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Review

Skeletal Muscle Stem Cells in Aging: Asymmetric/Symmetric Division Switching

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Abstract: In aged muscle, satellite cells' symmetric and asymmetric divisions are impaired, and intrinsic and extrinsic complex mechanisms govern these processes. This review presents many updated aspects regarding muscle stem cells' fate in normal and aging conditions. The balance between self-renewal and commitment divisions contributes to muscle regeneration, muscle homeostasis, aging, and disease. Stimulating muscle regeneration in aging could be a therapeutic target, but there is still a need to understand the many mechanisms that influence each other in satellite cells and their niche. We highlight here the general outlines regarding satellite cell divisions, the primary markers present in muscle stem cells, the aging aspects concerning signaling pathways involved in symmetric/asymmetric divisions, the regenerative capacity of satellite cells and their niche alteration in senescent muscle, genetics and epigenetics mechanisms implied in satellite cells aging and exercise effect on muscle regeneration in the elderly.

Keywords: satellite cells; muscle aging; asymmetric/symmetric divisions

Citation: Manole, E.; Gaina, G.; Ceafalan, L.C.; Hinescu, M.E. Skeletal Muscle Stem Cells in Aging: Asymmetric/Symmetric Division Switching. *Symmetry* **2022**, *14*, 2676. https://doi.org/10.3390/sym14122676

Academic Editors: Zonglin Li and John H. Graham

Received: 14 October 2022
Accepted: 8 December 2022
Published: 17 December 2022

1. Introduction

The satellite cells (SCs), skeletal muscle resident stem cells, are responsible for skeletal muscle growth and repair. In healthy muscle, they remain quiescent, but in response to injury or other stimuli, SCs activate, proliferate, and give rise to myoblast progenitors, which differentiate and fuse into multinucleated muscle fibers.

The asymmetric division of an SC gives rise to one stem cell, maintaining contact with basal lamina, and one differentiation competent progenitor, toward and in contact with the myofiber. Symmetric divisions are required for stem cell self-renewal, restoring the SC pool, as well as for myoblast proliferation to produce committed daughter cells for muscle regeneration [1,2]. The number of publications focusing on SC fate choice has recently increased. Data show the involvement of intrinsic and extrinsic factors in the mechanism of SC fate choice and the option for asymmetrical or symmetrical division. The study of the asymmetric SC niche has become significant, in need to establish the factors that determine the apicobasal polarity of cells and their mode of orientation [3–5]. Apico-basal divisions, perpendicular to the basal lamina, are associated with asymmetric fate [2]. Some of the regulators of this process are Notch3 and NUMB (biochemical regulators) and PAR complex (orients the mitotic spindle pole) [2,6]. Moreover, a recent study utilizing the zebrafish larval system [7] proved that this process occurs during muscle regeneration in vivo to generate the clonally related proliferative myoblast population required for muscle repair.

The symmetric divisions occur in a planar orientation, and they are regulated by WNT7a [8].

The aging process involves several intrinsic and extrinsic mechanisms that influence SC behavior. The involvement of muscle stem cells in the sarcopenia etiology is still debated [9]. It is known that the SC pool diminishes with age, but a fraction survives until very old

age [10], even with functional defects, which alter their regenerative ability. The extrinsic alterations appear first at the level from the environment (changes in signaling pathways); later, intrinsic alterations build up (genomic and metabolic impairment), which reduce SC ability to activate after injury. As a consequence, fibrous tissue build-up replaces the lost muscle fibers. Moreover, Arpke et al. showed in a comparative study on eight muscle groups in young and old mice (aged two years) that, unlike previous studies conducted only on locomotor muscles, self-renewal impairment with age is mainly acquired in the geriatric period, rather than being gradual over time, as previously thought [11].

This review aims to present an overview of the symmetrical and asymmetric divisions of SCs in aged muscle as basic mechanisms behind SC fate and balance between self-renewal and differentiation and highlight the latest research data in the field. The better we understand this phenomenon, the more we will be able to envisage possible strategies to slow down skeletal muscle degradation in aging.

