood[2] and Andrew A. Mercer[1]

Department of Microbiology and Immunology[1] and Department of Biochemistry[2], University of Otago, PO Box 56, Dunedin, New Zealand, e-Mail: wesda320@student.otago.ac.nz

Apoptotic cell death forms part of the host defence against virus infection. It is therefore not surprising that numerous viral inhibitors of apoptosis have been identified. We tested orf virus, a member of the poxvirus family, for its ability to inhibit apoptosis, and found that orf virus-infected cells are fully resistant to UV-induced changes in cell morphology, caspase activation and DNA fragmentation. Using a library of vaccinia - orf virus recombinants, we identified a candidate orf virus gene whose presence is linked with the inhibition of apoptosis. The 173 amino acid predicted protein (ORFV125) has no clear homologues in public databases. However, ORFV125 possesses a predicted mitochondrial-targeting motif, which, we were able to show, is necessary and sufficient to direct the viral protein to the mitochondria. Using a stable cell line expressing ORFV125, we determined that the viral protein alone could fully inhibit UV-induced DNA fragmentation, caspase activation and cytochrome *c* release. In contrast, we found that it is not able to prevent UV-induced activation of c-Jun NH2-terminal kinase, an event occurring upstream of the mitochondria. These observations suggest that ORFV125 acts at the mitochondria. Furthermore, these features are comparable with the anti-apoptotic properties of the mitochondrial regulator Bcl-2, indicating that ORFV125 may act in a similar manner. Further bioinformatics analyses reveal some sequence relatedness and secondary structure similarities with Bcl-2 family members, including some evidence of all four Bcl-2 homology domains. We propose that ORVF125 is a new anti-apoptotic member of the Bcl-2 family.

A transgenic non-invasive caspase detection system reporting apoptosis and differentiation during normal Xenopus development.

Willemarck Nicolas[1], Frans Van Roy[2] and Vleminckx Kris[1]

[1]Unit of Developmental Biology and [2]Molecular Cell Biology Unit, Department for Molecular Biomedical Research, VIB-Ghent University, Ghent, Belgium.
E-mail: Nicolas.Willemarck@dmbr.UGent.be

Apoptosis plays an essential role in animal development and homeostasis. Disorders of this process cause various pathologies, including autoimmune and neurodegenerative diseases. Caspases are central mediators of the apoptotic process, but there is growing evidence that these cysteine proteases also have apoptosis-independent functions, e.g. in the differentiation of lens fiber cells and erythrocytes.

Cell death has been extensively studied in cell culture and several assays are available to detect the effects of caspase activity. However, studies in whole organisms are not so easily performed and most of the assays to detect apoptotic cells or caspase activity *in vivo* are destructive. Because the activation of the caspase cascade is a relatively fast process, the developmental role of caspases is presumably greatly underestimated. Much of our current knowledge on the role of caspases during development is also based on phenotypic evaluation of specific knockout mice. However, because of molecular redundancy, these studies probably do not reflect all apoptotic and differentiation events involving caspase activation during development. For these reasons there is a clear and urgent need for assays that are able to report strong and mild caspase activation in a whole and living organism. Technologies based on intrinsic fluorescent proteins allow the non-invasive analysis of specific biological processes. Because of its external development, the transparency of its embryos and the ease to manipulating gene expression, *Xenopus* is ideally positioned to fulfill these needs.

We have designed and evaluated new non-invasive reporter systems that register caspase activation during apoptotic and differentiation events in living *Xenopus* embryos. These transgenic reporter systems are based on the generation of a fluorescent signal that is dependent on the activity of specific caspases. The offspring of the apoptosis-reporting transgenic line(s) will be used for hypothesis-driven experiments and to identify new roles of caspases during selected developmental processes.

Caspase-dependant cleavage of Beclin during apoptosis

Ellen Wirawan, Sigrid Cornelis en Peter Vandenabeele

Molecular Signalling and Cell Death Unit, Department of Molecular Biomedical Research, VIB, University of Ghent, Technologiepark 927, 9052 Zwijnaarde, B-9000 Ghent, Belgium

Beclin1 is a mammalian orthologue of Atg6/Vps30, a key component of the class III phosphatidyl inositol 3-kinase complex, that is essential for the formation of autophagosomes. Mammalian Beclin1 was first identified as a Bcl-2 interacting protein in addition to its participation in autophagosome formation. The interaction between the anti-apoptotic Bcl-2 protein and the autophagy protein Beclin is likely a point of convergence of the apoptotic and autophagic machinery. Indeed, complex interrelationships seem to exist between autophagy and the apoptotic cell death pathway. For example genetic inhibition of autophagy can activate apoptotic death in nutrient-starved mammalian cells and vice verse.

In the present study we demonstrate that apoptosis induced by IL-3 deprivation in Ba/F3 cells is associated with the cleavage of Beclin1, resulting in a ~20 kDa N-terminal fragment and a ~40 kDa C-terminal fragment. The N-terminal fragment is rapidly degraded by the 26 kDa proteasome, while the C-terminal fragment is very stable.

Beclin cleavage coincides with the activation of specific caspases (3, 7, 8 & 9) in IL-3 depleted Ba/F3 cells and treatment with the caspase inhibitor z-VAD.fmk completely prevented Beclin1 cleavage. Furthermore, cotransfection studies in 293T cells show that Beclin1 is cleaved upon overexpression of caspase 3, 8 and 9.

Currently, we are analysing the function of caspase-dependent cleavage of Beclin1 in stable Ba/F3 cell lines, overexpressing the C-terminal Beclin1 fragment or a mutant cleavage resistant Beclin1 protein.

Programmed cell clearance : role of recognition and detachment signals for macrophage engulfment of human neutrophils

Erika Witasp, Shouting Zhang, and Bengt Fadeel

Division of Biochemical Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, e-mail: bengt.fadeel@ki.se

Macrophages recognize apoptotic cells by a variety of "eat me" signals expressed on the cell surface. One of the most important signals for recognition and clearance of cell corpses is phosphatidylserine (PS) (Fadeel, Cell. Mol. Life Sci. 2003; 60:2575-2585). We established an in vitro co-cultivation system with M-CSF-stimulated human monocyte-derived macrophages (HMDMs) and human polymorphonuclear neutrophils (PMNs) isolated from peripheral blood to study PS-dependent phagocytosis. Surprisingly, engulfment of pre-chilled PMNs forced into massive synchronous apoptosis was not significantly different from uptake of viable cells despite a high degree of PS exposure on these cells. In contrast, PMNs undergoing spontaneous apoptosis upon overnight culture with similar levels of PS exposure were efficiently engulfed. We thus hypothesize that PS may be required, but not sufficient for macrophage clearance of PMNs. We also observed that phagocytosis of PMNs was diminished in the absence of serum, indicating that serum factor(s) are important for cell clearance in this model. Furthermore, CD31 (PECAM-1), a detachment signal for phagocytes, was highly expressed on freshly isolated PMNs and almost completely absent on the surface of cells undergoing spontaneous apoptosis, whereas expression was partially retained on temperature-arrested PMNs undergoing a rapid and synchronous burst of apoptosis. Our data suggest, therefore, that the sequential emergence of “eat-me” signal(s) and disabling/shedding of “don’t eat me” signal(s) may be required for efficient macrophage engulfment of PMNs. These studies could have important implications for our understanding of programmed cell clearance and its role in the resolution of inflammation.

Apoptosis-induced membrane expression of proteinase 3 during neutrophil apoptosis decreased the rate of phagocytosis by macrophages : a new pro-inflammatory role of membrane proteinase 3 in neutrophils.

Chahrazade Kantari*, Magali Pederzoli* and Véronique Witko-Sarsat
*equally contributed to the work

INSERM U507, Necker Hospital, Paris

Neutrophils are essential actors in the defense against pathogens. Because neutrophils contain highly toxic microbicidal mediators, their apoptosis, considered as a "clean disposal mechanism" is crucial for the resolution of inflammation. Proteinase 3 (PR3) and human neutrophil elastase (HNE)) are two serine proteinases homologues which are stored in the azurophilic granules of neutrophils. They have microbicidal properties and can mediate connective tissue degradation when they are released in the extracellular medium upon neutrophil degranulation. Because it has been previously described that serine proteinases (such as granzyme B) can be involved in apoptosis, we studied whether PR3 or HNE could be involved in neutrophil apoptosis. Using a model of a stably transfected mast cell line (RBL-2H3), we observed that apoptosis induced PR3 but not elastase membrane expression in the absence of degranulation, with a close relationship between PR3 and annexin-V membrane labeling. The aim of the present study was to test whether in human neutrophils, apoptosis induced PR3 membrane expression and whether PR3 could interfere with macrophage phagocytosis. We observed that, in neutrophils, PR3, but not elastase, was expressed at the plasma membrane during apoptosis. The membrane expression of PR3 did not result from degranulation since no increase in the expression of CD35 or CD63 was observed. Using streptolysine-O permeabilization, which permeabilizes only plasma membrane precluding the detection of granular proteins, we could visualize the extragranular pool of PR3. In contrast, no elastase or myeloperoxidase, which are restricted to the granular compartment, could be detected. We provide evidence using RBL/PR3, that apoptosis-induced membrane PR3 interferes with phagocytosis of the apoptotic RBL by macrophages.

This work was supported by Vaincre la Mucoviscidose (VLM) et l'Association de Recherche sur la Polyarthite Rhumatoide (ARP), Fondation pour le Recherche Médicale (FRM) and Amgen.

Up-regulated SPP1 in molecular pathway of cervical cancer

Y.F. Wong, T.H. Cheung, T.K.H. Chung

Department of Obstetrics & Gynaecology, The Chinese University of Hong Kong, Hong Kong

Though cervical cancer is highly curable when detected early, it remains the leading cause of gynecological cancer death worldwide. It is the most common malignancy of the genital tract in Hong Kong women. We have performed oligonucleotide microarray in an analysis of gene expression profiles obtained from cervical cancers in Hong Kong women and discovered the genes most aberrantly expressed in cervical squamous cell carcinoma. SPP1, secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte) located on 4q21-q25 is one of three genes that exhibited >4.0-fold increased expression in cervical cancers compared to their normal counterparts. Differentially expression of SPP1 has been further validated by quantitative RT-PCR and immunohistochemical staining on independent set of cancer and control specimens. In vitro study, we have obtained silencing of SPP1 expression in cervical cancer cell line HeLa cells by siRNA specific to SPP1. Fluorescent staining and flow-cytometer assessment have shown increased apoptosis of HeLa cells induced by SPP1 specific siRNA. The pathway analysis of cervical cancer expression data indicates that 4 genes including SPP1, VEGF, CDC2 and CKS2 are coordinately differentially regulated between cancer and normal. In this pathway, CKS2 up-regulates CDC2 gene, while CDC2 and VEGF up-regulate SPP1 each other. Taken together, our studies reveal that up-regulated SPP1 gene seems to be an endpoint in a molecular pathway of cervical squamous cell carcinoma, and this antiapoptosis-associated gene, together with VEGF, CDC2 and CKS2 may be as the candidate targets for molecular therapy of cervical cancer .

Direct and Specific Interaction of Bcl-xL with cytochrome c: Equilibrium Affinity and Binding Kinetics‡

M.Yadaiah, P.Nageswar Rao, P.Harish and Abani K Bhuyan

School of Chemistry, University of Hyderabad, Hyderabad 500046, India
e-mail: ch03ph05@uohyd.ernet.in

The mechanism by which Bcl-x_L, a member of the Bcl-2 family of proteins, regulates apoptosis is quite involved and less understood. Studies indicate that Bcl-x_L is capable of inhibiting the death inducing signaling complex, DISC (1). It has also been shown that overexpression of Bcl2/Bcl-xL inhibits mitochondrial membrane permeabilization (2). Additionally, earlier coimmunoprecipitation study has reported that Bcl-xL binds specifically to cytochrome *c* (3). However, no biochemical study demonstrating the direct interaction of Bcl-xL and cytochrome c has been reported to date. To address this problem, we have studied the binding of overexpressed Bcl-xL to cytochrome *c* by both immunoblotting and quanititative thermodynamic and kinetic methods biochemical methods at pH 7, 50 mM phosphate buffer, 22°C. Since the heme prosthetic group of cytochrome *c* was found to quench the fluorescence of Bcl-xL, we used optical absorption of cytochrome *c* as the probe, and analyzed the spectral changes in the presence of increasing concentrations of Bcl-xL. At pH 7, 100 mM NaCl, the 1:1 interaction between the two proteins is characterized by an equilibrium association constant, K_a=12.4×10^6 M^{-1}, which declines as the salt concentration increases. To determine the rate of interaction, we measured stopped-flow kinetics employing different stoichiometry of cytochrome *c* and Bcl-xL concentrations. The derived bimolecular rate constant for binding, k_{bi}= 0.24 μM^{-1} s^{-1}. These results that indicate fairly tight affinity of monomeric Bcl-xL for cytochrome *c* raise two important questions regarding the mechanism and molecular basis of this antiapoptotic interaction. Does Bcl-xL interact directly with cytochrome *c* within the DISC? If all of the cytosolic fraction of Bcl-xL is not translocated to mitochondria in response to apoptosis (4), could the persisting fraction act as the second arrest point for cytochrome c? Furthermore, the observed direct interaction suggests that the cytosolic Bcl-xL can indeed arrest cytochrome c under non-apoptotic situations where the latter may be translocated from the mitochondria.

References:

1. Wang, X., Zhang, J., Kim, H. P., Wang, Y., Choi, A. M. & Ryter, S. W. (2004) *FASEB J.* **18**, 1826-1833.
2. Breckenridge, D. G. & Xue, D. (2004) *Curr. Opin. Cell. Biol.* **16**, 647-652.
3. Kharbanda, S., Pandey, P., Schofield, L., Israels, S., Roncinske, R., Yoshida, K., Bharti, A., Yuan, Z., Saxena, S., Weichselbaum, R., Nalin, C. & Kufe, D. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 6939-6942.
4. Jeong. S., Game, B., Lee, Y., Hsu, Y., Ryu, S., Yoon, S. & Youle, R. J. (2004) *EMBO J.* **23**, 2146-2155.

‡ *Supported by the Department of Science & Technology (Grant 4/1/2003-SF), Government of India*

Expression of an uncleavable N-terminal RasGAP fragment *in vitro* and *in vivo* increases insulin secreting cells resistance towards apoptotic stimuli

Jiang-Yan Yang[§], Joël Walicki[§], Amar Abderrahmani[§], Marion Cornu[#], Gérard Waeber[§¶], Bernard Thorens[#], and Christian Widmann[§*]

[§] Department of Cellular Biology and [#]Department of Physiology, Faculty of Biology and Medicine, Lausanne University, Switzerland. [¶] Department of Internal Medicine, Lausanne University Hospital (CHUV), Switzerland.

Apoptosis of pancreatic beta cells is implicated in the onset of type 1 and type 2 diabetes. Consequently, strategies aimed at increasing the resistance of beta cells towards apoptosis could be beneficial in the treatment of diabetes. RasGAP, a regulator of Ras and Rho GTPases, is an atypical caspase substrate as it inhibits, rather than favors, apoptosis when it is partially cleaved by caspases-3 at position 455. The anti-apoptotic signal generated by the partial processing of RasGAP is mediated by the N-terminal fragment (fragment N) in a Ras-PI3K-Akt-dependent, but NF-kB-independent, manner. Further cleavage of fragment N at position 157 abrogates its anti-apoptotic properties. Here we demonstrate that an uncleavable form of fragment N protects the conditionally immortalized pancreatic insulinoma betaTC-tet cell line against various insults, including exposure to genotoxins, trophic support withdrawal, and incubation with inflammatory cytokines. Fragment N did not alter the insulin content and the ability of the cells to secrete insulin in response to glucose. These in vitro data indicate that fragment N protects beta cells without affecting their function. To evaluate that protective role of fragment N in physiological condition, we have developed a transgenic mice (Rip-N) expressing fragment N specifically in pancreatic beta cells. Rip-N transgenic animals have a non-fasted glycemia comparable to control wild type littermates. Importantly, primary islet prepared from Rip-N mice were significantly more resistant against cytokine-induced apoptosis compared to islets derived from wild type mice. Therefore the pathways regulated by fragment N represent promising targets for anti-diabetogenic therapy..

Keywords : Apoptosis, Beta cells, Caspases, Diabetes, RasGAP, Stress, Survival pathways.

Gene induction patterns of apopto-phagocytic genes in human macrophages

Gábor Zahuczky, Gyöngyike Májai, Goran Petrovski and László Fésüs

Cell Biology Laboratory, Department of Biochemistry and Molecular Biology, Medical and Health Science Center, University of Debrecen, Hungary

The third synapse is formed between the ~ 600 billion cells dying daily in the human body and the phagocytic cells responsible for their clearance. In order to study the molecular elements of the interaction between the dying and engulfing cells, a TaqMan Low Density Array has been designed and used for a systematic study. Through this approach the mRNA level of 95 apopto-phagocytic genes - including phagocytic receptors, integrins and other bridging molecules, members of various signaling pathways, effector molecules, cytokines – were parallel and quantitatively determined in human macrophages.

