Skeletal Muscle Degenerative Diseases and Strategies for Therapeutic Muscle Repair

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Abstract

Skeletal muscle is a highly specialized, postmitotic tissue that must withstand chronic mechanical and physiological stress throughout life to maintain proper contractile function. Muscle damage or disease leads to progressive weakness and disability, and manifests in more than 100 different human disorders. Current therapies to treat muscle degenerative diseases are limited mostly to the amelioration of symptoms, although promising new therapeutic directions are emerging. In this review, we discuss the pathological basis for the most common muscle degenerative diseases and highlight new and encouraging experimental and clinical opportunities to prevent or reverse these afflictions.

Keywords

myogenesis, muscular dystrophy, sarcopenia, cell therapy, gene therapy, muscle regeneration
SKELETAL MUSCLE: COMPOSITION, STRUCTURE, AND FUNCTION

Skeletal muscle is composed of thousands of muscle fibers, which are bundled together and attached to the skeleton by tendons (Figure 1). Skeletal muscle fibers (myofibers) are multinucleated and form during development by the fusion of mononucleated myoblasts. Myofibers are surrounded by a specialized plasma membrane, the sarcolemma, which transduces signals from motor neurons and other external stimuli into muscle fibers. Myofibers are surrounded by a layer of extracellular matrix (ECM) known as the basement membrane, which is composed of both an internal basal lamina and an external reticular lamina (1). The basal lamina associates closely with the sarcolemma, providing a protective niche in which muscle regenerative cells (satellite cells) reside. Satellite cells are unipotent adult stem cells that are activated in response to severe muscle damage to proliferate and differentiate, thereby forming myoblasts that can rebuild the muscle through fusion with one another or with residual myofibers. Satellite cells also possess potent self-renewal capacity, which ensures their persistence within the muscle and preserves the muscle’s ability to repair after injury.

The calcium-dependent contraction of muscle fibers requires a specialized cytoplasm (sarcoplasm) and modified endoplasmic reticulum (ER) [sarcoplasmic reticulum (SR)]. Transverse tubules (T-tubules) invaginate the sarcolemma to properly transduce action potentials and activate the SR (Figure 1). Myofibers contain abundant myofibrils, which act as contraction units and are surrounded by SR. Myofibrils are composed of thin myofilaments (actin) and thick myofilaments (myosin) whose calcium-dependent movement relative to one another produces muscle contraction. The

Figure 1
Skeletal muscle structure and composition. Skeletal muscle is composed of numerous bundles of muscle fibers. Each bundle consists of multiple fibers, and individual fibers encompass many myofibrils. Sarcomeres are the structural units of myofibrils and are made up of actin and myosin filaments. Abbreviation: T-tubule, transverse tubule.
organization of myofilaments into myofibrils underlies the normally striated appearance of skeletal muscle under light microscopy; thin filaments make up the light band (I-band), and thick filaments make up the dark band (A-band) (Figure 1). The Z-line defines the borders of each sarcomere, which is the structural unit of the myofibril (2).

Muscle contraction is induced by depolarization of the sarcolemma via action potential. This depolarization opens sarcoplasmic calcium release channels, increasing the intracellular calcium concentration and triggering actin-myosin-mediated contraction of sarcomeres. Protein assemblies known as costameres, which consist mainly of proteins contained within the dystrophin-glycoprotein complex (DGC) (3) and the integrin-vinculin-talin complex (4), transmit contraction forces from muscle fibers to the ECM and, eventually, to neighboring myofibers. Costameres align with the Z-line of peripheral myofibrils and physically link myofibrils to the sarcolemma.

**SKELETAL MUSCLE INJURY AND REGENERATION**

**Mechanisms of Muscle Injury: Acute Muscle Damage**

Acute damage to skeletal muscle can occur by physical or chemical insult. For example, sharp or blunt trauma, or excessively hot or cold temperatures, induces rapid myofiber necrosis. Likewise, envenomation by snakes or bees causes acute myonecrosis. Such venoms contain low-molecular-mass myotoxins (crotamine and myotoxin a) (5), phospholipases A2 (PLA2s) (6) and membrane-active cardiotoxins (7); bee venom alone contains melittin (8). All of these toxins cause depolarization and contraction of myofibers, and all except the low-molecular-mass myotoxins induce lysis of the sarcolemma (9). In contrast, PLA2s disrupt sarcolemmal integrity by hydrolysis of glycerophospholipids. Such membrane disruption provokes calcium influx, leading to hypercontraction, mitochondrial calcium overload, and activation of cytosolic calcium-dependent PLA2s and calpains (10). Myogenic satellite cells, nerves, and blood vessels appear unaffected by PLA2s (10). In addition, the basal lamina remains intact after envenomation, although some myotoxic and neurotoxic PLA2s may induce degeneration of nerve terminals (11, 12).

Cardiotoxins are single-chain small polypeptides that form pores in the muscle membrane (13). Myonecrosis induced by cardiotoxin and melittin involves rapid lysis of the sarcolemma and hypercontraction of sarcomeres following calcium influx (14). Muscle necrosis resulting from low-molecular-mass basic myotoxins is much slower and is caused by the induction of sodium influx via voltage-sensitive sodium channels in the sarcolemma. This sodium influx causes membrane depolarization, muscle contraction, and vacuolization of the SR. Nevertheless, these myotoxins cause no apparent sarcolemma or transverse tubule damage (15).

Ischemia/reperfusion also causes acute muscle damage. Prolonged periods of zero blood flow, which occur during organ-transplantation surgery, stroke, and hypovolemic shock, induce muscle dysfunction through the loss of cellular energy supplies and accumulation of potentially toxic tissue metabolites. Restoration of blood flow is essential to the rescue of ischemic muscle; however, reactive oxygen species (ROS) produced during reperfusion exacerbate tissue injury by directly damaging cellular macromolecules and promoting the infiltration of inflammatory leukocytes (16). Oxygen insufficiency during ischemia converts cellular metabolism to anaerobic pathways, initiating a cascade of reactions that generate large quantities of superoxide and other free radicals (17) and increase intracellular calcium concentrations. Calcium levels are further elevated following reperfusion, causing the activation of calpain and phospholipases. These molecules, in turn, can trigger proinflammatory mediator synthesis and leukocyte chemotaxis (18), which further damage the muscle through release of reactive oxygen and proteases, complement activation, and increased vascular permeability (19).
Mechanisms of Muscle Injury: Contraction-Induced Damage

Contraction of skeletal muscle can cause shortening (concentric contraction), lengthening (eccentric contraction), or no change (isometric contraction) of muscle length. Concentric contractions initiate movements, and eccentric contractions slow or stop them. During eccentric contraction, the opposing force exceeds the force generated by the muscle, leading to its elongation. Contraction-induced injury is most severe during eccentric contractions. Electron microscopy reveals regions of overstretched sarcomeres or half-sarcomeres, Z-line streaming and regional disorganization of myofilaments, and T-tubule damage in myofibers after lengthening contraction (20, 21). Weaker sarcomeres are overstretched during eccentric contraction, and repeated overextension of sarcomeres leads to their disruption. Disruption of multiple sarcomeres causes membrane damage, beginning with tearing of T-tubules and degradation of membrane components involved in excitation-contraction coupling (22). Increased intracellular calcium, promoted by damage to the SR or induction of stretch-activated channels, further drives muscle contractile protein degradation, as well as mitochondrial swelling and SR vacuolization (23). Depending on the extent of contraction-induced damage, myofiber necrosis may occur, which leads to a local inflammatory response associated with tissue edema and soreness.

Mechanisms of Muscle Injury: Inflammatory Myopathy

Inflammatory myopathies (IMs) represent the largest group of acquired and potentially treatable myopathies in people (24). This heterogeneous group of subacute, chronic, or sometimes acute muscle diseases commonly involves muscle weakness and inflammation, revealed in muscle biopsies (25). Four different types of IMs have been identified by histological, immunopathological, and clinical manifestations: dermatomyositis, polymyositis (PM), sporadic inclusion body myositis (sIBM), and necrotizing autoimmune myositis (NAM) (26).

Complement activation and the formation of membranolytic attack complexes that damage endothelial cells of the endomysial capillaries are two of the earliest events in dermatomyositis (27). Autoantibodies directed against endothelial cells of the endomysial capillaries may activate the complement system, causing endothelial cell lysis, perivascular inflammation, capillary destruction, and muscle ischemia (25). Thus, dermatomyositis decreases capillary density in the muscle, causing dilation of the remaining vessels, which attempt to compensate for the ischemic condition (28). Complement activation also triggers the expression of proinflammatory cytokines and the recruitment of CD4+ T cells, B cells, macrophages, and plasmacytoid dendritic cells to muscle (29, 30).

