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Heterogeneity in the muscle satellite cell population

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Abstract

Satellite cells, the adult stem cells responsible for skeletal muscle regeneration, are defined by their location between the basal lamina and the fiber sarcolemma. Increasing evidence suggests that satellite cells represent a heterogeneous population of cells with distinct embryological origin and multiple levels of biochemical and functional diversity. This review focuses on the rich diversity of the satellite cell population based on studies across species. Ultimately, a more complete characterization of the heterogeneity of satellite cells will be essential to understand the functional significance in terms of muscle growth, homeostasis, tissue repair, and aging.

Keywords

Satellite cell; heterogeneity; muscle; regeneration; lineage; stem cell

1. Introduction

Almost 50 years ago, Alex Mauro observed by electron microscopy the presence of mononucleated cells, which he termed “satellite cells”, that were intimately associated with skeletal muscle fibers of the frog [1]. Mauro noted that satellite cells (SCs) were “wedged” between the muscle fiber membrane and the basal lamina, and hypothesized that they could be involved in muscle regeneration.

SCs were subsequently identified in skeletal muscles of other vertebrates, including humans, and their involvement in muscle regeneration became unquestionable [2]. During regeneration, SCs break quiescence, undergo proliferative expansion, generate myoblasts and terminally differentiate by fusing to each other or with damaged fibers [3]. In growing muscles, SC progeny similarly fuse with existing fibers [4]. Importantly, a fraction of SCs do not terminally differentiate, but rather replenish the SC pool by self-renewal and reoccupy a position under the basal lamina [5]. SCs are rare in uninjured muscles, typically accounting for <2% of the nuclear content of muscle, but with higher estimates noted in specific muscles and species [6-7]. Nevertheless, they have remarkable proliferative potential and can efficiently repair even severely damaged muscles [8].

This historical, purely anatomical definition of SCs does not suggest any heterogeneity within the population. However, an increasing number of studies have indicated that the SC pool is more heterogeneous than originally anticipated. In this review we will provide a summary of the evidence supporting this idea, identifying different levels of heterogeneity.

2. Heterogeneity based on embryological origin

Different muscles exhibit distinct characteristics, including anatomical structure, contractile and metabolic properties, fiber composition, blood supply, pattern of innervation and embryonic origin. Moreover, they have different regenerative capacities [9] and are differentially affected in genetic disorders [10]. Numerous studies have begun to clarify the developmental, cellular and molecular bases of this diversification [11-13]. All the muscles in the trunk and limbs develop from the somites [14], whereas, in the head, only the muscles of the tongue,and some muscles of larynx and neck are believed to be somitic in origin [11]. Extraocular muscles (EOM) are derived from the prechordal and cranial paraxial mesoderm. The remaining head muscles (the branchiomeric muscles) control facial expression, pharyngeal function and jaw movements and originate from the paraxial unsegmented mesoderm and the lateral splanchnic mesoderm [11,15]. Molecular and biochemical properties distinguish head from body muscles, and there are even biochemical distinctions among muscles of the head [16]. Similarly body muscles are heterogeneous. Based on developmental and innervation pattern, it is possible to distinguish epaxial muscles of the back from hypaxial muscles, such as limb muscles and diaphragm [12,17] (Table 1). Lineage-tracing studies both in chick and mouse have disclosed differences in the origin of these different muscles groups. Importantly, extraocular, branchiomeric and somitic muscles (both in the head and in the body) follow distinct genetic programs during development [18-25].

Given the enormous diversity among muscles, it is not surprising that distinctions between the SCs residing within them would be found. In amniotes, the presence of SCs seems to be a common feature of all adult skeletal muscles, although the density of SCs differs among different muscle groups [26-28]. Beyond the simple property of number or density, evidence suggests that SCs resident in different muscle compartments are not identical but differ in terms of embryonic origin, lineage history, gene expression pattern and functional behavior in vitro and in vivo (see below).

Chick/quail chimera experiments, together with molecular and retroviral labeling experiments, showed that SCs of the limb (hypaxial) and back (epaxial) muscles are somitic in origin [29-31]. It has been proposed that cells expressing Pax3 and Pax7, which are resident in the central portion of the somitic dermomyotome, could be their embryonic ancestors [32-33]. SCs of the limb are believed to derive from $Pax3^{+}$ cells, which migrate from the lateral lip of the dermomyotome [31] and are initially negative for Pax7 but subsequently become dependent on Pax7 expression [32-36].The developmental origin of the SCs of other hypaxial muscles has not been evaluated carefully but is believed to be similar to that proposed for the hindlimbs. These observations suggest that the SCs of the body muscle derive from the same embryonic precursors as the muscles in which they reside.

