Fibrosis in DMD

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MVIMG# 7470: Fundamentals of Muscle Biology: Duchenne Muscular Dystrophy

April 3, 2014
What is fibrosis?

- Basic response of any organ that undergoes repetitive injury and inflammation.

- Characterized by the excessive deposition of extracellular matrix proteins (mainly collagens I and III, fibronectin) thus creating a scar.

- Leads to a disordered tissue structure, disruption of organ function, and ultimately organ failure.

- Major cause of mortality worldwide.

- No available FDA- or EMEA- approved anti-fibrotic therapies.
Impact on disease progression in DMD
Endomysial fibrosis is the main histopathological parameter that correlates with poor motor outcome in DMD patients
Consequences of Endomysial Fibrosis

Quadriceps muscle strength

DMD - LATE
Consequences of Endomysial Fibrosis

- Loss of tight association between muscle fibers and capillaries → decreased oxygenation and nutrients

- Decreased number of satellite cells → impaired regeneration

CD31 staining (brown) of capillaries

Control

DMD

C

p < 0.0001

C56+ cells per fiber

high fibrosis

low fibrosis

controls
Consequences of Endomysial Fibrosis

- Tissue contracture
- Increased tissue stiffness
  - inhibits the proliferation and differentiation of satellite cells
  - Enhances production of matrix proteins by fibrotic cells
  - Interferes with muscle contraction

Tomasek et al., 2002, Nature Reviews 3: 349
Variability in the rate of disease progression among patients, even if they have the same genetic mutation in the DMD gene or lack of dystrophin protein expression
Latent TGF-β-binding protein 4 modifies muscular dystrophy in mice

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LTBP4 Genotype Predicts Age of Ambulatory Loss in Duchenne Muscular Dystrophy

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Wendy M. King, PT,3 Alan Pestronk, MD,6 Julaine M. Florence, DPT,6
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ANN NEUROL 2013;73:481–488
The Fibroblast

- Versatile in shape
- Versatile in gene expression
- Versatile in function
  - Developing muscle:
    - Promote slow muscle myogenesis
    - Fetal to adult switch
    - Myoblast fusion
  - Adult muscle
    - Regulation of satellite cell self-renewal and differentiation
    - Tissue integrity

Lung fibroblasts

- Soft
- Stiff

Alpha-Smooth muscle actin

Myofibroblast / activated fibroblast

Fibroblast activation

Phenotype: Tissue homeostasis
- Migratory cell
- Matrix protein production, including specific forms of FN
- Production of TGF-β

Phenotype: Injured
- Contractile cell
- High matrix protein production
- Production of TGF-β
- Production of ROS

Phenotype: Apoptosis
Myofibroblasts are the key pathogenic cells in all fibrotic diseases

Research has focused on identifying:

- the factors that activate myofibroblasts
- the mechanisms that contribute to myofibroblast apoptosis
- the cellular origins of myofibroblasts
Fibroblast activation

**Stimulus**
- Immune cell released cytokines and TGF-β
- PDGF
- Stiff substrate
- Specific FN isoforms
- TGF-β

**Phenotype**
- Tissue homeostasis
  - Migratory cell
  - Matrix protein production, including specific forms of FN
  - Production of TGF-β
- Contractile cell
  - High matrix protein production
  - Production of TGF-β
  - Production of ROS
- Apoptosis
Fibroblast activation – impact of immune cells


Myofibroblast apoptosis

- **Fibroblast**
- **Proto-myofibroblast**
- **Differentiated myofibroblast**

**CCN1**
- Synergizes with TNFα, Jun, NADPH oxidase 1, or p38 to induce senescence or apoptosis via p53 and Rb

**TGF-β**
- SMAD3/FAK-dependent pathway
- P38 MAPK/PI3 kinase/Akt dependent signaling

Apoptosis
Cellular origins of fibroblasts/myofibroblasts

- Circulating Fibrocytes
- Endothelial to mesenchymal transition
- Epithelial to mesenchymal transition
- Mesenchymal progenitors/fibroblast and adipocyte precursors
- Pericytes
Genetic fate mapping experiments in several organs, including skeletal muscle, brain, kidney, lung skin and liver indicate that **mesenchymal progenitors** and **pericytes** are the precursors of myofibroblasts.