A common feature in the immunobiology of PM and sIBM is the ubiquitous expression of major histocompatibility complex 1 (MHC-1) antigen, which is normally undetectable on muscle fibers (31). CD8+ cytotoxic T cells attack MHC-1-expressing myofibers, causing fiber lesions. Spike-like processes from CD8+ cells and macrophages may also extend across the myofiber basal lamina and compress the fibers (32). Furthermore, perforin and granzyme, released from CD8+ cells, promote myofiber necrosis (33). Although there are significant immunobiological similarities between PM and sIBM, sIBM is additionally characterized by the presence of a strong degenerative process that involves rimmed vacuoles and intracellular deposition of β-amyloid and related molecules (34). Other degenerative phenomena in sIBM myofibers include an induced unfolded protein response, lysosome and mitochondrial abnormalities, and ER stress (34).

NAM is the least understood of the IMs. Macrophages are thought to be responsible for fiber necrosis in this disease, which causes high creatine kinase (CK) levels in patients’ blood. Neither infiltration of T cells nor expression of MHC-1 on muscle fibers has been detected in NAM (26); however, some patients harbor autoantibodies against signal recognition peptide,
which implies an antibody-mediated cause of the disease (35). Macrophage recruitment also supports antibody-dependent cell-mediated cytotoxicity in NAM. Although there have been cases of patients with cancer or active viral infection (e.g., HIV), it is unclear whether NAM can be triggered by these factors or not (24).

Mechanisms of Muscle Regeneration: Membrane Patch

Many eukaryotic cell types can repair minor membrane disruptions and restore plasma membrane integrity. Physiological disruptions to the plasma membrane occur frequently in skeletal muscle (36), and defects in membrane resealing can cause muscular dystrophy (37). The membrane repair process involves calcium-dependent exocytosis of intracellular vesicles, such as lysosomes and enlargeosomes, which form a membrane “patch” (Figure 2) (38, 39). Dysferlin, a protein that is present predominantly at the muscle surface and in cytoplasmic vesicles, is also a critical component in membrane repair (37); however, the mechanism underlying dysferlin’s involvement in this process remains poorly understood. Dysferlin may function as a calcium sensor, regulating vesicle-vesicle and vesicle-membrane fusion during membrane repair (40). Dysferlin also interacts with annexins A1 and A2 (41). Annexin A1 is required for membrane repair in HeLa cells. Adsorption of annexins to dysferlin and activation of calpains by oxidative stress lead to membrane patching and cytoskeletal remodeling. Dysferlin is critical for vesicle fusion and repair of the sarcolemma. Dysferlin deficiency results in impaired sarcolemmal integrity and muscle weakness in human and murine models.

Figure 2

Model for mechanism of membrane patching in muscle fibers. (a) In the intact muscle fiber, Mitsugumin 53 (MG53) is on sarcoplasmic vesicles in a reduced form, and the concentration of calcium in sarcoplasm is lower than in the extracellular matrix. (b) Upon membrane damage, exposure of the intracellular environment to oxidative agents leads to oxidation and oligomerization of MG53 proteins on sarcoplasmic vesicles. Increased calcium concentration around the injury site also activates calpain. Activated calpain may cleave annexin proteins, which subsequently mediate accumulation of vesicles near the site of damage. (c) Dysferlin senses the calcium that floods into the fiber and triggers fusion of accumulated vesicles. Activated calpain also degrades the cytoskeleton near the damaged area, making it easier for vesicles to fuse with plasma membrane. (d) Vesicles fuse with the sarcolemma and form a patch that seals the membrane. Abbreviation: SH, reduced sulfur moieties in MG53. Modified from Reference 262.
cells (42), but its mechanism of action is currently unclear. Binding of annexins to phospholipids in the presence of calcium, together with their ability to aggregate vesicles in vitro and interact with actin, fueled speculation about their involvement in vesicle-vesicle fusion and vesicle movement (43). In addition to triggering vesicle fusion, calcium influx after membrane injury also activates calpain, which likewise is necessary for membrane repair (44, 45) and may mediate the disassembly of the damaged actin cytoskeleton through degradation of cytoskeletal proteins such as talin and vimentin. Annexins are also proteolytic targets of calpain, and calpain-mediated cleavage of annexins may be critical for membrane repair (46). Recently, Mitsugumin 53, a muscle-specific tripartite motif family protein (TRIM72) that is present on the sarcolemma and intracellular vesicles, was implicated in nucleating the assembly of the repair machinery at injury sites (47, 48). This process is independent of calcium influx and triggered by changes in the intracellular oxidative environment (48). Figure 2 presents a simplified model of our current understanding of membrane patching in skeletal myofibers.

**Mechanisms of Muscle Regeneration: Muscle Satellite Cells**

Satellite cells are mononuclear cells with a high nucleus-to-cytoplasm ratio; they were first identified in electron micrographs on the basis of their distinct anatomical position between the sarcolemma and the basal lamina of muscle fibers (49). Satellite cells represent the endogenous source of muscle progenitor cells and account for the regenerative potential of adult muscle (50). Considering the infrequent turnover of myonuclei in adult muscle, satellite cells remain mitotically and metabolically quiescent throughout most of life. However, satellite cells are activated after muscle injury and in the context of chronic degenerative diseases (see the section titled Muscle Degenerative Diseases) (51–53). Damage to skeletal muscle results in the release of growth factors (GFs) and cytokines, such as hepatocyte growth factor; epidermal growth factor; platelet-derived growth factor BB; and members of the insulin-like growth factor (IGF) and fibroblast growth factor family (54, 55) from the ECM (56), myofibers, endothelial cells, interstitial cells (57), and leukocytes (58). Interaction among these GFs and receptors on quiescent satellite cells triggers satellite cell proliferation.

Quiescent satellite cells express various proteins, including Pax7, CD34, c-met, M-cadherin, syndecan-3, and syndecan-4 (59–61), that are important for their activation and proliferation in response to muscle damage (62). Activated satellite cells downregulate Pax7 expression and increase synthesis of the early myogenic regulatory factors MyoD and Myf5 (63). These activated cells undergo a rapid proliferation stage regulated in part by Notch signaling (64). Notch inhibition and activation of Wnt signaling can induce the progression of muscle satellite cells along the myogenic lineage to promote the production of fusion-competent myoblasts (65) and trigger the expression of late myogenic regulatory factors, including myogenin. Fusion of terminally differentiated myoblasts into myofibers marks the final stage of muscle regeneration.

Efficient repair of skeletal muscle after repeated injuries indicates that satellite cells must be replenished after muscle regeneration. Genetic fate-mapping studies strongly implicate satellite cells themselves as the endogenous source of such cell replacement; however, the mechanisms regulating satellite cell self-renewal are not fully understood. Some reports suggest that nonrandom segregation of DNA strands or asymmetric distribution of Numb, an inhibitor of Notch signaling, in the daughters of dividing satellite cells (66, 67) may drive asymmetric division of satellite cells and preservation of the satellite cell pool. A recent study (68) provided evidence suggesting that asymmetric division in satellite cells expressing high levels of Pax7 causes segregation of template DNA to daughter cells with a more immature phenotype, whereas daughters inheriting newly synthesized DNA acquire a more differentiated phenotype. This study also
Mechanisms of satellite cell activation and regulation during muscle repair. (a) During homeostasis, satellite cells (green) reside in close association with muscle fibers (red). Resting muscle also contains resident fibro-adipogenic precursors [FAPs (purple)]. (b) Damage to muscle induces myofiber degeneration and inflammation, beginning with infiltration by neutrophils and M1 macrophages (dark yellow) from blood vessels (red oval). (c) During the regenerative phase, elaboration of growth factors and cytokines by muscle fibers, infiltrating M2 macrophages (light yellow) and activated FAPs (purple with yellow border) activate satellite cells (green with yellow border) to proliferate and differentiate to form myoblasts (orange oval with yellow border) that exit the cell cycle and fuse with one another and with residual myofibers to replenish myofibers as well as the satellite cell pool (d).

FAPs: fibro-adipogenic precursors

In addition to soluble GFs and cytokines, satellite cell function is also regulated by infiltrating and interstitial cell populations, including recruited inflammatory cells (72) and resident fibro-adipogenic precursors (FAPs). In the absence of any recruited immune cells, satellite cell regenerative activity appears to be blocked (72); however, as discussed above, an overexuberant or unbalanced immune response can lead to myopathic tissue destruction that is not recoverable through satellite cell–mediated repair processes. Neutrophils appear to be the first immune cells recruited to damaged muscle (75). Their recruitment signals subsequent infiltration by M1 macrophages, followed by M2 macrophages (76, 77). M1 macrophages are efficient inducers and effectors of inflammatory processes, whereas M2 macrophages are more often involved in tissue repair, remodeling, and immunoregulation (78). Both neutrophils and macrophages participate in the clearance of myofiber debris at the injury site and in the production of inflammatory and immune-regulatory cytokines, but macrophages (particularly M2 macrophages) appear to have an additional, unique function in directly regulating muscle regeneration through induction of satellite cell activation and myoblast proliferation (79–81).

