In Vivo Imaging of Cell Death

7th Annual Training Course on Concepts and Methods in Programmed Cell Death

September 1, 2010, Ghent, Belgium

Francis G. Blankenberg, M.D.
Associate Professor of Radiology & Pediatrics
Stanford University

MIPS
Molecular Imaging Program at Stanford

LPCH
School of Medicine Department of Pediatrics

Stanford
School of Medicine Department of Radiology
Clinical Imaging Modalities for the Measurement of Apoptosis

- Annexin V (Annexin V-128) / PS binding agents
- Choline & Methylene/Methyl Lipid Proton Water Suppressed MR Spectroscopy
- ML-9 & ML-10? / GE (no published data)? Mechanism? Scramblase?
- No other Methods Tested in Humans
Imaging Apoptosis with Annexin

- Annexin V: endogenous human protein (36kD, 319 amino acids)
- Plasma concentration 2 ng/mL
- Binds with antibody-like (nanomolar) affinity to membrane bound phosphatidylserine (PS)
- PS is only exposed to circulating annexin at surface of stressed cells
  - PS externalized early in apoptotic cascade
  - PS accessible on inner leaflet of necrotic cells due to permeability
Annexin V

- 0 to 200 PS binding sites/cell on all viable cell types (exception: endothelial cells with about 50,000 sites/cell at rest)
- Number of PS binding sites per cell = $10^5$ to $10^6$ with apoptosis
- One annexin V binds up to 8 PS anionic head groups
- Annexin binding is associated with all types of cell death (necrosis/oncosis, mitotic catastrophe, cell senescence, pyroptosis, PARP-1 mediated cell death and autophagy).
Normal Plasma Membrane
Asymmetry

$\text{Ca}^{2+}$-Scramblase Activation

Floppase

Scramblase

Flippase

(inactivated by $\text{Ca}^{2+}$ release into the cytosol)

Aminophospholipid (PS or PE)

Cholinephospholipid (PC or Sphingomyelin)

$\text{Ca}^{2+}$ Release into Cytosol

ER & Mitochondria or via $\text{Ca}^{2+}$ ion channel activation within the plasma membrane

? L-type $\text{Ca}^{2+}$ channel lipid transport
Labeling of Annexin V Mutants via Endogenous Chelation Site

Ala-Gly-Gly-Cys-Gly-His-Annexin V-128.

+ Technetium-99m, SnCl₂, glucoheptonate

\[ \downarrow \] 37° x 60 min

\(^{99m}\text{Tc-Annexin V}\)

(\(~100 \mu\text{Ci/µg, >90% RCY, >95% RCP}\))

- No exogenous chelate needed
- Order of magnitude less kidney/bone marrow uptake than Hynic-Annexin allows for superior imaging

Tait et al., Bioconjugate Chem 2000; 11:918
Self-chelating Annexin V-128 is the structure of choice as random chelation with bifunctional moieties (i.e. HYNIC-annexin V) significantly degrades in vitro and in vivo binding to PS.

Phase I and II trials with GMP material planned for Spring of 2011 in patients with RA. Sponsored by Atreus Pharmaceuticals (Ottawa, Canada) in partnership with AAA (Advanced Accelerator Applications, Saint Genis Pouilly, France).

? Annexin V F-18 with site specific labeling
Early Diagnosis would allow for timely deployment of effective drug therapy

Early diagnosis of RA is essential to permit aggressive therapy with disease modifying drugs before loss of function

- Conventional radiography is an insensitive means of measuring changes in RA (1)
- Once joint destruction occurs, full function can never be restored
  - Current radiographic techniques provide detail of bone erosion and joint space narrowing “but only after clinical symptoms have been present for several months or even years.” (2)


Polyarticular Autoimmune Mediated Arthritis

CONTROL

ARTHritis

Prednisone
**Tc-99m Annexin Imaging of RA in Humans**

Spot views of Knees and hands in patient with RA using Tc-99m-hynic-annexin. Note marked synovial uptake in knees (and uptake in the tensor fascia lata of the right knee) as well as the carpal uptake in the right wrist.
Response to RA Therapy

Serial spot views of RA Patient with Acute Flare before and after treatment with Chimeric anti-TNF monoclonal antibody (Infliximab™). Note tenosynovitis at baseline which resolves.
ANNEXIN V IMAGING

PS can be expressed at low levels in a reversible fashion under condition of cell stress that does not necessarily commit a cell to apoptotic cell death. (transient myocardial of cerebral ischemia, RA / IBD (i.e. non-infectious inflammation, Infectious Inflammation)

Annexin imaging can therefore define territory at risk and potentially salvageable with prompt intervention.
Reversible PS Expression in Myocardial Ischemia


• Short periods of ischemia of the LAD (5 & 10 minutes) there was a 9 fold increase in PS binding of annexin V that persisted for 6 hours after reperfusion in both rabbit and murine models. This 9 fold increase was approximately half of that observed with infarction (30 minutes of ischemia).

• Regions of transient ischemia demostrated low levels of caspase-3 activation and PS expression without evidence of TUNEL +.
Reversible PS Expression in Myocardial Ischemia (continued)

Caspase -3 Staining

I/R = 10/180

I/R = 40/180

I/R = 0/0

Caspase -3 Staining
Reversible PS Expression in Myocardial Ischemia (continued)

• Annexin V Imaging can be used as a marker of cellular stress and tissues that maybe salvageable with prompt intervention.