2. Outline Regarding Satellite Cell Division

SCs are located underneath the basal lamina [4] in a niche next to other diverse cell types, such as immune cells, fibrogenic cells, vessel-associated cells, and myogenic differentiated cells. Muscle stem cells are mitotically quiescent in normal, inactive status, in the adult stage. After a muscle injury, the stem cells are quickly activated, proliferate, and differentiate into myoblasts. Muscle regeneration after injury is complex, and the cellular dynamics of all these types of cells occur in the stem cell niche with distinct spatial and temporal kinetics.

SCs can adopt two divergent fates, quiescent and differentiation states, for maintaining muscle tissue homeostasis. They have both symmetrical and asymmetrical divisions (Figure 1). Thus, the balance between self-renewal and differentiation to myoblasts in response to different stimuli should be maintained for efficient muscle growth or regeneration [12]. For a while, the total number of SCs remains constant after multiple cycles of regeneration [13].

During the life span, the number of satellite cells decreases, leading to a functional depreciation of skeletal muscle in the elderly [14,15]. Their regenerative potential is impaired, the maintenance of stem cell reservoir is compromised as well as the production of myoblasts; however, the precise mechanism is not yet fully understood. It has been observed in experiments on mice that SC depletion induces severe muscle damage and a reduced potential for regeneration [16]. The division pattern is altered, with many factors being implied: repetitive regeneration, cellular stress, inflammation, extracellular matrix, and stem cell niche changes. However, Shefer et al. [15] suggested that the myogenic potential of SCs is maintained with age, but their population is not fully replenished throughout life. Carlson and Conboy [14] showed that both the local environment of old differentiated skeletal muscle (with factors promoting myogenic differentiation and proliferation, which become depleted with age) and the systemic milieu (aged local niches containing inhibitors of adult stem cells and human embryonic stem cells) affect the regenerative potential of human embryonic stem cells like as mice post-natal myogenic progenitor cells.

It has been shown that the SC microenvironment plays a significant role in regulating stem cell identity and asymmetric divisions [17–19]. In several other tissues, stem cell polarity and spindle orientation relative to basal lamina establish the fate of daughter cells, having a planar orientation and symmetric divisions. Unlike these systems, in skeletal muscle stem cell divisions are asymmetric, having an apical-basal orientation and producing a stem cell (self-renewal) at the basal surface, covered by the basal lamina, and a committed daughter cell (pre-myogenic) on the apical surface, adjacent to the myofiber [17,18,20] (Figure 1).