Observations obtained with chick/quail transplantation experiments suggest that SCs of non-somitic head muscles also share a developmental origin with the muscles in which reside [19]. Homotopic transplantation of unsegmented quail mesoderm into the head of chick embryos revealed SCs of quail origin in newborn eye and branchiomeric muscles [19]. Additionally, a comparative lineage analysis has recently been performed by crossing several tissue-specific Cre-expressing mice with reporter lines. These analyses revealed that SCs in different compartments express different combinations of markers, suggesting distinct embryonic lineages [19]. Similar to the transplantation experiments, a strict correlation

between SCs and the muscles in which they reside is evident from these lineage studies [19] (Table 1).

2.1 Molecular characterization

Muscles of different developmental origin or fiber type composition present important differences in term of function and gene expression, identifying specific muscle “allotypes”. The categorization into different allotypes is supported by genome-wide expression analyses of whole muscles [37-38]. SCs in different muscles also differ in terms of gene expression. SCs sorted by FACS from mouse EOM express higher levels of Alx4, Pitx1, Pitx2 and Tcf21 compared to limb SCs [18]. Those from branchiomeric muscles also express higher levels of Tcf21 [18-19]. Pax3 and Lbx1 expression is lower in head SCs compared to those in limb [18]. Remarkably, EOMs are lost in mice in which the expression of Pitx2 is abolished [24-25]. In embryos in which both musculin and Tcf21 are genetically ablated, a subset of branchial muscles fails to develop [22], whereas in Pax3 and Lbx1 knock-out mice, limb myogenesis is affected [39]. Thus the differences in gene expression reflect the developmental ontology of SCs, indicating that the genetic requirements for SC generation could reflect the genetic programs governing the formation of the corresponding muscles.

Differences in gene expression have also been reported for SCs sorted from the diaphragm compared to limb muscles. In particular, SCs from the diaphragm express higher levels of Pax3 [40]. This observation correlates with evidence from mice in which the Pax3 locus drives the expression of reporter genes [34,41]. In these mice, only the SCs of the diaphragm, a subset of the forelimb and intercostal muscles, and the hindlimb gracilis muscle express the reporter [34].

2.2 Characterization in vitro and after transplantation in vivo

The finding that the molecular signature of SCs is different in different muscles raises the question whether this signature is intrinsic to the cells or depends on the environment. When SCs were sorted from adult mouse EOM, they failed to express EOM-specific markers such as Myh13 and Myh15 after differentiation in vitro [18]. Similarly, when sorted EOM SCs were transplanted into tibialis anterior (TA) muscles, they efficiently engrafted but failed to express EOM-specific markers [18]. These data suggest that the EOM SC phenotype is not dictated by intrinsic cues. However, this conclusion is challenged by the identification of a different pattern of gene expression in myogenic cell lines obtained from EOM and limb muscles [42], suggesting a hereditable EOM-specific signature.

SCs from mouse extensor digitorum longus (EDL) and masseter present differences in gene expression (i.e. Pax3, Lbx1, Tcf21) when freshly isolated and immediately assayed, and those differences are maintained when the cells are propagated in culture, again, indicating a heritable, muscle-specific signature [18-19,28]. When myoblasts isolated from feline masseter were isolated and induced to differentiate in vitro, expression of masticatory-specific genes became apparent [43]. Furthermore, when SC cultures were treated with BMP4, a greater expression of cardiac genes, such as Isl1 and Tbx20, was observed in branchiomeric compared to the limb cultures [19]. Finally, when feline (fast) masticatory muscles were transplanted into a fast limb muscle bed and allowed to regenerate and become innervated, the regenerated muscles still expressed masticatory MyHC [44]. This seems to be independent of innervation [45] and donor basal lamina [46]. Intriguingly, when the same muscles were transplanted in the slow soleus bed, only a few transplanted fibers expressed the masticatory MyHC [44]. These studies suggest that branchiomeric SCs are programmed to express a masticatory-specific set of myogenic genes, but that this program can be overridden by a slow, but not by a fast, muscle environment. A cell-autonomous signature characterizes not only the branchiomeric SCs, but also those of the diaphragm. Indeed, GFP^+ SCs sorted from the

diaphragm of $Pax3^{GFP/+}$ mice and engrafted into the GFP^{-} TA muscle retain their initial phenotype [41].

