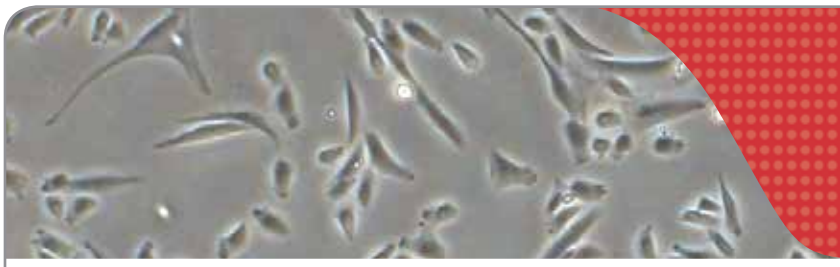

MINI-REVIEW

Myogenic Progenitor and Satellite Cells

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The Role of Satellite Cells in Myogenesis

Embryonic myogenesis in vertebrates is initiated by specific muscle progenitor cells that reside in the myotome. In mice, these events take place around embryonic day 8 concomitant with the expression of Myf5 by myogenic progenitor cells.[1-3] In post-natal muscle, myogenesis is driven by the expression of Pax3 and Pax7 by muscle satellite cells that normally reside just below the basal lamina in the stromal compartment in direct contact with the myofiber.[4-7] Satellite cells were first identified in muscle based on their localization and characteristic morphology.[8] Mauro speculated that satellite cells were dormant embryonic myoblasts that could readily recapitulate embryonic muscle development in response to injury in the adult. Several studies were reported thereafter suggesting that satellite cells were indeed able to sustain myogenic differentiation in vitro and in vivo [9-13] in response to normal growth and injury.[14-18] The self-renewal ability and myogenic differentiation potential of satellite cells were demonstrated by other groups, establishing these cells unequivocally as a population of myogenic adult stem cells.[19-21] These cells become activated and proliferate asymmetrically upon muscle injury, giving rise to myoblasts that fuse readily to existing myofibers,[22-26] as well as replenishing themselves to maintain a pool of satellite cells.[27,28]

The physical position of the satellite cell has also been shown to affect its functions. Its position along the surface of the myofiber under the basal lamina makes it prone to mechanical, electrical, and chemical signals emanating from the myofiber.[29-31] In addition, the composition of the basal lamina plays an important role in maintaining various properties associated with stem cell identity,[32] and likely plays an equally important role in maintaining satellite cell identity. The close proximity of satellite cells to the vascular structure is another important component of the satellite cell niche that allows them to be readily exposed to extrinsic factors in the circulation. Taken together, the physical location of the satellite cell within its niche likely influences the proliferative state (i.e. quiescence vs. activation) of these cells. Finally, it has been established that cell polarity, defined by the asymmetric distribution of receptors and adhesion molecules on the cell surface, dictates asymmetric satellite cell division within the niche through cell-cell and cell-ECM (extra cellular matrix) interactions.[32]

Although satellite cells are the main stem cell type involved in the formation of mature myofibers, other cell types have been shown to participate in the process. Of note are inflammatory and stromal cells. These cells appear to exert their functions through direct cell-cell contact with other cell types and through secretion of paracrine trophic factors that stimulate myogenesis. For an in depth review of these accessory cells and their functions in myogenesis, see Paylor et al.[33]

Molecular Regulation Of Satellite Cell Lineage Progression

The post-natal control of satellite cell myogenic lineage progression is strikingly similar to that observed during the embryonic stage,[34] and Pax7 has been identified as a marker of muscle satellite cells.[35] Although it's been widely demonstrated that Pax3 and Pax7 are expressed in quiescent and activated muscle satellite cells, their specific functions still need elucidation. Both Pax3 and Pax7 belong to the Pax family of proteins. They contain several domains, including an octapeptide motif, a paired domain, and a homeodomain allowing them to interact directly with DNA and other proteins.[15,36] Quiescent satellite cells express Pax3 and/or Pax7 in the absence of myogenic regulatory factors (MRFs), including Myf5 and MyoD, which are members of the basic-Helix-Loop-Helix (bHLH) transcription factors that play essential roles in execution of the myogenic program.[29] Myf5, the earliest expressed myogenic commitment gene, is a direct target of Pax3 and Pax7,[37,38] and an increase in Myf5 transcription in satellite cells marks the beginning of myogenic commitment. Regulation of Myf5 transcription by Pax7 is mediated by Carm1, an arginine methyltransferase that specifically methylates multiple arginine residues on the amino terminus of Pax7.[39] Methylated Pax7 recruits a histone 3 lysine 4 (H3K4) methyltransferase complex to regulatory enhancers and the proximal promoter of Myf5 to modulate its transcription. It has been proposed that methylation of Pax7 by Carm1 acts as a switch controlling the epigenetic induction of Myf5 during asymmetric satellite cell division and entry into the myogenic program.[39] To further demonstrate the function of Pax3 and Pax7 in vivo during mouse post natal development, Diao et al.[40] used a yeast two-hybrid screening approach. They