In addition to recruited immune cells, muscle-resident mesenchymal cells also appear to be critical for proper muscle repair. For example, skeletal muscle contains a unique population of Sca-1–expressing precursor cells, which can differentiate to form fibroblasts (82) and white or brown adipocytes (73, 83). Although these FAPs exhibit no intrinsic myogenic activity, they are potent inducers of myogenesis by satellite cells (Figure 3) (73, 74). Intriguingly, whereas undifferentiated FAPs promote myofiber formation, the presence of the FAPs: fibro-adipogenic precursors
of differentiated myofibers appears to inhibit FAP-mediated adipogenesis (74). Although the exact mechanisms by which this functional cross-antagonism is accomplished remain to be determined, studies have suggested a role for paracrine signaling [by soluble mediators such as IGF-1, Wnts, and interleukin (IL)-6] between FAPs and muscle satellite cells in the FAP-dependent promotion of myogenesis (73). In contrast, cocultures of FAPs with differentiated myotubes implicate direct cell-cell interaction in the inhibition of FAP-mediated adipogenesis by muscle fibers (74). Thus, in addition to alterations in satellite cell number and intrinsic signaling responses, numerous non-cell-autonomous inputs clearly influence the extent and efficacy of satellite cell-mediated muscle repair.

**MUSCLE DEGENERATIVE DISEASES**

**Genetic Diseases of Skeletal Muscle: Duchenne and Becker Muscular Dystrophies**

Duchenne muscular dystrophy (DMD) is the most common X-linked genetic disorder in humans; it affects one in 3,500 males. Most boys with DMD manifest symptoms within the first years of life. Progressive muscle weakening delays walking and causes repeated falls, leaving patients wheelchair bound, typically by ~12 years of age. Most patients experience premature death due to respiratory or cardiovascular failure in the second decade. Mutations in the dystrophin gene leading to genetic frameshift or loss of expression and complete absence of protein function are the cause of DMD (Table 1) (84). Dystrophin extends over 2.4 Mb of the X chromosome and represents the largest gene in the human genome. Point mutations in dystrophin are responsible for ~40% of DMD cases; the remaining ~60% are caused by large deletions or duplications in this gene (85).

Dystrophin is a structural protein, a component of the DGC (Figure 4) (86), and an essential part of the costamere. Dystrophin’s primary function is to link the myofiber cytoskeleton to the ECM and thereby stabilize the sarcolemma (87). Dystrophin binds cytoplasmic actin through its amino terminus as well as its rod-shaped domain, which is composed of 24 spectrin repeats and four hinge points (88). The C-terminal cysteine-rich domain of dystrophin directly binds to transmembrane β-dystroglycan protein. β-Dystroglycan is linked to the highly glycosylated α-dystroglycan (αDg), which completes the connection between the myofiber cytoskeleton and ECM by interacting with laminin in the basal lamina (89). Absence of functional dystrophin protein destabilizes the DGC, increasing the susceptibility of dystrophic muscle fibers to contraction-induced injury (90). Increased cytosolic calcium following mechanical stress, activation of proteases (particularly calpains), destruction of membrane constituents, and ultimately myofiber necrosis occur frequently in dystrophic muscles. Thus, satellite cells in these patients must support repeated rounds of regeneration in an attempt to compensate for damage. As the disease advances, satellite cells show reduced capacity for muscle regeneration, possibly due to proliferation-induced reductions in telomere length (91) or damage-associated cell attrition (92). Absent an adequate muscle regenerative response, fat and fibrotic tissue replace muscle fibers, leading to further weakening and wasting (93).

In addition to mechanical stress, other secondary mechanisms induce damage in dystrophic muscle. Loss of functional dystrophin leads to reduced expression and mislocalization of neuronal nitric oxide synthase (nNOS) from the sarcolemma (Figure 4) (94). Absence of nNOS signaling impairs blood supply to contracting muscles, exposing dystrophic muscles to continuous ischemic insult (95). Various immune cells are also recruited to the dystrophic muscle as a result of persistent damage; these cells can cause secondary damage through inflammatory responses and elaboration of ROS (96).

Like DMD, Becker muscular dystrophy (BMD) is also caused by mutations in dystrophin;
<table>
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<tr>
<th>Disease name</th>
<th>Protein (gene); disease subtype</th>
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<tr>
<td>Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD)</td>
<td>Dystrophin (DMD)</td>
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<tr>
<td>Myotonic dystrophy (DM)</td>
<td>Dystrophia myotonica protein kinase (DMPK); DM1 Zinc-finger nuclease 9 (ZFN9/CNB5); DM2</td>
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<td>Limb girdle muscular dystrophy (LGMD)</td>
<td>Myotilin (MYOT); LGMD1A Lamin A and C (LMA4); LGMD1B Caveolin-3 (CAV3); LGMD1C Calpain-3 (CAPN3); LGMD2A Dysferlin (DYSF); LGMD2B γ-Sarcoglycan (SGCγ); LGMD2C α-Sarcoglycan (SGCα); LGMD2D β-Sarcoglycan (SGCβ); LGMD2E δ-Sarcoglycan (SGCδ); LGMD2F Telethonin (TC4P); LGMD2G Tripartite motif–containing 32 (TRIM32); LGMD2H Fukutin related protein (FKRP); LGMD2I Titin (TTN); LGMD2J Protein-O-mannosyltransferase 1 (POMT1); LGMD2K Anoctamin 5 (ANO5); LGMD2L Fukutin (FCMD); LGMD2M Protein-O-mannosyltransferase 2 (POMT2); LGMD2N Protein O-linked mannose β1, 2-N-acetylglucosaminyltransferase (POMGnT1); LGMD2O Dystroglycan (DAG1); LGMD2P</td>
</tr>
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<td>Emery–Dreifuss muscular dystrophy (EDMD)</td>
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<td>Congenital muscular dystrophy (CMD)</td>
<td>Fukutin-related protein (FKRP); Walker–Warburg syndrome (WWS), Fukuyama CMD, or muscle–eye–brain disease Like-glycosyl transferase (LARGE); WWS, Fukuyama CMD, or muscle–eye–brain disease Fukutin (FCMD); WWS, Fukuyama CMD, or muscle–eye–brain disease Protein-O-mannosyltransferase 1 (POMT1); WWS, Fukuyama CMD, or muscle–eye–brain disease Protein-O-mannosyltransferase 2 (POMT2); WWS, Fukuyama CMD, or muscle–eye–brain disease Protein O-linked mannose β1, 2-N-acetylglucosaminyltransferase (POMGnT1); WWS, Fukuyama CMD, or muscle–eye–brain disease Laminin-2 (LAMA2) Collagen type VI, subunit α1 (COL6A1); Ullrich syndrome or Bethlem myopathy Collagen type VI, subunit α2 (COL6A2); Ullrich syndrome or Bethlem myopathy Collagen type VI, subunit α3 (COL6A3); Ullrich syndrome or Bethlem myopathy Integrin-α7 (ITGA7); Selenoprotein N1 (SEP1); rigid spine syndrome Laminin A and C (LMNA)</td>
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<td>Facioscapulohumeral muscular dystrophy (FSHD)</td>
<td>Double-homeobox protein 4 (Dux4); FSHD1A</td>
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Figure 4
Dystrophin glycoprotein complex (DGC). Dystrophin connects the muscle fiber cytoskeleton to the extracellular matrix (ECM) via interaction with actin filaments in the sarcoplasm and β-dystroglycan in the sarcolemma. β-Dystroglycan interacts with α-dystroglycan, which is connected to laminin in the ECM through its glycan moieties. The sarcoglycan-sarcospan complex is also a part of DGC that includes α-, β-, γ-, and δ-sarcoglycans and sarcospan. This subcomplex is connected to the ECM through interaction with biglycan. Dystrophin also interacts with α-dystrobrevin and α- and β-syntrophins through its C-terminal domain. Neuronal nitric oxide synthase (nNOS) is localized to the DGC via its interaction with syntrophins.

Myotonia: the inability to relax muscles after contraction

however, Becker mutations maintain the dystrophin reading frame. Thus, most BMD patients express a partially functional dystrophin protein, which lacks the internal spectrin repeats but contains the critical actin-binding and C-terminal domains (97). BMD patients show a milder phenotype and a more heterogeneous clinical manifestation of the disease. Some BMD patients remain ambulatory after theirforties, and a few can survive more than 60 years (98).

Genetic Diseases of Skeletal Muscle: Myotonic Dystrophy

Myotonic dystrophy type 1 [also known as dystrophia myotonica type 1 (DM1)] was first described by Hans Steinert in 1909 (99). DM1 affects more than 1 in 8,000 individuals worldwide but has an increased incidence (up to 1 in 500) in certain populations due to founder effects. DM1 is an autosomal dominant disease caused by a trinucleotide repeat expansion in the 3’ untranslated region (3’ UTR) of DMPK on chromosome 19 (100–102). Normal individuals have fewer than 30 repeats, and expansions above this range can initiate DM symptoms. Age of onset and disease severity appear to correlate with repeat expansion length. Typically, expansions greater than 1,000 repeats yield a severe congenital form of DM. DM repeats are unstable over time and across cell types, and repeat lengths in muscle, heart, brain, and other tissues can significantly exceed those measured in blood (103).

