Skeletal muscle regeneration and muscle progenitor cells

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Received: March 9, 2012 / Accepted: April 2, 2012

Abstract Skeletal muscle is the most abundant tissue in the mammalian body and is composed of multinucleated fibers that contract to generate force and movement. In addition, skeletal muscle has the ability to regenerate following severe damage by exercise, toxins or disease. Regeneration is possible because of the presence of mononucleated precursor cells called satellite cells. After injury, satellite cells are activated, proliferate, and fuse with the damaged fibers or fuse together to form new myofibers. A fraction of satellite cells self-renew and behave as muscle stem cells. Although satellite cells are the main players in muscle regeneration, a number of other cell types are also recruited to form new fibers or to modulate the behavior of satellite cells. Here we present an overview of current knowledge of regeneration focusing on muscle satellite cells and other stem cells and discussing promising stem cell therapy for diseases such as muscular dystrophy.

Keywords: skeletal muscle, regeneration, satellite cells, stem cells, muscular dystrophy

Skeletal muscle regeneration and muscle satellite cells

Normal skeletal muscle has a remarkable capacity to regenerate, and can go through rapid repair following muscle injury. This regeneration has been proposed to be mediated by satellite cells that are located beneath the basal lamina and adjacent to the plasma membrane of muscle fiber1). Normally, satellite cells comprise 2.5-6% of all nuclei of muscle fiber, and are mitotically quiescent. They are characterized by reversible mitotic arrest and reduced metabolic activity2). The paired-homeobox transcription factor, Pax7, was identified as the first quantifiable marker for muscle satellite cells in both the quiescent and activated state3). Pax7 and the closely related Pax3 play key roles in maintaining the proliferation of progenitors and preventing early differentiation. Recently, the generation of inducible-transgenic mice to conditionally remove Pax3 and Pax7 at critical time points in development and muscle regeneration has allowed for the elucidation of what functional roles they play in muscle satellite cell activation, fusion, development, and myofiber engraftment4). Pax7-deficient muscles have been demonstrated to have poor muscle regeneration following a cardiotoxin-induced skeletal muscle injury and most mutant mice expired shortly after birth, indicating that Pax7 is essential for normal skeletal muscle regeneration through the maintenance and regulation of muscle satellite cells5). Pax3-deficient mice have neural tube and cardiac chamber malformations, fail to form limb muscles, and die embryonically usually by day E10. Additionally, those same studies demonstrated that in the absence of Pax3, the paralog Pax7 failed to be induced in muscle progenitors, which implied that Pax3 was an essential regulator of Pax7 expression6).

Satellite cells also express M-cadherin, neural cell adhesion molecule 1 (NCAM1), vascular cell adhesion molecule 1 (VCAM1), c-Met, α7-integrin, and Syndecan-3/4 in mouse7). These markers are used to isolate and characterize the perspective satellite cells or myoblasts. In response to molecular triggers from exercise, injury or diseases, satellite cells are activated. Satellite cell activation involves several factors induced by damaged fibers and inflammatory cells including Leukemia inhibitory factor (LIF), Insulin growth factor (IGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF) and nitric oxide (NO)8-12).

Activated satellite cells, called myoblasts proliferate and migrate to the site of injury and fuse with themselves or damaged muscle fibers. Activation of satellite cells is regulated by the basic helix-loop-helix family of proteins (bHLH) that includes myogenic factor 5 (MYF5), myoblast determination protein (MYOD), muscle specific regulatory factor 4 (MRF4) and myogenin. The activation of satellite cells into myoblasts involves the up-regulation of Myf5 and MyoD. These factors exist during the proliferative phase. They are inactivated when myoblasts begin to differentiate into myotubes. Myf5 and MyoD drive the expression of MRF4 and myogenin, that are necessary for...
muscle cell fusion and transition into differentiation. A fraction of satellite cells self-renew and remain quiescent as satellite cells under normal physical conditions\(^1\). This is an essential capacity of satellite cells: i.e. to maintain the number of satellite cells ready to participate in repetitive muscle regeneration. One mechanism has been proposed: that asymmetric division result in the formation of daughter cells one of which returns to quiescence while the other undergoes myogenic activation and differentiation\(^1\). This regulatory mechanism is completely unknown, but seminal studies by Conboy and Rando have demonstrated that Notch signaling plays an essential role in the maintenance and activation of muscle satellite cells through interplay between Notch and its antagonist Numb. Activation of Notch1 through the cleavage of its intracellular domain by \(\gamma\)-secretase results in a proliferation and commitment of muscle satellite cells to myogenic fates and the asymmetric segregation of Numb\(^1\).