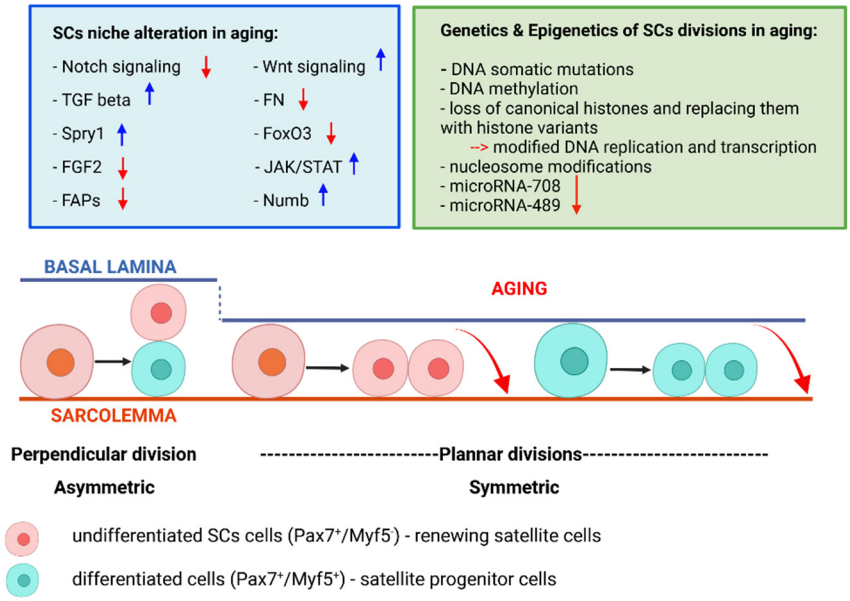

Figure 1. Satellite cell (SC) division types: asymmetric and symmetric. SC localization: between basal lamina (blue) and sarcolemma (light brown). In aging, the SC number decreases (red arrows), and as a consequence, the skeletal muscle regenerative capacity is compromised; the division pattern is changed. SC polarity relative to basal lamina establishes the fate of the daughter cells: perpendicular division, asymmetric, and planner division, symmetric. The asymmetric division has an apical-basal orientation, producing a self-renewal daughter stem cell ($Pax7^+/Myf5^-$) towards the basal lamina (pink), and a committed pre-myogenic cell ($Pax7^+/Myf5^+$) towards sarcolemma (light blue). SC symmetrical (plannar) divisions ensure their self-renewal in the niche. The committed cells give rise to the myogenic lineage. In the SC niche in aging, there are alterations of several factors, such as signaling pathways (Notch, Wnt, and JAK/STAT signaling) and proteins that are implied in signaling pathways (Spry1), growth factors (FGF2), cytokines (TGF beta), transcription factors (FoxO3), fibro-adipogenic progenitors (FAPs), and extracellular matrix proteins (FN). SC divisions in aged muscle are also perturbed by modified genetic and epigenetic mechanisms: DNA mutations, methylations, alterations in DNA replication and transcription due to modified histones, and down-regulation of some microRNAs, such as miR-708 and miR-489. Created with BioRender.com, (access on 16 October 2022).

Kuang et al. [2] observed that 10% of SCs are $Pax7^+/Myf5^-$. Within their niche, these cells have an asymmetric basal-apical oriented division, giving rise to a subpopulation of $Pax7^+/Myf5^-$ stem cells and a subpopulation of $Pax7^+/Myf5^+$ committed cells which differentiate in myoblasts.

There are also symmetric planar cell divisions of SCs (self-renewal), the daughter cells remaining in contact with both sarcolemma and basal lamina (Figure 1).

Thus, the SC niche proves to play an important role in directing the cell division of SCs towards symmetry or asymmetry (Figure 1). In this regard, although the literature contains many contradictory opinions on the effect of aging on SCs, if more intracellular or environmental alterations occur, Arpke et al. [11] demonstrated in a complex in vivo study that there is a negligible decline with age in the capability of transplanted satellite cells to generate new SCs or new muscle fibers, so there may be an environmental component acting on the rate of aging.

Another important determinant of asymmetric SC division is the proper orientation of proteins that organize the mitotic spindle (with an apical-basal orientation), such as PAR polarity complex and proteins sequestering fate determinants [12]. Niche environment and polarization of internal fate determinants influence this orientation.

Dystrophin, a sarcolemmal protein that stabilizes the myofiber, is an essential cofactor in cell polarity regulation during asymmetric division of SCs [21]. In senescent muscle

dystrophin is decreased [22], and we could speculate that this fact results, among others, in dysregulation of this type of SC division.

3. SC Characteristic Markers

SC in the quiescent state are responsible for skeletal muscle homeostasis and maintain the muscle stem cell pool throughout the life. After injury, SCs are activated, but only a part of them participate in muscle regeneration. The others revert to a quiescent state, preserving the balance of SC population [2,23] (Figure 2). Thus, SCs can be characterized by several markers more or less represented in these different situations, such as Paired box protein 7 (Pax7), a transcription factor, and protein Sprouty homolog 1 (Spry1). Figure 2 shows the activated SCs that proliferate and give rise to the myogenic lineage beginning to express myogenic regulatory factors (MRFs), such as myoblast differentiating factor (MyoD) and myogenic factor 5 (Myf5). Myoblasts are Pax7^{-}, Myf5^{+}, and MyoD^{+}. After differentiation in myocytes, myogenin (Myf4) begins to be expressed together with MyoD. The myogenic regulator factor 4 (Mrf4 or Myf6) [2,24–26] is present in myotubes and myofibers near the Myosin heavy chain (Figure 2).