Although the pathognomonic symptom of DM is myotonia, or the inability to relax muscles after contraction, DM affects multiple body systems and therefore is not exclusively a muscular dystrophy. Additional DM symptoms include muscle wasting and weakening, cardiac conduction block, smooth muscle dysfunction, so-called Christmas tree cataracts, insulin resistance, neuropsychiatric abnormalities,
hypersomnia, fatigue, sleep dysregulation, and testicular atrophy (99). Muscle wasting often occurs in the distal-to-proximal direction, first affecting hand, face, and tongue muscles, leading to grip weakness, ptosis, and speech difficulties. Weakness of the tibialis muscle leads to foot drop, and progressive weakness in all major skeletal muscle groups occurs over time. Although centralized myofiber nuclei, heterogeneous fiber cross-sectional areas, and fibrosis are commonly observed, regeneration and immune cell infiltration are not; thus, the central nuclei are believed to reflect processes that are unrelated to regeneration. Heart block in DM can cause atrial fibrillation and occasionally ventricular tachycardia, making sudden death a significant risk for DM patients; however, this risk can be mitigated by pacemakers or implantable cardioverter defibrillators. Smooth muscle dysfunction in DM can dysregulate gastrointestinal tract motility and cause gallbladder dysfunction. Executive functioning and other central nervous system functions, particularly involving the prefrontal cortex, are also especially affected in DM (99).

The penetrance of symptoms among DM patients is so wide ranging that it has been described as the most variable disease known to mankind (99). DM exhibits genetic anticipation, and its pattern of inheritance is similar to that of other microsatellite repeat diseases, such as Huntington’s disease and spinocerebellar ataxias. Often, entire families are diagnosed only after a hypotonic newborn, suffering from congenital DM1, inspires a cascade of diagnoses up the family tree (104). Haploinsufficiency of the DMPK protein, observed in DM1 patient tissue, was initially hypothesized to play a central role in DM1 pathogenesis, and epigenetic effects of expanded repeats on the expression of neighboring genes were also proposed to play a role (Figure 5). Yet, mice lacking DMPK develop only minor cardiac and muscle defects later in life, distinct from those observed in DM1 patients (105). Discovery of a second type of myotonic dystrophy, DM2, caused by a tetranucleotide (CCUG) repeat expansion in the first intron of the CNBP gene on chromosome 3q21, provided critical clues about molecular pathogenesis in DM (106). DM2 is estimated to account for less than 10% of all DM cases. Although the phenotype of DM2 patients is not identical to that of DM1 patients (muscle wasting occurs in a proximal-to-distal fashion, and symptoms are generally more mild than in DM1), DM2 patients also experience myotonia, cataracts, frontal balding, insulin resistance, and executive functioning difficulties, among other symptoms. The discovery of DM2, combined with the observation that nuclei of DM1 and DM2 cells contain CUG- or CCUG-rich RNA foci (107, 108), shifted the focus of investigators from cis to trans mechanisms, and to a model in which the pathogenic molecule may be the expanded CUG- or CCUG-containing RNA (Figure 5).

Transgenic mice expressing CUG repeats in the 3′ UTR of an unrelated gene (human skeletal actin) in a muscle-specific fashion exhibit myotonia, centralized muscle nuclei, and heterogeneous myofiber cross-sectional areas (109). Members of the Muscleblind-like family of RNA-binding proteins (MBNLs) bind to CUG repeat RNA in a length-dependent fashion, and MBNL1 knockout mice exhibit myotonia and centralized muscle nuclei (110, 111). Furthermore, the introduction of exogenous MBNL1 protein by an adeno-associated virus (AAV) into the muscle of CUG repeat–carrying mice significantly rescued myotonia (112).

The MBNLs were first discovered in Drosophila, where their loss results in defects in eye and muscle development (113). MBNLs can both repress and activate alternative mRNA splicing, and their endogenous binding sequences are similar to those of CUG or CCUG sequences (114). For example, aberrant inclusion of exon 7a of the chloride channel 1 (CLCN1) gene, which contains a premature stop codon and normally is repressed by MBNL, causes degradation and loss of CLCN1 at the sarcolemma, leading to myotonia (Figure 5) (115). Loss of MBNLs appears to account for ~80% of the splicing changes in
one mouse model of DM. This finding supports the model that expanded CUG/CCUG repeat expression functionally sequesters MBNLs, which leads to splicing changes that cause DM phenotypes (116). Several MBNL-dependent splicing events have been proposed to account for DM phenotypes, including Bin1 exon 7 splicing (muscle wasting) (117), insulin receptor exon 11 (insulin resistance) (118), CaV1.1 (muscle wasting) (119), and cardiac troponin T exon 5 (cardiac function) (120).

A separate line of investigation indicates that overexpression of CUG repeats may stabilize a distinct RNA-binding protein, CELF1 (121), via hyperphosphorylation by protein kinase C, which is aberrantly activated through unknown mechanisms by expanded CUG repeat expression in the context of the DMPK 3′ UTR (Figure 5) (122). CELF1 is elevated in DM1 patient tissues and myoblasts, and CELF1 over-expression in heart or muscle leads to cardiac defects and muscle wasting, respectively (123),
suggesting that CELF1 activation can mediate additional, MBNL-independent changes that may cause other DM1 symptoms.

Although it remains unclear exactly which events downstream of MBNL sequestration cause the plethora of DM phenotypes, it has been firmly established that DM is a disease of RNA toxicity. Thus, eliminating the expanded repeat RNA or preventing it from sequestering RNA-binding proteins is likely to represent a viable therapeutic strategy for both DM1 and DM2. Thus, similar approaches are being pursued for both diseases, although a small but increasingly recognized number of patients experience DM symptoms in the absence of expanded repeats in DMPK or CNBP (124). Further investigation is needed to determine the causative loci in this subset of patients and to evaluate whether similar molecular pathways may be perturbed.

Genetic Diseases of Skeletal Muscle: Limb Girdle Muscular Dystrophies

Limb-girdle muscular dystrophy (LGMD) refers to a phenotypically related group of dystrophies, each caused by a distinct genetic defect. All LGMDs show progressive muscle weakness beginning from the proximal limb muscles, elevated CK, and in some cases cardiomyopathy. Disease onset varies from early childhood to late adulthood. LGMD may be either autosomal dominant (LGMD1) or autosomal recessive (LGMD2) (125), and the causative genetic defect may occur in genes encoding DGC-associated proteins, sarcomemal proteins, muscle fiber enzymes, sarcomeric proteins, or nuclear lamina. In approximately one-third of LGMD patients, the mutated gene has yet to be identified. Autosomal dominant LGMD patients exhibit a milder phenotype, and the disease onset is typically in adulthood. Eight loci are known to cause LGMD1 so far, but only three genes, which encode myotilin (LGMD1A), lamin A and C (LGMD1B), and caveolin-3 (LGMD1C), have been implicated.

Mutations in the myotilin gene are responsible for LGMD1A (126). Myotilin is localized to the Z-line and interacts with α-actinin, which cross-links actins in Z-lines (127). LGMD1A muscle biopsies analyzed by electron microscopy exhibit extensive Z-line streaming. The mean age of onset of LGMD1A is 27 years, and some patients show a dysarthric pattern of speech in addition to the usual LGMD features (126).

Lamins A and C are intermediate filament proteins found in the nuclear membrane. Mutations in LMNA, which encodes lamin A and C, cause several different muscle diseases, including hypertrophic cardiomyopathy, congenital muscular dystrophy (CMD), familial partial lipodystrophy, Emery–Dreifuss muscular dystrophy (EDMD), and LGMD1B (125). Most LGMD1B patients exhibit cardiomyopathy. Skeletal myopathy is slowly progressive and mild in these patients, and their CK levels are slightly elevated (128). Given that lamin A and C are expressed in most postmitotic cells, the muscle specificity of these diseases is not well understood.

LGMD1C is caused by mutations in the CAV3 gene, which encodes the caveolin-3 protein (129). Caveolae are membrane invaginations involved in localizing proteins in the membrane. Caveolin-3 is expressed in muscle tissue (130) and interacts with dysferlin in the sarcolemma (131). On one hand, accumulation of dysferlin in the Golgi apparatus of cells lacking caveolin-3 led to the speculation that caveolin may help to transport dysferlin to the sarcolemma (132). On the other hand, a recent report indicates that caveolin-3 may associate with the calcium release complex in the sarcolemma (133). CK levels typically are increased in LGMD1C patients, but their disease phenotype is generally mild and some patients show no clinical muscle disease symptoms (125).

Mutations in the calpain-3 gene (CAPN3) cause LGMD2A, which accounts for 20% to 40% of LGMD cases. Calpain-3 is a muscle-specific intracellular calcium–activated protease associated with connectin and titin in the sarcomere (134), as well as with dysferlin (135). How mutations in calpain lead to muscular
dystrophy is poorly understood; deregulation of sarcomere remodeling (136), membrane repair (46), cytoskeleton–membrane interaction and cytoskeleton structure (137, 138), and apoptotic cell death (139) have been proposed as potential mechanisms.

