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Review

Skeletal muscle stem cells

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Abstract

Satellite cells are myogenic stem cells responsible for the post-natal growth, repair and maintenance of skeletal muscle. This review focuses on the basic biology of the satellite cell with emphasis on its role in muscle repair and parallels between embryonic myogenesis and muscle regeneration. Recent advances have altered the long-standing view of the satellite cell as a committed myogenic stem cell derived directly from the fetal myoblast. The experimental basis for this evolving perspective will be highlighted as will the relationship between the satellite cell and other newly discovered muscle stem cell populations. Finally, advances and prospects for cell-based therapies for muscular dystrophies will be addressed.

Introduction

Skeletal muscle is subject to constant injury resulting from weight bearing, exercise, and trauma, thereby requiring an ever-available, renewable source of cells for muscle repair and regeneration. Since its identification, the satellite cell has been a popular candidate for the adult skeletal muscle "stem cell" [1]. Residing dormant beneath the basal lamina of mature skeletal muscle fibers, this cell is ideally located for timely repair of degenerating muscle fibers. Additionally, these quiescent cells are activated to proliferate upon muscle injury, a necessary step towards generating sufficient numbers of myoblasts for muscle differentiation and myotube formation. However, the identification of multiple stem cell populations resident in skeletal muscle has added further complexity to understanding the process of muscle regeneration. In this minireview, we will briefly examine the molecular and morphological characteristics of the satellite cell, its role in muscle regeneration, and discuss outstanding questions regarding its origin, developmental potential, and uses in myoblast therapy.

Muscle Regeneration Parallels Myogenesis in the Embryo

Although the developmental origin of satellite cells remains unknown, in vertebrates, the majority of skeletal muscle progenitors arise in the somites. Somites are transient epithelial spheres that pinch off of the paraxial mesoderm lining both sides of the neural tube. Myogenic precursors are first identified in the dermomyotome, an epithelial layer located in the dorsal compartment of the somite. These precursors are characterized by their expression of the paired box transcription factors Pax-3 and Pax-7; in response to signals such as Wnts and Sonic hedgehog from surrounding embryonic structures, the myogenic determination genes *Myf-5* and *MyoD* are activated [3]. Coinciding with the down-regulation of *Pax* gene expression, muscle precursor cells committed to the skeletal muscle lineage (myoblasts) translocate to the subjacent myotome, where the muscle regulatory factors Myogenin and MRF4 direct differentiation and fusion into multinucleated myofibers.Satellite cells are first apparent towards the end of embryogenesis, and function as a pri-

mary source for the myogenic cells required for post-natal muscle growth [2].

In adult muscles, dormant, *Pax-7*-expressing satellite cells reside between the plasmalemma and basal lamina at frequencies that vary with age, muscle fiber type, and species [4]. The activation of satellite cells in vivo can be induced by muscle fiber injury brought on by acute injury [5-7], exercise [8-10], and denervation [11]. Upon injury, satellite cells are stimulated to re-enter the cell cycle to generate a pool of proliferating myogenic precursors analogous to the embryonic myoblasts, while the inflammatory response mounted by the immune system clears affected myofibers [2]. Recently, certain Wnt-family members were found to be up-regulated in muscle following injury, suggesting a parallel to myogenic signaling pathways in the embryo [12]. Additionally, up-regulation of *Myf-5* and *MyoD* occurs at the injury site in proliferating satellite cells indicating cell commitment [13-17]. *Pax-7* expression declines with the up-regulation of *MRF-4* and *Myogenin*, and differentiated myocytes fuse to new and existing fibers as part of the repair process. One of the hallmarks of regenerating myofibers is the centrally located position of the myonuclei; upon maturing, muscle fiber nuclei are located along the cell periphery [4]. Notably, repeated cycles of injury and regeneration do not appear to deplete satellite cell numbers, suggesting that these cells have the ability to self-renew [2].