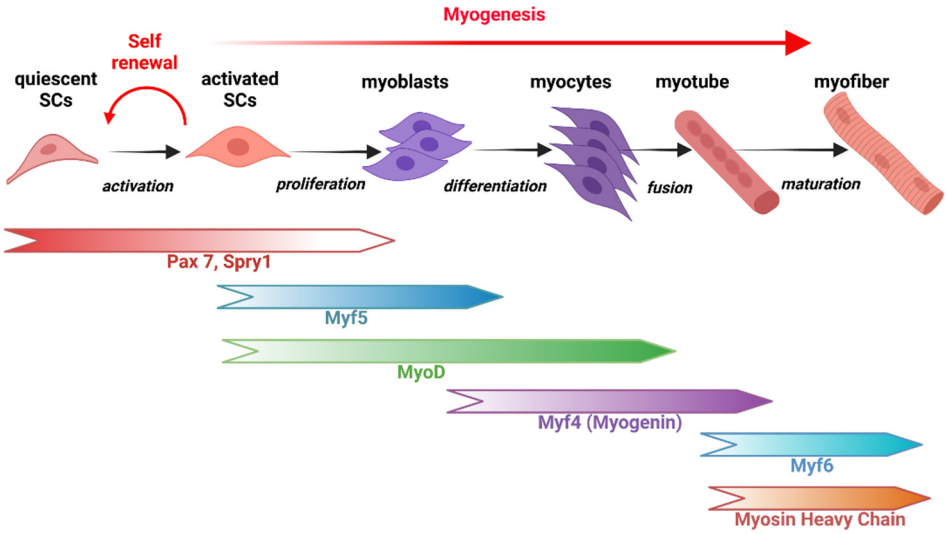

Figure 2. Quiescent satellite cells have high levels of Pax7 and Spry1. They begin to proliferate in response to muscle damage, injury, or disease. On the one hand, they restore the pool of satellite cells (Pax7^{+}). On the other hand, they activate, differentiate, and proliferate, turning into myoblasts (Myf5^{+}, MyoD^{+}), which differentiate into myocytes (MyoD^{+}, Myf4^{+}). The latter fuse and form myotubes (Myf6^{+}, MyHC^{+}), which turn into mature myofibers (Myf6^{+}, MyHC^{+}). Created with BioRender.com (access on 16 October 2022).

The skeletal muscle is capable of regeneration many times in healthy individuals. This process declines in degenerative disorders or aging. In addition to a genetic alteration in SCs, essential contributions to regeneration loss could be made by the alteration of the extracellular matrix composition, a disturbance of complex cellular interactions in skeletal muscle, and even some changes in systemic factors, such as the disappearance of the anti-aging hormone Klotho [27]. It was shown that Klotho protein counteracts canonical Wnt3a signaling in SCs [27]. Thus, systemic delivery of this protein could overcome, at least partially, the functional deficits of aged SCs [21].

Pax7 is a crucial regulator of SC survival, present in both quiescent and activated states. The quiescent SCs express Pax7 but no myogenic markers, such as MyoD. The activated SCs, which differentiate in myoblasts, present Pax7 and also MRFs. The myocytes that fuse to generate multinucleated fibers show a low expression of Pax7 [21] and a high level of MyoD [28,29]. Pax7^{+} SCs mark their commitment to the myogenic lineage by upregulating

Myf5. Thus, the transition to myoblasts is characterized by the presence of Myf5 and MyoD, along with a lower expression of Pax7.

Kuang et al. [2] characterized SC markers and showed that the satellite cell population is composed of hierarchal subpopulations of stem cells ($Pax7^+/Myf5^-$) and committed myogenic progenitors ($Pax7^+/Myf5^+$).

MRFs are myogenic regulatory factors activated during the regenerative process and participating in skeletal muscle differentiation. Although they are not mainly expressed in SCs, we mention them here to see how they vary in skeletal muscle recovery. We consider it is important to have an overview of the process of myofiber formation starting from SCs (Figure 2).

