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Feature Review

Satellite Cells in Muscular Dystrophy – Lost in Polarity

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Recent findings employing the *mdx* mouse model for Duchenne muscular dystrophy (DMD) have revealed that muscle satellite stem cells play a direct role in contributing to disease etiology and progression of DMD, the most common and severe form of muscular dystrophy. Lack of dystrophin expression in DMD has critical consequences in satellite cells including an inability to establish cell polarity, abrogation of asymmetric satellite stem-cell divisions, and failure to enter the myogenic program. Thus, muscle wasting in dystrophic mice is not only caused by myofiber fragility but is exacerbated by intrinsic satellite cell dysfunction leading to impaired regeneration. Despite intense research and clinical efforts, there is still no effective cure for DMD. In this review we highlight recent research advances in DMD and discuss the current state of treatment and, importantly, how we can incorporate satellite cell-targeted therapeutic strategies to correct satellite cell dysfunction in DMD.

Trends

Dystrophic muscle stem cells (satellite cells) are intrinsically impaired and directly contribute to DMD progression in *mdx* mice, demonstrating that DMD is also a stem cell disease.

Satellite cells express high levels of dystrophin, which is expressed during cell division to mediate cell polarity. In *mdx* satellite cells, establishment of polarity is impaired, resulting in the abrogation of asymmetric cell divisions, mitotic abnormalities, and inefficient generation of myogenic progenitors.

Recent advances in genome editing, myogenic cell reprogramming, and myogenic progenitor expansion protocols have important implications for genetic and muscle stem cell transplantation therapies.

Combinatorial approaches to restore endogenous satellite cell function with therapies that relieve fibrosis and inflammation associated with muscle degeneration may offer a potential cure for DMD.

Rethinking DMD

Muscular dystrophies represent a group of heterogeneous genetic diseases that are characterized by progressive skeletal muscle degeneration and weakening. The genes most commonly affected encode for structural proteins that are important for the maintenance of muscle fiber integrity. DMD is a severely debilitating and lethal disease affecting approximately 1 in 3500 male births [1]. Early signs of motor impairment and delays in motor-related milestones manifest between the ages of 2 and 5 years. Rapid disease progression and proximal muscle weakening result in patients being wheelchair-bound by the age of 12. At around 18 years of age, patients begin to suffer from cardiomyopathy. Eventual respiratory and cardiac failure is the leading cause of death around the 2nd or 3rd decades of life [2]. While the use of corticosteroids has aided in prolonging patient survival, there is still currently no effective cure for DMD.

DMD, the gene responsible for DMD, is located on the X chromosome and encodes dystrophin, a 427 kDa rod-shaped protein [3]. Spanning 2.5 Mb and comprising 79 exons, *DMD* is the largest known human gene and consequently is prone to mutations [4]. DMD is caused by frameshifting deletions, duplications, and nonsense point mutations that result in either complete loss of expression or the production of non-functional dystrophin protein [5]. Becker muscular dystrophy (BMD), which is less common than DMD, is caused by in-frame *DMD* mutations that generate a semi-functional form of dystrophin resulting in later onset of muscle weakening and a milder disease phenotype.

Dystrophin protein is primarily expressed in skeletal and cardiac muscle and to a lesser extent in smooth muscle as well as the brain [6]. Dystrophin functions as an essential component of the large oligomeric dystrophin–glycoprotein complex (DGC) [7,8]. The DGC acts to connect the actin cytoskeleton of the myofiber to the surrounding extracellular matrix through the sarcolemma. In the absence of dystrophin DGC assembly is impaired, which weakens the muscle

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fibers and renders them highly susceptible to injury. Muscle contraction-induced stress results in constant cycles of degeneration and regeneration [9]. Eventual accumulation of inflammation and fibrosis lead to progressive muscle weakening and loss of muscle mass and function [10].

For the past 20 years the role of dystrophin and its restoration in mature muscle fibers have been the primary focus of DMD research. Shifting the current paradigm, our laboratory recently showed that dystrophin is expressed in muscle satellite stem cells where it plays a vital role in defining **cell polarity** (see Glossary) and determining asymmetric cell division [11]. This review highlights the role of **satellite cells** in DMD, how misregulated cell polarity contributes to the mechanism of disease, and what we need to consider in light of these findings as we move forward towards therapeutic treatment of DMD.

