CORRELATION OF IN VIVO IMAGING with PATHOLOGY IN RODENT MODELS

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Goals for this lecture

1. Introduce methodology for in vivo imaging in rodents
2. Compare in vivo imaging to gross and histopathology
3. Provide examples of applications

Imaging modalities -Examples

- SPECT: Single photon emission computed tomography
- US: Ultrasound
- MR: Magnetic Resonance

Cardiac examples

- Myocardial hypertrophy
- Myocardial infarction
- Marfan's disease
- Atherosclerosis
- Cancer therapy-induced cardiac injury

CV phenotype-Hypertrophy

- Increased heart/body weight
- Increased heart weight/tibia length
- Increased cardiac myocyte cross section
- Biochemical markers- ANP, BNP, β myosin heavy chain
CV Phenotype: **Is the heart enlarged?**

- No **Cardiomegaly**
  - Yes **Infiltration**
    - No **Hypertrophy**
      - Yes **Eutrophy**
    - No **Adaptive**
      - Yes **Maladaptive**
  - No **Is the cardiomyocyte contractility normal?**
    - Yes
    - No

**Ultrasound**

*How can we measure hypertrophy in vivo?*

*What can we do with minimal budgets?*

Rat heart 2D

Pharmacology experiments

• Cardiomyocyte hypertrophy
• Karyomegaly
• Cell loss
• Fibrosis

Correlation of histopathology to \textit{in vivo} imaging

\textbf{Hypertrophy}
- Gross (ultrasound)
- Left ventricle free wall and septum measures thicker than control by ultrasound

\textbf{Hypertrophy}
- Histopathology
- Enlarged myofibers can be measured microscopically

Surgical manipulations to induce phenotypes- Myocardial infarction

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{surgicalManipulations.png}
\caption{Surgical ligature of the left anterior descending artery (ventricles, infarcted).}
\end{figure}
Correlation of histopathology to in vivo imaging

**Myocardial Infarction**
- Gross (ultrasound)
- LVFW or septum m-mode image has a flat wall showing no movement versus the unaffected normal wall showing normal contraction and relaxation.
- Damaged wall may measure thinner at infarct

**Myocardial Infarction**
- Histopathology
- Areas of cardiomyocyte loss and replacement fibrosis with inflammation

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Vevo 770™ High-Resolution In Vivo Imaging System

Developed for Rodents
30 micron resolution

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Phenotyping application:
Image valve and aorta in Mouse model for Marfan's disease

**D**

<table>
<thead>
<tr>
<th>Phenotype</th>
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<tbody>
<tr>
<td>Fbn1+/-</td>
<td></td>
<td></td>
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<tr>
<td>Fbn1-/-</td>
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Losartan rescues the phenotype
Marfan's mouse model

Wild-type | C1039G/- (Placebo) | C1039G/+ (Losartan)
C1039G/- (Losartan) | C1039G/+ (Losartan) | C1039G/+ (Losartan)
**Postnatal losartan: Aortic root growth**

![Graph showing postnatal losartan effects on aortic root growth](image)

- Wild-type: n = 11
- Placebo: n = 10
- Propranolol: n = 7
- Losartan: n = 5

<table>
<thead>
<tr>
<th>Growth (mm/6 months)</th>
<th>Wild-type</th>
<th>Placebo</th>
<th>Propranolol</th>
<th>Losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>p &lt; 0.0001</td>
<td>p = 0.001</td>
<td>p = 0.02</td>
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Losartan rescues aortic wall architecture and thickness

![Diagram showing aortic wall architecture](image)

**Phenotyping ApoE/- mice**

- Genetically modified to lack Apolipoprotein-E which is important in cholesterol metabolism
- Fed high fat diet
- Demonstrate similar lesion progression to humans

![Mouse image](image)

**Atherosclerosis Plaque Build-up in Common Carotid Arteries in Adult Mouse - ultrasound**

![Ultrasound images of atherosclerotic plaque](image)

- Normal
- Plaque
- 2-D

Image sequence courtesy of Gan et al., University of Gothenburg, Sweden, 2004

**Atherosclerotic Lesion**

![Diagram of atherosclerotic lesion](image)

- Dysplasia
- Fibrotic area
- Inflammation
- Tissue Build-up
- Lipid Core

**Ultrasound-Visualsonics- atherosclerotic plaque**

![Ultrasound images of atherosclerotic plaque](image)
Aorta- atherosclerosis in apolipoprotein E-deficient mice (Apoe/-/-)

Correlation of histopathology to \textit{in vivo} imaging

\textbf{Aortic Lesions}
- Gross (ultrasound)
- Dilated aorta with roughened irregular outline of the intima due to Atherosclerosis by ApoE KO
- SPECT- cell death (next example)
- Histopathology
- Thickened wall and accumulation of lipid, mononuclear cells and mineral, disrupting the intima and media
- TUNEL positive