demonstrated that Pax3 and Pax7 binding protein (Pax3/7BP) is ubiquitously expressed in the nucleus of satellite cells and acts as a bridge for Pax7 to recruit the H3K4 methyltransferase complex. This interaction establishes a bridging system between Pax7 and WDR5 that activates and maintains proliferation of these progenitor cells. In addition, Crist et al.[41] demonstrated that in adult muscle, quiescent satellite cells are primed for myogenic differentiation by transcribing Myf5 without activating the myogenic program. In these quiescent cells, Myf5 is sequestered to mRNP granules where miR-31 regulates its translation. Upon activation of satellite cells, mRNP granules dissociate, releasing Myf5 transcripts, thereby leading to a rapid accumulation of Myf5 protein and the onset of differentiation. Upon onset of differentiation, however, Pax7 expression is downregulated. In this regard, Pax7-null satellite cells failed to enter quiescence and promoted precocious differentiation,[42] and Pax7-null mice were not able to regenerate injured muscle fibers.[43-45]

Quiescent satellite cells, on the other hand, assume a sub-basal lamina position along the myofiber and maintain a MRF^- phenotype, thereby maintaining a pool of progenitor cells. Using a Cre-mediated lineage tracing system, it was demonstrated that satellite cells are heterogeneous; 10% are $Pax7^+/Myf5^-$, and 90% are $Pax7^+/Myf5^+$.[20] Further, this study demonstrated that $Pax7^+/Myf5^-$ cells could give rise to $Pax7^+/Myf5^+$ cells, suggesting a lineage relationship between these cells. Whereas $Pax7^+/Myf5^-$ cells were mostly involved in proliferation and maintenance of a pool of satellite cells, $Pax7^+/Myf5^+$ cells readily differentiated by upregulating the expression of MyoD. $MyoD^+$ cells gave rise to mononucleated myoblasts, which in turn downregulated expression of Pax7 and upregulated expression of Myogenin[25,26] and MyHC. $Myogenin^+/MyHC^+$ cells gave rise to myocytes that then fused together to form multinucleated myofibers. Taken together, the temporal expression of Pax3/Pax7 Myf5 MyoD Myogenin MyHC establishes the transcriptional network controlling lineage commitment, specification, progression, and differentiation of satellite cells.

Extrinsic Factors Regulating Satellite Cell Proliferation and Differentiation

Notch signaling has been implicated in several stages of myogenesis from embryonic myogenesis, to adult muscle formation and regeneration, to satellite cell function[46] as well in C2C12 cells.[47] In terms of satellite cell function, Notch signaling seems to promote quiescence. Notch3 is expressed in quiescent $Pax7^+/Myf5^-$ satellite cells, whereas its ligand, Delta1, is expressed in activated $Pax7^+/Myf5^+$ satellite cells.[20,48] Downregulation of Notch signaling through an RBP-J knockout completely ablated embryonic and post-natal satellite cells,[46,49] whereas downregulation of Delta1 led to a marked decrease in the number of satellite cells.[50]

Wnt signaling has also been shown to control myogenesis by upregulating Pax3, Myf5, and MyoD.[51–53] In aged muscles, however, activation of the Wnt signaling pathway led to a reduced maintenance of the satellite cell pool and a decrease in their differentiation ability by promoting fibrosis.[54]

Several other factors have been shown to regulate satellite cell biology, including NFκB, insulin growth factor (IGF), nitric oxide (NO), and myostatin.[55-58] Of particular interest, myostatin, a member of the transforming growth factor (TGF)-β family has a negative impact on muscle growth and regeneration. In this regard, downregulation of myostatin activity led to up to a 4-fold increase in muscle size.[57,59] Although the mechanism of myostatin activity is mostly unknown, there is evidence that myostatin is expressed by satellite cells to enhance self-renewal.[60,61] More recently, the reduced regenerative capacity of skeletal muscle in older mice was attributed to higher expression of JAK/STAT downstream targets. Targeted knockdown or pharmacological inhibition of Jak2 or Stat3 stimulated symmetric expansion of satellite cells and their engraftment in vivo.[62]

Myogenic Stem Cell-Based Therapy:

Stem cell therapy is attractive due to the ability of these cells to differentiate into multiple specialized cell types and their ability to self-renew, thereby providing a long-term effect. Over the years, many neuromuscular disorders that lead to muscular dystrophies have been reported.[63] Although the causes of these clinical and genetic dystrophies vary widely, a common effect is muscle wasting leading to atrophy. The most common dystrophy is Duchenne muscular dystrophy (DMD) caused by a mutation in the dystrophyn gene. DMD affects approximately 1 in 5,000 live male births.[64,65] DMD patients lose their mobility early in life, usually around their teenage years, and death by cardiac arrest or respiratory failure is usually the final outcome. Sarcopenia is another muscular dystrophy that affects the aging population leading to skeletal muscle loss and metabolic deregulation.[66]

Some of the first studies looking at implantation of minced adult muscle back into the muscle were promising and demonstrated that new myofibers originating from implanted cells were morphologically and functionally similar to those already present in the host muscle.[67,68] When similar transplantation experiments were performed using young minced muscle and aged hosts, and vice versa, muscle regeneration was only observed in young hosts,[69,70] thus suggesting an impact of aging on muscle regeneration and a role of the microenvironment in controlling skeletal muscle regeneration. These studies pioneered muscle cell-therapy for the treatment of muscular dystrophies. However, initial studies focused on derivation and expansion of adult myoblasts ex vivo before transplantation.[71–73] As a result, clinical trials performed in DMD patients resulted in little or no

improvement on strength of the treated muscle.[74,75] The poor outcome was attributed to the poor ability of these adult myoblasts to migrate beyond the injection site, and the poor survival of the injected cells.[76,77]

More recently, as an alternative to transplantation of myoblasts, researchers have turned to exploring the use of stem cells for muscle cell-based therapy. The self-renewal ability and myogenic potential of satellite cells make them a strong contender for stem cell therapy to treat muscle wasting and other muscular diseases. However, the inability to deliver these cells efficiently by intravenous injection, coupled with their poor survival and homing ability pose a serious limitation to their clinical use. Nevertheless, other populations with stem cell-like properties have been shown to have myogenic regenerative properties following injury. Mesoangioblasts, for example, are stem cells that normally associate with blood vessels and have a strong myogenic potential. They were shown to contribute to functional muscle fibers and increased mobility after intra-arterial injection into dystrophic muscles of dogs and mice.[78,79] Pericytes isolated from human skeletal muscle have also been shown to differentiate into myofibers after intra-arterial injection. This appears to occur more efficiently than with satellite cells,[80] presumably because, unlike satellite cells, pericytes are able to cross the endothelial barrier. Furthermore, some pericyte-derived cells were found occupying the physical position of satellite cells and to express the satellite cell marker M-cadherin, suggesting that pericytes are able to reconstitute the satellite cell compartment.[80] Nonetheless, the use of pericytes in cell-based therapy comes with some risks. These cells have multi-lineage potential and can differentiate into several mesenchymal tissues, including bone.[81,82] The implantation of these cells may increase the risk of arterial and muscle calcification, or lead towards their differentiation into non-muscle lineages.

As for the use of satellite cells in regenerative medicine, researchers have shown that when single myofibers containing no more than 7 satellite cells were transplanted into a host, more than 100 new muscle fibers could be generated in the engrafted area.[19] Reporter systems have also been used to isolate these cells for regenerative studies. The Pax3-GFP mouse, for example, allows for isolation of satellite cells by flow cytometry.[21] These cells were shown to express Pax7, to contribute to repair of the muscle fiber and to replenish the satellite cell pool in the engrafted area of dystrophic recipients. The Myf5-luciferase mouse allows for isolation of luciferase-positive satellite cells that were shown to proliferate well and to contribute to muscle fibers after intra-muscular injection into host muscle.[83] These implanted cells were able to contribute to the satellite cell compartment and were capable of self-renewal as demonstrated by re-isolation of $Pax7^+$/$Luciferase^+$ cells from engrafted animals. Finally, the generation of the Pax7-reporter mouse has also aided in the isolation of these cells[84,85] and allowed for better understanding of their regulation and function.[39,86,87]

Other stem cells have also been exploited for transplantation and regeneration of dystrophic muscle, including muscle-derived stem cells, mesoangioblasts, pericytes, embryonic stem cells, and induced-pluripotent stem cells.[88] These cells are beyond the scope of this review and won't be discussed here.

Methods for Isolation and Culture of Skeletal Muscle Satellite Cells

Deserved attention has been given to the understanding of satellite cell biology and their use in muscle regenerative medicine. Methods for isolation, and in vitro culture and expansion of skeletal muscle satellite cells have become standardized and are common practice in myogenic stem and progenitor cell laboratories. An excellent review of muscle satellite and progenitor cell isolation approaches, and alternative protocols to enrich and analyze these cells is presented by Danoviz and Yablonka-Reuveni[89] and in various other publications.[90–93]

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