Fiber- and Age-related Variation in Satellite Cell Frequency

Satellite cells were initially identified in frog leg muscles by electron microscopy [1], and subsequently have been identified in all higher vertebrates. In humans and mice, these quiescent [18], non-fibrillar, mononuclear cells are most plentiful at birth (estimated at 32% of sublaminar nuclei) [19]. The frequency declines post-natally, stabilizing to between 1 to 5% of skeletal muscle nuclei in adult mice [2]. Satellite cell frequency varies in different muscles, likely as a function of variation in fiber type composition (i.e. slow oxidative, fast oxidative, or fast glycolytic fibers). For example, the mouse soleus muscle, which is predominantly made up of slow oxidative fibers, has a higher number of satellite cells than the extensor digitorum longus (EDL) muscle, which primarily contains fast glycolytic fibers. Additionally, the absolute numbers of satellite cells increases in the soleus but not the EDL between 1 and 12 months of age, although the proportion of satellite cells decreases in both muscle types with increasing age [20]. In humans, the proportion of satellite cells in skeletal muscles also decreases with age, which may explain the decreased efficiency of muscle regeneration in older subjects [21]. Satellite cells from aged muscle also display reduced proliferative and fusion capacity, as well as a tendency to accumulate fat, all of which likely contribute to deteriorating regeneration capability [22,23]. That endurance training can offset the decline in satellite cell number with age suggests that poorer regeneration is not simply a result of limited replicative potential of older satellite cells [24].

Molecular Basis of Satellite Cell Activation and Differentiation

Several signals and growth factors have been implicated in promotion of satellite cell activation and proliferation (Figure 1). For example, the Notch signaling pathway, which is activated upon muscle injury, regulates satellite cell transition from quiescence to proliferation in single fiber cultures, thereby expanding the myoblast population in injured muscle [25]. Basic fibroblast growth factor (bFGF) stimulates satellite cell proliferation while inhibiting differentiation [2]. bFGF also promotes muscle regeneration in mdx mice [26], which undergo repeated cycles of degeneration and regeneration resulting from a mutation in the *dystrophin* gene; in humans, deficiency of dystrophin causes Duchenne muscular dystrophy [27,28]. In addition to expressing all known FGF receptors [29,30], satellite cells also express the tyrosine kinase receptor c-met [16,31]. The c-met ligand, hepatocyte growth factor/scatter factor (HGF/SF), is also a known activator of satellite cells [29,32].

Targeted deletion of the gene encoding the Forkhead/winged helix transcription factor Foxk1 [previously known as myocyte nuclear factor (MNF)], which is expressed in quiescent satellite cells, causes a severely runted phenotype, and cardiotoxin-induced muscle regeneration is delayed and accompanied by prominent accumulation of adipose cells, suggesting a defect in skeletal muscle commitment [33]. Interestingly, the myopathy associated with the *Foxk1* mutant is rescued when bred into a *p21*-null background. *p21* is up-regulated in *Foxk1*-null muscles, and while mice lacking this cyclin-dependent kinase inhibitor show a defect in satellite cell differentiation, double mutants exhibit normal muscle growth and regeneration, suggesting that p21 is a downstream target of *Foxk1* [34,35].

The muscle determination gene *MyoD* is also required for normal muscle regeneration [36]. Regenerating muscles in *MyoD*-null animals accumulate high numbers of mononuclear cells and have few differentiated myotubes; this phenotype is exacerbated in an mdx background, with $MyoD^{-/-}$; mdx muscles exhibiting severely reduced cross-sectional area and mass. *MyoD*-null animals exhibit increased numbers of satellite cells, suggesting that the cells fail to progress through the differentiation program and instead participate in self-renewal [36]. The abnormal proliferation observed with *MyoD*-null adult myoblasts and failure to up-regulate the muscle differentiation fac-