Many parallel genetic fate mapping studies show little or no evidence of direct differentiation of epithelial cells, endothelial cells, or circulating fibrocytes into myofibroblasts.
Mesenchymal progenitors

- Location: interstitium
- Main markers: PDGFR-α, Sca-1, CD34
- Differentiation potential:
  - Fibroblasts
  - Adipocytes
  - Osteogenic
  - Chondrogenic
Acute muscle injury

- Release trophic factors that support satellite cell expansion and myogenic differentiation
- Phagocytose dead cells and cellular debris
Muscular Dystrophy

- Mesenchymal progenitors:
  - Produce collagens
  - Differentiate into fibroblasts and adipocytes

Uezumi et al., 2014, Frontiers in Physiology, Vol 5, Article 68, p2
Pericytes

- Location: perivascular, around capillaries
- Main markers: PDGFR-β, NG2
- Activated by PDGF, VEGF, TGF-β
- Main function: Microvasculature homeostasis
- Differentiation potential:
  - Myogenic
  - Adipogenic
  - Osteogenic
  - Fibrogenic

(Duloroy et al., 2012, Nat. Med., 18:1262)
Pericyte activation

Figure 5. Schema of pericyte activation by a disease stimulus (based around kidney injury). In response to injury, pericytes become activated and detach from capillaries. This process requires bidirectional signaling between endothelial cells and pericytes. Epithelial cells can also signal to pericytes, and it is unknown whether pericytes signal to epithelial cells. In the presence of persistent injury, activated pericytes proliferate, migrate, and activate genes that give them the myofibroblast phenotype, including upregulated expression of pathological matrix genes, contractile machinery, and immune response genes. This process results in pathological matrix deposition in the virtual interstitial space; recruitment of inflammatory cells; and the loss of pericyte coverage of the endothelial cells, which causes an unstable endothelium that in turn leads to dysangiogenesis and, potentially, rarefaction. Abbreviation: CBM, capillary basement membrane.

Duffield et al., 2013, Annu. Rev. Pathol., 8: 241
Summary

- Fibrosis is a major determinant of disease progression in DMD
- Replaces muscle tissue and impairs the function of residual muscle fibers
  - Inhibition of satellite cell proliferation
  - Impaired interactions with the microvasculature
  - Stiffens the matrix
- Tight relationship between fibrotic and immune cells
- Treatment targets:
  - Immune modulation
  - Inhibition of differentiation of fibroblast progenitors
  - Inhibition of fibroblast differentiation into myofibroblasts
  - Induction of apoptosis/senescence of myofibroblasts
Anti-fibrotic treatment targets in DMD

- **Inflammation**
  - Nfk-B inhibition (Flavocoxid [Phase 1], VBP15 [preclinical])
  - TNF-α inhibition (BKT-104, cV1q, LMP420, etanercept [preclinical])

- **Pro-fibrotic pathways**
  - TGF-β (ACE inhibitors, Myostatin inhibitors [MYO-029, ACE-031, Follistatin])
  - ROS (CoQ10 [Phase 2/3], Sunphenon Epigallocatechin-Gallate [Phase 2/3], Catena)

- **Pro-regenerative pathways**
  - IGF-1 [Phase 2]
  - Tissue vascularization (Tadalafil, Sildenafil, PDE inhibitors)
Anti-fibrotic treatments are a challenge

Table 1. Therapeutics that are currently being tested or have been tested in fibrotic diseases (this list does not claim to be exhaustive)