MRFs regulate the expression of several genes encoding structural and regulatory proteins, and they also interact with distinct growth factors, Insulin-like growth factor-1 (IGF-1), for instance [30]. In aging, alterations of MRF genes induce modifications in MRF expression and activity, playing a role in muscle wasting. Moustogiannis et al. found a reduction in MRF expression in aged myoblasts [30], which have a delayed or reduced differentiation capability. However, the cells continued to have a myogenic differentiation potential. The authors observed that the delay in myoblast differentiation appears to affect in the same way the response of MRFs both early and late, involving a likewise impact of myoblast senescence on the total differentiation program and not a specific effect of a certain MRF in a particular stage of myogenesis. In this in vitro study, it was shown that IGF-1 isoforms, such as IGF-1Ea and IGF-1Eb, act differently in the myogenic program of aged muscle, the last one being reduced in aged myoblasts. This finding may show an as-yet unproven direct involvement of MRFs in this process [30].

MyoD, a key transcription factor and the most investigated MRF, is present in proliferating SCs. In the absence of MyoD, SCs showed an increased propensity for self-renewal rather than differentiation, which results in a deficit in muscle regeneration [31–33]. Myostatin, a growth and differentiation factor, is a negative regulator of myogenesis, and it was shown as an inhibitor of myoblast differentiation by down-regulating the expression of MyoD [34], mediated through Smad3 (mothers against decapentaplegic homolog 3). A subset of activated SCs downregulates MyoD and does not differentiate, maintaining an inactive state almost identical with the quiescence, process depending on Sprouty1 [21]. Myostatin is increased in aged muscle [33], so it is explicable that muscle mass decreases with the inhibition of cellular differentiation through myostatin/Smad3/MyoD signaling pathway.

The ***Myogenin (Myf4, MyoG)*** expression marks the myoblasts differentiation into myocytes [35,36] The reciprocal inhibition Pax7–Myf4 and MyoD occur to end the differentiation [37].

Myf5 is expressed in the most quiescent cells [38], and, as Mrf4 and MyoD, it has a determinant role in myogenic cell fate acquisition. It plays a crucial role in regulating SC self-renewal during muscle regeneration [38]. As shown above, quiescent SCs express Pax-7 and Myf5, and it is known that Myf5 is the first MRF seen upregulated in SCs, succeeded by MyoD, indicating a myogenic commitment to myoblasts [36].

A recent publication shows that **MRF4** (or ***Myf6)*** is a myogenic niche regulator inhibiting SC exhaustion by blocking their premature differentiation [39]. It promotes the expression of epidermal growth factor (EGF), a key myokine that blocks the SC premature differentiation. Thus, Myf6 determines a ligand–receptor interaction between SCs and their associated muscle fibers, transcriptionally regulating many muscle-secreted proteins, including myokines, such as EGF and VEGFA. This control is required to maintain the adult muscle SC pool in the Myf6 absence SCs interrupting their quiescent state. EGF is no longer produced, and EGFR signaling is deregulated, resulting in p38 MAPK signaling pathway upregulation and a premature SC differentiation is occurring. Consequently, the SC pool is reduced during life due to spontaneous exit from quiescence [39].

Sprouty 1 (Spry1), an inhibitor of the receptor tyrosine kinase (RTK) signaling pathway, is exclusively expressed in quiescent Pax7+SCs in normal adult muscle. In injured

muscle it is downregulated in myogenic cells that proliferate, and it returns to the initial state in regenerated muscle—in Pax7$^+$ quiescent SCs [40]. A mouse model with *SPRY1* deletion in SCs presented defects in the SC pool, Pax7+SCs not having the capacityy to return to a quiescent state, undergoing a process of apoptosis, resulting in a reduction in the number of these cells [40]. A defect in the *SPRY1* gene was shown by Bigot et al. in vitro [41]. They demonstrated in elderly muscle an impaired capacity of SC self-renewal due to increased *SPRY1* gene methylation, which determines in the aged muscle a Spry1 disruption, preventing the SC reversion to a quiescent state, and leading to a diminishing SC pool [41]. Chakkalakal et al. identified an age-dependent change in SC niche influencing SC quiescence and function [42]. Thus, they showed in vivo that relatively dormant aged SCs with a more quiescent phenotype express higher levels of Spry1, which is also an inhibitor of fibroblast growth factor (FGF) signaling. Interestingly, removing Spry1 in these cells results in increased FGF signaling, quiescence loss, depletion of satellite cells, and diminished muscle regenerative capacity. On the contrary, inducing a Spry1 overexpression or a reduced activity of niche-derived FGF prevents the depletion of SCs [42].