At first, we have investigated which of the genes in the above functional groups are up- or down-regulated during the differentiation of monocytes to macrophages. It was found that there is a significant change in the mRNA level of 37 genes: 27 (including receptors, bridging, signal and effector molecules and cytokines) were up- and 10 (including receptors, bridging molecules and a signal effector) were down-regulated. Comparing the expression patterns of human macrophages derived from different donors we have observed that different groups of genes can be distinguished according to their individual variability. We have not found specific correlation between the expression levels of clusters of genes and the phagocytic capacity of the macrophages toward apoptotic neutrophils. Upon glucocorticoid treatment the phagocytic capacity of macrophages increases, which is, according to our results, accompanied by the induction of specific receptors and bridging molecules.

Since the phagocytic machinery of the phagocytes consists of highly redundant molecular mechanisms, we were especially interested in whether there is a difference in the pattern of genes activated during the phagocytosis of cells dying through different types of cell deaths that is by autophagy, anoikis induced autophagy or UV induced apoptosis. Our results indicate that there are genes which are activated in macrophages independently of the type of engulfed particles, while other genes are activated only in the case of the phagocytosis of particular type of dying cells. This means that the parallel existing phagocytic pathways are differentially engaged depending on which types of dying cells they are encountered.

Proteasome inhibition causes severe growth retardation in mice through specific induction of apoptosis in stem-like chondrocytes

Farasat Zaman, Victoria Menendez-Benito, Andrei S Chagin, Emma Eriksson, Masaharu Takigawa, Bengt Fadeel, Nico P. Dantuma, Dionisios Chrysis, Lars Sävendahl

Department of Woman and Child Health, Pediatric Endocrinology Unit, Astrid Lindgren Children's Hospital, Karolinska Institutet, Stockholm, Sweden, Department of Cell and Molecular Biology, The Medical Nobel Institute, Karolinska Institutet, Stockholm, Sweden, Department of Biochemistry and Molecular Dentistry, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan and Institute of Environmental Medicine, Division of Biochemical Toxicology, Karolinska Institutet, Stockholm, Sweden.
E-mail:: Farasat.Zaman@ki.se

Proteasome inhibitors, a novel class of anti-cancer drugs, are relatively well tolerated and have recently been introduced in the clinic for the treatment of multiple myeloma. The tumor selectivity and low toxicity of proteasome inhibitors are surprising given the crucial role of the ubiquitin/proteasome system in a multitude of vital cellular processes. Here, we show that systemic administration of low doses of the proteasome inhibitor MG262 tissue specifically impairs the ubiquitin/proteasome system in growth plate chondrocytes. Importantly, young mice displayed severe growth retardation not only during but also 45 days after a short period of treatment (10 days) with the proteasome inhibitor. Dysfunction of the ubiquitin/proteasome system was accompanied by induction of apoptosis of stem-like chondrocytes in the growth plate. These results were recapitulated in cultured rat metatarsal bones suggesting a direct effect of proteasome inhibitors in the growth plate. Similarly, induction of apoptosis was also detected in chondrocyte-derived cell lines; an effect associated with activation of the caspase cascade and up-regulation of p53. Suppression of p53 with siRNA partly rescued chondrocytes from apoptosis. Collectively, our observations emphasize the need to carefully scrutinize the in vivo effects of proteasome inhibitors since essential cell populations may be selectively targeted which, in turn, may cause long lasting and irreversible side effects in cancer patients, especially in children.

Induction of osteoclasts apoptosis by using a decoy oligodeoxynucleotide in vivo mimicking a region of distal promoter C of ER alpha gene

[1]Letizia Penolazzi, [1]Margherita Zennaro, [1]Ercolina Bianchini, [2] Eros Magri, [1]Elisabetta Lambertini, [1]Elisa Tavanti, [1]Roberto Gambari and [1]Roberta Piva

[1]*Department of Biochemistry and Molcular Biology, Ferrara University, Italy*
[2]*Department of Experimental and Diagnostic Medici, Ferrara University, Italy*
E-mail: piv@unife.it

Osteoclasts (OCs) are multinucleated cells derived from hematopoietic stem cells and are the sole bone resorbing cells, through different mechanisms which are not fully characterized.These cells are essential for skeletal development and remodelling, consequently the molecular characterization of osteoclast differentiation is of potential clinical importance. Of particular interest is the role of estrogen receptor alpha (ER) protein that mediates the action of estrogen. In particular, it suppresses OCs formation and activity and inhibits OCs-mediated bone resorption in part by stimulating OCs to undergo apoptosis through a receptor-mediated genomic action. We demonstrated, for the first time, that a specific decoy approach designed for increasing ER expression induced apoptosis of human primary osteoclasts but not of osteoblasts. ER expression was increased by interfering with the activity of a negative transcription factor and by removing it with a powerful decoy oligonucleotide (RA4-3') mimicking a region of distal promoter C of ER gene. The observed effect was associated with increase of the levels of Caspase-3 and Fas death receptor. In particular, our results are of interest to develop a valuable strategy to modify cellular phenotype and to design new therapeutic approaches both for osteopenic disorders and bone metastatic control. At this purpose, the clinical orthodontic offers a good opportunity to study in vivo the efficacy of our approach. In fact, the increased number and activity of OCs is involved not only in the regulation of alveolar bone resorption during orthodontic tooth movement, but also in the origin of dental problems in diseases of OCs activation affecting the maxillo-mandibular bone. We designed in vivo experiments aimed at regulating alveolar bone resorption. Six Wistar male rats were subjected to orthodontic forces, in combination or not with RA4-3' decoy treatment by using a split-mouth design. Examination of paraffin sections of the excised molars showed that orthodontic forces caused a percentage of apoptotic OCs that appears to be highly potentiated by RA4-3', but not by scramble ODN. These data confirm the results obtained in vitro with human OCs from peripheral blood, suggesting an important correlation between ER and the possibility to modulate OCs functions.

Crucial role of PTEN in regulation of ROS generation and neuronal apoptosis

Yuan Zhu[1,2], Patrick Hoell[1], Barbara Ahlemeyer[3] and Josef Krieglstein[1]

[1]Institut für Pharmakologie und Toxikologie, Philipps-Universität Marburg, Ketzerbach 63, 35032, Marburg, Germany; [2]Klinik für Neurochirurgie, Universitätsklinikum Gissen and Marburg, Baldingerstrasse, 35043, Marburg, Germany and [3]Institut für Anatomie und Zellbiologie, Universitätsklinikum Giessen and Marburg, Aueweg 123, 35385, Giessen, Germany. E-mail: zhu@med.uni.marburg.de

The highly frequent mutation of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) in various cancers has attracted much attention from researchers to study the role of this molecule in tumor genesis. As an important tumor suppressor, the pro-apoptotic function of PTEN has been linked to its capacity to antagonize the PI3K/Akt signaling pathway. However, less data are available concerning its role in neurodegeneration in which apoptotic processes are also involved. In the present study, we attempted to study the role and the underlying mechanism of PTEN in neuronal apoptosis. Using primary rat hippocampal cultures, staurosporine (STS, 100 nM) induced a time-dependent apoptosis, accompanied by a massive production of reactive oxygen species (ROS), release of cytochrome c and activation of caspase 9 and 3. However, the expression of PTEN, and the levels of phospho-PTEN and phospho-Akt were not changed at any of the tested time points (0.5 h-24 h) after STS stimulation, suggesting that the protein level as well as the phosphorylation status of PTEN were not related to the process of apoptosis. Interestingly, immunostaining revealed a punctate intracellular distribution of PTEN from 2 h to 8 h after adding STS. Double labeling and Western blotting of mitochondrial fraction demonstrated a mitochondrial location and accumulation of PTEN, respectively, after challenging with STS. Furthermore, we provided evidence for the first time that PTEN was associated with Bax in the absence and the presence of STS. Of note, the STS-induced massive increase in the cellular level of ROS, release of cytochrome c and activation of caspase 3 were inhibited in cultured hippocampal cells when PTEN was knocked down by a specific antisense. Moreover, knockdown of PTEN significantly protected hippocampal cells from apoptotic damage. These findings demonstrated that PTEN is a crucial mediator of mitochondria-dependent apoptosis, and thus could become a molecular target for interfering with neurodegenerative diseases.

Long term effect of nicotinic acethylcholine receptor activation and downstream signaling in neurons

Elena Ziviani*, Daniele Bano*, Giordano Lippi*†, Michele Zoli† and Pierluigi Nicotera*

**MRC toxicology Unit, University of Leicester, Hodgkin Building, Lancaster Road, Leicester LE1 9HN,UK †Department of biomedical science, section of physiology, University of Modena and Reggio Emilia, Italy.*

Although nicotinic acetylcholine receptors (nAChRs) are widely present also in the central nervous system (CNS), their cellular functions and physiological significance are not yet fully understood. Recently, *in vivo* and *in vitro* studies have suggested a possible neuroprotective role for nAChRs. Exposure to nicotine, an agonist at nAChRs, has been shown to protect from neurototoxicity induced by NMDA, glutamate and potassium withdrawal, and to prevent β–amyloid induced neurotoxicity (Kihara et al., 2001 and Zamani and Allen, 2001). Furthermore, stimulation of nAChRs has been shown to delay the ageing process of nigrostriatal neurons, increase neurotrophic factor levels and up-regulate the expression of NGF receptors in the brain. These observations may provide a basis for the findings that cigarette smoking is negatively correlated with Parkinson's disease and positively correlated with the delayed onset of Alzheimer's disease (AD). In conjunction with this, nicotine has been shown to affect a wide variety of biological functions including gene expression (Yildiz, D. 2004).

Here we show that rat cortical neurons treated with 50μM nicotine for 24h are protected against NMDA induced exitotoxicity. The same treatment can alter gene expression, as reflected by activation of the transcription factor CREB. Supporting this hypothesis, we found that nicotine selectively up-regulates the expression of ryanodine receptor isoform 2, a calcium channel present in the endoplasmic reticulum whereas it does not effect the expression of the other calcium-transporting (CaTP) and calcium-buffering proteins (CaBP).

Ryanodine receptor 2 up-regulation is mirrored by a significant modification of the calcium response upon caffeine stimulation.

Poster List
(alphabetically)

Supported by EC within the framework of the Marie Curie
Conferences and Training Courses

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Supported by EC within the framework of the Marie Curie
Conferences and Training Courses

Poster List
(by session)

Supported by EC within the framework of the Marie Curie
Conferences and Training Courses

Session 1: Autophagy

Poster	Title	Presenting author
P-1	Accumulation of oxidative damage is responsible for autophagic programmed cell death of senescent cells	Abbadie, Corinne
P-2	Dexamethasone induces apoptotic cell death through NMDA receptor activation in cultured cerebellar granule cells, but does not act through NMDA receptor subunits 2a or B or L-type Ca channels.	Aden, Petra
P-3	HAMLET (Human alpha-lactalbumin made lethal to tumor cells) triggers rapid macroautophagy in tumor cells	Aits, Sonja
P-19	Mechanisms of cell death induced by adenoviral E1A deletion mutants in ovarian cancer	Baird, Sarah
P-56	Autophagic cell death by photodamage to the endoplasmic reticulum	Dewaele, Michael
P-58	CoBRA regulates autophagy during development of the nervous system in mammals	Di Bartolomeo, Sabrina
P-69	Gtdap-1 monitors the disappearance of the gonads during stress-inducing processes in planarians	Felix, Daniel A.
P-85	Gtdap-1 is involved in autophagy and autophagic programmed cell death in planarians	González-Estévez, Cristina
P-105	Elucidating the mechanism by which DT-IL3 kills Acute Myelogenous Leukemia cells	Horita, Henrick
P-130	Inhibition of caspases shifts propionibacterial SCFA-induced cell death from apoptosis to autophagy	Lan, Annaig
P-139	Selective elimination of mitochondria from living cell induced by inhibitors of bioenergetic functions.	Lyamzaev, Konstantin
P-151	Selective clearance of macrophages in atherosclerotic plaques via drug-induced nonapoptotic cell death	Martinet, Wim
P-168	Dramatic increase of cardiomyocytes necrosis in blockade of programme pathways of cell death	Nagibin, Vasyl
P-184	A model inducible system to examine the toxic effects of α-synuclein on human neuronal cells	Pavlaki, Maria
P-206	Cis-platinum treatment of subconfluent NIH/3T3 cultures induces a form of autophagic cell death in polyploid cells	Sciola, Luigi
P-256	Caspase-dependent cleavage of Beclin during apoptosis	Wirawan, Ellen

Session 2: Development

Poster	Title	Presenting author
P-84	Inspected by p53	Godlewski, Michal
P-88	Interaction of apoptosis signal transduction and capacitation in human spermatozoa	Grunewald, Sonja
P-92	Die and let live – mechanisms of apoptosis in postnatal development of intestinal mucosa	Hallay, Natalia
P-93	Characterisation of a putative poly(ADP-ribose)polymerase in the ascomycete Podospora anserina	Hamann, Andrea
P-132	DC-developed from CD34+ cells used as vaccine for possible anticancer therapy	Lazarova, Paula
P-152	Cell death in the primary enamel knot - mitochondrial pathways	Matalova, Eva
P-163	Bcl-2 and Bcl-XL are indispensable for the late phase of mast cell development	Möller, Christine
P-198	Localization of Active Caspase-6 in the Kinetochores Influences the Timing of Mitosis Progression	Rodriguez-Nieto, Salvador
P-203	Tissue transglutaminase promotes alternative cell death of erythrocytes	Sarang, Zsolt
P-212	Amazing journey down the tube – enterocyte turnover along the gastro-intestinal tract in young rats	Slazak, Paulina
P-213	The effect of leptin and ghrelin on survivin expression in the small intestinal mucosa of neonate piglets	Slupecka, Monika
P-226	Adenosine A2A Receptor-Mediated Cell Death of Mouse Thymocytes Involves Adenylate Cyclase, Bim and is negatively regulated by Nur77	Szondy, Zsuzsa
P-236	Ligation of RARα enhances glucocorticoid-induced apoptosis of T cells by facilitating glucocorticoid receptor mediated transcription	Tóth, Katalin
P-255	A transgenic non-invasive caspase detection system reporting apoptosis and differentiation during normal Xenopus development.	Willemarck, Nicolas
P-263	Proteasome inhibition causes severe growth retardation in mice through specific induction of apoptosis in stem-like chondrocytes	Zaman, Farasat

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Session 3: DNA damage and cancer

Poster	Title	Presenting author
P-5	Mechanisms of doxycycline-induced cytotoxicity and anoikis on human bronchial epithelial cells	Andreau, Karine
P-10	The effect of 5-FU on of Jurkat E6 cells with respect to bcl-2 family proteins	Aresvik, Dina
P-11	Induction of apoptosis in rat prostate cancer cells following treatment with difluoromethylornithine and roscovitine	Arisan, Elif Damla
P-12	New derivatives of doxorubicin are potent inducers of erythroid differentiation of human Chronic Myelogenous Leukemia (CML) cell line K562	Arkusinska, Justyna
P-15	VDAC1-based peptides interaction with Hexokinase I as a new approach for cancer therapy	Arzoine, Laetitia
P-24	Effect of the anthracycline Sabarubicin on the mitochondrial compartment of the human ovarian tumor cell line A2780	Bellarosa, Daniela
P-25	Acute apoptosis by cisplatin requires induction of reactive oxygen species but is not associated with damage to nuclear DNA	Berndtsson, Maria
P-26	Protection from apoptosis, arrest at the G2/M checkpoint and stimulation of DNA repair are main mechanisms underlying drug resistance induced by the BCR/ABL oncogene	Blasiak, Janusz
P-28	Survivin splice variant roles in caspase-3 dependent apoptosis	Boidot, Romain
P-29	“Lethal kiss” on a dielectrophoresis (DEP) based Lab-on-a-chip device: two-color real-time determination of cytotoxic T-lymphocyte activity	Borgatti, Monica
P-33	Overexpression of alphaVbeta3 integrin protects human laryngeal carcinoma cell line from cisplatin-induced apoptosis	Brozovic, Anamaria
P-36	A novel plant toxin, persin, with in vivo activity in the mammary gland, induces Bim-dependent apoptosis in human breast cancer cells	Butt, Alison
P-37	ApoJ/clusterin is a suppressor of the metastatic phenotype in neuroblastoma and a novel effector of apoptosis in immortalized and prostate cancer cells	Caccamo, Alessandro E.
P-39	Daxx-Brg1/BAF complexes participate in the regulation of transcription and apoptosis	Cermak, Lukas
P-41	Alternative mediators of apoptosis in toxic induced liver injury	Codorean, Eleonora
P-44	Insight into the role of Elongator in DNA-damage induced apoptosis	Cornez, Isabelle
P-48	Inhibitor of Apoptosis proteins (IAPs) and apoptotic signalling in Malignant Mesothelioma	Czerwionka, Inez
P-51	Reduced tumor growth in vivo and increased c-Abl activity in PC3 prostate cancer cells overexpressing the Shb adapter protein.	Davoodpour, Padideh
P-54	Effects of Cisplatin and Quercetin combination in N-ras transformed myoblast cells	Demiroglu-Zergeroglu, Asuman
P-55	Target genes to sensitize ovarian cancer cells to cisplatin	Dettori, Daniela
P-65	Photochemically mediated delivery of AdhCMV-TRAIL augments the TRAIL-induced apoptosis in colorectal cancer cell lines.	Engesaeter, Birgit