LGMD2B is a type of dysferlinopathy in which defective membrane repair may lead to extensive myonecrosis. Dysferlin is a 230-kDa transmembrane protein and, as discussed above, has been implicated in membrane patching (37). Dysferlin-deficient cells in patients and mouse models show accumulation of submembranous vesicles and membrane discontinuities, implicating a defect in vesicle-membrane fusion (140, 141). Impaired fusion and differentiation of dysferlin-null myoblasts in vitro further support a role for this protein in myogenesis (142). Interestingly, recent data indicate that genetic manipulations that correct defects in membrane resealing in certain assays may be insufficient to correct muscle pathology in dysferlin-deficient mice, raising the possibility that additional pathological mechanisms may contribute to this disease (143). Mutations in dysferlin can cause LGMD2B, Miyoshi myopathy (MM), or distal myopathy with onset in the anterior tibial muscles (144). LGMD2B patients exhibit proximal myopathy and substantially elevated CK levels, and disease onset usually occurs in the patients’ twenties. An additional symptom in LGMD2B that distinguishes it from the other LGMDs is infiltration of inflammatory immune cells into muscle (145). Interestingly, identical mutations can cause LGMD2B or MM symptoms, which suggests that genetic modifiers or environmental factors may influence disease pathology (146).

The sarcoglycans are N-glycosylated transmembrane proteins that form a heterotrimeric glycoprotein subcomplex within the DCG (147). Six sarcoglycan proteins have been cloned thus far, and the major proteins existing in the muscle sarcolemma are the α-, β-, γ-, and δ-sarcoglycans. Mutations in the γ-, α-, β-, and δ-sarcoglycans cause LGMD2C, -D, -E, and -F, respectively. Defects in any of the sarcoglycans, except γ-sarcoglycan, destabilize the entire DCG, which suggests that the α-, β-, and δ-sarcoglycans are closely associated (148, 149). Loss of dystrophin also leads to the disappearance of sarcoglycan subunits from DGC, but mutations in sarcoglycan genes do not interfere with the localization of dystrophin. Sarcoglycanopathies usually have a childhood onset and greater severity than that of other LGMDs. Cardiomyopathy is present in all sarcoglycanopathies, although it is rarer in LGMD2D (150).

The sarcomeric proteins telethonin and titin are mutated in LGMD2G and LGMD2J, respectively. Titin is an enormous protein that spans half the length of the sarcomere and provides a scaffold for myofibrils. Telethonin is a substrate of titin kinase, which binds to its N terminus and provides spatially defined binding sites for sarcomeric proteins to help assemble the sarcomere (151). LGMD2H results from deficient sarcomere recycling. TRIM32, the gene responsible for LGMD2H, encodes an ubiquitin ligase that marks sarcomeric proteins for degradation by the proteasome (152). Recently, a putative calcium-activated chloride channel, Anoctamin 5 (ANO5), was identified as the defective gene in LGMD2L. This report also suggests that ANO5 may function in the dysferlin-dependent muscle membrane repair pathway (153).

Mutations in proteins involved in dystroglycan glycosylation are known to cause CMDs, Walker–Warburg syndrome (WWS), or muscle–eye–brain disease. Nonetheless, specific mutations in some of these genes, including FKRP (LGMD2I), POMT1 (LGMD2K), fukutin (LGMD2M), POMT2 (LGMD2N), and POMGnT1 (LGMD2O), can also cause LGMD (154). LGMD2P, another type of LGMD, is caused by disruption of dystroglycan (DAG1).

**Genetic Diseases of Skeletal Muscle: Facioscapulohumeral Muscular Dystrophy**

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common form of
muscular dystrophy and was first described in 1884 (155). FSHD patients typically experience symptoms first in the skeletal muscles of the face (facio), scapula (scapulo), and upper arms (humeral) but ultimately develop progressive muscle weakness throughout the body (155). Like myotonic dystrophy, FSHD is inherited in an autosomal dominant fashion, but the genetics of the disease have proven challenging to unravel. More than 95% of FSHD cases are associated with deletion of the tandemly repeated D4Z4 units at the subtelomeric region of chromosome 4q35 (FSHD1) (156). Other, rarer forms of FSHD also exist, but the genetic bases for these variants either are dissimilar to FSHD1 or remain unclear.

Whereas normal individuals have 11–100 D4Z4 repeats at the end of chromosome 4, FSHD1 patients have 10 or fewer (157). Translocations between these repeat units and a similar repeating unit on the subtelomeric region of chromosome 10q occur frequently. Although contractions occur within the 10q repeats, these never lead to FSHD (157). A particular polymorphism found exclusively on chromosome 4, downstream of the last repeating DUX4 unit, is essential for manifestation of FSHD1, which explains why 10q contractions fail to cause disease. This polymorphism encodes a polyadenylation signal that stabilizes DUX4 transcripts that normally would be degraded (158). Thus, aberrantly stabilized expression of DUX4 may be the causative event in FSHD pathogenesis.

Overexpression of DUX4 in various cell types leads to apoptosis, but aberrant DUX4 expression has been reported in only a minority of cells. Yet, several DUX4 transcriptional targets appear to be robustly upregulated in FSHD muscle relative to control muscle, suggesting that relatively restricted DUX4 misexpression can lead to widespread changes in gene expression, possibly through the release of secreted factors and immunomodulators (159). Proposed strategies for mitigating FSHD pathology include downregulation or repression via a dominant negative splice isoform of aberrant DUX4 expression (159).

**Genetic Diseases of Skeletal Muscle: Emery–Dreifuss Muscular Dystrophy**

EDMD is caused by mutations in nuclear membrane proteins. EDMD can be X-linked or autosomal; it causes progressive muscle weakness, contractures, and cardiac defects (160). X-linked EDMD was mapped to the emerin gene (EMD) (161), and mutations in LMNA and in the nesprin-1 and nesprin-2 genes (SYNE1 and SYNE2) cause autosomal dominant and autosomal recessive EDMD, respectively (Table 1) (162–164). Emerin localizes to the inner nuclear membrane and interacts directly with nuclear lamin proteins (Figure 6) (165). Emerin also binds to barrier-to-autointegration factor (BAF), and BAF oligomers directly associate with chromatin (166). Furthermore, nuclear lamins are indirectly linked to the actin cytoskeleton via SUN and nesprin proteins (167). Thus, emerin, lamin, nesprin, and other nuclear proteins are considered to be involved in organizing chromatin structure, thereby providing a scaffold for proteins involved in gene regulation and linking the nuclear skeleton to the cytoskeleton. Both lamin and emerin are expressed in all tissues, and the particular impact of mutations in these genes on skeletal muscle is not well understood. One hypothesis (the mechanical-stress hypothesis) holds that contraction-induced forces to which striated muscles are specifically exposed cause myonuclear damage and ultimately death in muscle cells with defective nuclear membrane proteins (168). Another hypothesis (the gene-expression hypothesis) suggests that LMNA and EMD mutations alter specific chromatin sites, thereby deregulating muscle differentiation, mechanotransduction, and apoptosis-associated genes. Of course, these two hypotheses are not mutually exclusive; defects in nuclear membrane proteins may cause both increased sensitivity to contraction force and aberrant gene expression.

**Genetic Diseases of Skeletal Muscle: Congenital Muscular Dystrophies**

Infants with hypotonia, muscle weakness, histological manifestation of dystrophic
myopathy, and delayed motor development are diagnosed with CMD. CMDs are a genetically heterogeneous group of neuromuscular disorders that vary widely in severity. In some forms of CMD, neurologic features result in involvement of the eye and brain as well. On the basis of the genes found to be mutated in CMD patients thus far, and the function of their protein products, CMDs can be classified into five groups. The first and most-studied group is caused by abnormalities in the glycosylation state of the αDg protein (169). αDg undergoes extensive O-linked glycosylation, and defects in protein glycosylation interfere with its interaction with laminin (170), thereby disrupting the link between the myofiber cytoskeleton and the ECM. Mutations in six genes (LARGE, fukutin, FKRP, POMT1, POMT2, and POMGnT1) cause reduced αDg glycosylation in CMD patients (171). POMT1, POMT2, and POMGnT1 are enzymes responsible for transferring O-mannosyl glycans to αDg. LARGE, fukutin, and FKRP may also be involved in αDg glycosylation; however, a detailed understanding of their mechanism of action is lacking (172). Mutations in these genes can lead to WWS, Fukuyama CMD, or muscle–eye–brain disease, according to the severity of the effect on αDg glycosylation.

The second group of CMDs results from defects (a) in genes encoding the ECM proteins laminin-211/merosin (LAMA2) and collagen type VI (COL6A1, COL6A2, and COL6A3) or (b) in ITGA7 (which encodes integrin-α7, the merosin receptor) present on the sarcolemma (173). These genetic defects also disrupt the association between the fiber contraction machinery and the basal lamina. Allelic mutations in each of the genes encoding different subunits of collagen type VI (COL6A1, COL6A2, and COL6A3) can cause severe Ullrich CMD or mild Bethlem myopathy.

