Myostatin and muscular dystrophy

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Duchenne muscular dystrophy

Common (1/3500 boys)
X-linked inheritance
Female carriers may exhibit some symptoms
Progressive skeletal muscle wasting beginning at around age 3
Characteristic changes in posture; lordosis, pseudohypertrophy of calves
“Gower’s maneuver” (climbing up from a sitting position)
Usually confined to a wheelchair by age 10-12
Progressive difficulty breathing due to wasting of diaphragm muscle
Death usually before age 30
Pathology

Definitive diagnosis by muscle biopsy
Muscle fibers variable in size
Necrotic fibers with significant inflammation
Evidence of regenerating muscle fibers; satellite cells proliferate to form new myoblasts, fusion into new muscle fibers with central nuclei
High serum levels of creatine kinase (also often true in asymptomatic female carriers)

Muscular dystrophies and related degenerative diseases

Duchenne muscular dystrophy
All skeletal muscles are affected
Caused by null or severe hypomorphic mutations in the gene encoding dystrophin, often frame-shift or nonsense mutations or large deletions

Becker muscular dystrophy
Less severe, later onset, about 1/20,000 boys
Usually in-frame deletions or point mutations in dystrophin

Emery-Dreifuss muscular dystrophy
Wasting of shoulders and upper arms first, often with cardiomyopathy
Two genetically distinct forms, both nuclear proteins (lamin A/C, emerin)

Limb girdle muscular dystrophies
Pelvic and shoulder girdles affected first
Genetically heterogeneous group of diseases, several sarcoglycans

Congenital muscular dystrophy
Genetically heterogeneous group, symptoms present at birth
~50% due to deficiency in laminin α2

About 40 neuromuscular diseases tracked by mdausa.org include genetic and autoimmune causes
Molecules deficient in various muscular dystrophies

Identified by positional cloning in affected families
Mechanical attachment of skeletal muscle contractile apparatus to extracellular matrix
Possible roles in cell signaling via NOS

Dystrophin distribution in normal and dystrophic muscle

Note female carriers often have some affected fibers due to mosaic X inactivation; elevated serum creatine kinase, sometimes muscle weakness
Treatment modalities

Options for degenerative diseases are usually poor
Supportive therapy includes vitamins, corticosteroids (prednisone), breathing exercises

Approaches in development:
  Conventional gene therapy with various vectors
    Minigenes
  Alternative gene therapies
    Gene repair
    Exon skipping
    Utrophin upregulation
  Cell-based therapies
    Myoblast transplant
    Stem cell transplants - satellite cells, bone marrow
  Therapies to increase muscle mass
    Myostatin

Dystrophin

Enormous gene/protein: >3500 amino acids, ~170 nm long
79 exons spread over 2.6 Mbp
Most mutations in large spectrin-like coiled coil domain

Animal models for DMD:
mdx mouse (nonsense mutation in exon 23)
  Pathology does not mimic human disease
  Fiber degeneration, compensation by regeneration
  Myopathy grows less severe later in life
  Normal lifespan

CXMD golden retriever dogs
  Exon skipping, frame shift due to splice site mutation
  Severe muscle degeneration; better model
Conventional gene therapy for DMD

Delivery of full-length gene with adenovirus, herpes simplex virus, or plasmid vectors
High transduction levels are required; work OK in regenerating mouse muscle but persistence is poor
Inflammatory response is a particular problem since degenerating muscle is already inflamed
Viral vectors not yet used in human patients; phase I trial for plasmid vector currently underway
Minigenes mimic Becker, can be carried by simpler and more efficient vectors. Alleviate degeneration in mdx mice

Alternative genetic approach: exon skipping

Related to mechanism of “reversion” in DMD**
Antisense oligonucleotides block splice site recognition
Temporary effect in mdx mice and cultured human myotubes

Utrophin as a substitute?
Ubiquitously expressed dystrophin paralog
Found in mature skeletal muscle fibers at neuromuscular junctions; associates with acetylcholine receptors
Expression upregulated in mdx mice and protein relocates to a dystrophin-like pattern; double knock-out mice have pathology more similar to human DMD

Cell-based therapies (transplantation)
Myoblasts can be readily cultured from donor biopsy samples
Human trials; repeated injections into biceps muscle over 6 months gave some fibers (1-10%) expressing donor-derived dystrophin but no clinical improvement

Mendell et al., 1995, NEJM 333: 832
Stem cells?

Satellite cells are rare and difficult to isolate, probably cannot home properly. In mdx mice, no difference between myoblast transplantation and satellite cell transplantation

Bone marrow stem cells???

Bone marrow contains multipotent mesenchymal progenitor cells as well as hematopoietic stem cells

mdx mice have dystrophin-positive fibers with donor nuclei after BMT

DMD patient who had received BMT for X-linked immunodeficiency had donor nuclei persisting in muscle after 13 years, but no expression of donor-derived dystrophin (and there are always a few revertant fibers…)

(Gussoni et al., 2002, J. Clin Inv. 110: 807)

Animals reconstituted with a single HSC have some GFP-positive myofibrils: plasticity of adult stem cells?

Corbel et al., 2003, Nature Medicine, 9:1528
BUT: this is due to fusion of a myeloid cell with a regenerating muscle fiber and not due to trans-differentiation

Note similar adhesion and signaling pathways in myoblast fusion and in monocyte fusion to form osteoclasts, also macrophage fusion

Camargo et al., 2003, Nature Medicine, 9:1520

A different approach: Target “booster genes” that affect disease progression
Myostatin as a candidate to increase muscle mass and regeneration in DMD

Note regeneration appears to compensate early in disease progression
Candidate for regulation: MYOSTATIN
TGF-β family member, cloned by degenerate PCR
Expressed exclusively in skeletal muscle at all stages of development

McPherron et al., 1997, Nature 387: 83

Myostatin knockout supermice

30% larger than littermates
“Abnormal body shape with pronounced shoulders and hips”
Skeletal muscle mass increases 2-3X
Hyperplasia and hypertrophy (note different dominant negative alleles can separate these two effects)

McPherron et al., 1997, Nature 387: 83

Also myosotatin defects in double-muscled cattle
McPherron and Lee, 1997
PNAS 94: 12457

PAPER 1: And in humans!
Myostatin KO improves muscle mass and grip strength in *mdx* mice

Blue - mdx  
Red - mdx, Mstn-/-  
Yellow - wild type  
Green - Mstn -/-  

PAPER 2: Blockade with antibody has a similar beneficial effect

Wagner et al., 2002, Annal. Neurol. 52:832

Myostatin requires proteolytic processing for activity: possible targets for small molecules?

Propeptide binds active sulfhydryl-linked dimer in latent complex  
Protease inhibitors, stable propeptide analogs, or receptor antagonists might work

Where else might myostatin blockade be useful? Muscle wasting for HIV+ men is correlated with increased serum myostatin levels

Gonzales-Cadavid et al., 1998, PNAS 95: 14938