P-74	p400 function is important for the ability of E1A to downregulate EGFR and to induce apoptosis in tumour cells.	Flinterman, Marcella
P-75	The BH3-only protein Bim in the mitochondrial apoptotic pathway	Forro, Gaby
P-78	Promotion of TRAIL-induced apoptosis in HeLa cells expressing Bcl-2	Gasparian, Marine
P-79	Engagement of alpha2beta1 integrin reduces doxorubicin-induced apoptosis in leukemic T cell	Gendron, Steve
P-89	Anti-apoptotic properties and localization of different isoforms of cytoskeleton-related Hax-1 protein	Grzybowska, Ewa
P-90	c-FLIP-mediated resistance to death receptor-induced apoptosis in pancreatic cancer cell lines	Haag, Christian
P-91	Epigenetic control of programmed cell death in lung carcinoma cells resistant to conventional anticancer treatments	Hajji, Nabil
P-96	Noscapine Induce Apoptosis via Activation of Caspases-2,-3,-6,-8,-9 in Fas-independent manner in myeloblastic leukemic K562 cells	Heidari, Nastaran
P-97	Real-time analysis of initiator caspases activation and activity during TRAIL induced apoptosis in human cancer cells	Hellwig, Christian
P-98	Identification of small molecules that induce apoptosis in a Myc-dependent manner and inhibit Myc-driven transformation	Henriksson, Marie
P-99	Differential p53, Ca2+ and reactive oxygen responses in stress-induced apoptosis signaling by oxaliplatin and cisplatin	Hernlund, Emma
P-100	Proteasome inhibitors efficiently reactivate TRAIL-induced apoptosis in malignant glioma	Hetschko, Holger
P-104	The effects of some polycyclic aromatic hydrocarbons (PAHs) on apoptosis-related cell signalling may have implications for their mutagenic and carcinogenic potential	Holme, Jørn A.
P-106	Bcl-xAK, a novel Bcl-x splice product lacking a BH3 domain triggers apoptosis in human melanoma cells when applying a Tet-off adenoviral expression system	Hossini, Amir M.
P-107	RHAU, a stress-responding RNA helicase that represses transcription of DAPK	Iwamoto, Fumiko
P-108	Terfenadine, an H1 histamine receptor antagonist, induces calcium dependent DNA-damage and apoptosis in human melanoma cells by acting on the cells apart from H1 histamine receptors.	Jangi, Sh Muhialdin
P-109	TRAIL – an exogenous and endogenous tumour growth factor	Jeremias, Irmela
P-112	XIAP targeting sensitizes Hodgkin´s Lymphoma cells for cytolytic T cell attack	Kashkar, Hamid
P-114	Induction of apoptosis by plumbagin through ROS-mediated inhibition of topoisomerase II	Kawiak, Anna
P-121	Chemokine receptors CXCR4 and CCR7 mediate inhibition of anoikis in cancer cells	Kochetkova, Marina
P-124	Apoptosis induced by iron deprivation in tumor cells	Kovar, Jan
P-127	Death or Survival? Differential effects of NF-kappaB on apoptosis induced by DNA-damaging agents.	Kulms, Dagmar
P-137	Modulation of Bcl2 expression by gemcitabine and nimesulide in pancreatic tumor cell lines	Longoni, Biancamaria
P-142	Nutlin-3, a hdm2 antagonist, suppresses cell proliferation in	Magrini, Roberta

	human p53 wild-type glioblastoma cell lines	
P-146	MDM4 function: a proapoptotic activity for a negative regulator of p53	Mancini, Francesca
P-147	Investigating the role of PIDD in cell death and survival	Manzl, Claudia
P-154	Hsp90 inhibitor radicicol changes the mode of cisplatin-induced cell death.	Mateva, Rada
P-155	Effects of oxazaphosphorines on DNA degradation in leukaemic cells	Mazur, Lidia
P-157	"Role of the Transcription Factor Prep1 in Apoptosis"	Micali, Nicola
P-159	DNA damage checkpoint control programs interfere with p14ARF induced apoptosis	Milojkovic, Ana
P-166	Role of protein phosphatase 4 in the regulation of apoptosis and cell cycle	Mourtada-Maarabouni, Mirna
P-170	Differences in the anticarcinogenic mechanisms of bexarotene and N-(4-hydroxyphenyl)retinamide in Cutaneous T-cell lymphoma	Nieto-Rementeria, Naiara
P-173	Identification of a Second Transactivation Domain in the Carboxyl Terminus of p73: Cell, Promoter and Process Specificity	Nyman, Ulrika
P-175	Investigation the tumorsuppressor potential of BH3-only proteins Bmf and Bad in c-Myc induced lymphomagenesis.	Olsson, Anna
P-176	Effects of oxazaphosphorines on DNA damage in the erythropoietic system	Opydo, Malgorzata
P-178	Role of lipid rafts in intrinsic versus extrinsic apoptosis signaling in B lymphoma cells: implications for cancer treatment	Ottosson, Astrid
P-180	Mathematical Modeling of Apoptotic Caspase Activation	Pace, Vincenza
P-181	Molecular mechanisms of resistance of human colorectal COLO 205 cancer cells to extrinsic apoptosis	Pajak, Beata
P-185	Sphingosine kinase: Apoptosis regulating enzyme and chemotherapy target in prostate cancer	Pchejetski, Dimitri
P-196	Is Apoptosis a Cause or Effect in Myocardial Reperfusion Injury and Donor Heart Preservation?	Robicsek, Francis
P-199	PEA-15/PED is phosphorylated in response to glucose withdrawal and protects glioblastoma cells from glucose deprivation-induced cell death	Roth, Wilfried
P-205	Search by virtual screening techniques for XIAP inhibitors which target BIR3 domain.	Schyschka, Lilianna
P-208	Antisense treatment against anti-apoptotic genes induces apoptosis and acts as chemoadjuvant in head and neck cancer.	Sharma, Himani
P-209	Molecular events associated with alpha-lipoic acid-induced apoptosis of hepatoma cells	Simbula, Gabriella
P-215	p21 blocks irradiation-induced apoptosis downstream of mitochondria by inhibition of cyclin-dependent kinase-mediated caspase-9 activation	Sohn, Dennis
P-218	ATM deficiency triggers resistance to Fas-induced apoptosis in lymphoid cells	Stagni, Venturina
P-222	The caspase-generated fragments of PKR cooperate to activate full length PKR and inhibit translation	Suin, Vanessa
P-225	TRAIL induces tumour cell apoptosis exclusively through DR4 or DR5	Szegezdi, Eva

P-227	Proliferation and apoptosis in relation to treatment response in pituitary adenomas	Tanase, Cristiana
P-229	Non-apoptotic programmed cell death induced by a copper(II) complex in human cancer cells	Tardito, Saverio
P-230	High Antitumor Activity of Novel Glycosylated Indolocarbazole Is Determined by Strong Intercalation Into DNA	Tatarskiy, Victor
P-233	Regulation of BARD1 subcellular localization and apoptotic function	Tembe, Varsha
P-234	Bone microenvironment-related growth factors and zoledronic acid modulate differentially the PTHrP expression in PC-3 prostate cancer cells.	Tenta, Roxane
P-238	RelA/NF-kB subunit binds the bax gene promoter and inhibits p73-induced bax transcription in CD28-costimulated T lymphocytes	Tuosto, Loretta
P-240	Balance between pro-survival and death signals – important determinant of colon cancer cell sensitivity to TRAIL-induced apoptosis	Vaculova, Alena
P-241	Caspase-2 in ovarian cancer cells	Vakifahmetoglu, H.
P-243	Caspase-3 and Caspase-3s roles in etoposide-induced apoptosis.	Végran, Frédérique
P-245	Caspase-mediated cleavage of 5-lipoxygenase during apoptosis in T lymphoma cells	Villanova, Federica
P-247	The cell cycle inhibitor p57Kip2 promotes apoptotic mitochondrial changes and cell death.	Vlachos, Pinelopi
P-248	Targeting XIAP overcomes apoptosis resistance of pancreatic carcinoma cells in vitro and suppresses tumor growth and invasion in vivo	Vogler, Meike
P-249	TRAIL Increases Caspase Activity and Apoptosis in Primary Human Hepatocytes	Volkmann, Xandra
P-250	Synthetic phospholipid analogues induce apoptotic cell death through death ligand independent formation and activation of FADD/caspase-8 DISC complexes	von Haefen, Clarissa
P-253	Sensitization of Hepatocytes by Methyltransferase-Inhibitor 5-Azacytidine against Cytokine-mediated Cell Death	Weiland, Timo
P-259	SPP1 induced apoptosis in molecular pathway of cervical cancer	Wong, Yick Fu
P-260	Direct and Specific Interaction of Bcl-xL with cytochrome c: Equilibrium Affinity and Binding Kinetics	Yadaiah, Madasu

Session 4: ER stress

Poster	Title	Presenting author
P-7	Bcl-2 and Bcl-XL display differential pro-survival effects in different cell compartments in response to different cytotoxic stimuli.	Anguissola, Sergio
P-13	Noxa mediates p53-independent ER stress-induced apoptosis of neuroectodermal tumours	Armstrong, Jane
P-14	Bax and the mitochondrial permeability transition cooperate in the release of cytochrome c during endoplasmic reticulum stress induced apoptosis	Armstrong, Jeffrey
P-20	Endoplasmic reticulum-dependent apoptosis in acetaminophen-induced liver injury	Banhegyi, Gabor
P-22	Alphavirus and apoptosis: The apoptotic pathways triggered in mammalian cells as a response to Semliki Forest virus infection.	Barry, Gerald
P-43	The NF-kB Pathway Mediates Fenretinide-Induced Apoptosis In SH-SY5Y Neuroblastoma Cells.	Corazzari, Marco
P-47	Endoplasmic Reticulum Stress induced Caspase activation	Cuffe, Lorraine
P-82	Induction of apoptosis by Nbk via an ER pathway depends on Bax but is independent of Bak	Gillissen, Bernhard
P-119	A novel activity of Bak at the endoplasmic reticulum.	Klee, Martina
P-122	ER stress-induced apoptosis is independent of the death receptor pathway but sub-lethal ER stress sensitizes cells to death receptor stimuli by up-regulating DR5	Koehler, Barbara
P-128	Caspase-4 is involved in apoptosis induced by endoplasmic reticulum (ER) stressors and polyglutamine aggregates	Lakshmanan, U.
P-129	Metacaspases and Bax-Inhibitor I as regulators of programmed cell death in plants.	Lam, Eric
P-134	Endoplasmic reticulum stress induces calcium-dependent permeability transition, mitochondrial outer membrane permeabilization and apoptosis	Lemaire, Christophe
P-138	Fenretinide-induced apoptosis via endoplasmic reticulum stress-New targets for melanoma therapy.	Lovat, Penny
P-149	Endoplasmic Reticulum Stress and Apoptosis in the Liver of Scorbutic Guinea Pigs	Margittai, Eva
P-164	Defect of Ubiquitin/Proteasome-ERAD Stimulates Autophagic Pathway and ER Stress-mediated Cell Death Pathway.	Momoi, Takashi
P-190	In vitro and in vivo evidence of ER stress and ER-mediated cell death during exposure to nephrotoxic drugs.	Peyrou, Mathieu

Session 5: Inflammation

Poster	Title	Presenting author
P-21	TRAIL is Required for Virus Elimination in Chronic HCV Infection	Bantel, Heike
P-27	Defective apoptosis of peripheral blood lymphocytes in hyper-IgD and periodic fever syndrome	Bodar, Evelien
P-31	The role of calpains in Feline Calicivirus-induced apoptosis	Brennan, Benjamin
P-42	Cathepsin D is a key initiator protease during neutrophil apoptosis	Conus, Sébastien
P-49	Apoptotic neutrophils release macrophage migration inhibitory factor upon death receptor stimulation	Daryadel, Arezoo
P-50	The cationic host defence peptide LL-37 mediates contrasting effects on apoptotic pathways in different primary innate immune cells	Davidson, Donald
P-61	Notch regulates the cytokine-dependent survival of activated T-cells	Divya, P
P-62	GILZ (Glucocorticoid Induced Leucine Zipper) is a mediator of glucocorticoid-induced apoptosis	Donato, Valerio
P-64	Critical roles of PUMA and A1 in the regulation of mast cell apoptosis and survival	Ekoff, Maria
P-66	Mitochondrial signalling pathway involved in monocytic oxidized LDL-induced apoptosis	Ermak, Natalia
P-68	Stromal-derived factor-1 (SDF-1) blocks constitutive apoptosis of WHIM syndrome neutrophils.	Fadeel, Bengt
P-71	Characterization of a RIP1-containing high molecular weight complex following oxidative stress	Festjens, Nele
P-86	YopP of Yersinia enterocolitica induces apoptotic and necrosis-like cell death in murine dendritic cells	Gröbner, Sabine
P-102	p30 – a new cleavage product at the CD95 Death-Inducing Signalling Complex (DISC)	Hoffmann, Julia
P-110	CKIP-1 interacts with the intracellular domain of TNF and participates in the inflammatory response of macrophages	Juhasz, Kata
P-111	The pro-survival gene A1 is transcriptionally regulated by NFAT in mast cells	Karlberg, Mats
P-113	Dynamic molecular regulation of phagocytosis in the apoptotic cells by macrophages	Katona, Klara
P-115	Caspase-1 and TRIM16/EBBP: Two key regulators of inflammation and interleukin-1 secretion in keratinocytes	Keller, Martin
P-116	Structure-function analysis caspase-1/CARD-mediated NF-kappaB activation	Kersse, Kristof
P-120	Novel proteins interacting with death receptor-6 (DR6 / TNFRsf21)	Klima, Martin
P-125	Macrophages use different internalization mechanisms to clear apoptotic and necrotic cells without activation of NF-kB transcription factor	Krysko, Dmitri
P-141	The cytomegalovirus mitochondrial inhibitor of apoptosis (vMIA) is required for virus replication and cross-species infection	Mack, Claudia
P-143	PPARgamma dependent regulation of human macrophages in phagocytosis of apoptotic cells	Majai, Gyongyike

P-156	Identification of a Serum Factor Required for Augmented Phagocytosis of Apoptotic Cells	McColl, Aisleen
P-158	The pro-inflammatory cytokine TNFalpha critically regulates macrophage clearance of apoptotic neutrophils.	Michlewska, Sylwia
P-169	Hepatoprotection by Cyclosporin A in Experimental Hepatitis. Sorting Desensitization of the Mitochondrial Permeability Transition Pore from Immunosuppression	Nicolosi, Luca
P-182	Mitochondria as therapeutic target for the treatment of fungal infections. Implications for the effector function of the immune system	Pardo, Julian
P-183	Antirheumatic biologic agents induce leukocyte-dependent apoptosis of synovial fibroblasts.	Pattacini, Laura
P-192	Apoptosis induction in pig macrophages by African swine fever virus correlates to virulence of the agent	Portugal, Raquel
P-194	Glucocorticoids and Control of Cell Growth: Role of GILZ	Riccardi, Carlo
P-195	Life and death of a neutrophil: The role of NF-κB dependent and independent survival proteins in neutrophil apoptosis.	Riley, Nicola
P-210	Mast cells induce cell death via secreted granzyme B	Simon, Markus M
P-244	Glucocorticoids and NF-kB inhibition: glucocorticoid-induced leucine zipper (GILZ)/NF-kB interaction	Velardi, Enrico
P-257	Programmed cell clearance : role of recognition and detachment signals for macrophage engulfment of human neutrophils	Witasp, Erika
P-258	Apoptosis-induced membrane expression of proteinase 3 during neutrophil apoptosis decreased the rate of phagocytosis by macrophages : a new pro-inflammatory role of membrane proteinase 3 in neutrophils	Witko-Sarsat, Véronique
P-261	Expression of an uncleavable N-terminal RasGAP fragment in vitro and in vivo increases insulin secreting cells resistance towards apoptotic stimuli	Yang, Jiang-yan
P-262	Gene induction patterns of apopto-phagocytic genes in human macrophages	Zahuczky, Gábor
P-264	Induction of osteoclasts apoptosis by using a decoy oligodeoxynucleotide in vivo mimicking a region of distal promoter C of ER alpha gene	Zennaro, Margherita