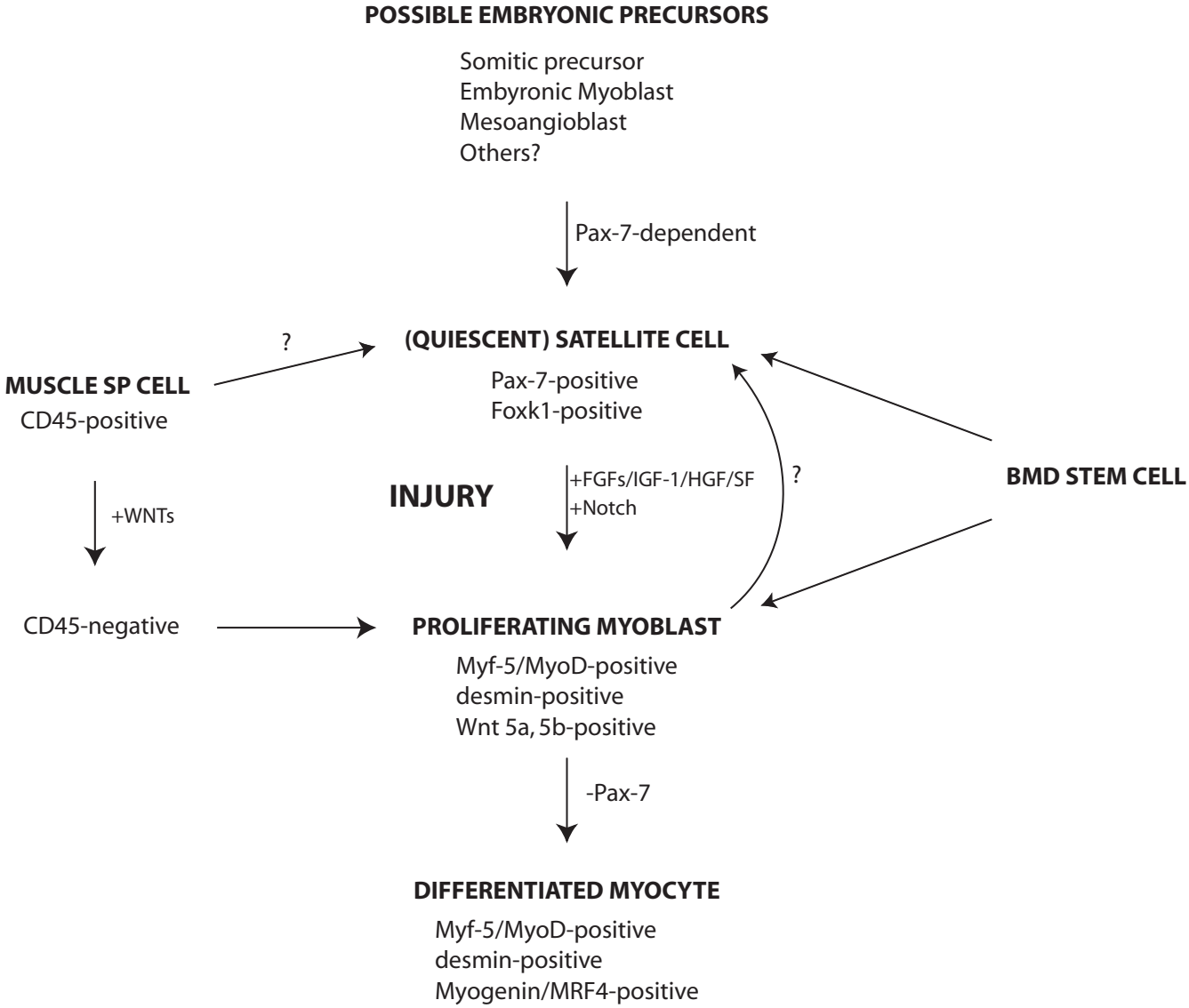

Figure 1
Model for the development, activation, and maintenance of the satellite cell. Upon skeletal muscle injury, quiescent satellite cells expressing *Pax-7* and *Foxk1* are activated to proliferate, up-regulating the myogenic determination factors, *MyoD* and *Myf-5* [13-17], the myoblast marker *desmin* [16,79], and *Wnts 5a* and *5b* [12]. Satellite cell activation is regulated by the Notch signaling pathway [25], and proliferation is stimulated by a number of growth factors, including basic FGF, insulin-like growth factor-1, and HGF/SF [26,32,80]. Transition from proliferation to differentiation, which is accompanied by the down-regulation of *Pax-7* [57] and up-regulation of *Myogenin* and *MRF-4* [13,14,16], is dependent on both *MyoD* [36] and the Foxk1 pathway [33,34]. Candidate satellite cell progenitors, which must activate *Pax-7* for satellite cell development [57], include embryonic myoblast precursors, fetal myoblasts, and vessel-associated mesoangioblasts, the latter of which exhibits strong myogenic potential [60-62]. Additionally, bone marrow-derived (BMD) stem cells can contribute directly to quiescent satellite cells and regenerating muscle fibers following injury [54,65,68], and muscle SP cells have been used with some success in myoblast transplantation experiments into dystrophic muscle [53,54]. Importantly, members of the Wnt family of secreted glycoproteins can convert SP cells favoring the hematopoietic fate into highly myogenic cells [12].

tors MRF-4 or Myogenin under differentiation conditions support this hypothesis [37,38]. In addition, *MyoD*-null satellite cells express increased levels of *Myf-5* [37,38]. In embryos lacking *MyoD*, myogenesis is dependent on *Myf-5* and vice versa: while single mutant embryos have normal muscles at birth, *MyoD*$^{-/-}$; *Myf-5*$^{-/-}$ double mutant embryos fail to develop myoblasts or myotubes [39-41]. Given the defects in muscle regeneration observed in adult *MyoD* mutants, it is evident that the functional redundancy between *MyoD* and *Myf-5* that ultimately rescues embryonic muscle development is not sufficient to rescue myogenesis in injured muscle.