\textbf{SPECT/CT}

Methods to increase in vivo contrast

CT scan of a mouse- a vascular contrast agent was injected in the tail vein to heighten the contrast of the blood vessels

Fenestra=contrast agent

Tc^{99m}-HYNIC-Annexin-V and Apoptosis

\begin{itemize}
  \item Live
  \item Early Apoptotic
  \item Plasma membrane
  \item Phosphatidyl serine
  \item Annexin V- TdT
\end{itemize}
Background

- ApoE^{-/-} mice on high fat diets are treated with Tc^{99m} labeled Annexin V and imaged (SPECT)
- Hot spots are hypothesized to be vulnerable atherosclerotic plaques
- Autoradiography is performed post-mortem on the dissected aortas
- Not all atherosclerotic plaques appear hot on the autoradiographs…

Methods

- After imaging, animals were sacrificed and vessels were fixed by perfusion with 10% buffered formalin
- Dissected aortas were photographed, dyed, sectioned according to “hot” or “cold” plaques, imbedded in paraffin, and placed on slides
- Cell death was visualized using In Situ Cell Death Detection Kit, Fluorescein, from Roche Applied Science
- Slides were analyzed with the fluorescence microscope
Toxicity induced phenotypes

- Doxorubicin
- Used for 30 years to treat cancer
- Major side effect - cardiac toxicity

Chronic study - Progressive Cardiomyopathy in Doxorubicin treated mouse

M-mode of the left ventricle

Cytoplasmic vacuolation in cardiomyocytes

- Myocardial cytoplasmic vacuolation
- Doxorubicin induces dilation of rough endoplasmic reticulum and T-tubules (EM)

Nuclear imaging of cell death

SPECT-Tc $^{99m}$Tc Annexin-v labeling

Phenotyping application: in an acute study, image heart and assess function in +/- and +/- cbr mice
• MR: Magnetic Resonance

Image multiple mice simultaneously
N Bock, N Konyer and R Henkelman
An array of four birdcage coils in hexagonal shields for imaging at 1.5 T.

Images of 4 mice

Two types of small animal MR imaging

- Fixed specimens
  - Whole animals-adults, embryos
  - Isolated tissues
- Live animals
  - Monitor a disease process by serial imaging

Some potential applications

- Perfuse-fixed specimens
  - Phenotyping GEM adults or embryos
  - Identifying toxicities – Acetaminophen-hepatic necrosis or carbonyl sulfide neurotoxicity in rats
  - Rat teratology
  - Identifying carcinogenesis liver and lung
- Live animals
  - Monitor a disease process by serial imaging

Visualization of Rat Brain Lesions Caused by Demoic Acid

M. E. Lester, D.S. et al., Toxicologic Pathology 28(1):100-104 (2000)
(Lesion is located in the pyriform and endopyriform cortex)
Body: 695.6
GI tract volume: 442.1
Liver volume: 427.9
Brain volume: 203.8
Pancreas volume: 146.6
Kidney volume: 123.0
Lung volume: 106.2
Stomach volume: 104.4
Colon volume: 91.1
Spleen volume: 79.0
Spleen volume: 79.0
Nasal turbinate: 51.3
Aorta volume: 54.1
Semi-mature testes: 53.3
Mesenteric node: 41.2
Mature testes: 41.2
Hyalinizing gland: 41.2
Nasal turbinates: 49.3
Spleen volume: 26.8
Adipose volume: 24.4
Femur volume: 16.0
Intestinal gland: 15.9
Peroxidase gland: 15.9
Femur volume: 9.4
Aorta volume: 7.3
Thymus volume: 6.6
Mesenteric node: 5.6
Bulbourethral gland: 4.4
Kidney volume: 2.9
Adrenal volume: 1.8
Ureter volume: 1.6
Bladder volume: 0.4
Thymus gland: 0.4
Adipose volume: 0.4
Lung volume: 0.2
Thyroid gland: 0.4
Iliac node volume: 0.2

All units in mm$^3$

Rat Teratology studies
Non-destructive
40 organs visualized intact
Organ substructures
Inherently 3-dimensional
Linear
Volumetric
Measurements
Digital images allow for
Consultation
Combined with other
modalities

Lung tumors
Liver hyperplastic foci
Carbonyl Sulfide (500 ppm) 2 week exposure
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Additional References

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Websites:
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  http://www.eumorphia.org/EMPriSS/servlet/EMPriSS.Frameset
- MRC Mutagenesis Program
  http://www.mgu.har.mrc.ac.uk/facilities/mutagenesis/mutabase/
- mouse.wustl.edu/index.htm

Rat heart 2D