Session 6: Neurodegeneration

Poster	Title	Presenting author
P-4	Role of presenilin-dependent gamma-secretase activity in the control of p53-mediated cell death in Alzheimer's disease	Alves da Costa, Cristine
P-9	Mcl-1 is a Tightly Regulated Key Player in Neuronal Cell Death	Arbour, Nicole
P-18	Activation of caspase-8 by Tumor Necrosis Factor Receptor 1 (TNFR1) is necessary for OGD-mediated apoptosis in cultured cortical cells	Badiola, Nahuai
P-53	AMPA receptor activation can induce both apoptotic and necrotic cell death in primary cultured motoneurons.	De Paola, Massimiliano
P-59	Parvovirus H-1 triggers a type II cell death in permissive gliomas through a Bcl-2 resistant and cathepsin B dependent mechanism	Di Piazza, Matteo
P-70	Cell stress and death response in animal and cellular models of Alzheimer Disease	Ferraro, Elisabetta
P-81	p53-dependent antiapoptotic function of synphilin-1 is mediated by its caspase-3 derived C-terminal product	Giaime, Emilie
P-126	Large-conductance potassium channel activators in neuroprotection	Kulawiak, Bogusz
P-140	Nucleophosmin regulates Bax-mediated apoptosis.	MacDonald, David
P-153	Genotype-dependent priming to self- and xeno-cannibalism in heterozygous and homozygous lymphoblasts from patients with Huntington disease	Matarrese, Paola
P-160	A potential role for Cdk5 in apoptotic events following stroke in man	Mitsios, Nicholas
P-172	The phycotoxin okadaic acid induces apoptotic death via oxidative stress in cultured glial cells.	Novelli, Antonello
P-186	Gene and protein expression changes in human brain following acute ischemic stroke: identification of potential novel mediators of apoptosis	Pennucci, Roberta
P-188	p25alpha; induces alpha-synuclein dependent cell death in a cellular model for neurodegenerative disorders	Petersen, Christine Lund
P-193	Detailing the role of Bax translocation, cytochrome c release and perinuclear clustering of the mitochondria in the killing of HeLa cells by TNF.	Pucci, Bruna
P-200	Cybrids with mtDNA mutations causing Leber's hereditary optic neuropathy are sensitized to apoptotic death induced by a mitochondrial oxidative stress	Rugolo, Michela
P-202	Calpains are involved in the activation of both AIF and caspase-12 that mediate apoptosis in retinal degeneration	Sanges, Daniela
P-204	State of proteins B23/nucleophosmin and UBF in HeLa cells during apoptosis induced by alpha-TNF/emetine	Sautkina, Elena
P-217	Vap-1/SSAO-mediated Methylamine Oxidation Induces Apoptosis on Vascular A7r5 and Huvec Cell Types.	Sole, Montse
P-219	Pyk2 involvement in membrane depolarization-promoted cerebellar granule neuron survival	Strappazzon, Flavie
P-228	Structure-based design of non-peptidic small molecular inhibitors of caspase-3	Tanuma, Sei-ichi

P-232	Sensory Cell Death in the Inner Ear	Taylor, Ruth
P-252	Stress activation protein kinases: key signalling molecules as therapeutic targets for hair cell death in the injured inner ear	Wang, Jing
P-266	Long term effect of nicotinic acethylcholine receptor activation and downstream signalling in neurons	Ziviani, Elena

Session 7: Organelle cross-talk

Poster	Title	Presenting author
P-6	Collagen VI muscular dystrophies from animal model to human therapy: mitochondria as targets for therapeutic intervention in muscle cell death	Angelin, Alessia
P-16	Lysosomal mechanisms of death in HTC rat hepatoma cells	Autelli, Riccardo
P-23	Overexpression of both catalytically active and -inactive cathepsin D by cancer cells enhances apoptosis-dependent chemo-sensitivity	Beaujouin, Mélanie
P-30	Dissecting the pro-apoptotic function of the caspase substrate HDAC4	Brancolini, Claudio
P-32	CD95L-independent activation-induced cell death of primary lymphocytes is mediated by cleaved HPK1	Brenner, Dirk
P-35	Glucocorticoid-induced apoptosis in ALL cells is mediated through lowered glucose uptake and utilization	Buentke, Eva
P-38	Selective targeting of cyclin D1 by bile acids influences their ability to modulate apoptosis in primary rat hepatocytes	Castro, Rui Eduardo
P-46	Involvement of nucleolin in Tumor Necrosis Factor-induced cell death	Cottyn, Anneleen
P-57	Random mutagenesis screen in yeast reveals novel residues required for Bak oligomerization and pro-apoptotic activity	Dewson, Grant
P-63	A new caspase-independent pathway to apoptosis that affects lysosomes is triggered by the proapoptotic Bcl-2-related protein Nbk/Bik in human melanoma cells	Eberle, Jürgen
P-67	Engineering NAIP-BIR3 to interact with SMAC type peptides	Es-haghi, Ali
P-76	OPA1 Controls Apoptotic Cristae Remodelling Independently from Mitochondrial Fusion	Frezza, Christian
P-77	Identification and characterization of novel PTP inhibitors	Gambalunga, A.
P-83	The N-terminal conformation of Bax regulates cellular commitment to apoptosis.	Gilmore, Andrew
P-87	Characterization of molecular pathways involved in the apoptotic defects of collagen VI knockout mice, a model of human muscular dystrophies	Grumati, Paolo
P-95	Identification of a link between Calmodulin kinase II and Jun-terminal kinase by mapping Ca2+-dependent apoptosis pathways	Havelka Mandic, Aleksandra
P-101	Pro-apoptotic signaling induced by IFN-alpha	Hjortsberg, Linn
P-136	VDAC and Bak recombinant proteoliposomes induce caspase 9 activation in cancer cell lines	Liguori, Lavinia
P-161	A role for the DED domain –containing DEDD in cell-cycle and cell-size regulation	Miyazaki, Toru
P-167	A fraction of cytochrome c is retained in mitochondria until advanced stages of apoptosis	Mussche, Sylvie
P-174	Investigating the role of serine proteases in leukaemic cell death	O'Connell, Ailish
P-177	Assembly of pro-apoptotic Bcl-2 family proteins in the yeast outer mitochondrial membrane	Ott, Martin
P-179	Novel approaches to evaluate the alteration in membrane traffic induced by FasL signalling.	Ouasti, Sihem

P-187	Comparison of detection methods of cell death and cell viability after exposure to Doxorubicin, Actinomycin D and Mitomycin C in vitro	Penttinen, Piia
P-197	Ursodeoxycholic acid modulates hepatocyte apoptosis through nuclear steroid receptors	Rodrigues, Cecília Maria
P-201	Tumor Necrosis Factor-induced cell death is mediated via the Receptor for Advanced Glycation End products.	Salliau, Stefanie
P-207	Nucleolus disassembly in mitosis and apoptosis: dynamic redistribution of phosphorylated-c-Myc, fibrillarin and Ki-67	Scovassi, Anna Ivana
P-216	Changes in the organization of the Golgi apparatus and the endoplasmic reticulum during apoptosis	Soldani, Cristiana
P-224	Murine cytomegalovirus m41 protein protects from apoptosis induced by PKC inhibitors	Syta, Maren
P-235	Role of tissue transglutaminase in the process of phagocytosis of apoptotic cells	Toth, Beata
P-237	Magnesium: A New Player in the Apoptotic Signalling Cascade?	Trapani, Valentina
P-239	The influenze of EHV-1 on cytoskeleton in vitro	Turowska, A.
P-242	Single-cell image analysis of cellular localization and colocalization of endonuclease G, AIF and AMID in living cells	Varecha, Miroslav
P-251	Involvement of full-length RasGAP in the anti-apoptotic response induced by its cleavage fragments.	Walicki, Joël
P-254	A novel Bcl-2-like inhibitor of apoptosis is encoded by the parapoxvirus, orf virus	Westphal, Dana

Session 8: ROS

Poster	Title	Presenting author
P-8	Modulation of ceramide metabolism as a possible strategy for N-(4-hydroxyphenyl)retinamide resistance tumor cells	Apraiz, Aintzane
P-17	Role of reactive oxygen and nitrogen species in stimulation of inflammatory response and Fas mediated programmed cell death of polymorphonuclear leukocytes in epidemic dropsy patients	Babu, Ch. Kishore
P-34	Oxidative stress by ascorbate/menadione association kills K562 human chronic myelogenous leukaemia cells and inhibits its tumour growth in nude mice.	Buc Calderon, Pedro
P-40	Mitochondria are involved in development of oxidative stress	Chernyak, Boris
P-52	AOPP and RCOs as indicators of Oxidative Stress in Hemodialysis and Peritoneal Dialysis Patients	De Cal, Massimo
P-60	In vitro and in vivo effects of Trolox on arsenic mediated toxicity	Diaz, Zuanel
P-72	p66Shc promotes T-cell apoptosis by inducing mitochondrial dysfunction and impaired Ca2+ homeostasis	Finetti, Francesca
P-73	Identification of PP1alpha as a caspase-9 regulator in IL-2 deprivation induced apoptosis	Fleischer, Aarne
P-94	A novel permeability transition inhibitor with antioxidant properties protects ischemic heart by inhibiting both apoptotic and necrotic cell death	Hanto, Katalin
P-103	Apoptosis versus differentiation induced by interaction of butyrate and unsaturated fatty acids in normal and transformed colonic epithelial cells	Hofmanová, Jirina
P-117	Regulation of FBP1 expression and changes in glucose metabolism are crucial for an appropriate response to DNA damage and aging in S.cerevisiae	Kitanovic, Ana
P-118	Mechanisms involved in apoptosis of HT-29 cells after 5-LOX pathway modulation followed by photodynamic therapy with hypericin	Kleban, Ján
P-123	KATP channel openers uncouple oxidative phosphorylation of cardiac mitochondria in situ	Kopustinskiene, Dalia Marija
P-131	The mammalian circadian clock gene Per2 modulates cell death in response to oxidative stress	Langmesser, Sonja
P-133	Overexpression of Epstein-Barr virus latent membrane protein 1 (LMP1) in EBV Latency III B-cells induces caspase 8 mediated apoptosis: role of CD95.	Le Clorennec, Christophe
P-135	GSH depletion, protein S-glutathionylation and mitochondrial transmembrane potential hyperpolarization are early events in initiation of cell death induced by a mixture of isothiazolinones in HL60 cells.	Leonini, Alessandra
P-144	Old Yellow Enzymes; new contrasting roles for old enzymes in oxidative stress and programmed cell death in yeast	Makris, Antonios
P-145	A chemically standardized alcoholic extract of Withania somnifera induce apoptosis in HL60 cells through ROS generation and elicits cell -mediated immune responses associated with a Th1-dominant polarization in BALB/c mice	Malik, Fayaz

P-148	Reactive oxygen species and PKC delta: a crosstalk aimed at triggering apoptosis of MYCN amplified neuroblastoma cells	Marengo, Barbara
P-150	Menadione-induced apoptosis is affected by low frequency 50 Hz magnetic field in murine fibroblast cells.	Markkanen, Ari
P-162	Loss of HtrA2 results in increased mitochondrial stress, enhanced ROS production and perturbation of calcium homeostasis.	Moisoi, Nicoleta
P-165	Could Fatty Acids act as surrogate Free Radical Scavengers?	Moraitis, Christos
P-171	Pro-apoptotic activity of the naturally occurring compound, 13-Hydroxy-15-oxozoapatlin.	Nigro, Patrizia
P-189	Reduction of the cardiolipin per se do not mimic the effects on cell death associated with CL remodelling in Barth syndrome lymphoblasts	Petit, Patrice X.
P-191	Reactive oxygen species affects structural and functional unity of mitochondrial network	Pletjushkina, Olga
P-211	Mitoptosis, elimination of mitochondria	Skulachev, Vladimir
P-214	Survival signaling by C-RAF: mitochondrial ROS and Ca2+ are critical targets	Smigelskaite, Julija
P-221	Internalization is essential for the anti-apoptotic effects of exogenous thymosin beta-4 on human corneal epithelial cells	Su, Yeu
P-223	Nitric oxide as a mediator in regulation of hypoxia inducible factor-1alpha accumulation under normoxic conditions and low oxygen availability	Sumbayev, Vadim
P-231	P53-independent apoptosis induced by peroxynitrite in Jaws II bone marrow cells is inhibited by Bcl2	Taylor, Emma
P-246	Inhibition of apoptosis by induction of hypoxia-inducible factor-1. Cell survival in the face of injury.	Vissers, Margret
P-265	Crucial role of PTEN in regulation of ROS generation and neuronal apoptosis	Zhu, Yuan

Authors Index

Supported by EC within the framework of the Marie Curie
Conferences and Training Courses

Supported by EC within the framework of the Marie Curie
Conferences and Training Courses

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Participants List

Supported by EC within the framework of the Marie Curie
Conferences and Training Courses

Supported by EC within the framework of the Marie Curie
Conferences and Training Courses

Abbadie Corinne
Institut de Biologie de Lille
UMR8161
1 rue du Pr. Calmette
59021 Lille, France
corinne.abbadie@ibl.fr

Agostinis Patrizia
Catholic University of Leuven
Molecular & Cell Biology
Herestraat 49
3000 Leuven, Belgium
patricia.agostinis@med.kuleuven.be

Alves da Costa Cristine
IPMC-CNRS, UMR6097
660 Route des Lucioles
6560 Valbonne, France
acosta@ipmc.cnrs.fr

Andreau Karine
Université Paris 7
Laboratoire de Cytophysiologie et Toxicologie Cellulaire
2 place jussieu
75251 Paris cedex 05, France
karine.andreau@paris7.jussieu.fr

Anguissola Sergio
Royal College of Surgeons in Ireland (RCSI)
Dept. of Physiology and Medical Physics
123 St. Stephen's Green
Dublin2 Dublin, Ireland
sanguissola@rcsi.ie

Arai Satoko
The University of Tokyo
Div. of Molecular Biomedicine for Pathogenesis, CDBIM, Faculty of Medicine
7-3-1 Hongo, Bunkyo-ku
113-0033 Tokyo, Japan
sarai@m.u-tokyo.ac.jp

Aden Petra
Neuropathologic Section
Dep. of Pathology, Ulleval University Hospital
0407 Oslo
407 Oslo, Norway
p.k.aden@medisin.uio.no

Aits Sonja
Institute of Laboratory Medicine
Section of Microbiology, Immunology and Glycobiology
Sölvegatan 23
22362 Lund, Sweden
Sonja.Aits@med.lu.se

Andera Ladislav
Institute of Molecular Genetics AS CR
Cell Signaling and Apoptosis
Videnska 1083
14220 Prague 4, Czech Republic
andera@biomed.cas.cz

Angelin Alessia
University of Padova
Departement of Biomedical Sciences
Viale G.Colombo, 3
35121 Padova, Italy
alessia.angelin@unipd.it

Apraiz Aintzane
University of the Basque Country
Sarriena s/n
48940 Leioa, Spain
ggbapgaa@lg.ehu.es

Arbour Nicole
University of Ottawa
Biochemistry, Microbiology and Immunology
451 Smyth Rd, rm 2454
K1H 8M5 Ottawa, Canada
narbo081@uottawa.ca

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Aresvik Dina
Rikshospitalet
Department of Pediatric Research
Sognsvannsveien 20
27 Oslo, Norway
diana.aresvik@studmed.uio.no

Arisan Elif Damla
Halic University
Molecular Biology and Genetics
Halic University, Science and Literature Fac.
Ahmet Vefik Pasa Cad. No1
34280 Istanbul, Turkey
damlaarisan@gmail.com

Arkusinska Justyna
Medical University of Lodz
Molecular Biology of Cancer
6/8 Mazowiecka Street
92-215 Lodz, Poland
jarkusinska@op.pl

Armstrong Jeffrey
National University of Singapore
Biochemistry
8 Medical Drive
117597 Singapore, Singapore
bchjsa@nus.edu.sg

Armstrong Jane
Newcastle University
Northern Institute for Cancer Research
Paul O'Gorman Building, Medical School,
Framlington Place
NE2 4HH Newcastle Upon Tyne, United Kingdom
J.L.Armstrong@ncl.ac.uk

Arzoine Laetitia
Ben-Gurion University of the Negev
Life Sciences
P.O.Box 653
84105 Beer Sheva, Israel
arzoinel@bgu.ac.il