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Company</th>
<th>Target/MOA</th>
<th>Indication</th>
<th>Phase/notes</th>
<th>Clinical Trials.gov identifier</th>
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<tbody>
<tr>
<td>Pirfenidone</td>
<td>Intermune</td>
<td>p38/TGFβ inhibitor</td>
<td>IPF</td>
<td>Approved in Europe and Asia, phase III in USA (ongoing)</td>
<td>NCT01366209</td>
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<td>Fresolimumab</td>
<td>Sanofi</td>
<td>Anti-TGFβ monoclonal antibody</td>
<td>Diffuse systemic sclerosis</td>
<td>Phase I (recruiting)</td>
<td>NCT01284322</td>
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<td>LY2382770</td>
<td>Lilly</td>
<td>Anti-TGFβ monoclonal antibody</td>
<td>FSOS, IPF</td>
<td>Phase II (recruiting)</td>
<td>NCT01665391</td>
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<tr>
<td>STX-100</td>
<td>Biogen Idec</td>
<td>Anti-αvβ3 monoclonal antibody</td>
<td>Diabetic kidney disease; diabetic nephropathy, diabetic glomerulosclerosis</td>
<td>Phase II (recruiting)</td>
<td>NCT0113901</td>
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<td>Macitentan</td>
<td>Actelion</td>
<td>Endothelin receptor antagonist ET-A and ET-B</td>
<td>IPF</td>
<td>Phase II (fail)</td>
<td>NCT00903331</td>
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<tr>
<td>Bosentan</td>
<td>Actelion</td>
<td>Endothelin receptor antagonist ET-A and ET-B</td>
<td>IPF</td>
<td>Phase III (fail)</td>
<td>NCT00631475</td>
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<td>Ambrisentan</td>
<td>Gilead</td>
<td>Endothelin receptor antagonist selective for ET-A</td>
<td>IPF</td>
<td>Phase III (fail)</td>
<td>NCT00879229</td>
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<td>RE-021</td>
<td>Retrophin</td>
<td>Selective endothelin type 3 receptor antagonist</td>
<td>FSOS</td>
<td>Phase II (not yet open)</td>
<td>NCT01613118</td>
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<td>FG-3019</td>
<td>Fibrogen</td>
<td>Anti-CTGF</td>
<td>Liver fibrosis due to HBV</td>
<td>IPF</td>
<td>NCT01217632</td>
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<td></td>
<td></td>
<td>(ongoing)</td>
<td>NCT01262001</td>
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<td></td>
<td>(phase II, ongoing, with promising preliminary results)</td>
<td>NCT00125385</td>
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<td></td>
<td></td>
<td>(not yet recruiting)</td>
<td>NCT00782561</td>
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<td></td>
<td></td>
<td>(terminated)</td>
<td>NCT00891393</td>
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<td>(ongoing)</td>
<td>NCT01181245</td>
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<td>PF-06473871</td>
<td>Pfizer</td>
<td>Antisense CTGF</td>
<td>Hypermotrophic skin scarring</td>
<td>Phase II (recruiting)</td>
<td>NCT01730339</td>
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<td>RXI-109</td>
<td>RXI Pharmaceuticals</td>
<td>CTGF RNAi</td>
<td>Dermal scar prevention</td>
<td>Phase I (ongoing) Phase I (recruiting)</td>
<td>NCT0140912</td>
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<td>SAR156597</td>
<td>Sanofi</td>
<td>Bi-specific IL-4/IL-13 mAB</td>
<td>IPF</td>
<td>Phase I (recruiting)</td>
<td>NCT01529853</td>
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<td>Tralokinumab</td>
<td>MedImmune</td>
<td>IL-13 inhibition</td>
<td>IPF</td>
<td>Phase II (ongoing)</td>
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<td>DAX576</td>
<td>Novartis</td>
<td>IL-13 inhibition</td>
<td>Pulmonary fibrosis secondary to SSc</td>
<td>Phase II - Terminated due to SAE</td>
<td>NCT00581907</td>
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<td>Rilonacept</td>
<td>Regeneron</td>
<td>IL-1 trap</td>
<td>IPF</td>
<td>Phase II (terminated)</td>
<td>NCT01266135</td>
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<tr>
<td>CTN0 888</td>
<td>Centocor</td>
<td>MCP-1(CCL2) inhibition</td>
<td>SSc</td>
<td>Phase II (recruiting)</td>
<td>NCT01538719</td>
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<td>Etanercept</td>
<td>Pfizer/Amgene</td>
<td>TNF inhibition</td>
<td>IPF</td>
<td>Phase II (completed)</td>
<td>NCT00782601</td>
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<td>Actimmune</td>
<td>Intertrum</td>
<td>Human interferon-γ</td>
<td>IPF</td>
<td>Phase II (failed)</td>
<td>NCT00603869</td>
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<tr>
<td>Interferon-α interferon-α</td>
<td>Intertrum</td>
<td>Oral IFNα</td>
<td>IPF</td>
<td>Phase II (completed)</td>
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<td>PRM-151</td>
<td>Promedior</td>
<td>Recombinant pentraxin-2</td>
<td>IPF</td>
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<td>(phase II, terminated)</td>
<td>NCT00800885</td>
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<tr>
<td>PRM-151</td>
<td>Promedior</td>
<td>Recombinant pentraxin-2</td>
<td>IPF</td>
<td>Phase I (completed, improvements in FVC and 6MWT)</td>
<td>NCT01254409</td>
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<td>Belimumab</td>
<td>GlaxoSmithKline</td>
<td>Anti-BAFF mAB</td>
<td>Scarring in trabeculectomy</td>
<td>Phase II (completed)</td>
<td>NCT01064817</td>
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<td>Pomalidomide</td>
<td>Celgene</td>
<td>Multiple; anti angiogenic and immunomodulatory</td>
<td>Membranous glomerulonephritis</td>
<td>Phase II (recruiting)</td>
<td>NCT01610492</td>
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<tr>
<td>IW001</td>
<td>United Therapeutics</td>
<td>Collagen V solution as immunomodulator</td>
<td>SSc</td>
<td>Phase II (not yet recruiting)</td>
<td>NCT01135199</td>
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<td>Phase II (recruiting)</td>
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<td>Phase I (completed)</td>
<td>NCT0199887</td>
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