Pathology of Neuromuscular Disease
Part 1: muscle

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MUSCLE BIOPSY
DESCRIPTION OF SPECIMENS, PROCEDURES & STAINS

- 2 blocks of skeletal muscle, **frozen** in isopentane cooled in liquid nitrogen. 12 μm thick sections are cut using a cryostat.

- The following *routine* stains are done:

  - **Basic histopathological** stains: H & E and Gomori trichrome
  - **Special Stains**: oil red O, PAS, Congo red.
  - **Enzyme Histochemistry**: NADH, SDH, COX, and ATPase, at pH 9.4, 4.6, 4.2. (Myophosphorylase, MAD, acid phosphatase if needed)
  - **Immune staining**: carried out if needed
    - CD3, CD4, CD8, CD20 and CD68 cell markers, MAC
    - dystrophin (dys 1, 2, 3), sarcoglycans (α, β, γ, δ), dystroglycans (α, β), dysferlin, caveolin 3, laminin alpha 2 (merosin), utrophin, spectrin, collagen VI
    - specific antibodies for protein aggregates

- EM piece placed in glutaraldehyde for further processing
- A separate piece of muscle frozen for biochemical/genetic studies
H&E and Gomori Trichrome

Give wide range of information:
✓ Necrosis
✓ Regeneration
✓ Fiber size – atrophy/hypertrophy
✓ Inflammation
✓ Fibrosis
✓ Structural changes
✓ Organelle changes
Examples of tissue handling artifacts
### Useful Histochemical Reactions of Skeletal Muscle Cells

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Cellular localization</th>
<th>Source of Reaction</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH-tetrazolium</td>
<td>Intermyofibrillar*</td>
<td>Enzyme in mitochondria,</td>
<td></td>
</tr>
<tr>
<td>reductase</td>
<td>perinuclear, Subsarcolemmal</td>
<td>SR, T-tubules</td>
<td>poor</td>
</tr>
<tr>
<td>Succinic dehydrogenase</td>
<td>Intermyofibrillar*</td>
<td>Enzyme in mitochondria</td>
<td>excellent</td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
<td>Intermyofibrillar*</td>
<td>Enzyme in mitochondria</td>
<td>excellent</td>
</tr>
<tr>
<td>Myofibrillar ATPase</td>
<td>Intermyofibrillar</td>
<td>Myosin or actomyosin</td>
<td>good</td>
</tr>
</tbody>
</table>

**Images:**
- NADH
- SDH
- COX
- ATPase, 9.4
Necrosis

Factors triggering necrosis in muscle cells:

- Lengthening contractions
  - dystrophic muscle particularly vulnerable
- Ischemia
  - dermatomyositis
- Energy deprivation
  - Glycolytic defects
- Toxic agents
  - Cardiotoxin, neutoxin, statins

In the course of necrosis:

- Plasma membrane becomes permeable
  -- Ca\(^{++}\) entry, activation of phospholipases, proteases (calpains)
- Some DAG complex- lost early; by 24 hrs dys lost
- Activation of compliment cascade, diffuse cytoplasmic appearance of lytic C5-9 (MAC) within muscle

Segmental Necrosis
Dermatomyositis-acute stage (Ischemic necrosis)
**Phagocytosis**

- Starts ~ 6 to 8 hrs after the fiber passed the point of no return
  - sarcolemmal and myonuclear dissolution (earliest change), followed by gradual dissolution of contractile elements
  - what is not destroyed: Basal Lamina & Satellite Cells

- In surviving stumps- T tubule dilatation
- Abundant MFs within endomysium

*Acid phosphatase*
Patterns of inflammation

Perivascular inflammation
- Variation in muscle fiber size
- Small rounded fibers

Perivascular & Perimysial inflammation
- Mononuclear cell

Endomysial inflammation
Often associated with focal invasion of muscle fibers
Temporal sequence of inflammatory and regenerative events following muscle injury:

- Neutrophils
- Inflammatory macrophages CD68+
- Anti-inflammatory macrophages CD163+
- Satellite cells activation, proliferation and fusion
- Embryonic MyHC
- NMJ formation
- Adult fast MyHC
- Slow MyHC

Myofiber growth and embryonic MyHC expression in regenerating skeletal muscle

Satellite Cells

- Muscle specific stem cells located beneath the basal lamina of the myofiber
- Pax7-useful marker for quiescent SCs
- Prevalence = r S/M
- Major role in
  - Natural growth
  - Muscle maintenance, work hypertrophy
  - Regeneration
- Proliferative/differentiating processes lead transformation into myoblast/myotubes in necrotic segments
- Limit of their mitotic cycles?

Model for satellite cell self-renewal and differentiation

Activated satellite cells in necrotic fibers
IDEAL MUSCLE FIBER REGENERATION

1. Surviving segment
   - Satellite cell
   - Myoblasts (activated satellite cells)
   - Macrophage
   - Basal lamina
   - Plasma membrane

2. Necrotic segment
   - Regenerating Myotubes

3. Surviving segment
   - Full restoration of the normal fibre calibre

Karpati, G; 2008
Histological Features of Regenerating Muscle

- Eosinophilic cytoplasm, reflecting high content of ribosomes
- Nuclei tend to be pale and large
- Relative excess of glycogen and mitochondria (early)
- Emb & Neo forms of myCH
- Diffuse cytoplasmic desmin stain
Muscle Fiber Regeneration
Muscle Fiber Regeneration
ABERRATIONS OF MUSCLE FIBER REGENERATION