Muscle Stem Cell Plasticity

Interestingly, while traditionally thought to be committed to the skeletal muscle fate, it is now evident that muscle stem cells, including satellite cells, are multipotent. For example, bone morphogenetic protein (BMP) treatment activates osteogenic markers while down-regulating *MyoD* in C2C12 myoblasts, an immortalized cell line derived from mouse limb muscle [42,43]. Additionally, treatment with thiazolidinediones and fatty acids converts C2C12 cells to the adipogenic cell fate [44]. Primary myoblast cultures from adult muscles respond similarly to C2C12 cells in the presence of strong osteogenic and adipogenic inducers; interestingly, satellite cells derived from intact single fiber cultures (and thought to be more representative of true myogenic stem cells) spontaneously form adipocytes and osteocytes when cultured on Matrigel, a soluble basement membrane matrix lacking strong osteogenic or adipogenic signals [45]. The finding that undifferentiated cells in adult myoblast cultures co-express *MyoD*, *Runx2*, and *PPARγ*, key regulators for myogenesis, osteogenesis, and adipogenesis, respectively, supports the hypothesis that satellite cells have a multipotential predisposition [46].

The plasticity of muscle stem cells has also been demonstrated using ex vivo approaches. Muscle stem cells isolated via serial preplating enrich for a population of cells which, in addition to contributing to regenerating myofibers when injected directly into dystrophic muscle, are detected in differentiated vascular and nerve cells [47,48]. Furthermore, these cells, which express the myoblast markers *desmin* and *MyoD*, are sufficient to completely heal skull defects in vivo when engineered to express BMP [49]. These muscle-derived stem cells are also capable of reconstituting bone marrow in lethally irradiated mice [50].

Another muscle-based stem cell with hematopoietic potential is the muscle side population (SP) cell, which can be isolated based on its specific exclusion of the vital dye Hoechst 33342 [51]. Initially sorted from bone marrow derived (BMD) stem cells by FACS analysis and observed to possess the majority of hematopoietic stem cell activity in bone marrow [52], SP cells have since been identified in a variety of tissues, including skeletal muscle, brain, heart, spleen, kidney and lung, although they are notably absent in peripheral blood [53]. It is important to note that the relationship between these different SP populations, and whether or not they derive from a common precursor, remains to be determined. Muscle SP cells reconstitute bone marrow in lethally irradiated mdx mice, although less efficiently than BMD SP cells. Interestingly, donor-derived nuclei also appear in regenerating muscle fibers after bone marrow reconstitution, indicating a contribution by the hematopoietic system in muscle repair [51,54]. The heterogeneity of muscle stem cells is underscored by the observation that SP cells within normal, uninjured skeletal muscle can be distinguished as positive for the hematopoietic marker CD45 (and poorly myogenic) or CD45-negative (a population that readily differentiates along the myogenic pathway) [55]. The CD45-positive subpopulation of cells has also been shown to contribute to neo-vascularization in regenerating muscle, whereas the CD45-negative population does not [56]. Interestingly, *Wnts* 5a, 5b, 7a and 7b, which are up-regulated in myoblasts and myofibers of regenerating muscle, convert the normally resistant CD45-positive muscle SP fraction to the myogenic program; this property to induce a switch in fate could contribute to the recruitment of much-needed progenitors upon injury [12].