3. Heterogeneity based on fiber association

The multinucleated myofibers that comprise each muscle are, themselves, heterogeneous. On the basis of anatomical and functional properties, two major groups of fibers can be identified: extrafusal and intrafusal. Extrafusal fibers are far more numerous, are innervated by motor neurons, and are responsible for the maintenance of posture and locomotion. Within the extrafusal fiber population, further heterogeneity exists (see below). Intrafusal fibers are important for the control of muscle contraction by monitoring changes in muscle length, which are then transduced into proprioceptive signals by sensory neurons [47]. Below we will discuss heterogeneity of SCs based on their association with either extrafusal or intrafusal fibers.

3.1 Extrafusal fibers: fast vs. slow

Adult extrafusal fibers are heterogeneous based on their speed of contraction, which depends mainly on the ATPase activity of the predominant myosin isoform that is expressed, and are categorized broadly as being either “fast” or “slow” contracting fibers. In rodents, a single slow and three adult fast myosin heavy chain (MyHC) genes have been identified [48].

The characteristics of the mature fibers are indisputably influenced by extrinsic factors such as functional demands, electrical stimulation, and hormones [49]. Nevertheless, work of several investigators also suggests that fiber type is dependent, at least in part, on the intrinsic properties of the progenitors which contributed to their generation [12].

Several differences in SCs associated with fast or slow fibers/muscles have been reported, including differences in the proliferation rate and differentiation capacity of their progeny in culture [50-52]. Moreover, a higher adipogenic potential was observed in cells isolated from the slow soleus than from predominantly fast muscles in the rat [53]. Myoblasts from fast or slow muscles differ in the levels of expression of many genes, such as AChE [54], FGF-receptors [55], and Pax7 [56]. Notably, all these differences were observed in vitro and thus in absence of innervation or a specific milieu in vivo, further suggesting an intrinsic diversity of “slow” and “fast” SCs.

Many studies have evaluated the pattern of myosin expression in cultures of myotubes derived from SCs associated with fast or slow contracting fibers. Myogenic cells from avian [51,57] and rodent [50,58-59] fast muscles or fast fibers clearly give rise to myotubes expressing exclusively fast MyHC whereas cells from slow muscles/fibers give rise to myotubes expressing slow MyHC and fast MyHC. Additionally, by engineering three-dimensional muscle constructs from myogenic cells isolated from rat TA and soleus, differences in the dynamics of contraction were reported, indicating that the cells from fast and slow muscles give rise to functionally distinct differentiated muscle cells in vitro [60]. Intriguingly, it has been reported that, in contrast to birds and rodents, human fast and slow muscles/fibers give rise to myogenic clones almost all of which co-express fast and slow MyHC [61-62].

The capacity of different subpopulation of SCs to maintain a specific phenotype for multiple cycles of replication suggests an epigenetic control of SC heterogeneity. Although hereditable, epigenetic modifications can be altered under specific stimuli; the observation that quail SC-derived myoblasts can change over time in culture, giving rise to myotubes with different patterns of MyHC expression [51], and that the in vitro conditions can affect the differentiation program in rodent cells [59], reveal a certain degree of plasticity in the SCs. Indeed, transplantation experiments indicate that myoblasts can express their intrinsic program if they

differentiate alone, but that this program is overridden upon fusion with a fiber that expresses a different phenotype [63].

Nevertheless, there is considerable evidence of intrinsic differences between SCs from fast and slow muscles [64-66]. When clones of SC-derived myoblasts with the capacity to differentiate into both fast and slow myotubes in vitro were transplanted into the limb buds of developing embryos, they generated fibers co-expressing fast and slow MyHC [66]. Moreover, in experiments in which the rat soleus was injured and transplanted to the bed of the EDL, the signature of the SCs of the soleus was revealed by the presence of fibers expressing slow MyHC when analyzed after 3 months [65]. However, this signature could not be observed after 6 months, suggesting that the slow phenotype was stable, but not irreversible, and that extrinsic influences such as the innervation pattern could eventually override the intrinsic program [67]. These observations also suggest that innervation can efficiently reprogram mature regenerated fibers but not SCs themselves. Indeed, when fast or slow muscles were stimulated by slow or fast innervation patterns, respectively, a large shift of fiber-type was observed in the muscles, but the in vitro differentiation predisposition of the SCs was not changed [68].