1. Regenerated segment is of smaller caliber than the rest of the fiber

2. Forked fibers due to incomplete lateral fusion of myotubes

3. Surviving stump Independent regenerated fiber
   Multiple independent fibers due to lack of fusion of myotubes with the surviving stump

4. Empty basement membrane sleeve due to lack of regeneration
LGMD2A:
- caused by mutations in the CAPN3, encoding Ca²⁺-activated cysteine protease
- role in sarcomere assembly, turnover and maintenance
- in Calpainopathy there is a good correlation between age, duration of symptoms and degree of fibrosis
- microRNA dysregulation leads to inability of Pax7-positive SCs to transit from proliferation to differentiation resulting in impaired regeneration and fibrosis in LGMD 2A
Satellite Cells in Dystrophic Process (calpainopathy)

A

<table>
<thead>
<tr>
<th>Biopsies</th>
<th>n</th>
<th>Age</th>
<th>DD</th>
<th>FG</th>
<th>SC/type1</th>
<th>SC/type2</th>
<th>SC/fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>0.117</td>
<td>0.196</td>
<td>0.147</td>
</tr>
<tr>
<td>Group 2</td>
<td>3</td>
<td>19.7+2.7</td>
<td>7.7+3.3</td>
<td>1+0.0</td>
<td>0.134+0.032</td>
<td>0.210+0.076</td>
<td>0.168+0.051</td>
</tr>
<tr>
<td>Group 3</td>
<td>9</td>
<td>37.8+4.8</td>
<td>19.7+3.6</td>
<td>3.1+0.2</td>
<td>0.189+0.054</td>
<td>0.298+0.087</td>
<td>0.205+0.052</td>
</tr>
<tr>
<td>Group 3  LF</td>
<td>5</td>
<td>36.4+2.8</td>
<td>19.4+3.6</td>
<td>2.8+3.6</td>
<td>0.093+0.018*</td>
<td>0.236+0.124</td>
<td>0.109+0.029+</td>
</tr>
<tr>
<td>no LF</td>
<td>4</td>
<td>39.5+11.0</td>
<td>20.0+7.6</td>
<td>3.5+0.5</td>
<td>0.310+0.092*</td>
<td>0.374+0.130</td>
<td>0.325+0.080+</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>45.7+5.0</td>
<td></td>
<td></td>
<td>0.081+0.001</td>
<td>0.056+0.010</td>
<td>0.065+0.006</td>
</tr>
</tbody>
</table>

B

Fibrosis Grade and Satellite Cell Number in Calpainopathy Muscle

C

Fibrosis Grade and Satellite Cell Number in Lobulated and non-Lobulated Biopsies

D

E
Fiber Hypertrophy and Satellite Cells

Multinucleated hypertrophic cells following AAV1.CMV.follistatin gene therapy
Follistatin induces muscle hypertrophy through:
- SC proliferation, Mstn and Act inhibition
- Overexpression in muscle lead to increased DNA & muscle protein content and increased fiber size
- The nuclei are contributed to by satellite cells that the muscle fiber incorporates as it grows in size.
Dystrophinopathies

**Immune stains:**

- DMD: Exon 55-63 duplication
- BMD: Exon 19-29 duplication
C09-103 Inclusion body myositis

Structural abnormalities: vacuoles
Inclusion Body Myositis (IBM)

- The term IBM coined in 1971 by Yunis & Samaha
- Histopathologic differentiation from PM by:
  - vacuolated fibers
  - Nuclear and cytoplasmic fibrillary inclusions, which are congophilic
Structural abnormalities: vacuoles

C09-103, IBM, Congo red stain
Acid phosphatase

C09-115
Adult onset acid maltase deficiency

Structural abnormalities: vacuoles
Structural Abnormalities: Tubular Aggregates
Structural Abnormalities: Protein Aggregate Myopathy (PAM)

Myofibrillar Myopathies
- Desmin
- αB-crystallin (HSP20)
- Myotilin
- ZASP (Z-band alternatively spliced PDZ)
- Filamin (filamin C)
Structural Abnormalities: Protein Aggregate Myopathy (PAM)
Organelle change: Mitochondria content and distribution

C10-33 Mitochondrial myopathy
Organelle change: Mitochondria content and distribution

C10-33 Mitochondrial myopathy
Organelle change: Mitochondria content and distribution

C10-33 Mitochondrial myopathy
Organelle Change: Mitochondria content and distribution

C05-97 Thymidine Kinase 2 deficiency
Muscle Fiber Types

Myofibrillar ATPase
**Muscle Fiber Types**

- **STO/type I** (high lipid, mito.)
- **FTG/type IIA**
- **FTO/type IIB** (high glycogen)

ATPase 4.6
Fiber Types and Performance

Endurance Athletes

ATPase, 4.6

Weight lifters

ATPase, 4.2
Neurogenic Changes
Group atrophy and muscle fiber type grouping
Group atrophy and muscle fiber type groupings
DM1

> CTG_{4-37} repeats in the terminal exon of DMPK gene

DM2 (PROMM)

> 104 to 176 bp CCTG repeats in intron 1 of exon of ZNF9 gene
Centronuclear Myopathy: X-linked
- Onset, infancy with severe hypotonia
- Mutations in MTM1
- Protein expressed in sarcolemma, I band, T-tubule triads, associated with endosomes
- Role in muscle fiber maturation

Centronuclear Myopathy: Autosomal recessive
- Onset, infancy, childhood, adult

Centronuclear Myopathy: Autosomal Dominant
- Onset, adolescence and adult
- Mutations in DNM2
- Protein associated with MTs, binds to BIN1, implicated in endocytosis and cell motility