Syndecans are proteoglycans relevant for muscle development and regeneration and they are expressed in proliferating myoblasts but are lost during myogenesis [43]. Syndecans 3 and 4 had been reported to play roles in muscle development and regeneration. Syndecan-3 was shown to promote SC self-renewal, and syndecan-4 is very important for skeletal muscle differentiation, regulating myoblast fusion and myogenic transcription factors [44,45]. Syndecan-3-/- mice have improved muscle regeneration after injury, and the SCs of syndecan-4-/- mice cannot activate, proliferate and differentiate [44,46]

Other markers located in the SC plasma membrane are: α7β1 integrin, the laminin receptor, specifically expressed in SCs on the basal surface (toward the basal lamina) [47,48], M-cadherin, the cell adhesion molecule, specifically expressed on the apical surface of SCs (toward the muscle fiber) [49,50], Calcitonin-Receptor (CALCR), which maintains SCs in a quiescent state [51], C-X-C Chemokine Receptor type-4 (CXCR4), having a role in cell proliferation [52], Vascular Cell Adhesion Molecule 1 (VCAM1), regulating fusion of muscle cells [53], and CD34, marking mainly the quiescent SCs together with Myf5 and M-cadherin [54]. Regarding CD34 expression in aging muscle tissue, it was reported that there are two quiescent stem cell states, one CD34High, with genuine state properties, and the other CD34Low, committed to myogenic differentiation, primed state [55]. Thus, the genuine-quiescent state could be preserved into later life, disappearing in extreme old age due to the acquisition of primed-state characteristics.

4. Signaling Pathways Involved in Symmetric/Asymmetric SC Divisions

In muscle aging, there is a decline in the number of SCs due to extrinsic and intrinsic factors. The balance between cell quiescence, proliferation, and apoptosis is disturbed. In summary, several signaling pathways involved in SC divisions (Figure 1), which are disorganized in senescent muscle, are described as follows.

The exhaustion of SCs is determined by getting the cells out of the quiescent state, one of the reasons being the action of fibroblast growth factor 2 **(FGF2)**, which has an increased expression in the aged muscle, breaking SC quiescence. FGF2 also inhibits Spry1 [10]. **TGFα** (transforming growth factor alpha) and **Notch** with a disturbed activity determine changes in SC activation and differentiation. An aberrant fibrotic response in aged muscle is produced by an increased **Wnt** signaling under the influence of various systemic factors. Increased activation **of JAK/STAT** (Janus kinases/signal transducer and activator of transcription proteins) signaling in old SCs also leads to myogenic commitment limiting the regeneration [10]. **BMP/TGFα** (bone morphogenetic protein/transforming growth factor) signaling promotes skeletal muscle cell differentiation. **SPACL1** (SPACL-like protein 1), a protein found in extracellular space, activates this pathway in C2C12 mouse muscle cells, binding BMP7 [56]. **PAR** (partitioning defective) **complex**, an important regulator of asymmetric SC divisions, activates the **p38a/b MAPK** (p38 mitogen-activated protein kinase) pathway, which triggers MyoD transcription [12].

The Notch signaling pathway, one of the most important signaling pathways, is a cell transduction pathway with a significant role in many cellular functions [57]. It is a well-known negative regulator of myogenic differentiation, also controlling the asymmetric SC division. Sun et al. [58] found in an in vitro experiment that Notch activity is essential for maintaining the expression of Pax7 in quiescent undifferentiated myoblasts after they exit the cell cycle. This signaling pathway is activated when Notch ligands (transmembrane proteins present at the surface of a signal-sending cell) bind to Notch receptors [58]. In mammals, five Notch ligands (Delta-like and Jagged) and four Notch receptors exist. After the interaction between ligand and receptor, a sequential cleavage of the receptor occurs, and the intracellular domain of the receptor (NICD, Notch intracellular domain) translocates to the nucleus where NICD forms a complex with a transcription factor, CBF1, and the coactivator Mastermind, activating target gene expression [58]. A study on mice showed that genetic disruption of Notch receptors resulted in embryonic lethality or developmental defects of multiple organs, including skeletal muscle [59,60]