DMD Is Also a Stem Cell Disease

Satellite cells are the adult stem cells of skeletal muscle and are defined by their distinct anatomical location between the basal lamina and sarcolemma of the muscle fiber [12]. Satellite cells are responsible for postnatal muscle growth and are indispensable for regeneration in response to muscle injury [13–16]. In healthy muscle, satellite cells remain quiescent in their niche until activated by triggers such as exercise or trauma. Upon activation, satellite cells enter the cell cycle and are able to rapidly proliferate to generate myogenic progenitors, also known as myoblasts, which subsequently fuse together or with damaged myofibers to regenerate and repair the injured muscle [17].

The precise contribution of satellite cells to the mechanism of DMD disease progression has remained an outstanding question within the muscle field. Because dystrophin expression was not detected in primary myoblasts [18,19], it was presumed that satellite cells were also lacking in dystrophin expression. Thus, any effect on satellite cell dysfunction was thought to be an indirect one, emerging as a consequence of the dystrophic environment. One widely accepted view has been the concept of muscle stem cell 'exhaustion' caused by repetitive cycles of muscle degeneration and regeneration [20,21]. This model suggests that satellite cells are ultimately unable to keep up with the high regeneration demand in a dystrophic muscle context, resulting in eventual loss of regenerative capacity.

Incompatible with the stem cell exhaustion model, multiple studies have reported an increase in the number of satellite cells observed in dystrophic muscle. Analysis of muscle biopsies from DMD patients ranging from 2 to 7 years of age revealed that satellite cell numbers were elevated in dystrophic muscle compared to controls for all age groups [22]. Another study demonstrated that satellite cell content was dramatically and specifically increased in type I muscle fibers of DMD patients with advanced disease [23]. Recent studies examining single myofibers isolated from ***mdx* mice** – a commonly used mouse model for DMD harboring a naturally occurring null mutation in the *Dmd* gene [24] – also found elevated satellite cell numbers in fibers from young to old *mdx* mice relative to age-matched wild-type controls [11,25,26]. These results collectively suggest that the impaired regenerative capacity of dystrophic muscle cannot simply be due to an exhaustion of muscle stem cells.

Notably, the *mdx* mouse model has a modest dystrophic phenotype and does not accurately recapitulate human DMD disease. Early work examining *mdx* mice that lack MYOD, a critical myogenic determination factor expressed by committed satellite cells, established that *mdx:MyoD*$^{-/-}$ mutant mice display severe myopathy, thus demonstrating that loss of MYOD greatly exacerbates the *mdx* degenerative phenotype [27]. More recently, Sacco and colleagues introduced a telomerase null mutation into *mdx* mice to generate double-knockout mice that concomitantly lacked dystrophin expression and telomerase activity [21]. Remarkably, these mice exhibit severe muscular dystrophy that more closely resembles human DMD pathology.

Glossary

Apicobasal: the axis that extends from the apical to basal side of the cell or tissue.
Autophagy: a cellular pathway to degrade and recycle nutrients from intracellular organelles and proteins.
Cell polarity: the asymmetric spatial organization of cellular components within a cell.
Centrosome: the organelle from which microtubules are organized for formation of the mitotic spindle.
***In situ* proximity ligation assay:** a highly sensitive fluorescence-based method to detect protein interactions or protein modifications (e.g., protein phosphorylation) in tissue or cells.
***mdx* mice:** a mouse model for Duchenne muscular dystrophy (DMD) that arose from a spontaneous mutation generating a premature stop codon within exon 23 of the dystrophin gene. These mice exhibit robust muscle degeneration of the limb and diaphragm muscles and display a milder disease phenotype compared to human DMD patients.
Mitotic spindle: a cytoskeletal structure responsible for segregating chromosomes into two daughter cells during cell division.
Neuroblast: a neural stem cell that is able to undergo several rounds of mitosis to generate diverse type of neurons, which are postmitotic cells.
Neurocyte: any type of differentiated postmitotic nerve cell.
Par complex: an evolutionarily conserved protein complex composed of PAR3, PAR6, and aPKC that is essential for establishment of cell polarity.
Planar: the axis within the plane of the cell or tissue that is perpendicular to the apicobasal axis.
Planar cell polarity (PCP): the coordinated alignment of cellular components along the plane of the cell or tissue.
Satellite cells: adult stem cells of skeletal muscle.
Self-renewal: a fundamental property of stem cells to generate identical progeny either by symmetric or asymmetric cell divisions.
Senescence: a permanent state of cell cycle arrest.