**Figure 6**

Nuclear matrix proteins involved in Emery–Dreifuss muscular dystrophy. Emerin acts as a bridge between chromatin and the nuclear lamina. Nuclear lamin interacts with the SUN dimer, which is indirectly linked to the actin cytoskeleton via nesprin. Thus, mutation of any of these genes interrupts the normal linkage between the cytoskeleton and the nuclear matrix. Abbreviation: BAF, barrier-to-autointegration factor.
Sarcopenia:

Sarcopenia, the progressive loss of skeletal muscle mass and strength as a result of advancing age, is a present and growing global health concern; it affects ~25% of individuals older than 70 and 40% of individuals older than 80. Individuals suffering from sarcopenia experience decreased independence, including a progressive loss of the ability to perform normal activities of daily living and a significantly reduced quality of life. The underlying molecular mechanisms that contribute to the onset and progression of sarcopenia remain ill defined. Studies have implicated an increase in chronic inflammation, both systemically and in the muscle tissue itself; alterations in metabolic processes that lead to increased insulin resistance and activation of catabolic pathways; accumulation of genotoxic DNA damage; mitochondrial dysfunction and oxidative stress; loss of motor neurons leading to motor-unit remodeling and denervation-induced atrophy; and deficits in satellite cells that impair the normal muscle regenerative response (reviewed in References 179 and 180). Strategies to reverse sarcopenia have focused largely on behavioral interventions. Introduction of resistance or endurance training slows sarcopenia in elderly subjects, enhancing muscle function and even increasing the apparent content of muscle satellite cells (181). Hormone therapy also has proven effective in some instances, which has prompted the initiation of large-scale human trials to evaluate the impact of testosterone supplementation on muscle function. Finally, studies aimed at understanding the age-related decline in muscle regenerative potential implicate both local influences [including Notch signaling (182) and TGF-β (183)] and systemic influences (184, 185). Results from these studies could eventually be translated into pharmacological interventions to boost muscle repair potential in elderly individuals.

THERAPEUTIC POSSIBILITIES FOR MUSCLE WASTING DISEASES

Current treatment options for muscular dystrophy are disappointingly limited and focus mainly on managing symptoms and suppressing the immune and inflammatory response (186, 187). Therapeutic approaches that aim instead to cure these disorders have been a subject of research for many decades and can be grouped broadly into two categories on the basis of their strategic approach. The first category seeks to repair or replace the mutated gene, whereas the second aims to reduce the impact of the mutation by activating alternative pathways or intervening downstream to correct the pathological consequences. Each of these strategies presents unique advantages and challenges, and past experiences have helped inform and focus the direction of future research and the design of future clinical trials. Here we discuss several promising therapeutic avenues, including cell transplantation, gene supplementation or correction, and oligonucleotide and small-molecule delivery, each of which has been considered as a basis for curative treatment of muscle disease.
Therapeutic Possibilities: Cell Transplantation

Because satellite cells represent a robust and exclusive source of new myofibers during normal muscle regeneration, these cells and their derivatives have long been considered attractive targets for cell-replacement therapy in muscle. In this approach, cells from an unaffected donor, or gene-corrected autologous cells (see the section titled Therapeutic Possibilities: Gene Supplementation or Correction), could be infused into patients, where they would presumably produce donor-engrafted muscle fibers carrying the normal allele of the affected gene, thereby reconstituting gene function. Indeed, this strategy of precursor cell transplantation has been successful in the treatment of some hematopoietic disorders, wherein bone marrow transplantation now represents a relatively common (although certainly not risk-free) clinical intervention. However, limitations in the numbers of satellite cells that can be obtained from human muscle, and the lack of viable methods to expand these cells ex vivo, have thus far restricted clinical application of this approach and have simultaneously spurred consideration of alternative sources of cells for transplantation. Early clinical trials evaluated the efficacy of transplanted myoblasts, generated by long-term culture from explants of donor muscle and injected directly into the muscle. However, these trials yielded largely disappointing results (188), perhaps due to significant cell loss upon transplantation, caused by the death of up to 90% of the transferred cells within days of transplantation (189). Progress in the ability to isolate and expand primitive satellite cells, which may show enhanced survival ability after transplantation (92), will probably be essential in reinvigorating this conceptually attractive therapeutic approach. Also, such enhancements in satellite cell acquisition should be coupled with improvements in cell-delivery strategies, given that satellite cells cannot currently be delivered systemically and do not migrate far from the site of intramuscular injection. These limitations pose a daunting challenge for the delivery of donor cells to affected muscles throughout the body.

In addition to satellite cells, non-satellite cell populations that reside in muscle have been considered as alternative cell-therapy vehicles. Particularly encouraging (190) have been studies in dog models with cultured mesangioblasts, which may be related to blood vessel-associated pericytes (191), and exhibit broad differentiation potential in culture, including production of cells expressing markers of the skeletal muscle, smooth muscle, and vascular lineages (191–193). Trials are ongoing to assess the efficacy of these cells in human patients. An attractive attribute of mesangioblasts, as well as the probably related populations of CD133+ (194) and muscle-derived “side-population” cells (195), for cell therapy is their apparent ability to home from the circulation into dystrophic muscle tissue (190 196), which allows them to be delivered via vascular, rather than intramuscular, injection. Similar promise for vascular delivery of muscle regenerative cells was excited by observations that transfusion of donor bone marrow cells may lead to detectable contributions of these cells in skeletal myofibers (197, 198); however, further evaluation of such approaches indicated that the rate of engraftment was far below that predicted to be necessary for therapeutic effect (82, 199). Indeed, in a DMD patient who received a bone marrow transplant for coincident severe combined immunodeficiency, although evidence of rare donor cell engraftment in muscle was found, no improvement in dystrophic phenotype could be attributed to the transplanted cells (200).

In an effort to overcome the pervasive limitations in obtaining adequate numbers of immunologically matched donor cells when working with adult somatic cells for muscle regenerative medicine, several groups have attempted to derive engraftable muscle precursor cells from pluripotent stem cell sources. Such cells, including embryonic stem cells (ESCs), derived from human embryos, and induced pluripotent stem cells (iPSCs), derived by transcription factor–dependent “reprogramming” of differentiated somatic cells,
can be propagated indefinitely in culture and can, in principle, differentiate into any cell type in the body (201, 202). Thus, a robust strategy for producing muscle precursors from these cells would provide an inexhaustible source of donor cells for transplant. Moreover, when coupled with gene-correction strategies, iPSCs generated in a patient-specific manner would produce immunologically matched donor cells that could eliminate, or at least reduce, the threat of graft destruction due to recognition by the host immune system. However, a major challenge in realizing the potential of pluripotent stem cells for skeletal muscle therapy has been the difficulty of deriving fully mature, “adult” somatic cells from ESCs or iPSCs (203). Nonetheless, important advances have been made, including the demonstration that transient induction of the satellite cell–associated transcription factors Pax3 and Pax7, or cell sorting with satellite cell–specific surface markers, in differentiating mouse ESCs or iPSCs can promote the recovery of myogenic precursors, which have been successfully engrafted in models of DMD and FSHD (204–208).

**Therapeutic Possibilities: Gene Supplementation or Correction**

Rather than relying on transplanted cells as vehicles for complementing defective alleles in muscular dystrophy patients, some investigators in the area of muscle regenerative medicine have focused instead on achieving direct gene therapy in affected muscle fibers through exogenous delivery of a normal copy of the mutated gene or, more recently, by introduction of genome-modifying nucleases that may enable in situ gene repair. Most gene-delivery approaches have employed recombinant viral vectors, particularly adenoviruses, because they can carry very large inserts (a significant challenge when attempting genetic complementation of the largest gene in the human genome!), and AAVs, because of their relatively high efficiency of transduction in skeletal muscle and their low immunogenicity (187). However, even AAVs are susceptible to antiviral host immune responses, which may necessitate host immunosuppression and can prevent repeated gene-delivery attempts (186). Attempts have also been made to produce pared-down versions of dystrophy genes (e.g., mini- and microdystrophin) that could provide at least partial restoration of gene function and enable packaging of target sequences into AAV, as well as retro- or lentiviral vectors (186). Finally, strategies that may support the transfer of entire regions of the human chromosome, including those areas that encompass the human dystrophin gene and its regulatory elements, have been pursued using human artificial chromosomes, which can be introduced into target cells and maintained episomally to support tissue-specific expression of the exogenous gene (209).