3.2 Intrafusal fibers

Intrafusal fibers express genes specific to embryonic myogenesis [47]. They are thus believed to persist in a relatively immature state. Studies suggest that this peculiarity could reflect the existence of unique types of intrafusal myogenic progenitors with distinct differentiation programs [47]. Nevertheless, it is not known to what extent spindle fiber SCs are intrinsically predetermined. Indeed, sensory innervation seems to be important for proper spindle fiber formation and studies involving the transplantation of growing muscles indicate that intrafusal SCs show great plasticity, as their MyHC expression can be respecified towards the extrafusal muscle fiber phenotype by foreign motor innervation [69-70].

The idea of the existence of intrafusal SCs with specific characteristics is corroborated by the report of the expression of Pax3 in a subpopulation of intrafusal fiber-associated SCs [71]. It remains undetermined if this difference in Pax3 expression is intrinsic or due to the innervation of the intrafusal fibers. Similar to the SCs in the EOM [72], a small fraction of the SCs in the spindle fibers could also be mitotically active in absence of external insults. In this sense, it is interesting to note that both EOM and spindle fibers contain a particularly high density of SCs [73-74].

4. Heterogeneity based on postnatal stage

Prenatally, myogenic progenitors establish the patterning of skeletal muscle [12]. After birth, skeletal muscle undergoes robust growth associated with the general growth of the organism. During this phase, muscle fibers increase in size by the fusion of post-natal progenitors. During this phase, it is difficult to equate the sublaminar cells, which are proliferative, with the entirely quiescent SCs of mature muscle. In mature, adult muscle, SCs can be activated in response to specific stimuli and are involved in the maintenance of muscle homeostasis and in the response to external insults or changes of functional demand [75]. Although relatively stable, skeletal muscle progressively changes with age, and those changes are accompanied also by changes in the SC population [76]. Characterizations of SCs in the young (growing), adult and aged muscle environment are considered below.

4.1 Postnatal growing muscle

Sublaminar cells can first be detected during late fetal stage [77]. It is generally believed that SCs and their progeny are responsible for the postnatal growth of the muscles. This idea is supported by the observation that myogenic cells, located beneath the basal lamina, can be

frequently observed during postnatal growth. Moreover, newborn myoblasts give rise to functional SCs when transplanted in the adult muscle [78]. As in the adult (see below), distinct subsets of SCs have been proposed for growing muscle [79-80].

With regard to the presence of SCs in the neonatal period, clear differences have been observed between newborn and adult myogenic cells. Newborn myoblasts engraft less efficiently than their adult counterparts [81]. However, they regenerate fibers that are largely peripherally nucleated, whereas regenerated adult mouse skeletal muscles are normally centrally nucleated [82]. Moreover, the activation of myoblasts during neonatal growth may be different from that occurring during adult muscle regeneration [83]. Additionally, a cell-intrinsic difference between neonatal progenitors and adult SCs in their Pax7-dependency has been recently demonstrated [35].

Importantly, studies indicate that the pool of myogenic cells present in juvenile muscles is more complex than initially predicted. First, until approximately 3 weeks of age in the mouse, a subset of the myogenic progenitors in growing muscles are actively proliferating or committed to terminal differentiation [84]. This is distinct from the adult where essentially all the progenitors are quiescent, undifferentiated SCs in absence of an external injury or stimuli. Furthermore, although evidence suggests that immediately after birth the majority of myoblasts exhibit characteristics distinct from fetal myoblasts in vitro [85], it is not known when prenatal progenitors are no longer present in muscles. Finally, the recent description of myogenic $Pw1^{+}/Pax7^{-}$ interstitial cells ("PICs") in postnatal growing muscle further increases the level of complexity [86].

4.2 Biochemical and functional heterogeneity of adult SCs

There is increasing evidence that there is SC heterogeneity beyond that conferred by the muscle in which they reside or the fiber type with which they are associated. Indeed, SCs present in the same muscle and even on the same fiber can express different markers and exhibit different functions, as described below.

