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>Intermembranar*</td>
<td>Enzyme in mitochondria,</td>
<td></td>
</tr>
<tr>
<td>reductase</td>
<td>perinuclear,</td>
<td>SR, T-tubules</td>
<td>poor</td>
</tr>
<tr>
<td></td>
<td>Subsarcolemmal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinic dehydrogenase</td>
<td>Intermembranar*</td>
<td>Enzyme in mitochondria</td>
<td>excellent</td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
<td>Intermembranar*</td>
<td>Enzyme in mitochondria</td>
<td>excellent</td>
</tr>
<tr>
<td>Myofibrillar ATPase</td>
<td>Intermembranar</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
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

Satellite cell

Desmin IF
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 and 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 Ca\(^{2+}\) 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

| fiber type specific distribution of satellite cells in calpainopathy biopsies |
|---|---|---|---|---|---|---|---|
| Biopsies | n | Age | DD | FG | SC/type1 | SC/type2 | SC/fiber |
| Group 1 | 1 | 10 | 1 | 1 | 0.117 | 0.196 | 0.147 |
| Group 2 | 3 | 19.7 ± 2.7 | 7.7 ± 3.3 | 1 ± 0.0 | 0.134 ± 0.032 | 0.210 ± 0.076 | 0.168 ± 0.051 |
| Group 3 | 9 | 37.8 ± 4.8 | 19.7 ± 3.6 | 3.1 ± 0.2 | 0.189 ± 0.054 | 0.298 ± 0.087 | 0.205 ± 0.052 |
| Group 3 LF | 5 | 36.4 ± 2.8 | 19.4 ± 3.6 | 2.8 ± 3.6 | 0.093 ± 0.018* | 0.236 ± 0.124 | 0.109 ± 0.029+ |
| Group 3 no LF | 4 | 39.5 ± 11.0 | 20.0 ± 7.6 | 3.5 ± 0.5 | 0.310 ± 0.092* | 0.374 ± 0.130 | 0.325 ± 0.080+ |
| Control | 3 | 45.7 ± 5.0 | | | 0.081 ± 0.001 | 0.056 ± 0.010 | 0.065 ± 0.006 |

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

II1

II2

II3

d

a

b

c

non-L. Grade 3.5

L. Grade 2.8

Satellite Cell

Muscle Fiber

Pax7-positive Nuclei/Muscle fiber

Non-L, Grade 3.5

L, Grade 2.8

Pax7-positive Nuclei/Muscle fiber

* p < 0.05

+ p < 0.01

a, b, c, d: satellite cells in different stages of differentiation

II1, II2, II3: fiber types

Muscle fiber structure and satellite cells distribution.
Fiber Hypertrophy and Satellite Cells

Multinucleated hypertrophic cells following AAV1.CMV.follistatin gene therapy

Fiber Size Distribution

- WT-FT
- 12 wks-FT
- 36wks-FT
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.
Immune stains: Dystrophinopathies

DMD
Exon 55-63 duplication

BMD
Exon 19-29 duplication
Structural abnormalities: vacuoles

C09-103 Inclusion body myositis
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
Structural abnormalities: vacuoles

C09-115
Adult onset acid maltase deficiency

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

C05-97 Thymidine Kinase 2 deficiency
Muscle Fiber Types

Myofibrillar ATPase
Muscle Fiber Types

PAS

Type I

Type II

FTG

STO

STO/type I (high lipid, mito.)

FTG/type IIA

FTO/type IIB (high glycogen)

ATPase 4.6
Fiber Types and Performance

Endurance Athletes

Weightlifters

ATPase, 4.6

ATPase, 4.2
Neurogenic Changes
Group atrophy and muscle fiber type grouping
Group atrophy and muscle fiber type groupings
> CTG$_{4-37}$ repeats in the terminal exon of DMPK gene

> 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