A second and emerging approach in the gene therapy realm has been to attempt direct correction of the mutated allele(s) in the patient’s own cells. This process could, in theory, be accomplished in situ or by genetic modification in autologous somatic cells or patient-specific iPSCs, which would otherwise be genetically matched to individual patients and could be transplanted therapeutically to restore gene function in patient muscles. Early efforts toward this goal have employed site-specific zinc-finger nucleases (ZFNs), which are experimentally engineered DNA-binding proteins that are modified by fusion to the Fok1 nuclease domain. Site-specific nuclease activity, directed by the ZFN DNA-binding domain, is used to induce a strand break in the target genomic sequence, which can then be repaired by nonhomologous end joining or, in the case of therapeutic gene correction, by homologous recombination with a normal donor sequence to generate a gene-corrected allele. However, despite more than 15 years of development and recent progress in the application of some of these technologies in clinical trials aimed at suppressing HIV-1 in vivo, ZFNs remain cumbersome to design and employ, due in part to the context specificity of ZFN sequences, nonspecific DNA binding that can lead to off-target gene cleavage, and a relative stranglehold on the technology by a single biotech company that has
limited ZFNs’ availability and greatly increased their cost for research and clinical studies (210). Happily, the emergence of a related technology, based on DNA-binding virulence factors produced by plant pathogens, may circumvent these obstacles. Transcription activator–like effector nucleases (TALENs), like ZFNs, exhibit sequence-specific binding to target DNA sequences; yet, unlike ZFNs, TALENs appear to be considerably more approachable in design and may show greater efficacy with lower cellular toxicity (210).

However, in all of these approaches, a persistent concern even with the use of autologous cells is that the ectopic or induced expression of a gene not normally present in patient cells could provoke an autoimmune response, which would lead to clearance of the gene-corrected cells. Indeed, some studies have supported the idea that induced expression of dystrophin can stimulate both humoral and cellular immune responses (211). Overcoming such immunological barriers represents a significant challenge for the future of gene-therapy approaches in dystrophic disease.

**Therapeutic Possibilities:**

**Recombinant Protein Administration**

Different groups have attempted direct administration of a few recombinant proteins to ameliorate DMD symptoms, mostly in the *mdx* mouse model of the disease. IGF-I is a hormone produced by many different tissues, including skeletal muscle, and is elevated in dystrophic muscle (212). IGF-I is implicated in stimulation of satellite cell proliferation and differentiation during muscle regeneration, growth, and hypertrophy (213). Administration of recombinant IGF-I protein to *mdx* mice reportedly increases the resistance of the extensor digitorum longus and soleus muscles to fatigue (214). In addition, *mdx* mice treated with recombinant IGF-I showed increased resistance of the diaphragm muscle to fatigue and enhanced specific force output compared with untreated controls (215). IGF-I administration also increased muscle force–producing capacity and size in laminin-deficient mice (216).

Proteins inhibiting myostatin also have been reported to ameliorate symptoms when administered to *mdx* mice. Myostatin (*GDF8*) is a member of the TGF-β superfamily and a negative regulator of muscle growth. Mutations in myostatin cause increased muscle mass in mice (217), cattle (218), and humans (219). Delivery of a monoclonal antibody against myostatin into *mdx* mice resulted in increased body weight, muscle mass, and strength; however, resistance to contraction-induced injury was not improved according to ex vivo evaluations (220). Another study used a myostatin propeptide to inhibit myostatin in *mdx* mice and reported improvements in muscle size and strength but not in susceptibility to eccentric contraction–induced damage (221). However, systemic or intramuscular injection of laminin-111 into *mdx* mice was reported to prevent exercise-induced muscle damage, increase the expression of integrin-α7 as part of the costamere, and reduce serum CK to normal levels (222). Laminin-111 also reportedly enhances myoblast transplantation into immunodeficient *mdx* mice when used as a coadjuvant (223).

Utrophin is the autosomal homolog of dystrophin, and these two proteins share significant structural similarities (224). Utrophin is upregulated in *mdx* mice, and utrophin overexpression both prevents and ameliorates disease symptoms in dystrophic mice (225, 226). Systemic injection of a recombinant TAT-μ-utrophin protein [a truncated form of utrophin that lacks parts of the internal rod-shaped domain and is conjugated to the arginine-rich TAT (transactivator of transcription) cell–penetrating peptide] into *mdx* mice resulted in improved histopathology, increased specific force production, and reduced force drop after eccentric exercise (227). TAT-μ-utrophin also improved pathophysiology when administered to dystrophin/utrophin double-knockout mice (228).

A recent report suggests that recombinant human biglycan (rhBGN) may upregulate utrophin in the sarcolemma in *mdx* mice (229). Biglycan is an ECM protein that binds αDg and
α- and γ-sarcoglycans and is both critical for timely muscle regeneration (230) and important for neuromuscular junction stability (231). Mdx mice treated with rhBGN also upregulated some DGC components, and interestingly, muscles from these mice showed a reduced drop in force after multiple rounds of eccentric contractions in vitro, which suggests increased resistance to contraction-induced injury.

**Therapeutic Possibilities: Oligonucleotide-Mediated Approaches**

Oligonucleotide-mediated approaches for the muscular dystrophies offer the advantage of specificity for targets and for mechanisms of action, whether based on RNA interference (RNAi), antisense, splicing modulation, or steric hindrance (Figure 7). Oligonucleotides

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**Figure 7**

Oligonucleotide-mediated therapeutic approaches for muscular dystrophies. Aside from RNA interference–mediated approaches, at least three major oligonucleotide-based approaches are under active investigation; these approaches include splice–site modulation, antisense via steric blocking, and antisense via gap-mer function. Several of these approaches have been effective in clinical and preclinical studies of Duchenne muscular dystrophy (DMD) and dystrophia myotonica type 1. Abbreviations: MBNL, Muscleblind-like family of RNA-binding protein; mRNA, messenger RNA; pre-mRNA, precursor mRNA.
have been used in the context of DMD to alter splice site usage in order to modulate the dystrophin open reading frame (232); positive results have recently been obtained in the clinic. These antisense oligonucleotides are basically designed to mask the splice site for mutated or additional exons, thereby removing these exons from the messenger RNA and creating an internally deleted protein that maintains its crucial N- and C-terminus-associated functions. Such shortened forms of dystrophin are found in BMD patients, whose disease is usually much milder than DMD. Phosphorodiamidate morpholino oligomer (PMO) and 2′-O-methylphosphorothioate (2′OMP) are two types of antisense oligonucleotides that have been used in DMD exon skipping studies and clinical trials. Both PMO and 2′OMP contain modifications that make them more biologically stable and resistant to nucleases. 2′OMPs designed to target exon 23 of the DMD gene in mdx mice restored dystrophin expression in skeletal muscle when injected intramuscularly (233) or intravascularly (234). This promising result led to testing of a 2′OMP targeting exon 51 of the DMD gene (PRO051) in clinical trials. Intramuscular injection of PRO051 resulted in dystrophin expression in 64% to 97% of muscle fibers at levels between 17% and 35% of normal fibers (235). A Phase I/II clinical trial for PRO051 has also been performed by subcutaneous injection of the compound in patients; the injections led to the expression of dystrophin in a dose-dependent manner without apparent adverse effects (236). A Phase III clinical trial of PRO051 is under way.

PMOs are effective in skipping exon 23 of the DMD gene in mdx mice (237, 238). The MDEX Consortium and Sarepta (formerly AVI BioPharma) tested the efficacy of a 30-mer morpholino (AVI-4658 or eteplirsen) in skipping exon 51 of human DMD in patients. Intramuscular injection of AVI-4658 (239) and systemic delivery of the morpholino in clinical trials led to exon 51 skipping and expression of dystrophin when higher morpholino doses were used. A Phase II clinical trial for eteplirsen is now ongoing (clinicaltrials.gov identifier: NCT01396239), and Sarepta has begun preclinical studies that use a drug to skip exon 50 of the human DMD gene (AVI-5038).

A unique feature of DMD is the leakiness of the muscle membrane, which facilitates delivery of macromolecules to skeletal muscle, a challenge that must still be overcome in many other muscular dystrophies. Exon skipping of chloride channel exon 7a has been achieved in a mouse model of myotonic dystrophy and leads to rescue of myotonia (240). Steric blocking approaches have also been successful in myotonic dystrophy in displacing MBNL from expanded CUG repeats by targeting the RNA directly with CAG repeat oligonucleotides (241, 242), and RNase H–competent gap-mers are effective in degrading expanded CUG repeat–containing transcripts (243). Still, a significant challenge facing oligonucleotide-mediated approaches is delivery; solutions to this problem could usher in a new era of rational and specific therapies.

**Therapeutic Possibilities: Small-Molecule Therapy**

The first small molecules used to treat DMD patients were anti-inflammatory compounds from the family of glucocorticoid corticosteroids. Deflazacort, prednisone, and prednisolone have been most commonly used in the clinic. In some cases, treated patients showed improved muscle strength, prolonged ambulation, and slowed disease progression; however, these interventions are not a cure for the disease, and major side effects, including hypertension, diabetes, weight gain, and cataract, present obstacles to their prescription (244, 245).

Approximately 10–15% of DMD cases are caused by mutations that introduce premature stop codons (246). Some chemicals can interact with ribosomal subunits to cause the translational machinery to skip such nonsense mutations by introducing an amino acid in those positions instead. Differences in the context of nucleotide sequence surrounding premature and normal stop codons allow for specificity of action of these compounds (247). Gentamicin, an aminoglycoside antibiotic that promotes
such ribosomal “read-through,” can induce dystrophin expression in mdx muscle to up to 20% of normal levels (248); however, this compound has not been effective in human trials (249).