4.2.1 Markers—For years, electron microscopy remained the only way to identify SCs. Over the past decades, molecular markers have been found which are sufficiently specific to allow SC identification at the light microscope level in muscle sections or single fiber preparations. Some of the markers are membrane proteins, which therefore can be used to isolate SCs with FACS, although in most cases, combinations of positive and negative markers are required to obtain a high purity. Mutant mice in which the expression of reporter genes is under the control of SC-specific genes also represent a way to identify and isolate SCs. A list of recognized SC markers is provided in Table 2.

Some markers characterize specific subpopulations of SCs. For example, not all the SCs express CD34 or M-cadherin, have an active Myf5 locus, or have previously expressed Myf5 [87-88]. Only specific subsets of quiescent SCs express Itgß1 or CXCR4 [89] or the "side population" (SP) cell marker ABCG2 [90]. Single cell expression studies also suggest variability in the SC pool [91-92]. Importantly, many markers are expressed at low level and therefore some of the heterogeneity might arise due to detection problems. Indeed, different results have been obtained when different techniques have been used, as exemplified by studies of Myf5, M-cadherin and Sca1 [40,88,90,92-94]. Additionally, only in rare cases have differences in gene expression been linked with functional heterogeneity [87,89-90]. Finally, due to the complexity of the SC niche [76], some of the differences in gene expression could simply reflect transient states of SCs under the influence of local cues.

4.2.2 Activation, proliferation and lineage progression—In early studies of injured rat muscles, $desmin^{+}$, $MyoD^{+}$ and $myogenin^{+}$ myoblasts were observed within 12 hours after

injury, but SC proliferation was not detected until 24 hours [95]. This temporal pattern suggested the existence of two populations of precursor cells in adult muscles: more committed SCs, which are ready for immediate differentiation without preceding through cell division, and more immature SCs, which undergo mitosis before generating daughter cells capable of differentiating [95]. Similar to embryonic myogenic progenitors [96-97], SCs have been shown to enter the myogenic program by inducing either MyoD or Myf5 [92,98-99] (Figure 1). It is not known if SCs follow a different developmental program depending on the predominant expression of MyoD or Myf5, although the delayed differentiation reported for MyoD$^{-/-}$ myoblasts [100] and the precocious differentiation observed in Myf5$^{-/-}$ myoblasts [101] may suggest that this is the case. Several studies also indicate a variability in terms of morphology, adhesion properties, proliferation, clonogenic capacity, survival, differentiation and fusion ability in the myogenic precursors isolated by enzymatic digestion from single muscles [93, 102-105].

The capacity of SCs to differentiate into alternative lineages, such as fibrogenic, adipogenic and osteogenic lineages, has been suggested by in vitro analyses of cells associated with muscle fibers [106-112]. Only in the case of the fibrotic conversion has the SC origin been confirmed by lineage tracing, thus ruling out the possibility of contaminating cells associated to the fiber [106]. Intriguingly, recent work with mouse SCs suggests that only a fraction of SCs possess a transdifferentiation potential, thus linking intrinsic SC heterogeneity to multipotentiality [107,113]. The recent observation that in human muscles a subpopulation of cells expressing the myogenic marker CD56 and co-expressing CD34 contains cells with adipogenic potential further supports this possibility [114].

4.2.3 Self-renewal and quiescence—The idea that SCs can both terminally differentiate into multinucleated fibers and self-renew was definitively confirmed only recently by experiments in which few SCs were transplanted and shown to contribute both to muscle regeneration and to the replenishment of the undifferentiated SC pool [27]. Different models, not necessarily mutually exclusive, implying stochastic events and/or asymmetric cell divisions, can explain how the progeny of SCs can adopt these divergent fates [115].

Undifferentiated subpopulations of cells that can self-renew and give rise to terminally differentiated myotubes have been identified in cultures of single muscle precursors established from adult muscles [116-117]. Further studies on single fiber-associated SCs in suspension also confirmed that a subset of activated SCs will retain Pax7 expression, turn off the expression of MyoD, return to quiescence and thus renew the stem cell pool [118-119] (Figure 1). These studies indicate that SCs can adopt alternative fates but do not discriminate whether all SCs have this capacity or if stem-cell like properties reside only in a subset of cells.

