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Review

Fibrosis development in early-onset muscular dystrophies: Mechanisms and translational implications

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ABSTRACT

Duchenne muscular dystrophy (DMD) is one of the most devastating neuromuscular genetic diseases caused by the absence of dystrophin. The continuous episodes of muscle degeneration and regeneration in dystrophic muscle are accompanied by chronic inflammation and fibrosis deposition, which exacerbate disease progression. Thus, in addition of investigating strategies to cure the primary defect by gene/cell therapeutic strategies, increasing efforts are being placed on identifying the causes of the substitution of muscle by non-functional fibrotic tissue in DMD, aiming to attenuate its severity. Congenital muscular dystrophies (CMDs) are early-onset diseases in which muscle fibrosis is also present. Here we review the emerging findings on the mechanisms that underlie fibrogenesis in muscular dystrophies, and potential anti-fibrotic treatments.

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1. Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a progressive neuromuscular disorder linked to the X chromosome. It is a highly aggressive, fatal myopathy, with an early onset, that affects about 1 in 3500 live male births, being the most common inherited muscle disease of childhood. The afflicted boys have an average life span not extending beyond 25 years of age. There is currently no cure for DMD, so that boys with DMD only can be offered palliative care measures to prolong survival [1–3]. The degenerative nature of the

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disease means that a strategy of reversal of the process is highly unlikely to succeed; rather, a successful intervention is more likely to occur by blocking the pathogenic mechanisms. This requires an in-depth understanding of the molecular pathways, during both normal function and in misregulated situations.

DMD is caused by mutations in the dystrophin gene that prevent functional dystrophin protein from being produced (giving rise to either mutated or shortened versions of the protein) [4]. Dystrophin is a large structural protein that stabilizes the muscle fiber sarcolemma. Without it, fibers become vulnerable to contractions and undergo cycles of necrosis and repair until muscle is replaced by fat and fibrous tissue. DMD also involves failure of the muscle stem cells (also called satellite cells), which normally are quiescent and activate in response to injury to further proliferate, differentiate and fuse to form new muscle fibers, or self-renew to reconstitute the quiescent stem cell pool. However, in DMD, the constant cycles of degeneration-regeneration are thought to exhaust the satellite cell pool over time. Very interestingly, recent studies have shown that, in addition to myofibers, satellite cells express dystrophin and loss of this protein alters the regenerative capacity of these cells in dystrophic muscles, supporting the assumption of exacerbated muscle wasting in DMD by impaired regeneration owing to intrinsic satellite cell dysfunction [5].

DMD is characterized by extensive fibrosis, which is an excessive deposition of extracellular matrix (ECM) components leading to loss of tissue function due to changes in the quality and/or amount of the components of this ECM [6]. It also involves abnormal repair processes, comprising chronic cycles of myofiber necrosis and repair with a sustained infiltration of mononuclear cells in muscle tissue. The causes of fibrous tissue deposition are not well understood. Nevertheless, accumulating evidence unravels inflammation as a driver of fibrosis, with several cell types contributing to ECM accumulation. In this review, we will discuss the recent advances on this fibrogenic axis in DMD and in congenital muscular dystrophies (CMDs).

2. The EMC lifecycle

Under normal growth and repair conditions, ECM components are deposited to provide a structural scaffold for new tissue. Several growth factors promote ECM deposition, including transforming growth factor beta (TGFβ), connective tissue growth factor (CTGF), and the renin-angiotensin system (RAS). In addition to these molecules, normal muscle repair also requires factors that regulate the proteolytic degradation of the ECM during regeneration for fiber growth.