A high-throughput screen of ∼800,000 chemicals, performed by PTC Therapeutics®, identified ataluren (PTC-124®) as a compound that efficiently induced nonsense mutation read-through. Ataluren, which has no structural similarity with aminoglycosides, restores dystrophin expression in cultured myotubes from mdx mice and DMD patients. When administered to mdx mice in vivo, ataluren improved muscle-specific force and resistance to contraction-induced injury and restored dystrophin expression in up to 25% of fibers (250). Phase I trials of PTC-124 revealed that the compound is well tolerated; however, three Phase II clinical trials were terminated in March 2010 when the predetermined primary outcome (the 6-min walk test) was not achieved. A Phase III clinical trial (clinicaltrials.gov identifier: NCT01247207) is currently recruiting DMD and BMD participants who have previously been exposed to ataluren.

Another small molecule, BMN-195 (SMT-C1100), emerged from a screen for chemicals that upregulate the expression of utrophin. Daily administration of BMN-195 to mdx mice ameliorated dystrophic pathology (251), but when tested in a Phase I clinical trial, plasma concentrations of this compound failed to reach the required level (see http://phx.corporate-ir.net/phoenix.zhtml?c=106657&p=irol-newsArticle&ID=1455247&highlight). However, no adverse effects were reported for BMN-195, and Summit PLC is working to get a new formulation of the compound back into clinical trials.

Recently, Kawahara et al. (252) used a zebrafish model of DMD to screen a small-molecule library to identify compounds that ameliorate dystrophic pathology in newborn fish. The most potent chemical identified in this study was aminophylline, reported to be a nonselective phosphodiesterase (PDE) inhibitor (253). Interestingly, the skeletal muscle structure of affected dystrophin-null fish was restored after treatment with aminophylline for 26 days, although no dystrophin expression was detected in the muscle. The mechanism underlying this compound’s activity is not understood, but it may relate to elevation of intracellular cyclic AMP (cAMP) levels and activation of cAMP-dependent protein kinase (252). These authors also reported that sildenafil citrate, a PDE5 inhibitor, influences muscle pathology in dystrophin-null zebrafish. Sildenafil citrate was previously shown to reverse cardiomyopathy in mdx mice, possibly via activation of cyclic GMP (cGMP)-dependent pathways (254). Tadalafil, another PDE5 inhibitor, reportedly improves the histopathology of dystrophic muscle in mdx mice when administrated prenatally (255); however, existing studies on the effects of PDE5 inhibitors on dystrophic muscle have evaluated compound administration only in the early stages of life, and it remains unclear whether these molecules can reverse disease phenotype if animals are treated after disease onset. Both sildenafil citrate and tadalafil are in clinical trials for DMD and BMD patients (clinicaltrials.gov identifiers: NCT01350154, NCT01070511, and NCT01359670).

Small-molecule approaches for the treatment of myotonic dystrophy have focused primarily on either disrupting RNA-MBNL interactions or addressing symptoms of DM. Several compounds displace MBNL from expanded CUG repeats or suppress CUG repeat-dependent pathology (256–259), and some have proven efficacious in animal models (257, 259). Approaches to identify these compounds include the use of high-throughput screening methods, such as fluorescence resonance energy transfer and fluorescence anisotropy. A modular approach also has been pioneered in which molecules that target short RNA motifs with moderate affinity are assembled onto a peptoid-like backbone to increase affinity and specificity (260). Mexiletine, a US Food and Drug Administration–approved antiarrhythmic sodium channel blocker, has been used to treat myotonia and other DM symptoms (261); a clinical trial is under way to
formally investigate mexiletine’s potentially positive effects in this context.

CONCLUSIONS AND PERSPECTIVES

The great variation in the etiology and pathology of skeletal muscle diseases presents many challenges to their efficient diagnosis and treatment; however, recent advances show promise for applying both patient-specific and generic therapies to ameliorate symptoms and, potentially, to reverse disease course. Further understanding of the molecular and biochemical bases for myofiber loss in these genetically diverse degenerative diseases will certainly help uncover new strategies for effective intervention to promote healthy muscle function throughout life.

SUMMARY POINTS

1. Damage to skeletal muscle may occur throughout life due to exercise and contraction, acute physical and chemical insult, abnormalities in the immune system, or muscle degenerative diseases.
2. Skeletal muscle has significant endogenous repair capacity, including the abilities to repair small disruptions in muscle fiber membranes and to produce new myofibers from resident muscle stem cells following injury.
3. Muscular dystrophies include a wide range of genetic muscle degenerative diseases caused by deficiencies in stability, repair, or gene-expression regulation in muscle fibers.
4. Some muscular dystrophies arise from mutations in ubiquitously expressed genes; the reasons for the muscle specificity of these genetic disruptions remain mysterious.
5. Although most muscular dystrophies are caused by mutations in protein-coding genes, myotonic dystrophy arises from expanded nucleotide repeats in noncoding RNAs, which cause pathological sequestration of proteins involved in mRNA splicing.
6. Current therapeutic approaches for muscular dystrophies are based on ectopic introduction of the normal gene, endogenous repair of the mutated gene, or amelioration of symptoms achieved by triggering alternative or redundant pathways.
7. High-throughput screening for therapeutically beneficial compounds has identified some small molecules with potential clinical applicability in muscular dystrophy patients.
8. Skipping of mutated exons in DMD has been promising, and results from clinical trials hold promise for improved therapies for many patients with relevant genetic lesions.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED


59. Identifies Pax7 as a key regulator of satellite cells; Pax7+/− skeletal muscle is depleted of satellite cells and shows defective postnatal muscle growth.

68. Shows that Pax7(high) satellite cells perform asymmetric cell divisions and that daughter cells that retain the template DNA strands express stem cell markers.


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239. Reports that intramuscular injection of AVI-4658 had no adverse effects in DMD patients and caused expression of dystrophin in injected muscles.


Contents

Pathogenesis of Langerhans Cell Histiocytosis
   Gayane Badalian-Very, Jo-Anne Vergilio, Mark Fleming, and Barrett J. Rollins ......................................................... 1

Molecular Pathophysiology of Myelodysplastic Syndromes
   R. Coleman Lindsley and Benjamin L. Ebert ................................................................. 21

The Role of Telomere Biology in Cancer
   Lifeng Xu, Shang Li, and Bradley A. Stohr ............................................................ 49

Chromosome Translocation, B Cell Lymphoma, and Activation-Induced Cytidine Deaminase
   Davide F. Robbiani and Michel C. Nussenzweig ...................................................... 79

Autophagy as a Stress-Response and Quality-Control Mechanism: Implications for Cell Injury and Human Disease
   Lyndsay Murrow and Jayanta Debnath ................................................................. 105

Pathogenesis of Antineutrophil Cytoplasmic Autoantibody–Associated Small-Vessel Vasculitis
   J. Charles Jennette, Ronald J. Falk, Peiqi Hu, and Hong Xiao ...................................... 139

Molecular Basis of Asbestos-Induced Lung Disease
   Gang Liu, Paul Cheresh, and David W. Kamp .............................................................. 161

Progressive Multifocal Leukoencephalopathy: Why Gray and White Matter
   Sarah Gheuens, Christian Wiethrich, and Igor J. Koralnik ........................................ 189

IgA Nephropathy: Molecular Mechanisms of the Disease

Host Responses in Tissue Repair and Fibrosis
   Jeremy S. Duffield, Mark Lupher, Victor J. Thannickal, and Thomas A. Wynn ................................. 241
Cellular Heterogeneity and Molecular Evolution in Cancer
Vanessa Almendro, Andriy Marusyk, and Kornelia Polyak ........................................ 277

The Immunobiology and Pathophysiology of Primary Biliary Cirrhosis
Gideon M. Hirschfield and M. Eric Gershwin ................................................................. 303

Digital Imaging in Pathology: Whole-Slide Imaging and Beyond
Farzad Ghaznavi, Andrew Evans, Anant Madabhushi, and Michael Feldman ....................... 331

Pathological and Molecular Advances in Pediatric
Low-Grade Astrocytoma
Fausto J. Rodriguez, Kah Suan Lim, Daniel Bowers, and Charles G. Eberhart .......................... 361

Diagnostic Applications of High-Throughput DNA Sequencing
Scott D. Boyd .................................................................................................................. 381

Pathogenesis of the Viral Hemorrhagic Fevers
Slobodan Paessler and David H. Walker ........................................................................... 411

Skeletal Muscle Degenerative Diseases and Strategies for Therapeutic Muscle Repair
Mohammadsharif Tabebordbar, Eric T. Wang, and Amy J. Wagers ................................. 441

The Th17 Pathway and Inflammatory Diseases of the Intestines, Lungs, and Skin
Casey T. Weaver, Charles O. Elson, Lynette A. Fouser, and Jay K. Kolls ............................ 477

Indexes
Cumulative Index of Contributing Authors, Volumes 1–8 ........................................ 513
Cumulative Index of Article Titles, Volumes 1–8 .......................................................... 517

Errata
An online log of corrections to Annual Review of Pathology: Mechanisms of Disease articles may be found at http://pathol.annualreviews.org