The hypothesis that SCs are heterogeneous in terms of self-renewal is supported by evidence from transplantation experiments. Only a small subpopulation of cultured myoblasts was shown to efficiently survive transplantation and contribute to regeneration, exhibiting stem cell-like properties [120]. This subpopulation is characterized by a slow rate of replication in vitro but efficiently expands in vivo [120]. Experiments involving freshly sorted SCs or fiber associated SCs showed a high rate of successful engraftment, suggesting that the expansion of these cells in culture before transplantation reduces their regenerative capacity, possibly selecting against cells with stem-cell properties [41]. Moreover, when single sorted SCs are transplanted, only a small percentage engraft [91]. It is not known if these latter observations simply reflect a stochastic survival of the cells after transplantation or a true heterogeneity.

The behavior of muscle stem cells under specific experimental conditions further suggests the presence of subsets of SCs with different self-renewal ability. Myogenic cells that present different proliferative/regenerative potentials and that can be differentially activated by distinct

types of injury were identified on the basis of their sensitivity to irradiation [121]. The finding suggests that an altered environment possibly selects for specific subpopulations of SCs with different self-renewing ability [121].This idea was supported by the observation that the aging process may select for subpopulations of SCs with high self-renewal ability [122].

Recently, non-random DNA segregation was shown to be strongly associated with divergent fates of self-renewing SCs: in the great majority of the cases, the daughter cell inheriting the older templates retained the more immature phenotype, whereas the daughter inheriting the newer templates exhibited signs of differentiation [123-124]. The observation that asymmetric segregation of parental DNA occurs in a fraction of SCs or their progeny with frequencies ranging from as low as 7% [123] to as high as 50% [124], albeit under different experimental conditions, suggests that the ability to segregate parental DNA non-randomly may be a property of a subset of SCs.

$Cd45^{-}/Sca1^{-}/Mac1^{-}/ß1integrin^{+}/CXCR4^{+}$ SCs represent a subset of fiber-associated cells with high myogenic properties and self-renewal capacity after transplantation [89,94]. Transplanted cells not only self-renew, but also give rise to $CXCR4^{-}/ß1integrin^{-}$ and $CXCR4^{-}/ß1integrin^{+}$ cells, suggesting that $ß1integrin^{+}/CXCR4^{+}$ may be the more primitive stem cells [89]. In this sense, as the majority of the $Pax7^{+}$ cells associated with the fiber are $CXCR4^{+}$ and $ß1integrin^{+}$ [89], it would be interesting to compare their ability to self-renew with the smaller $CXCR4^{-}$ and/or $ß1integrin^{-}$ fractions. Similarly, a small fraction of SCs expressing the side population marker ABCG2 and Sca1 were identified [90]. They represent 3-10% of the Syndecan-4^{+} cells, co-express the SC marker Pax7, and apparently engraft more efficiently than the rest of the SC pool [90]. The requirement of Sprouty1 for the return to quiescence of a subset of SCs after injury further suggest the presence of distinct fractions in the pool of self-renewing SCs [125].

Based on studies using mice expressing Cre under the control of the regulatory regions of Myf5 (Myf5Cre) intercrossed with Rosa26YFP reporter mice, it was concluded that approximately 10% of the $Pax7^{+}$ SCs have never expressed Myf5 by virtue of being YFP^{-} [87]. Transplantation revealed that these cells extensively contributed to the SC reservoir, whereas YFP^{+} cells only occasionally gave rise to SCs. Both YFP^{+} and YFP^{-} SCs efficiently contributed to fiber regeneration [87]. Importantly, exogenous Wnt7a stimulated the expansion of YFP^{-} “satellite stem cells” [126]. The genetic strategy, such as using Myf5Cre, represents a powerful approach to disclose SC heterogeneity, but there are several caveats that need to be borne in mind. First, YFP^{-} cells could result from inefficient recombination. Additionally, the presence of YFP does not imply that cells co-express Myf5. It is possible that a subset of the YFP^{+} SCs no longer expresses Myf5 and thus may be functionally equivalent to the YFP^{-} cells. It is also important to note that a subset of the YFP^{+} SCs in vivo could be derived from pre- or perinatal myoblasts and not from YFP^{-} SCs. Similar lineage studies with MyoDCre mice revealed that almost all the SCs are derived from myoblasts expressing MyoD pre- or perinatally [127]. These observations raise some questions about the homogeneity of the (Myf5Cre)YFP^{+} population of SCs. Future studies will determine the developmental history of SCs with regard to Myf5 and MyoD expression and whether a distinct subset is more stem-like.