Autelli Riccardo
Section of General Pathology
Experimental Medicine and Oncology
Corso Raffaello 30
10125 Torino, Italy
riccardo.autelli@unito.it

Babu Ch. Kishore
Industrial Toxicology Research Centre
Food Toxicology Laboratory
PO Box#80, M.G.Marg
226001 Lucknow, India
kishorebio@yahoo.co.in

Baccarini Manuela
Max F Perutz Laboratories, University of Vienna
Dr-Bohr-Gasse 9
1060 Vienna, Austria
manuela.baccarini@univie.ac.at

Badiola Nahuai
Instituto de Neurociencias, Universidad Autónoma de Barcelona
Bioquímica y Biología Molecular
Facultad de Medicina, Edificio M
8193 Bellaterra, Barcelona, Spain
nahuai.badiola@uab.es

Baehrecke Eric
University of Maryland Biotechnology Institute
Center for Biosystems Research
5115 Plant Sciences Building
MD 20742 College Park,
baehreck@umbi.umd.edu

Baird Sarah
Molecular Oncology Unit
Cancer Research UK Clinical Centre
Barts and The London School of Medicine and Dentistry, Charterhouse Square
EC1M 6BQ London, United Kingdom
sarah.baird@cancer.org.uk

Balakireva Maria
Institut Curie
Inserm U528
26 rue d'Ulm
75005 Paris, France
maria.balakireva@curie.fr

Banhegyi Gabor
Semmelweis University
Dept. of Medical Chemistry
POB 260
1444 Budapest, Hungary
banhegyi@puskin.sote.hu

Bantel Heike
Medical School Hannover
Gastroenterology, Hepatology and Endocrinology
Carl Neuberg Str.01
30529 Hannover, Germany
bantel.heike@mh-hannover.de

Barry Gerald
Centre for Infectious Diseases
Summerhall
EH9 1QH Edinburgh, United Kingdom
g.m.barry@sms.ed.ac.uk

Bartek Jiri
Institute of Cancer Biology, Danish Cancer Society
Strandboulevarden 49
DK-2100 Copenhagen, Denmark
jb@cancer.dk

Bartkova Jirina
Danish cancer society, Institute of cancer biology
CCC
Strandboulevarden 49
DK-2100 Copenhagen, Denmark
jib@cancer.dk

Beaujouin Mélanie
INSERM U540, 'Molecular and Cellular Endocrinology of Cancers'
60 rue de Navacelles
34090 Montpellier, France
beaujoin@montp.inserm.fr

Bellarosa Daniela
Menarini Ricerche
Pharmacology
V. Tito Speri 10
40 Pomezia, Italy
pharmacology@menarini-ricerche.it

Berndtsson Maria
Karolinska Institute
Oncology/Pathology
Cancer Center Karolinska, Department of Oncology/Pathology, Karolinska Institute and Hospital
17176 Stockholm, Sweden
Maria.Berndtsson@ki.se

Blank Michael
Sackler Faculty of Medicine, Tel Aviv University
Department of Human Molecular Genetics and Biochemistry
Ramat Aviv 69978 Tel Aviv
69978 Tel Aviv, Israel
blankmic@post.tau.ac.il

Blasiak Janusz
University of Lodz
Department of molecular Genetics
Banacha 12/16
90-237 Lodz, Poland
januszb@biol.uni.lodz.pl

Blenis John
Harvard Medical School
Cell Biology
240 Longwood Ave.
2115 Boston, USA
jblenis@hms.harvard.edu

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Bodar Evelien
University Medical Centre St Radboud
General Internal Medicine 463
Postbus 9101
6500 HB Nijmegen, The Netherlands
e.bodar@aig.umcn.nl

Boidot Romain
Centre Georges-Francois Leclerc
Molecular Genetics Laboratory
1, rue du Professeur Marion
21079 Dijon, France
r-boidot@club-internet.fr

Borgatti Monica
Ferrara University
Biochemistry and Molecular Biology
via Fossato di Mortara, 74
44100 Ferrara, Italy
brgmnc@unife.it

Brancolini Claudio
Università degli Studi di Udine
Scienze e Tecnologie Biomediche
p.le Kolbe 4
33100 Udine, Italy
cbrancolini@makek.dstb.uniud.it

Brennan Benjamin
University of Surrey
School of Biomedical and Molecular Sciences
University of Surrey
GU2 7XH Guildford, United Kingdom
b.brennan@surrey.ac.uk

Brenner Dirk
German Cancer Research Center (DKFZ)
Division of Immunogenetics
INF 280
69121 Heidelberg, Germany
d.brenner@dkfz.de

Broemer Meike
The Breakthrough Breast Cancer Research Centre
Apoptosis lab
The Institute of Cancer Research, 237 Fulham Road
SW3 6JB London, United Kingdom
meike.broemer@icr.ac.uk

Brozovic Anamaria
Institute Rudjer Boskovic
Bijenicka 54
10000 Zagreb, Croatia
brozovic@irb.hr

Brust Diana
Institute of Molecular Biosciences
Department of Molecular Developmental Biology
Max-von-Laue Straße 9
60438 Frankfurt am Main, Germany
brust@em.uni-frankfurt.de

Buc Calderon Pedro
Université catholique de Louvain
Departement de sciences pharmaceutiques
avenue E. Mounier, 73
1200 Bruxelles, Belgium
calderon@pmnt.ucl.ac.be

Buentke Eva
The Karolinska Institute
Department of Oncology-Pathology
Cancer Centre Karolinska, CCK, R8:03
SE-17176 Stockholm, Sweden
eva.buentke@ki.se

Butt Alison
Garvan Institute of Medical Research
Cancer Research Program
384 Victoria Street
NSW 2010 Darlinghurst, Australia
abutt@garvan.org.au

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Büttner Sabrina
Institut for Molecular Biosciences
Karl-Franznes University Graz
Universitätsplatz 2
8010 Graz, Austria
sabrina.buettner@uni-graz.at

Caccamo Alessandro E.
Faculty of Medicine and Surgery
Experimental Medicine, Section of Biochemistry
Via Volturno 39
43100 Parma, Italy
emanuelealessandro.caccamo@unipr.it

Carmona-Gutierrez Didac
Institut for Molecular Biosciences
Karl-Franzens University Graz
Universitätsplatz 2
8010 Graz, Austria
didac.carmonagutierrez@uni-graz.at

Castro Rui Eduardo
Faculty of Pharmacy, University of Lisbon
Centro de Patogenese Molecular
Av. das Forças Armadas
1600-083 Lisbon, Portugal
castro_rui@hotmail.com

Cecconi Francesco
Dulbecco Telethon Institute, University of Tor Vergata
Dept. of Biology
Via della Ricerca Scientifica
133 Rome, Italy
francesco.cecconi@uniroma2.it

Cermak Lukas
Institute of molecular genetics
Laboratory of cell signaling and apoptosis
Videnska 1083
14220 Prague, Czech Republic
cermak@leuko.biomed.cas.cz

Chernyak Boris
A.N. Belozersky Institute, Moscow State University
Bioenergetics
A.N. Belozersky Institute, Moscow State University, Building "A"
119992 Moscow, Russia
bchernyak@yahoo.com

Chiara Federica
Università degli Studi di Padova
Dipartimento di Scienze Biomediche
viale G. Colombo 3
35121 Padova, Italy
fchiara@bio.unipd.it

Chumakov Peter
Lerner Research Institute, The Cleveland Clinic Foundation
Department of Molecular Genetics
9500 Euclid Ave
OH 44195 Cleveland, USA
chumakp@ccf.org

Codorean Eleonora
Victor Babes National Institute of Pathology
Pathology
Spl. Independentei, No. 99-101
50096 Bucharest, Romania
imunotox@vbabes.ro

Columbano Amedeo
University of Cagliari
Department of Toxicology
9124 Cagliari, Italy
columbano@unica.it

Comoglio Paolo M.
Institute for Cancer Research and Treatment
University of Turin School of Medicine
Strada Provinciale 142
10060 Candiolo (Torino), Italy
paolo.comoglio@ircc.it

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Conus Sébastien
Department of Pharmacology
University of Bern
Friedbühlstrasse 49
3010 Bern, Switzerland
sebastien.conus@pki.unibe.ch

Corazzari Marco
National Institute for Infectious Deseases
Lab. Cell Biology
Via Portuense 292
149 Rome, Italy
corazzari@inmi.it

Cornez Isabelle
University of Liege
Laboratory of Medical Chemistry
CHU Tour de pathologie +3 B23
4000 Liege, Belgium
icornez@student.ulg.ac.be

Corradi Valentina
ospedale san bortolo
nephrology
via rodolfi 37
36100 vicenza, Italy
corradivalentina@yahoo.it

Cottyn Anneleen
UGENT
Department of Medical Protein Research
A. Baertsoenkaai 3
9000 GENT, Belgium
anneleen.cottyn@ugent.be

Cuffe Lorraine
NCBES
Biochemistry
Newcastle road
n/a Galway, Ireland
lorrainecuffe@gmail.com

Cursi Silvia
EBRI Fondazione Santa Lucia
laboratory of Cell signalling
Via del Fosso di Fiorano, 64
143 roma, Italy
silvia.cursi@uniroma2.it

Czerwionka Inez
University of Western Australa
Anatomy and Human Biology
35 Stirling Highway, Crawley
6009 Perth, Australia
iczerwionka@anhb.uwa.edu.au

Daniel Peter
University Medical Center Charité
Clinical and Molecular Oncology
Augustenburger Platz 1
13353 Berlin, Germany
pdaniel@mdc-berlin.de

Daryadel Arezoo
Pharmacology
University of Bern
Friedbühlstrasse 49
3010 Bern, Switzerland
arezoo.daryadel@pki.unibe.ch

Davidson Donald
University of Edinburgh
MRC Centre for Inflammation Research
Queen's Medical Research Institute, W2.03, 47
Little France Crescent,
EH16 4TJ Edinburgh, United Kingdom
Donald.Davidson@ed.ac.uk

Davoodpour Padideh
Medical Cell Biology
Medical cell biology
Biomedicum Box 571
75123 Uppsala, Sweden
padideh.davoodpour@medcellbiol.uu.se

Supported by EC within the framework of the Marie Curie
Conferences and Training Courses

De Cal Massimo
san bortolo hospital
nephrology
via rodolfi 37
36100 vicenza, Italy
mamo00@libero.it

De Paola Massimiliano
Mario Negri Institute for Pharmacological Research
Biochemistry and Molecular Pharmacology
via Eritrea 62
20157 Milan, Italy
depaola@marionegri.it

De Zio Daniela
IRCCS Fondazione Santa Lucia
Via del Fosso di Fiorano 64
143 Rome, Italy
dezios@yahoo.com

Debatin Klaus-Michael
University Children"s Hospital
Dpt. Of Pediatrics and Adolescent Medicine
Eythstr. 24
89750 Ulm, Germany
klaus-michael.debatin@medizin.uni-ulm.de

Declercq Wim
VIB-University Ghent
Department for Molecular Biomedical Research
Technologiepark 927
9000 Gent, Belgium
wim.declercq@dmbr.Ugent.be

Demiroglu-Zergeroglu Asuman
Gebze Institute of Technology
Department of Biology
Gebze Yuksek Teknoloji Enstitusu, Fen fakultesi, Biyoloji bolumu, Istanbul Yolu, No:101
41400 Gebze/Kocaeli, Turkey
ademiroglu@gyte.edu.tr

Desagher Solange
Institute of Molecular Genetics of Montpellier, UMR 5535
Mechanisms of neuronal death
1919 route de Mende
34000 Montpellier, France
solange.desagher@igmm.cnrs.fr

Dettori Daniela
Institute for Cancer Research and Treatment
Laboratory of Cancer Genetics
Strada provinciale 142, km 3.95
10060 Candiolo, Torino, Italy
daniela.dettori@ircc.it

Dewaele Michael
Catholic University of Leuven (KUL)
Molecular & Cell Biology, Faculty of Medicine
Herestraat 49, box 901
3000 Leuven, Belgium
Michael.Dewaele@med.kuleuven.be

Dewson Grant
Walter and Eliza Hall Institute
1G Royal Parade
3050 Melbourne, Australia
dewson@wehi.edu.au

Di Bartolomeo Sabrina
University of Rome Tor Vergata
Department of Biology
Via della Ricerca Scientifica
133 Rome, Italy
sdibartolomeo@dti.telethon.it

Di Piazza Matteo
German Cancer Research Center (DKFZ)
Tumor Virology Division (F010)
Im Neuenheimer Feld 280
D-69120 Heidelberg, Germany
m.dipiazza@dkfz.de

Di Stefano Anna
Biological Chemistry
Molecular Biology
Via Fiorentina,1
53100 Siena, Italy
distefano@unisi.it

Dilek Güner
University Medical Center Charité
Clinical and Molecular Oncology
Lindenberger Weg 80
13125 Berlin, Germany
dguener@gmx.de

Domenicotti Cinzia
General Pathology
Experimental Medicine
via L. B. Alberti, 2
I-16132 Genoa, Italy
cdomeni@medicina.unige.it

Donato Valerio
Section of Pharmacology
Clinical & Experimental Medicine
Via del Giochetto
6122 Perugia, Italy
valeriodonato80@libero.it

Dosenko Victor
Bogomoletz Institute of Physiology
Experimental Cardiology
Bogomoletz Str., 4
1024 Keiv, Ukraine
dosenko@biph.kiev.ua

Eisenberg Tobias
Institut for Molecular Biosciences
Karl-Franzens University Graz
Universitätsplatz 2
8010 Graz, Austria
tobias.eisenberg@uni-graz.at

Diaz Zuanel
Lady Davis Institute for Medical Research
McGill University
3775 Cote Saint Catherine Road
H3S 3T3 Montreal, Quebec, Canada
zuanel.heredia@mail.mcgill.ca

Divya P
National Centre for Biological Sciences
Cellular Signaling and Organization
Bellary Road,
560065 Bangalore, India
pdivya@ncbs.res.in

Domnina Lidia
Moscow State University
N.A. Belozersky Institute of Physico-Chemical Biology
Slaviansky bulvar, b.11/1, ap.58
121352 Moscow, Russia
domnina@mbio.genebee.msu.ru

Donzé Olivier
Apotech Corporation
22 Chemin des Croisettes
1066 Epalinges, Switzerland
odonze@apotech.ch

Eberle Jürgen
Charité
Dermatology and Allergy - Skin Cancer Center
Charité
Campus Benjamin Franklin, Hindenburgdamm 30
12203 Berlin, Germany
juergen.eberle@charite.de

Ekoff Maria
Karolinska Institutet, Clinical Immunology and Allergy Unit
Medicine
KS L2:04 Solna
17176 Stockholm, Sweden
maria.ekoff@ki.se

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Engesaeter Birgit
Institute for Cancer Research
Department of Tumor Biology
Montebello
310 Oslo, Norway
birgite@labmed.uio.no

Es-haghi Ali
Institute of Biochemistry and Biophysics
molecular biology
Institute of Biochemistry and Biophysics,
University of Tehran ,Enghelab Ave. , Tehran ,
Iran P.O. Box: 13145-1384
13145-1384 Tehran, Iran
eshaghi@ibb.ut.ac.ir

Felix Daniel A.
University of Barcelona
Department of Genetics
Av. Digaonal 645 Edifici Annex 1a Planta
8028 Barcelona, Spain
dafex@tiscali.ch

Festjens Nele
Ghent University-Flanders Interuniversity
Institute for Biotechnology
Molecular Biomedical Research
Technologiepark 927
9052 Ghent, Belgium
Nele.Festjens@dmbr.UGent.be

Fimia Gian Maria
National Institute for Infectious Diseases "L.
Spallanzani"
Cell Biology
Via Portuense 292
146 Rome, Italy
fimia@inmi.it

Fleischer Aarne
Centro de Investigación del Cancer
Campus Miguel de Unamuno
37007 Salamanca, Spain
afbutch@yahoo.de

Ermak Natalia
Necker Hospital
Biochemistry A
149, rue de Sèvres
75015 Paris, France
ermakn@necker.fr

Fadeel Bengt
Karolinska Institutet
Institute of Environmental Medicine
Nobels väg 13
171 77 Stockholm, Sweden
bengt.fadeel@ki.se

Ferraro Elisabetta
IRCCS Fondazione Santa Lucia
Via del Fosso di Fiorano 64
143 Rome, Italy
ferraroeli@yahoo.it

FESUS Laszlo
University of Debrecen
Biochemistry and Miolecular Biology
Nagyerdei krt. 98.
H-4010 Debrecen, Hungary
fesus@indi.dote.hu

Finetti Francesca
University of Siena
Evolutionary Biology
via A. Moro, 2
53100 Siena, Italy
finetti4@unisi.it