Degradation of the ECM involves the large family of matrix metalloproteinases (MMPs), which are calcium-dependent zinc-containing proteolytic enzymes. Degradation is required for facilitating migration of myogenic, inflammatory, vascular and fibroblastic cells to damaged tissue. MMPs are tightly controlled not only by their expression and release but also at the activation step. In damaged muscle, MMPs are mainly released by infiltrating cells, after which they must be activated by proteolytic cleavage of the inactive precursors, in concert with their corresponding inhibitors. The diverse MMP family includes collagenases (MMP-1, MMP-8, and MMP-13), gelatinases (MMP-2 and MMP-9) stromelysins (MMP-3, MMP-7, MMP-10 and MMP-11), membrane-type metalloproteinases (MMP-14 to -17, MMP-24, and MMP-25), and metalloelastase MMP-12 [7]. MMP signaling is essential in muscle regeneration, as demonstrated by the detrimental effect of their inhibition on satellite cell function [8]. The plasminogen activation (PA) system is an enzymatic extracellular cascade that degrades fibrin (among other molecules). The PA system is necessary for matrix turnover and cell migration during tissue repair. The zimogen plasminogen is converted into the active enzyme, plasmin, by two plasminogen activators (PAs): tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). Inhibitors of the PA system include the plasminogen activator inhibitor 1 (PAI-1) and alpha2-antiplasmin, which operate at the level of the PAs or plasmin, respectively [9]. The MMP and PA systems can interact and amplify or synergize their activities to mediate ECM remodeling during tissue repair. Both the MMPs and the PA proteolytic systems have been implicated in the regulation of inflammation, fibrosis and myogenesis during the muscle regeneration process after injury and in muscular dystrophy (see below).

3. Fibrosis and its molecular effectors in DMD

Fibrosis impairs function and reduces the overall amount of muscle tissue, and results from chronic degeneration and impaired regeneration of affected myofibers in DMD dystrophic muscle with no functional dystrophin protein. Changes in how satellite cells interact with their surrounding environment can delay muscle repair and alter regeneration and inflammation, thereby accelerating disease progression and fibrosis development.

One of the most potent fibrogenic factors is TGFβ [10,11] (see Fig. 1). This is initially generated as a latent precursor of one of the three isoforms: TGFβ1, TGFβ2, and TGFβ3 [12]. Latent TGFβ is stored in the ECM and is activated by tissue damage or specific growth signals (reviewed in [13,14]). Activated TGFβ binds to a heterodimeric complex comprised of a TGFβ type I receptor molecule (also called activin linked kinase 5, or ALK5) and a TGFβ type II receptor. TGFβ plays an important regulatory role in regenerating muscle after injury and can also be produced by infiltrating inflammatory, mesenchymal, and tissue-specific cells (reviewed in [15,16]). It is also activated in the dystrophic muscle of boys with DMD and in *mdx* mice (the mouse model of DMD) [12,17–19].

Activated TGFβ stimulates fibroblasts to produce ECM proteins, such as collagen and fibronectin. Signaling through the canonical TGFβ pathway in normal fibroblasts starts with ALK5, which phosphorylates the transcription factors Smad2 and -3, allowing these to bind the co-Smad Smad4. This Smad complex is translocated to the nucleus, where it activates transcription of profibrotic genes [20,21]. A genetic mutation that reduced Smad signaling in transgenic mice improved skeletal muscle and cardiac function in the context of dystrophy-increased TGFβ [22]. TGFβ may also signal through other intracellular transducers besides Smad2/3, such as the Ras/MEK/ERK pathway, the p38 MAPK pathway, the c-abl pathway and JNK, which then work as intracellular signaling mediators [11,23]. Through these alternative signaling pathways, gene expression can be modified in a promoter-selective fashion, as these transducers are required in divergent processes such as collagen type I expression, ECM contraction (resulting from the mechanical forces exerted by fibroblasts on the surrounding ECM), and myofibroblast differentiation [24].

The activity of TGFβ signaling can also be altered in skeletal muscle by misregulation of other interfering pathways, with detrimental results. For instance, decreasing the insulin-like growth factor (IGF) signaling in IGF-1R(+/−) heterozygous mice resulted in impaired muscle regeneration, reduced expression of MyoD and myogenin, and increased expression of TGFβ1, α-smooth muscle actin (α-SMA), and collagen I, and ultimately, in fibrosis [25]. In myoblasts, treating with IGF-1 inhibited TGFβ1-stimulated Smad3 phosphorylation and increased phosphorylated-AKT (P-AKT)–Smad3 interactions, which blocked the nuclear translocation of Smad3 and in turn reduced the expression of fibrotic genes. Conversely, reducing IGF-1R levels led to diminished levels of P-AKT, which allowed Smad3 to dissociate and be transported to