• Annexin V Imaging is therefore far more sensitive than potentially “specific” markers of apoptosis or necrosis (i.e. caspase-3, PARP-1, or markers of scramblase activity, ML-9).

• Annexin V binding and internalization via PS exposure maybe a way to selectively deliver anti-apoptotic drugs do regions of ischemic disease even after prompt reperfusion.
Hypoxic Ischemic Injury in a Preterm Infant

Coronal View: 1st day of life
Normal?

Coronal View: day 21

Sagittal View: day 21

Coronal View: day 21
PVL
Neonatal Rabbit Model of Hypoxic-Ischemic Injury

ADC Map after 60 mins hypoxia

ADC Map after recovery

T₁ W post Gd immediately prior to annexin V imaging

D’Arceuil et al. Stroke 2000
Two Hours After Hypoxia

(A) Post

(B) R Lat

(C) Ex vivo

(D)
(-) staining with TUNEL in neonatal rabbit brain after following 2 hours of global hypoxia

Neurons

Astrocytes
A) Co-localization of annexin and neuronal marker (MAP2B)

B) Localization of annexin V and astrocytic cell marker (GFAP)
Minocycline is a member of the tetracycline family of antibiotics with both anti-inflammatory and anti-apoptotic bacterial properties.

Minocycline treatment reduces microglial activation

![Graph showing the number of IB4 positive cells/HPF over different time points (1d, 3d, 7d, 30d) with control and minocycline treatment groups. The graph indicates a significant reduction in IB4 positive cells with minocycline treatment compared to control at all time points.]
Acute vs Subacute Stroke

Pt #001 Annexin V SPECT Day 3

Pt #001 F/U Annexin V SPECT Day 30
Multifocal Stroke: Age of Cerebellar Lesion Indeterminate on DWI and Acute Occipital Visual Field Loss
Alzheimer’s vs Multi-infarct Dementia

Annexin V Imaging on Positive on Day 1 /
Blood Cultures not positive till Day 9 for Streptococcal Endocarditis

A 79 year old woman with a right hip prosthesis for 15 years.

A 78 year old woman with a history of a sub-capital fracture of her left femur, leading to AVN and THR.

A 76 year old man with a painful right knee prosthesis and signs of loosening on plain x ray.

**False Positive Bone Scan/ True Negative Annexin V Scan**

**True Positive Bone Scans/ True Positive Annexin V Scans**

A 79 year old woman with a right hip prosthesis for 15 years.

A 78 year old woman with a history of a sub-capital fracture of her left femur, leading to AVN and THR.
“Initiators” of Protein Synthesis  $\text{eIFs} = \text{[eukaryotic initiation factors-2, 2B and 4G]}$

Inhibitor of Protein Synthesis  $\text{PERK} = \text{[protein kinase RNA (PRK); PRK like ER kinase]}$

$\text{eIF2} \text{ GDP} \rightarrow \text{Phosphorylated-eIF2} \text{ GDP} \text{ eIF2B}$

$\text{GTP}$

$\text{GDP/GTP exchange}$

$\text{eIF2B}$

$\text{eIF2} \text{ GTP}$

$\text{PERK}$

$\text{PERK phosphorylation of eIF2}$

$\text{Apoptosis}$

$\text{Choline}$

$\text{Inhibition of Protein Synthesis}$

$\text{Activated Caspase 3/7}$

$\text{N-FRAG}$

$\text{M-FRAG}$

$\text{C-FRAG}$

$\text{Met-tRNAi}$

$\text{mRNA} + \text{eIF4G}$

$\text{Ribosome}$

$\text{“Inhibition of Protein Synthesis”}$

$\text{“Cleavage of eIF4G”}$

$\text{“Protein Synthesis”}$

$\text{“Choline Production”}$

$\text{“GDP/GTP exchange”}$

$\text{eIF2}$

$\text{GTP}$

$\text{Met-tRNAi}$

$\text{“Protein Synthesis”}$

$\text{“Choline Production”}$
Detection of Apoptosis with In Vitro Lipid Proton Spectroscopy

Quantification of Apoptosis with In Vitro Lipid Proton Spectroscopy

Doxorubicin Time Course (200ng/ml)

Normal Murine Liver Proton MRS

3 hr after Cycloheximide (50 mg/kg i.p.)

Courtesy of Helen D'Arceuil, Brain Ross, Pratip Bhattacharya / April 2010
HMRI Spectroscopy Unit : California Institute of Technology / Pasadena, CA
Sagittal contrast-enhanced fat suppressed MR images & MR spectra of the right breast in a 32-year-old woman with invasive ductal carcinoma.

75% of the objective responders showed a decrease in [tCho] at day 1 after therapy whereas 92% of non-responders showed no change or an increase at the same time point.

Conclusions from the Most Recent Proton MRS Studies

Apoptosis correlates with a rapid (within 3-48 hours) \textit{increase} in CH$_2$/CH$_3$ peak intensity ratio as well as an accumulation of TAGs (triacylglycerides) in cytoplasmic lipid droplets.

Apoptosis also correlates with a rapid (within 12-48 hours) \textit{decrease} in total choline signal intensity (t-Cho at 3.2 ppm) that directly reflects a decrease in global protein synthesis within an apoptotic cell.
Conclusions from the Most Recent Proton MRS Studies

The ratio $\text{CH}_2 / \text{t-Cho}$ has the highest dynamic range of any MRS variable taking advantage of increases in methylene (mobile lipid) and decreases in total choline that both coincide with the early stages (within the first 3 to 48 hours of the start of therapy) of apoptotic cell death.