Flinterman Marcella
King's College London
Head and Neck Oncology Group
The Rayne Institute, 123 Coldharbour Lane
SE5 9NU London, United Kingdom
marcella.flinterman@kcl.ac.uk

Floc'h Nicolas
Institut de Génétique Moléculaire montpellier
1919, route de mende
34293 Montpellier, France
nicolas.floch@igmm.cnrs.fr

Forge Andrew
University College London
Centre for Auditory Research, UCL Ear Institute
332, Gray's Inn Road
WC1X 8EE London, United Kingdom
a.forge@ucl.ac.uk

Forro Gaby
University Medical Center Charité
Clinical and Molecular Oncology
Lindenberger Weg 80
13125 Berlin, Germany
gabriella.forro@charite.de

Frezza Christian
Venetian Institute for Molecular Medicine
Via Orus 2
35129 Padova, Italy
cfrezza@dti.telethon.it

Fulda Simone
University Children's Hospital
Eythstr. 24
89075 Ulm, Germany
simone.fulda@uniklinik-ulm.de

Fussi Heike
Institut for Molecular Biosciences
Karl-Franzens University Graz
Universitätsplatz 2
8010 Graz, Austria
fussihe@uni-graz.at

Gambalunga Alberto
University of Padova (Italy)
Department of Biomedical Sciences,
Via Colombo 3
35121 Padova, Italy
albertogambalunga@msn.com

Gasparian Marine
Shemyakin and Ovchinnikov Institute of
Bioorganic Chemistry, RAS, Moscow, Russia
Protein Engineering
16/10 Miklukho-Maklaya
117997 Moscow, Russia
marine_gasparian@yahoo.com

Gendron Steve
Laval University
Rheumatology and Immunology Research
Center
2705 Blvd Laurier, local T1-49
G1V 4G2 Ste-Foy, Quebec,, Canada
steve.gendron@crchul.ulaval.ca

Gevaert Kris
Ghent University
Biochemistry and Medical Protein Research
A. Baertsoenkaai 3
B9000 Ghent, Belgium
kris.gevaert@ugent.be

Ghidoni Riccardo
Dept. Medicine
Via A. di Rudinì, 8
20142 Milano, Italy
riccardo.ghidoni@unimi.it

Giaime Emilie
IPMC CNRS-UMR6097
660 route des Lucioles
6560 Valbonne, France
giaime@ipmc.cnrs.fr

Gillissen Bernhard
University Medical Center Charité, Berlin, Germany
Clinical and Molecular Oncology
Lindenberger Weg 80
13125 Berlin, Germany
Bernhard.gillissen@charite.de

Gilmore Andrew
Wellcome Trust Centre for Cell Matrix Research
Faculty of Life Sciences, University of Manchester
A.3034 Michael Smith Buidling, Oxford Road
M13 9PT Manchester, United Kingdom
agilmore@manchester.ac.uk

Godlewski Michal Marek
Warsaw Agricultural University
Physiological Sciences
Nowoursynowska 159
02-776 Warsaw, Poland
mickgodl@hotmail.com

González-Estévez Cristina
University of Barcelona
Department of Genetics
Av. Digaonal 645 Edifici Annex 1a Planta
8028 Barcelona, Spain
crisgonzalez@ub.edu

Gougeon Marie-Lise
Institut Pasteur
Infection and Epidemiology
28 rue du Dr. Roux
75015 Paris, France
mlgougeo@pasteur.fr

Gröbner Sabine
Institute for Medical Microbiology and Hygiene
University of Tübingen
Elfriede-Aulhorn-Straße 6
72076 Tübingen, Germany
sabine.groebner@med.uni-tuebingen.de

Gross Atan
The Weizmann Institute
Biological Regulation
Herzel St
76100 Rehovot, Israel
atan.gross@weizmann.ac.il

Grumati Paolo
University of Padova
Departments of Histology, Microbiology and Medical Biotechnologies
Viale G.Colombo, 3
35121 Padova, Italy
paolo_grumati@yahoo.com

Grunewald Sonja
University of Leipzig
European Academy of Andrology Training Center
Philipp-Rosenthal-Str. 23-25
4103 Leipzig, Germany
sonja.grunewald@medizin.uni-leipzig.de

Grzybowska Ewa
Cancer Center Institute
Molecular Biology
Roentgena 5
02-781 Warsaw, Poland
ewag@coi.waw.pl

Haag Christian
University Children´s Hospital
Eythstrasse 24
89075 Ulm, Germany
christian.haag@uniklinik-ulm.de

Hajji Nabil
Institute of Environmental Medicine
Cell & Molecular Biology Toxicology and Neurotoxicology
Karolinska Institute Box 210
S-171 77 Stockholm, Sweden
nabil.hajji@ki.se

Hallay Natalia
Faculty of Veterinary Medicine, Warsaw Agricultural University
Department of Physiological Sciences
Nowoursynowska 159
02-776 Warsaw, Poland
mickgodl@hotmail.com

Hanto Katalin
Department of Biochemistry and Medical Chemistrty
Faculty of Medicine, University of Pecs
Szigeti u.12.
7624 Pécs, Hungary
hantokata@yahoo.com

Havelka Mandic Aleksandra
Karolinska Institute
Department of Oncology and Pathology
Cancer Centrum Karolinska, R8:03, Karolinska Hospital
17176 Stockholm, Sweden
aleksandra.mandic@cck.ki.se

Hellwig Christian
Royal College of Surgeons in Ireland
Physiology and Medical Physics
York Street Building
D-2 Dublin, Ireland
chellwig@rcsi.ie

Hernlund Emma
Karolinska Institute
Dept of Oncology and Pathology
CCK R8:03
S-171 76 Stockholm, Sweden
Emma.Hernlund@ki.se

Hjortsberg Linn
Karolinska Institutet
Oncology and Pathology
Cancer Centre Karolinska, R8:03
S-171 76 Stockholm, Sweden
Linn.Hjortsberg@ki.se

Hamann Andrea
Molecular Biosciences
Biosciences
Max-von-Laue-Str. 9
40438 Frankfurt, Germany
a.hamann@em.uni-frankfurt.de

Haslett Chris
University of Edinburgh
MRC Centre for Inflammation Research
47 Little France Crescent
EH16 4TJ Edinburgh, United Kingdom
c.haslett@ed.ac.uk

Heidari Nastaran
institute of biochemistry and biophysics
IBB
Ibb,University of Tehran,
1417614411 Tehran, Iran
heidari@ibb.ut.ac.ir

Henriksson Marie
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology (MTC)
Box 280
SE-171 77 Stockholm, Sweden
marie.henriksson@ki.se

Hetschko Holger
Johann Wolfgang Goethe University Clinics Frankfurt
Experimental Neurosurgery
Theodo-Stern-Kai 7
60590 Frankfurt, Germany
h.hetschko@med.uni-frankfurt.de

Hoffmann Julia
German Cancer Research Center
Division of Immunogenetics
INF 280
69120 Heidelberg, Germany
julia.hoffmann@dkfz-heidelberg.de

Hofmanová Jirina
Institute of Biophysics, Academy of Sciences of the Czech Republic
Laboratory of Cytokinetics
Královopolská 135
612 65 Brno, Czech Republic
hofmanova@ibp.cz

Holme Andrea
Yong Loo Lin School of Medicine
National University Medical Institutes
Redox Biology and Apoptosis Group, 10 Medical Drive, CRC NUS
117597 Singapore, Singapore
nmihal@nus.edu.sg

Hossini Amir M.
University Medical Center Charite
Clinical and Molecular Oncology
Lindenberger Weg 80
13125 Berlin, Germany
amir.hossini@charite.de

Ivanova Saska
Jozef Stefan Institute
Biochemistry and Molecular Biology
Jamova 39
1000 Ljubljana, Slovenia
saska.ivanova@ijs.si

Iwamoto Fumiko
Friedrich Miescher Institute for Biomedical Research
Maulbeerstrasse 66
4058 Basel, Switzerland
fumiko@fmi.ch

Jänicke Reiner
Institute of Molecular Medicine
University of Düsseldorf
Universitätsstrasse 1, Bldg. 23.12
40225 Düsseldorf, Germany
janicke@uni-duesseldorf.de

Holme Jørn A.
National Institute of Public Health
Division of Environmental Medicine
Postbox 4404 Nydalen
403 Oslo, Norway
jorn.holme@fhi.no

Horita Henrick
University of Colorado at Denver and Health Sciences Center
Pharmacology
P. O. Box 6511 MS 8303
80045 Aurora, USA
henrick.horita@uchsc.edu

Inohara Naohiro
University of Michigan Medical Center
Department of Pathology
1500 E. Medical Center Dr.
48109 Ann Arbor, MI, USA
ino@umich.edu

Ivanova Olga
Moscow State University
A.N. Belozersky Institute of Physico-Chemical Biology
Akad. Bochvara st., b.8, ap.49
123098 Moscow, Russia
ivanova@mbio.genebee.msu.ru

Jangi Sh Muhialdin
Faculty of Medicine and Dentistry/ University of the basque Countryu
Cell Biology and Histology
Leioa
48940 Vizcaya, Spain
gcbmuxxj@lg.ehu.es

Jeremias Irmela
Junior Research Group Apoptosis
Gene Vectors
Marchioninistrasse 25
81377 Munich, Germany
Irmela.Jeremias@gsf.de

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Juhasz Kata
Biological Reseach Certer, Hungarian Academy of Sciences
Biochemistry
Temesvari krt. 62.
6726 Szeged, Hungary
juhaszk@brc.hu

Karlberg Mats
Karolinska Institutet
Medicine
Karolinska university hospital Solna, L2:04
171 76 Stockholm, Sweden
mats.karlberg@ki.se

Kass George
University of Surrey
School of Biomedical & Molecular Sciences
Staghill
GU2 7XH Guildford, United Kingdom
g.kass@surrey.ac.uk

Kaufman Randal
HHMI @ University of Michigan
Biological Chemistry
1150 W. Medical Ctr. Dr., MSRB II, Room 4570
48109-0650 Ann Arbor, USA
kaufmanr@umich.edu

Keller Martin
Institute of Cell Biology, ETH Zurich
Schafmattstrasse 18, HPM D44
CH-8093 Zurich, Switzerland
martin.keller@cell.biol.ethz.ch

Kersse Kristof
University Ghent-VIB
Department for molecular biomedical research
Technologiepark 927
9052 Gent-Zwijnaarde, Belgium
kristof.kersse@dmbr.ugent.be

Karawajew Leonid
Robert-Rossle-Clinic, Charite, Humboldt University Berlin
Lindenberger Weg 80
13122 Berlin, Germany
karawajew@rrk.charite-buch.de

Kashkar Hamid
Med. Microbiol. and Immunol., University of Cologne
Goldenfelsstr. 19-21
50935 Köln, Germany
h.kashkar@uni-koeln.de

Katona Klara
University of Debrecen, Medical and Health Science Center
Biochemistry and Molecular Biology
Egyetem ter 1, Life Science Building, POB 6.
4010 Debrecen, Hungary
katonak@indi.biochem.dote.hu

Kawiak Anna
Intercollegiate Faculty of Biotechnology, University of Gdansk & Medical University of Gdansk
Department of Biotechnology
Kladki 24
80-822 Gdansk, Poland
kawiak@biotech.univ.gda.pl

Kerr Lorraine
The University of Edinburgh
Astellas CNS Research
The Chancellor's Building, 49 Little France Crescent
EH16 4SB Edinburgh, United Kingdom
Lorraine.Kerr@ed.ac.uk

Kitanovic Ana
Institute for Pharmacy and Molecular Biotechnology, University of Heidelberg
Molecular Biology
Im Neuenheimer Feld 364
69120 Heidelberg, Germany
Ana.Kitanovic@urz.uni-heidelberg.de

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Kleban Ján
Institute of biology and ecology, Faculty of Science, University of P.J. Šafárik
Department of cell and molecular biology
Moyzesova 11
040 01 Košice, Slovakia
jankleban@yahoo.com

Klee Martina
Centro de Investigación del Cáncer - Lab 18
Campus Miguel de Unamuno, Universidad de Salamanca
37007 Salamanca, Spain
mklee@usal.es

Klima Martin
Institute of Molecular Genetics
Laboratory of Cell Signaling and Apoptosis
Videnska 1083
14220 Prague, Czech Republic
klima@leuko.biomed.cas.cz

Kochetkova Marina
University of Adelaide
School of Molecular and Biomedical Sciences
North Tce
5005 Adelaide, Australia
marina.kochetkova@adelaide.edu.au

Koehler Barbara
Royal College of Surgeons in Ireland
Physiology and Medical Physics
York Street
Dublin 2 Dublin, Ireland
bkohler@rcsi.ie

Kopustinskiene Dalia Marija
Insitute for Biomedical Research
Kaunas University of Medicine
Eiveniu 4
LT-50009 Kaunas, Lithuania
daliammk@mail.lt

Kovar Jan
Institute of Molecular Genetics
Department of Cell Signalling and Apoptosis
Videnska 1083
14220 Prague 4, Czech Republic
kovar@biomed.cas.cz

Krammer Peter
Deutsches Krebsforschungszentrum (DKFZ)
Tumorimmunology Program
Im Neuenheimer Feld 280
D-69120 Heidelberg, Germany
p.krammer@dkfz.de

Kroemer Guido
CNRS
FRE2939
Institut Gustave Roussy, PR1
94805 Villejuif, France
kroemer@igr.fr

Krysko Dmitri
Ghent University
of Human Anatomy, Embryology, Histology and Medical Physics
Godshuizenlaan 4
9000 Ghent, Belgium
dmitri.krysko@ugent.be

Kulawiak Bogusz
Nencki Institute of Experimental Biology
Cellular Biochemistry
3 Pasteur St.
02-093 Warsaw, Poland
b.kulawiak@nencki.gov.pl

Kulms Dagmar
Institute of Cell Biology and Immunology
University of Stuttgart
Allmandring 31
70569 Stuttgart, Germany
Dagmar.Kulms@izi.uni-stuttgart.de

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Kumar Sharad
Institute of Medical and Veterinary Science
Haematology
PO Box 14, Rundle Mall
5000 Adelaide, Australia
sharad.kumar@imvs.sa.gov.au

Lakshmanan Umayal
Institute of Molecular and Cell Biology
Cell Death and Human Disease Lab
61, Biopolis Drive, Proteos,
138673 Singapore, Singapore
mcbul@imcb.a-star.edu.sg

Lam Eric
Rutgers University
Biotech Center
59 Dudley Rd
8901 New Brunswick, USA
Lam@aesop.rutgers.edu

Lan Annaig
INRA
UMR 1253 INRA–Agrocampus Sciences et Technologie du Lait et de l'Oeuf
65 rue de Saint Brieuc
35042 RENNES, France
annaig.lan@rennes.inra.fr

Langmesser Sonja
Institute of Biochemistry
Department of Medicine
Rue du Musée 5
1700 Fribourg, Switzerland
sonja.langmesser@unifr.ch

Lassus Patrice
Institute of Molecular genetics of Montpellier
CNRS
1919, route de Mende
34293 Montpellier, France
patrice.lassus@igmm.cnrs.fr

Lazarova Paula
District University Hospital "St. Anna"
Clinical Laboratory
100 Tzar Osvoboditel Blvd.
BG-9002 Varna, Bulgaria
paula.lazarova@bulgaria.com

Le Clorennec Christophe
UMR CNRS 6101 "Physiologie de la réponse Immune et des Lymphoprolifération"
2, Rue du Docteur Marcland
87025 LIMOGES, France
christophe.le-clorennec@etu.unilim.fr

Lemaire christophe
LGBC, CNRS-UMR 8159
Mitochondrial protein complex and apoptosis team
University of Versailles, 45 avenue des Etats unis
78035 Versailles, France
clemaire@genetique.uvsq.fr

Leonini Alessandra
Biological Chemistry
Molecular Biology
Via Fiorentina,1
53100 Siena, Italy
leonini7@unisi.it

Liaudet-coopman Emmanuelle
Inserm unit 540 'Molecular and Cellular Endocrinology of Cancers'
60 rue de navacelles
34090 Montpellier, France
liaudet@montp.inserm.fr

Liguori Lavinia
Université Joseph Fourier
Laboratoire Européen HumProTher - GREPI
Laboratoire d'Enzymologie - CHU - BP 217
38043 Grenoble, France
lavynia@email.it

Longoni Biancamaria
university of Pisa
Department of Oncology, Transplantation and Advanced Technologies in Medicine
via Roma 52
56100 Pisa, Italy
biancam@dfb.unipi.it

Lovat Penny
University of Newcastle upon Tyne
School of Clinical and Laboratory Sciences
2nd Floor Leech Building, The Medical School, Framlington Place
NE2 4HH Newcastle upon Tyne, United Kingdom
p.e.lovat@ncl.ac.uk