4.3 Aging muscle

In aged skeletal muscles, many aspects of the regenerative response are impaired [106, 128-130]. This correlates with an age-dependent decrease in the functionality of the SCs, and possibly with a decrease in SCs numbers, although there is debate about the magnitude and functional significance of any observed changes in SC number with age [131]. The niche in which the SCs reside is profoundly modified during aging [76]. Alteration both in the local and systemic environment of SCs impacts their function, accounting for cell-extrinsic changes

in addition to any cell-intrinsic changes that accompany aging and contribute to impaired SC functionality [131]. Once established, cell-intrinsic changes may be irreversible or may in fact be reversible [132], depending on the nature of those changes. Either way, these changes could represent an additional source of SCs heterogeneity.

The transcriptional profile of SCs (or their progeny) changes with age [133]. Myogenic cells from aged muscles showed a delayed response to activation cues and an initially reduced proliferative expansion in vitro [103,130,134-137]. Intriguingly in the aged muscle, a reduced expression of the Notch-ligand Delta affects Notch signaling and results in a reduced proliferation of myogenic progenitors and impaired regeneration [130]. TGFβ, Wnt, and IGF pathways have also implicated in the impaired SCs proliferation and myogenic progression associated with the aging process [138-141]. Furthermore, impaired differentiation ability in vitro has been reported for aged SCs [122,142]. Evidence suggests an increased tendency of aged SCs to enter alternative differentiation programs by adopting an adipogenic and fibroblastic fate [106,143], and SCs may be more prone to undergo senescence or apoptosis with aging [144]. Finally, besides affecting the functionality of SCs, the aged environment possibly selects for specific subpopulations of SCs with distinct regenerative potential [122]. Collectively, these observations disclose multiple levels of diversification between muscle stem cells present in the young and old muscle.

5. Concluding remarks

An increasing amount of evidence indicates, as described herein, that SCs are heterogeneous by many criteria. Nevertheless many unanswered questions remain. How are different subpopulation of SCs related to one another from lineage and functional perspectives? Which roles do different subpopulations exert under normal conditions of growth, homeostasis and regeneration? How are the properties and functionality of SCs or specific subsets of SCs affected by pathological conditions? In the setting of diseases, extrinsic influences, different from those acting in healthy adult muscle, may impart both reversible and irreversible changes to SCs, as recently described for aging muscle [131]. These processes remain poorly understood and require further investigation.

Muscle regeneration depends primarily, if not exclusively, upon SCs. However, other cell types have been shown to have myogenic potential [145-146], although in most cases the physiological relevance remains unclear. Even more uncertain is the relationship of these cells with SCs. Intriguingly, bone marrow derived cells [147-148], SP cells [149], mesenchymal cells from the synovia [150], $AC133^{+}$ cells [151], pericytes [152], mesoangioblasts [153] and even embryonic stem cells [154] and induced pluripotent stem cells (iPS) [155] (or the progeny of all of these populations) have been found in the SC position or to express SC markers after transplantation. Although in certain cases (for example for ES cells) the capacity to generate SC-like cells is not meant to model a natural process, the physiological contribution to the SC pool by other cell types is worthy of future investigation. A better understanding of the origin and heterogeneity of SCs, together with further studies of the mechanisms driving their self-renewal, quiescence, proliferation and differentiation, will all be important steps for harnessing the potential of adult stem cells in order to apply them, ultimately, to regenerative medicine.

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Abbreviations

SC	satellite cell
EDL	extensor digitorum longus
TA	tibialis anterior
EOM	extra-ocular muscle
MyHC	myosin heavy chain

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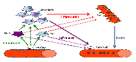

Figure 1. Schematic representation of SC myogenic progression

Upon activation, SCs (initially Pax7+/MyoD−) can enter the myogenic program by either inducing Myf5 or MyoD expression. Most of the cells subsequently express both determination genes and terminally differentiate by inducing Myogenin (Mgn+) expression and then fusing to form multinucleated fibers. A fraction of the SCs self-renew by down-regulating MyoD. Dashed lines represent possible but not proven connections. A fraction of SCs possibly differentiate without entering the cell cycle (see text). The newly formed fiber on the right is depicted as having centrally placed nuclei to reflect the process that occurs during muscle regeneration as opposed to homeostatic turnover of mature myonuclei by SCs which occurs normally at a very low level.