The Developmental Origin of Satellite Cells

A recent study of the *Pax-7*-null mouse revealed that this paired box transcription factor is essential for satellite cell formation. In addition to exhibiting severe muscle deficiency at birth and premature lethality, *Pax-7* mutants are completely devoid of satellite cells [57]. However, while this observation demonstrates the requirement for *Pax-7* in satellite cell formation, it remains to be seen whether the satellite cell arises from a pre-determined myoblast in the dermomyotome, a fetal myoblast, or from a non-somitic progenitor. Satellite cells may originate from specified *Pax-7*-positive cells prior to the activation of *Myf-5* and *MyoD*, and thus represent a true precursor to the myogenic lineage. Alternatively, satellite cells may arise from determined myoblasts which, instead of differentiating, continue to proliferate until withdrawing from the cell cycle and taking up residence beneath the basal lamina of myofibers. While relatively little is known about the cis regulation of the *Pax-7* gene, the extensive characterization of *Myf-5* and *MyoD* regulatory elements [3,58] can be used to determine if satellite cells originate from a *Myf-5* or *MyoD*-positive population by in vivo cell tracing. Interestingly, while *Pax-7*-null animals lack satellite cells, the muscle SP population remains intact, although exhibiting increased hematopoietic potential; Pax-7 may direct specification of pluripotent SP cells to satellite cells [57,59].

The observation that various non-muscle stem cells can participate in skeletal muscle regeneration has expanded the candidate pool for the satellite cell precursor. For example, myogenic potential has been demonstrated in vivo by mesoangioblasts, which are vessel-associated stem cells [60-62], neural stem cells [63], and, as mentioned previously, bone marrow cells [64,65].

Bone marrow cells have long been known to have myogenic potential [66,67]. Direct injection of β-galactosidase-positive bone marrow cells into cardiotoxin-injured muscle gives rise to labeled myofibers, although at a lower frequency than injected satellite cells [64]. Interestingly, bone marrow cells contribute directly to regenerating myofibers in lethally irradiated mdx bone marrow transplant recipients [68]. Surprisingly, in the absence of myogenic induction, a subset of bone marrow cells in mdx mice are positive for both early and late myogenic markers including Pax3, MyoD, and myosin heavy chain, suggesting that muscle commitment and differentiation are underway [69]. Also intriguing is the finding that GFP-labeled BMD cells take up residence beneath the basal lamina of skeletal muscle fibers in irradiated transplant recipients following injury (in this case, an exercise model), with subsequent injury provoking an increased contribution of BMD cells to regenerated muscle fibers [65]. This suggests that satellite cells are maintained in regenerating fibers through self-renewal as well as replenishment from the bone marrow. It remains to be seen what proportion of satellite cells arise anew with each round of injury, and whether multiple rounds of injury results in a complete turnover of host satellite cells with donor bone marrow cells.

Advances in Muscle Stem Cell Therapy

The use of muscle stem cells for therapeutic purposes holds much promise for treatment of diseases affecting skeletal muscle, including muscular dystrophy [70]. Dystrophic muscles that receive myoblast transplants exhibit some dystrophin-positive myofibers, and persistence of donor fibers in regenerated muscles is observed [71-73]. However, certain roadblocks hinder the efficacy of this therapy, including the limited migration of donor cells into dystrophic muscle and problems with poor donor cell survival and inefficient myogenic contribution. Advances have been made in identifying chemotactic factors and cell surface molecules that enhance the migration of transplanted cells [74-76]. In addition, careful selection of donor cells has been shown to enhance efficiency of rescue and cell survival in transplant hosts. In particular, Huard and colleagues have found that their serial preplated muscle stem cell cultures display enhanced proliferative capabilities and readily contribute to regenerating muscle while failing to trigger a strong immune response [47,77]. Furthermore, CD45-positive muscle SP cells also contribute to regenerating muscle with high efficiency [54,59].

The use of bone marrow transplants for treating muscular dystrophy has been contemplated as an alternative therapy to myoblast injection and, as mentioned previously, BMD cells do contribute to regenerating muscle. In fact, bone marrow derived nuclei have been identified in muscle biopsies from a 15-year-old patient who received a bone marrow transplant at age 1, and was diagnosed with a mild case of Duchenne muscular dystrophy at age 12 [78]. While this demonstrates the longevity of transplanted cells in muscle, it remains to be seen whether the contribution of these cells to regenerating muscle is responsible for the mild form of the patient's disease. Intriguingly, intra-arterial injection of wild-type mesoangioblasts into mice suffering from limb girdle muscle dystrophy results in complete functional recovery of all affected muscles [62]. This presents a promising solution to difficulties encountered with myoblast transplantation therapy, and makes all muscles accessible for treatment. This is especially important for the treatment of essential muscles such as the diaphragm, impairment of which results in severe respiratory problems.

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