**Satellite cells and other cell types during muscle regeneration**

As stated, satellite cells are the main players in regeneration of injured muscle while various other cell types are also recruited to help form new myofibers or to modulate the behavior of satellite cells by secreting cytokines. After acute muscle injury, neutrophils and macrophages appear, beginning to invade and phagocytose muscle debris. Macrophages support satellite cell survival by cell-cell contact and the release of soluble factors\(^1\). In addition, macrophages promote satellite cell proliferation and differentiation, indicating that macrophages are important not only for the necrosis but also the induction of muscle regeneration\(^2,3\). Recent studies revealed that fibroadipogenic progenitor cells can differentiate into adipocyte as well as fibroblasts, promote the terminal differentiation of myoblasts, and prevent the adipogenic differentiation of mesenchymal stem cells\(^4,5\). This evidence strongly suggests that muscle regeneration relies on complex cellular interactions among myogenic and nonmyogenic cells.

**Myogenic progenitor cells and muscle disease**

Duchenne muscular dystrophy (DMD) is characterized by a lack of dystrophin protein at the sarcolemma of muscle fibers, resulting in progressive muscle weakness associated with chronic degeneration and regeneration of skeletal myofibers\(^21-23\). DMD has been reported to show the loss of satellite cells regenerative capacity due to continual needs for satellite cell proliferation. Transplantation of normal myogenic cells into dystrophin-deficient muscle was an attractive therapeutic approach. Indeed, it has been demonstrated that transplanted satellite cells or myoblasts contribute to muscle regeneration giving rise to skeletal muscle fibers expressing functional dystrophin protein\(^24-30\). However, myoblast transplantation has several limitations, including immune rejection, poor cellular survival rates, and limited dissemination of the injected cells. Alternative stem or progenitor cells with myogenic potential are being investigated.

In addition to satellite cells, other myogenic progenitor cells within skeletal muscle, that are multipotent and capable of differentiating into several cell types, have been identified. These include muscle side population (SP) cells\(^31,32\), muscle-derived stem cells (MDSC)\(^33\), multipo-
tent adult precursor cells (MAPC)\textsuperscript{34}, myogenic-endothelial progenitors\textsuperscript{35}, CD133\textsuperscript{+} cells,\textsuperscript{36} mesoangioblasts\textsuperscript{37}, and pericytes\textsuperscript{38}. These cells have been shown to have the potential to differentiate into skeletal muscle \textit{in vitro} and \textit{in vivo}. A specific character of these cells is that unlike satellite cells or myoblasts, they can maintain their stem-cell potential when systematically delivered and pass through vascular walls into muscles.

Muscle SP cells, which are defined as the cell fraction that efficiently effluxes Hoechst 33342 dye on FACS analysis and are thought to be multipotent, can engraft into injured muscle of mice following intravenous or intra-arterial delivery and gave rise to muscle satellite cells\textsuperscript{30}. SP cells also act as paracrine cells, and their secreted factors promote the proliferation of myogenic cells located nearby\textsuperscript{39,40}.

CD133\textsuperscript{+} cells, which normally localize in the interstitial space between muscle fibers, are able to migrate through the blood vessel wall. When injected into dystrophic deficient mice (mdx), the CD133\textsuperscript{+} cells can migrate toward myofibers, contribute to the muscle fiber repair, and restore satellite cells\textsuperscript{30}. Mesoangioblasts have been identified in the wall of the mouse embryonic dorsal aorta. These cells can proliferate extensively and differentiate into different types of mesoderm. Arterial delivery of these cells in dystrophic dogs generates many dystrophin expressing fibers, resulting in amelioration of muscle dystrophic morphology\textsuperscript{37}. Although the molecular mechanisms by which these multipotent stem cells can differentiate into myofibers remain to be worked out, it is likely that they will provide new therapeutic strategies to enhance muscle repair.

Conclusion and perspective

Despite positive results from myogenic stem cell transplantation in mice and dogs, stem cell therapy in human muscular dystrophy is still an elusive goal. However, new strategies using stem cell therapy combined with gene transfer therapy is promising. Recent studies have shown that CD133\textsuperscript{+} cells that are genetically engineered to modify the defective dystrophin gene by using exon skipping result in producing a functional protein and improving muscle functions\textsuperscript{41}. Mesoangioblasts that are corrected using a human artificial chromosome containing the entire human dystrophin gene injected into mice also result in producing a functional protein and improving muscle functions\textsuperscript{42}. Although there are still many issues to be resolved before clinical utility, stem cell based therapies for muscle injury and diseases are very promising. In addition to the characterization and isolation of myogenic progenitor cells, it is necessary to identify the mechanisms of regeneration. We hope that a better understanding of the mechanisms on muscle regeneration and myogenic progenitor cells through experimental research will provide potential cell based therapies to ameliorate muscle problems caused by injury, toxins or myopathies and ultimate care for muscle diseases.

References

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