Table 1

Heterogeneity of SCs based on lineage history and muscle of origin

SCs resident in different muscle groups originate from different embryonic tissues and can be discriminated according to their lineage history, based on Cre/loxP-mediated cell tracing experiments. Indicated Cre driver mice express Cre recombinase under the control of the regulatory sequences of specific genes and can be used for genetic labeling of precursor cells in vivo. A "+" indicates that SCs of the individual muscle are derived from, or induced to have been derived from (see notes), progenitors of the lineage that his mapped by the specific Cre driver.

Muscle group	Developmental origin	Lineage (Cre-drivers)						References
		Myf5Cre	MyoDCre	Pax3Cre	MCre[2]	Mesp1Cre	Isl1Cre	
EOM	Cranial unsegmented mesoderm	+	+	−	−	+	−	[18-19,127]
Branchiomeric	Cranial unsegmented mesoderm	+	+[1]	−	−	+	+	[18-19]
Tongue (hypaxial)	Occipital somites	+	+[1]	+	+[1]	+	−	[19]
Limbs (hypaxial)	Somitic dermomyotome	+	+	+	+	−	−	[18-19,31, 87,127]
Inter-limb (hypaxial)	Somitic dermomyotome	+[1]	+	+[3]	+[1]	−[1]	−[1]	[127]
Back (epaxial)	Somitic dermomyotome	+[1]	+[1]	+	−	−[1]	−[1]	[30-31]

[1] Not formally proven, but deduced from lineage studies on the corresponding muscle.

[2] Hypaxial Pax3-enhancer [31].

[3] $Pax3^{nlacz}$/Pax3GFP are transcriptionally active in interlimb SCs (see text).

Table 2

Quiescent SC markers

A list of markers expressed in quiescent SCs is provided together with information regarding their expression after activation and their usage for prospective isolation by FACS.

Gene/Protein	Active SC[1]	FACS	Comments	References
Membrane				
Syndecan 3-4	+	yes (7% are CD31+)	nearly all SCs	[90,156]
Integrin-α7	+	with Itgβ1+/Lin–[2] or CD34+ /Lin–[2] or Syn4+/Lin–[2]	nearly all SCs	[87,91,125-126,157]
Integrin-β1	+	with Itga 7+/ Lin–[2] or CXCR4+/Lin–[2]	~90% of SCs	[87,89]
CXCR4	+	with1 Itgβ+ /Lin–[2]	~80% of SCs	[89,94]
CD34	+/−	with Itgα7+/ Lin–[2]	majority of SCs	[88,91,126, 158]
Vcam1	+	with Lin–[2,3]	nearly all SCs	[159-160]
SM/C2.6	+	yes		[161]
NCAM1 (CD56)	+	yes	used for FACS in human	[162]
Calcitonin receptor	~	n.t.[5]	great majority of SCs	[157,163]
Nrn1	+	n.t.[5]		[160]
Igsf4a	+	n.t.[5]		[160]
Cadherin 15 (Mcad)	+	n.t.[5]	majority of SCs	[88]
c-Met	+	n.t.[5]	nearly all SCs	[99]
Caveolin-1	+	n.t.[5]	nearly all SCs	[157]
Nucleus				
Pax7	+	$Pax7^{GFP}$	nearly all SCs	[36,93]
Pax3	+	$Pax3^{GFP}$	subsets of muscles	[41]
Myf5	+	$Myf5^{Cre}/R26R^{STOP\text{-}YFP}$	80-90% of SCs[4]	[87-88]
Foxk1 (Mnf)	+		switch of isoform during activation	[164]
Msx1	−			[92]
Sox8-9	+			[165]
Cytoplasm				
Nestin	−	$Nestin^{GFP}$	endogenous protein differs from $Nestin^{GFP}$	[40]
Desmin	+		higher in differentiation	[40,166]
Secreted				
Myostatin	~			[92,167]
Bdnf	+		64% of diaphragm SCs	[168]

[1] Expression after activation: + present; − not expressed; ~ expressed at low level or unclear;+/− switch of isoform [88] before down-regulation [158].

[2] Lin−: negative selection for CD45 (hematopoietic); CD31 (endothelial) and in certain cases Sca1 and/or CD11b.

[3] Unpublished.

[4] Data obtained with $Myf5^{nlacz}$ and $Myf5^{Cre}$ mice.

[5] Not tested.