MacDonald David
The University of Edinburgh
Astellas CNS Research
The Chancellor's Building, 49 Little France Crescent
EH16 4SB Edinburgh, United Kingdom
D.C.Macdonald@sms.ed.ac.uk

Madeo Frank
IMB
Universitaetsplatz 2
8010 Graz, Austria
Frank.Madeo@Uni-Graz.at

Magrini Roberta
Siena Biotech
Cancer Biology Unit
Via Fiorentina 1
53100 Siena, Italy
rmagrini@sienabiotech.it

Makris Antonios
Mediterranean Agronomic Institute of Chania
Natural Products & Biotechnology
Alsylion agrokepion, Makedonias 2
73100 Chania, Greece
antoniosmakris@yahoo.gr

Look A. Thomas
Dana-Farber Cancer Institute
Pediatric Oncology, Mayer-630
44 Binney Street
2115 Boston, USA
THOMAS_LOOK@dfci.harvard.edu

Lyamzaev Konstantin
A. N. Belozersky Institute, Moscow State University
Vorob'evi gori, A. N. Belozersky Institute, bild "A"
119992 Moscow, Russia
Lyamzaev@mail.ru

Mack Claudia
Robert Koch-Institute
Viral Infections
Nordufer 20
13353 Berlin, Germany
gehrkec@rki.de

Magiera Magda
Institute of Molecular Genetics of Montpellier, UMR 5535
Mechanisms of neuronal death
1919 route de Mende
34000 Montpellier, France
magda.magiera@igmm.cnrs.fr

Majai Gyongyike
University of Debrecen
Biochemistry and Molecular Biology
Egyetem ter 1
4010 Debrecen, Hungary
mgyongyi@indi.dote.hu

Malik Fayaz
Regional Research Laboratory (Counsil of Scientific & Industrial Reserach,CSIR)
Pharmacology
Canal Road, Jammu, Jammu and Kashmir
180001 Jammu, India
malikfayaz@gmail.com

Malorni Walter
Istituto Superiore di Sanita
Drug Research and Evaluation
Viale Regina Elena 299
161 Rome, Italy
malorni@iss.it

Mancini Francesca
Lab. Molecular Oncogenesis - regina Elena Cancer Inst.
Via delle Messi D'Oro, 156
158 Roma, Italy
mancini@ifo.it

Manzl Claudia
Div. of eperimental Pathophysiology and Immunology, Insbruck Medical University
Laboratory of comparative immunology
Fritz-Pregl-str. 3
A-6020 Innsbruck, Austria
claudia.manzl@i-med.ac.at

Marengo Barbara
G. Gaslini
Largo Gaslini, 5
I-16100 Genoa, Italy
bmare2002@yahoo.it

Margittai Eva
Semmelweis University
Dept. of Medical Chemistry
POB 260
1444 Budapest, Hungary
meva@puskin.sote.hu

Markkanen Ari
University of Kuopio
Department of Environmental Sciences
P.O.Box 1627
FI-70211 Kuopio, Finland
ari.markkanen@uku.fi

Martinet Wim
University of Antwerp
Pharmacology
Universiteitsplein 1
2610 Wilrijk, Belgium
wim.martinet@ua.ac.be

Martinou Jean-Claude
University of geneva
Department of cell biology
30 Quai Ansermet
1211 Geneva, Switzerland
jean-claude.martinou@cellbio.unige.ch

Matalova Eva
Institute of Animal Physiology and Genetics
Laboratory of Animal Embryology
Veveri 97
602 00 Brno, Czech Republic
matalova@iach.cz

Matarrese Paola
Istituto superiore di sanita'
Drug research and evaluation
Viale Regina Elena 299
161 Rome, Italy
paola.matarrese@iss.it

Mateva Rada
Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria,
G. Bonchev str., 21
1113 Sofia, Bulgaria
mateva@obzor.bio21.bas.bg

Mazur Lidia
Institute of Zoology, Jagiellonian University
Department of Animal Physiology, Lab. Exp. Hematol.
R. Ingardena 6
30-060 Cracow, Poland
maz@zuk.iz.uj.edu.pl

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

McColl Aisleen
Queen's Medical Research Institute
Inflammatory Cell Biology
47 Little France Crescent
EH16 4TJ Edinburgh, United Kingdom
s0571550@sms.ed.ac.uk

Mérino Delphine
INSERM U517
University of medicine
7 Boulevard Jeanne d'Arc
21 000 DIJON, France
merinodf@yahoo.fr

Micheau Olivier
INSERM U517
mort Cellulaire et Cancer
Faculté de Médecine et de Pharmacie
F21078 Dijon, France
omicheau@u-bourgogne.fr

Milojkovic Ana
University Medical Center Charité
Clinical and Molecular Oncology
Lindenberger Weg 80
13125 Berlin, Germany
ana.milojkovic@charite.de

Miyazaki Toru
The University of Tokyo
Div. of Molecular Biomedicine for
Pathogenesis, CDBIM, Faculty of Medicine
7-3-1 Hongo, Bunkyo-ku
113-0033 Tokyo, Japan
tm@m.u-tokyo.ac.jp

Möller Christine
Karolinska institutet
Department of medicine
Clinical immunology and allergy unit, KS L2:04
17176 Stockholm, Sweden
christine.moller@ki.se

Melino Gerry
Medical Research Council
Toxicology Unit
Leicester, UK
gm89@leicester.ac.uk

Micali Nicola
Scientific Institute San Raffale, DIBIT
Department of Molecular Biology and
Functional Genomics
Via Olgettina 58
20132 Milan (Italy), Italy
Micali.Nicola@HSR.it

Michlewska Sylwia
The Queen's Medical Research Institute
(University of Edinburgh)
MRC Centre for Inflammation Research
47 Little France Crescent
EH16 4TJ Edinburgh, United Kingdom
s.michlewska@sms.ed.ac.uk

Mitsios Nicholas
Manchester Metropolitan University
Biology, Chemistry and Health Science
John Dalton Building, Chester Street
M1 5GD Manchester, United Kingdom
n.mitsios@mmu.ac.uk

Moisoi Nicoleta
MRC Toxicology unit
Cell Death Regulation
University of Leicester, Hodgkin Building
LE1 9HN Leicester, United Kingdom
nm105@le.ac.uk

Momoi Takashi
National Institute of Neuroscience
Divisions of Development and Differentiation
4-1-1 Ogawahigashi, Kodaira
187-8502 Tokyo, Japan
momoi@ncnp.go.jp

Supported by EC within the framework of the Marie Curie
Conferences and Training Courses

Moraitis Christos
AEA Technology, c/o GlaxoSmithKline, Harlow, Essex, UK
Environment
71 Sunnyside Road, Epping, Essex (personal address)
CM16 4JN Epping, United Kingdom
cmoraitis@aol.com

Moretti Fabiola
Inst. Neurobiology and Molecular Medicine - CNR
Via delle Messi D'Oro, 156
158 Roma, Italy
moretti@ifo.it

Mourtada-Maarabouni Mirna
Institute for Science and Technology in Medicine
School of Life Sciences
Keele University
ST5 5BG Keele, United Kingdom
bia19@biol.keele.ac.uk

Mussche Sylvie
Ghent University
Anatomy, Embryology, Histology and Medical Physics
Godshuizenlaan 4
9000 Ghent, Belgium
s.mussche@ugent.be

Nagibin Vasyl
Bogomoletz Institute of Physiology
Experimental cardiology
Bogomoletz str. 4.
1024 Kiev, Ukraine
nvasya@ukr.net

Nieto-Rementeria Naiara
University of the Basque Country
Cell biology and histology
barrio Sarriena s/n
48940 Leioa, Spain
gcbniren@lg.ehu.es

Nicolosi Luca
University of Padova
Biomedical Sperimental Sciences
Viale G. Colombo, 3
35121 Padova, Italy
luca.nicolosi@unipd.it

Novelli Antonello
University of Oviedo
Psychology
Plaza Feijoo s/n
33003 Oviedo, Spain
anovelli@uniovi.es

Nigro Patrizia
University of Salerno
Department of Pharmaceutical Sciences
Via Ponte Don Melillo
84084 Fisciano, Italy
panigro@unisa.it

O'Connell Ailish
national university of ireland galway, ireland
biochemistry
orbsen building ncbes, university road, galway ireland
co galway galway, Ireland
ailish_o@yahoo.ie

Nyman Ulrika
Karolinska Institutet
Institute of Environmental Medicine, Division of Toxicology
Nobels väg 13
17177 Solna, Sweden
ulrika.nyman@imm.ki.se

Olsson Anna
Div. of experimental Pathophysiology and Immunology, Innsbruck Medical University
Laboratory of comparative immunology
Fritz-Pregl-str. 3
A-6020 Innsbruck, Austria
anna.olsson@i-med.ac.at

O'Connor Carla
Royal College of Surgeons in Ireland
Department of Physiology and Medical Physics
York House, York Street, Dublin 2
D2 Dublin, Ireland
carlaoconnor@rcsi.ie

Opydo Malgorzata
Institute of Zoology
Department of Animal Physiology
R. Ingardena 6
30-060 Krakow, Poland
magios@interia.pl

Ottosson Astrid
Institute of enviromental medicine, Karolinska Institutet
Deparment of biochemical toxicology
Nobelsväg 13
171 77 Stockholm, Sweden
astrid.ottosson.439@student.ki.se

Pace Vincenza
University of Calabria
Department of Cell Biology
Arcavacata
87030 Rende (CS), Italy
vincenza_pace@yahoo.it

Pajak Beata
Warsaw Agricultural University
Department of Physiological Sciences
Nowoursynowska 159
02-776 Warsaw, Poland
bepaj@wp.pl

Pardo Julian
MPI for Immunobiology
Metschnikoff
Stuebeweg 51
79108 Freiburg, Germany
pardo@immunbio.mpg.de

Ott Martin
Institute for Environemental Medicine,
Karolinska Institute
Division of Toxicology
Nobelsväg 13
17177 Stockholm, Sweden
martin.ott@ki.se

Ouasti sihem
the University of Manchester
Faculty of Life Sciences
Stopford building, Oxford Road
M139PT Manchester, United Kingdom
sihem.ouasti@manchester.ac.uk

Paidassi Helena
Institut de Biology Structurale
Laboratoire d'enzymologie moleculaire
41, rue J. Horowitz
38027 Grenoble, France
helena.paidassi@ibs.fr

Paleeva Anna
N.N.Blokhin Cancer Center
Institute of Carcinogenesis
24 Kashirskoye shosse
115478 Moscow, Russia
evanjelista@mail.ru

Pattacini Laura
Ospedale Santa Maria Nuova
Clinical Pathology
Viale Risorgimento, 50
42100 Reggio Emilia, Italy
laura.pattacini@asmn.re.it

Pchejetski Dimitri
Institute of pharmacology and structural biology
Dept. of Functional proteomics and therapeutic targets
205 route de Narbonne
31000 Toulouse, France
dpshezhet@mail.ru
dimitri.pchejetski@ipbs.fr

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Pavlaki Maria
Foundation for Biomedical Research of the Academy of Athens (IIBEAA)
Division of Basic Neurosciences
4, Soranou tou Efessiou Street
11527 Athens, Greece
mpavlaki@bioacademy.gr

Pennucci Roberta
Manchester Metropolitan University
School of Biology, Chemistry and Health Science
John Dalton Tower, Chester Street
M1 5GD Manchester, United Kingdom
r.pennucci@mmu.ac.uk

Pelicci Piergiuseppe
European Institute of Oncology
Experimental Oncology Dept.
Via Ripamonti, 435
20141 Milan, Italy
piergiuseppe.pelicci@ifom-ieo-campus.it

Petersen Christine Lund
Medical Biochemistry
University of Aarhus
Ole Worms Allé bygn 1.170
8000 Aarhus C, Denmark
clp@biokemi.au.dk

Penttinen Piia
National Public Health Institute
Department of Environmental Health
P.O.Box 95
70701 Kuopio, Finland
piia.penttinen@ktl.fi

Peyrou Mathieu
UPEI
Biomedical Sciences
550 University Avenue
C1A 4P3 Charlottetown, Canada
mpeyrou@upei.ca

PETIT Patrice X.
Institut Cochin
Genetic and Physiopathology
24 rue du Faubourg Saint-Jacques
75013 Paris Paris, France
pxpetit@cochin.inserm.fr

Pletjushkina Olga
A.N. Belozersky Institute, Moscow State University
Moscow State University, Lab. building "A"
119992 Moscow, Russia
pletjushkina@mail.ru

Piacentini Mauro
University of Rome, "Tor Vergata"
Department of Biology
Via della Ricerca Scientifica
133 Rome, Italy
mauro.piacentini@uniroma2.it

Portugal Raquel
Faculdade de Medicina Veterinária
Doenças Infecciosas, CIISA
Avenida da Universidade Técnica
1300-477 Lisboa, Portugal
raqport@fmv.utl.pt

Porcelli Anna Maria
Università di Bologna
Dipartimento di Biologia
Via Irnerio 42
40126 Bologna, Italy
porcelli@alma.unibo.it

Rasola Andrea
Università degli Studi di Padova
Dipartimento di Scienze Biomediche
viale G. Colombo 3
35121 Padova, Italy
andrea.rasola@unipd.it

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Pucci Bruna
San Raffaele La Pisana Tosinvest
Molecular and cellular pathology
Via dei Bonacolsi
163 Roma, Italy
brunapucci@yahoo.com

Reef Sharon
Weizmann Institute of Science
Molecular Genetic
P.O. Box 26 Rehovot
76100 Rehovot, Israel
sharon.kahan@weizmann.ac.il

Richter Antje
IGT-Labor,Robert-Rössle-Klinik
Charite Berlin-Buch
Lindenberger Weg 80
13125 Berlin, Germany
antje.richter@charite.de

Rigou Patricia
Institut de Génétique Moléculaire
Département de Pharmacologie, Inserm U716
27 rue Juliette Dodu
75010 Paris, France
patricia.rigou@yahoo.fr

Rippo Maria Rita
Polytechnic University of Marche
Dept. of Molecular Pathology and Innovative Therapies
Via Ranieri, 1
60100 Ancona, Italy
m.r.rippo@univpm.it

Robicsek Francis
Carolinas Heart Institute, Carolinas Medical Center
Dept. of Cardiovascular and Thoracic Surgery
1000 Blythe Blvd.
28203 Charlotte, NC, USA
tjohn@sanger-clinic.com

Riccardi Carlo
University of Perugia
Dept of Clinical and Experimental Medicine - Sect. Pharmacology
Via del Giochetto
6122 Perugia, Italy
riccardi@unipg.it

Richter Anja
IGT-Labor, Robert-Rössle-Klinik
Charite Berlin-Buch
Lindenberger Weg 80
13125 Berlin, Germany
anja.richter@charite.de

Riley Nicola
Queen's Medical Research Institute (University of Edinburgh)
MRC Centre for Inflammation Research
47 Little France Crescent
EH16 4TJ Edinburgh, United Kingdom
s0459710@sms.ed.ac.uk

Robbins Ian
Institute of Molecular Genetics of Montpellier, UMR 5535
Mechanisms of neuronal death
1919 route de Mende
34000 Montpellier, France
ian.robbins@igmm.cnrs.fr

Rodrigues Cecília Maria
Faculty of Pharmacy, University of Lisbon
Centro de Patogénese Molecular
Av. das Forças Armadas
1600-083 Lisbon, Portugal
cmprodrigues@ff.ul.pt

Rossi Adriano
University of Edinburgh
MRC Centre for Inflammation Research
47 Little France Crescent
EH16 4TJ Edinburgh, United Kingdom
a.g.rossi@ed.ac.uk

Rodriguez-Nieto Salvador
Institute of Environmental Medicine. Karolinska Institute.
Toxicology and Neurotoxicology
Box 210. Nobelsvägen, 13.
17177 Stockholm, Sweden
salvador.rodriguez-nieto@ki.se

Roth Wilfried
German Cancer Research Center
Molecular Neuro-Oncology
Im Neuenheimer Feld 280
69120 Heidelberg, Germany
W.Roth@dkfz.de

Rugolo Michela
University of Bologna
Detp. of Biology
Via Irnerio 42
40126 Bologna, Italy
rugolo@alma.unibo.it

Sanges Daniela
Telethon Institute of Genetics and Medicine (TIGEM)
V. P. Castellino, 111
80131 Naples, Italy
sanges@tigem.it

Sautkina Elena
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry Russian Academy of Sciences
Department of Structural Biochemistry
Ul. Miklukho-Maklaya 16/10
117997 Moscow, Russia
vla@ibch.ru

Sciola Luigi
University of Sassari
Physiological Biochemical and Cellular Sciences
Via Muroni 25
7100 Sassari, Italy
sciola@uniss.it