It was shown that SCs contacting basal lamina displayed elevated expression of the Notch-3 receptor, whereas committed progenitors presented a Delta-1 ligand upregulation [2]. Thus, the newly divided cells Myf5^{+} have had a high level of Delta-1, whereas Myf5^{-} cells showed a low level of Delta-1. Furthermore, Sun et al. [58] showed that MyoD may stimulate Delta-1 (or Delta-like 1) expression, leading to up-regulation of Notch signaling in neighboring Pax7^{+}/MyoD^{-} cells. It is presumed that Delta-1 ligand and Notch-3 receptor interaction stimulates the Notch signaling pathway activation, having a positive role in SC self-renewal.

It was demonstrated that in old muscle, the upregulation of Delta ligand is diminished, resulting in a decrease in the regenerative potential of SCs, a phenomenon that could be reversed by indirectly inducing the Notch activity [61]. In addition, the exposure of aged SCs to young mice serum rejuvenates the SC response, suggesting that SCs do not lose their ability to participate in muscle regeneration [61].

Notch also controls SC adhesion to the niche and the composition of the extracellular matrix by direct transcriptional regulation [62].

Inhibition of Notch signaling pathway results in the loss of Pax7^{+}/Myf5^{-} population [2]. An inhibitor of Notch signaling is **Numb**, a membrane-bound protein. It interacts with the intracellular domain of Notch, preventing its translocation to the nucleus [63]. Conboy and Rando [64] showed that an increased Numb expression led to an attenuation of Notch signaling in SCs, resulting in the commitment of progenitor cells to the myoblast cell fate with the expression of Pax7, MRFs, and desmin. This study evidenced that Numb was asymmetrically localized in actively dividing cells, leading to an asymmetric cell division and, of course, to divergent cell fates of resulting cells. Thus, the balance between Notch-Numb controls cellular homeostasis and cell fate determination.

Wnt signaling is antagonistic with Notch signaling, contributing to myogenic commitment and differentiation of SCs [65]. This is possible by a temporal switch from Notch to Wnt signaling in myogenic progenitors, the muscular tissue expressing an increase in Wnt. Bracket al. revealed in a study on mice with muscle injury that the crosstalk between these pathways occurs via GSK3beta, which is activated by Notch and inhibited by Wnt in the canonical Wnt signaling cascade [65]. Wnt7a activates the Wnt pathway through β-catenin. Moyle et al. have shown on isolated muscle fibers that Wnt7a controls the SC symmetric division via the noncanonical Wnt/planar cell polarity pathway [5].

During aging, Wnt signaling increases, altering the muscle stem cell fate and enhancing fibrosis [66]. Thus, in aged mice, SCs tend to convert from a myogenic lineage to a fibrogenic one.

Forkhead box protein O3 (FoxO3) is a member of the FoxO family of transcription factors involved in stem cell regulation. It was found that FoxO3 is required for SC self-renewal in muscle regeneration [67]. FOXO3 gene is expressed in quiescent SCs. The FOXO3 deficient SCs proliferate and differentiate more rapidly, thus resulting in reduced

self-renewal of the SC pool. Furthermore, FOXO3 regulates the Notch signaling pathway and deletion of the FOXO3 gene, resulting in reduced levels of Notch signaling in SCs.

5. Regenerative Capacity of Skeletal Muscle in Aging

The regenerative capacity of skeletal muscle declines with aging, resulting in a low capacity to produce myoblasts [6]. The symmetric and asymmetric cell division is impaired, and aged SCs are more susceptible to undergoing apoptosis than the young ones [68]. The number of SCs during aging remains controversial, with studies showing a decrease in their number, and others showing the contrary that this number is not diminished in aged mice compared with the young ones [69]. However, evaluating the number of SCs is indeed difficult as long as these cells are very rare and their number differs depending on the location and type of skeletal muscles [69].