Rudka Tomasz
KULeuven and VIB
Department of Human Genetics
Herestraat 49 bus 602
3000 Leuven, Belgium
Tomasz.rudka@med.kuleuven.be

Salliau Stefanie
UGENT
Department of Medical Protein Research
A. Baertsoenkaai 3
9000 GENT, Belgium
stefanie.salliau@ugent.be

Sarang Zsolt
University of Debrecen
Biochemistry and Molecular Biology
Egyetem ter 1
4010 Debrecen, Hungary
sarang@indi.dote.hu

Schyschka Lilianna
Pharmaceutical Biology
Department of Pharmacy
Butenandtstr. 5-13
81377 Munich, Germany
lilianna.schyschka@cup.uni-muenchen.de

Scovassi anna ivana
IGM-CNR
via abbiategrasso 207
27100 Pavia, Italy
scovassi@igm.cnr.it

Sharma Himani
All India Institute of Medical Sciences
Biochemistry
R.No. 3027A, Dept of Biochemistry, AIIMS
110029 New Delhi, India
himanisharma@rediffmail.com

Sevrioukova Irina
University of California Irvine
Molecular Biology and Biochemistry
3205 McGaugh Hall
92697-3900 Irvine, USA
sevrioui@uci.edu

Shtil Alexander
N.N.Blokhin Cancer Center
Institute of Carcinogenesis
24 Kashirskoye shosse
115478 Moscow, Russia
shtilaa@yahoo.com

Simmen Thomas
University of Alberta
Department of Cell Biology
Medical Sciences Building, Room 5-65
T6G 2H7 Edmonton, Canada
Thomas.Simmen@ualberta.ca

Simon Markus M
MPI for Immunobiology
Metschnikoff
Stuebeweg 51
79108 Freiburg, Germany
simon@immunbio.mpg.de

Slack Ruth
University of Ottawa
Cellular and Molecular Medicine
451 Smyth Road
K1H8M5 Ottawa, Canada
rslack@uottawa.ca

Slupecka Monika
The Kielanowski Institute of Animal Phsiology and Nutrition
Department of Gastrointestinal Physiology
Instytucka 5
05-110 Jablonna near Warsaw, Poland
monika_slupecka@wp.pl

Simbula Gabriella
Unit of Oncology and Molecular Pathology
Toxicology
Via Porcell,4
9124 Cagliari, Italy
gsimbula@unica.it

Simon Hans-Uwe
University of Bern
Dept. of Pharmacology
Friedbuehlstrasse 49
CH-3010 Bern, Switzerland
hus@pki.unibe.ch

Skulachev Vladimir
Belozersky Institute of Physico-Chemical Biology
Bioenergetics
Moscow State University, Leninskie Gory 1
119992 Moscow, Russia
skulach@belozersky.msu.ru

Slazak Paulina
Faculty of Veterinary Medicine, Warsaw Agricultural University
Department of Physiological Sciences
Nowoursynowska 159
02-776 Warsaw, Poland
mickgodl@hotmail.com

Sluyser Mels
The Netherlands Cancer Institute H4
Editorial Office APOPTOSIS
Plesmanlaan 121
1066 CX Amsterdam, The Netherlands
m.sluyser@planet.nl

Sohn Dennis
Institute of Molecular Medicine
University of Düsseldorf
Universitätsstrasse 1
40225 Düsseldorf, Germany
Dennis.Sohn@uni-duesseldorf.de

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Smigelskaite Julija
Daniel Swarovski Research Laboratory
Department of General and Transplant Surgery/Innsbruck Medical University
Innrain 66
6020 Innsbruck, Austria
julijasmigelskaite@yahoo.com

Soldani Cristiana
Laboratorio di Biologia Cellulare e neurobiologia
Dipartimento di Biologia Animale-Universita' di Pavia
Piazza Botta 10
27100 Pavia, Italy
soldani@unipv.it

Strappazzon Flavie
INSERM 0108 Laboratoire Neurodégénérescence et Plasticité
Pavillon de Neurologie, CHU de Grenoble, BP-217
38O49 Grenoble, France
strappazzonflavie@hotmail.com

Strettoi Enrica
Istituto di Neuroscience del CNR
Medicine
Area della Ricerca CNR, Via Giuseppe Moruzzi 1
56100 Pisa, Italy
enrica.strettoi@in.cnr.it

Sugawara Masahiro
Greater Los Angeles VA Hospital
Medical Service (111M)
11301 Wilshire Blvd.,
90073 Los Angeles, California, USA
msugawar@ucla.edu

Sumbayev Vadim
University of Aarhus
Department of Molecular Biology
Gustav Wieds Vej 10C
8000C Aarhus, Denmark
sumbayev@yahoo.co.uk

Sole Montse
Universitat Autònoma de Barcelona
Dpt. Bioquímica i Biologia Molecular. Institut de Neurociències
Edifici M, Campus UAB
8193 Bellaterra (Barcelona), Spain
montserrat.sole@uab.es

Stagni Venturina
EBRI Fondazione Santa Lucia
Laboratory of cell signalling
Via dell fosso di fiorano 64
143 Rome, Italy
venturinastagni@yahoo.it

Strasser Andreas
The Walter and Eliza Hall Institute
Molecular genetics of cancer
1G royal parade
3050 VIC Parkville, Australia
strasser@wehi.edu.au

Su Yeu
Institute of Biopharmaceutical Science
National Yang-Ming University, Li-Nong St. Sec. 2, No. 155, Shi-Pai, Taipei, Taiwan, R.O.C.
11221 Taipei, Taiwan
yeusu@ym.edu.tw

Suin Vanessa
Pasteur Institut
Cellular Microbiology
Rue Engeland, 642
Bruxelles (Uccle) 1180, Belgium
vsuin@pasteur.be

Syta Maren
Robert Koch-Institute
FG12 Viral Infections
Nordufer 20
13353 Berlin, Germany
SytaM@rki.de

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Szegezdi Eva
National University of Ireland, Galway
National Centre for Biomedical Engineering Science
University Rd.
0 Galway, Ireland
eva.szegezdi@nuigalway.ie

TACNET Pascale
institut de biologie structurale
LEM
41 Rue Jules Horowitz
38027 Grenoble, France
tacnet@ibs.fr

Tanase Cristiana
Victor Babes National institute of Pathology
Pathology
Spl. Independentei, No. 99-101
50096 Bucharest, Romania
bioch@vbabes.ro

Tardito Saverio
University of Parma
Experimental Medicine, Unit of Clinical and General Pathology
Via Volturno 39,
43100 Parma, Italy
saverio.tardito@nemo.unipr.it

Taylor Emma
Queen's Medical Research institute
MRC Centre for Inflammation Research
47 Little France Crescent
EH16 4TJ Edinburgh, United Kingdom
etaylor1@staffmail.ed.ac.uk

Tembe Varsha
Westmead Millennium Institute
Westmead Institute for Cancer Research
Darcy Road P.O. Box 412
2145 Sydney, Australia
varsha_tembe@wmi.usyd.edu.au

Szondy Zsuzsa
University of Debrecen
Department of Biochemistry and Molecular Biology
Nagyerdei krt.98
H-4012 Debrecen, Hungary
szondy@indi.dote.hu

Takács Viktor
Eötvös Lóránd University
Department of Anatomy, Cell and Developmental Biology
1/C Pázmány Péter stny.
H-1117 Budapest, Hungary
takacsvik@gmail.com

Tanuma Sei-ichi
Tokyo University of Science
Department of Biochemistry, Faculty of Pharmaceutical Sciences, and Genome & Drug Research Center
2641 Yamazaki, Noda
278-8510 Chiba, Japan
tanuma@rs.noda.tus.ac.jp

Tatarskiy Victor
N.N.Blokhin Cancer Center
Institute of Carcinogenesis
24 Kashirskoye shosse
115478 Moscow, Russia
tatarskii@gmail.com

Taylor Ruth
Centre for Auditory Research, The Ear Institute
University College London
332 Gray's Inn Road
WC1X 8EE London, United Kingdom
Ruth.R.Taylor@ucl.ac.uk

Tenta Roxane
University of Athens, Medical School
Experimental Physiology
Voutsina 63, Cholargos
15561 Athens, Greece
roxane@med.uoa.gr

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Torch Sakina
INSERM 0108 Laboratoire
Neurodégénérescence et Plasticité
Pavillon de Neurologie BP 217
38043 Grenoble, France
Sakina.Torch@ujf-grenoble.fr

Tóth Katalin
University of Debrecen
Biochemistry and Molecular Biology
Nagyerdei krt.98.
H-4028 Debrecen, Hungary
tkati@indi.dote.hu

Tschopp Jurg
University of Lausanne
Biochemistry
Chemin des Boveresses 155
1066 Epalinges, Switzerland
jurg.tschopp@unil.ch

Turk Boris
Jozef Stefan Institute
Biochemistry and Molecular Biology
Jamova 39
1000 Ljubljana, Slovenia
Boris.Turk@ijs.si

Vaculova Alena
Institute of Biophysics, Academy of Sciences of the Czech Republic
Kralovopolska 135
61265 Brno, Czech Republic
vaculova@ibp.cz

Vandenabeele Peter
Ghent University-Flanders Interuniversity Institute for Biotechnology
Molecular Biomedical Research
Technologiepark 927
9052 Ghent,
Peter.Vandenabeele@dmbr.ugent.be

Toth Beata
University of Debrecen
Biochemistry and Molecular Biology
Egyetem ter 1
4012 Debrecen, Hungary
tbeata@indi.dote.hu

Trapani Valentina
Università Cattolica del Sacro Cuore
Istituto di Patologia Generale
largo Francesco Vito 1
168 Rome, Italy
valentinatrapani@hotmail.com

Tuosto Loretta
University of Rome "La Sapienza"
Dept. Cellular Biology and Development
Via dei Sardi 70
185 Rome, Italy
loretta.tuosto@uniroma1.it

Turowska Agnieszka
Warsaw Agricultural University
Department of Preclinical Sciences
Ciszewskiego 8
02-786 Warsaw, Poland
aturowska@alpha.sggw.waw.pl

Vakifahmetoglu Helin
Karolinska Institutet
Department of Toxicology in Institute of environmental Medicine
Nobelsv. 13
127 47 Stockholm, Sweden
Helin.vakifahmetoglu@imm.ki.se

Vanderhaeghen Pierre
University of Brussels ULB
IRIBHM
808 Route de Lennik
1070 Brussels, Belgium
pvdhaegh@ulb.ac.be

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Varecha Miroslav
Centre for Biomedical Image Analysis (CBIA)
Faculty of Informatics, Masaryk University
Botanicka 68a
60200 Brno, Czech Republic
mvara@fi.muni.cz

Velardi Enrico
University of Perugia
Department of Clinical and Experimental Medicine, Section of Pharmacology
Via del Giochetto
6122 Perugia, Italy
enrico_velardi@yahoo.it

Verna Jean-Marc
INSERM 0108 Laboratoire
Neurodégénérescence et Plasticité
Pavillon de neurologie, BP 217
38O43 Grenoble, France
Jean-Marc.Verna@ujf-grenoble.fr

Vissers Margret
Christchurch School of Medicine and Health Sciences, University of Otago
Pathology Dept
PO Box 4345
8005 Christchurch, New Zealand
margret.vissers@chmeds.ac.nz

Vogler Meike
Children´s Hospital
University of Ulm
Eythstr. 24
89075 Ulm, Germany
meike.vogler@medizin.uni-ulm.de

Vollmar Angelika
University of Munich
Department of Pharmacie
Butenandtstr. 5-13, B
81377 Munich, Germany
angelika.vollmar@cup.uni-muenchen.de

Végran Frédérique
Centre Georges Francois Leclerc
Molecular Genetics
1 rue du Pr Marion
21079 Dijon, France
fvegran@club-internet.fr

Velasco Guillermo
Complutense University
Biochemistry and Molecular Biology I
School of Biology, Anexo Building, 1st floor,
Lab 2, Avda Complutense s/n
28040 Madrid, Spain
gvd@bbm1.ucm.es

Villanova Federica
Polytechnic University of Marche, Ancona
Department of Molecular Pathology and Innovative Therapies
vai Ranieri 1
60100 Ancona, Italy
villanova_f@libero.it

Vlachos Pinelopi
Institute of Environmental medicine, Karolinska Institute
Toxicology and Neurotoxicology
Nobels väg 13
171 77 Stockholm, Sweden
pinelopi.vlachos@imm.ki.se

Volkmann Xandra
Medical School Hannover
Gastroenterology, Hepatology and Endocrinology
Carl Neuberg Str.01
30625 Hannover, Germany
volkmann.xandra@mh-hannover.de

von Haefen Clarissa
University Medical Center Charite
Clinical and Molecular Oncology
Lindenberger Weg 80
13125 Berlin, Germany
clarissa.von-haefen@charite.de

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Walicki Joël
Department of Cellular Biology and Morphology
Rue du Bugnon 9
1005 Lausanne, Switzerland
joel.walicki@unil.ch

Wang Jing
INSERM
INSERM U 583
80 rue Augustin Fliche
34400 Montpellier, France
jingwang@montp.inserm.fr

Wang Zhao-Qi
Leibniz Institute for Age Research - Fritz Lipmann Institute (FLI) Jena, Germany
Beutenbergstrasse 11
7745 Jena, Germany
zqwang@fli-leibniz.de

Weiland Timo
University of Konstanz
Chair of Biochemical Pharmacology
Universitätsstrasse 10
78457 Konstanz, Germany
Timo.Weiland@uni-konstanz.de

Westphal Dana
University of Otago
Microbiology and Immunology
720 Cumberland Street
9001 Dunedin, New Zealand
wesda320@student.otago.ac.nz

Widmann Christian
Department of cellular biology and morphology
Bugnon 9
1005 Lausanne, Switzerland
Christian.Widmann@unil.ch

Willemarck Nicolas
Ugent - VIB1
Molecular Biomedical Research
Technolgiepark 927
9052 Zwijnaarde Gent, Belgium
Nicolas.Willemarck@DMBR.UGent.be

Wirawan Ellen
VIB, University of Ghent
Department of Molecular Biomedical Research
Technologiepark 927
9052 Zwijnaarde, Belgium
Ellen.Wirawan@dmbr.Ugent.be

Witasp Erika
Karolinska Institutet
Institute of Environmental Medicine, Division of Biochemical Toxicology
Nobels väg 13
171 77 Stockholm, Sweden
erika.witasp@ki.se

Witko-Sarsat véronique
INSERM U507
Necker Hospital
161 rue de Sèvres
75015 Paris, France
witko-sarsat@necker.fr

Wong Yick Fu
The Chinese University of Hong Kong
Obstetrics & Gynaecology
Prince of Wales Hospital, Shatin
N.T. Hong Kong, Hong Kong
b360710@mailserv.cuhk.edu.hk

Wright Marianne
Pediatric Research Institute, UiO
Rikshospitalet-Radiumhospitalet Medical Center
Sognsvannsveien 20
N-0027 Oslo, Norway
marianne.wright@medisin.uio.no

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Yadaiah Madasu
University of Hyderabad
School of chemistry
PO. Central University
500046 Hyderabad, India
ch03ph05@uohyd.ernet.in

yang Jiang-yan
Deparment of cellular biology and morphorlogy
university of Lausanne
Rue du Bugnon 9
1005 Lausanne, Switzerland
jiang-yan.yang@unil.ch

Zahuczky Gábor
University of Debrecen, Medical and Health Science Center
Biochemistry and Moloecular Biology
Nagyerdei Krt 98.
4032 Debrecen, Hungary
nkfp@indi.biochem.dote.hu

Zaman Farasat
Karolinska Institutet
Women and Child Health
Pediatric Endocrinology, Q2:08, Astrid Lindgren Children Hospital
17176 Stockholm, Sweden
Farasat.Zaman@ki.se

Zennaro Margherita
University of Ferrara
Biochemistry and Molecular Biology - Molecular Biology section
Via Fossato di Mortara, 74
44100 Ferrara, Italy
margheritazennaro@yahoo.it

Zhivotovsky Boris
Institution of enviromental Medicine, Karolinska Institutet
Division of Toxicology
Nobelsväg 13
SE-171 77 Stockholm, Sweden
Boris.Zhivotovsky@imm.ki.se

Zhu Yuan
Department of Neurosurgery, Hospital of Philipps-University Marburg
Baldingerstrasse
35043 Marburg, Germany
zhu@med.uni-marburg.de

Ziviani Elena
MRC
Toxicology unit
lancaster road
LE1 9HN leicester, United Kingdom
ez5@le.ac.uk

Supported by EC within the framework of the Marie Curie Conferences and Training Courses

Supported by EC within the framework of the Marie Curie
Conferences and Training Courses