Both intrinsic (modified signaling pathways, altered metabolism, DNA damage, oxidative stress) and extrinsic (extracellular components, inflammatory responses, and interactions between different cell types in the stem cell niche) factors contribute to the entry of SCs into a pre-senescent phase, with affected self-renewability [70,71]

A recent in vitro study on C2C12 cells, concerning the effects of myoblast aging on skeletal myogenesis, showed G0/G1 cell cycle arrest, increased activity of β-galactosidase (SA-b-Gal), DNA damage, a rising number of apoptotic cells, and an increase in the expression of aging-related proteins and cell cycle inhibitors p16 and p21 [30]. Aged myoblasts presented a reduction in the expression of MRFs, a decrease in metabolic factors, an increased expression of inflammatory factors, and marked atrophy, leading to a disrupted myogenic lineage and to a diminished differentiation capacity of these senescent muscle cells.

Evano et al. also showed, in an ex vivo study, that it is a switch between asymmetric and symmetric SC divisions, they can alternate, and this process is regulated by an intrinsic counting mechanism and extrinsic communications among sister cells [72]. They contradict the conclusions of Kuang et al. [2] that only Notch signaling between sister myogenic cells mediates asymmetric cell fates and suggest that it is a more complex mechanism. Moreover, it was proposed that the post-mitotic midbody remnants after cytokinetic abscission, occurring in the cleavage furrow during the cell division, are released in the extracellular space or integrated only in one of the daughter cells. Thus, they are asymmetrically inherited and degraded between the two daughter cells [73,74]. This asymmetric receiving of midbodies serve as signaling platforms which can control SC proliferation and fate [72]. It can be speculated that in aged muscle in which the divisions of SCs are affected, the midbodies could also influence these conditions by feedback.

6. The SC Niche Alteration in Aging with Impact on SC Divisions

The orientation and position of SCs, between the sarcolemma and basal lamina, which form the niche, give the polarity of the niche and establish the internal cell polarity of SCs. They interact with extracellular matrix constituents in the niche [12]. Quiescent SCs are highly polarized, and some adhesion proteins are expressed on the basal versus apical cell surface, influencing this quiescent state of SCs and cell polarity. Among these adhesion proteins can be listed integrins A7 and B1, dystroglycan on the basal surface, and M-cadherin and NCAM on the sarcolemmal surface [12].

The signals from other cells found in the niche regulate the SC function, and alterations in this environment affect the SC quiescence and activation. The aging niche has an important contribution to the SC degradation, and this was demonstrated by Conboy et al. in an experiment on mice with blood exchange between a young mouse and an aged one (heterochronic parabioses), exposing old mice to factors present in young serum [63]. They observed the restoration of the Notch signaling activation and an increase in the proliferation and regenerative capacity of aged satellite cells. Diminished activation of Notch in aged muscle is due to insufficient up-regulation of the Notch ligand Delta. A

study on mice showed that inhibition of Notch impaired regeneration of young muscle, whereas forced activation of Notch restored the regenerative potential of old muscle [6].

The myofiber signaling also influences the SC activity. In addition to the Notch activation loss, aged muscle produces excessive transforming growth factor beta (TGF-beta), which induces high levels of Smad3 in SCs, interfering with their regenerative capacity. The experimental attenuation of TGF-beta/phosphorylated Smad3 in old, injured muscle restores regeneration to satellite cells [75].

Another niche factor expressed by muscle fiber that directly influences the SC quiescence and function is fibroblast growth factor 2 (FGF2). It was revealed in mice that aged quiescent SCs express a high level of Spry1, a FGF signaling inhibitor. Removing Spry1 from old SCs under homeostatic conditions increases FGF signaling, following a loss of SC quiescence, a depletion of stem cells and a diminished regenerative capacity of SCs. On the contrary, reducing niche-derived FGF activity by inhibiting FGF2 signaling or over-expressing Spry1 in SCs prevents their depletion [42].

In the aged SC niche, the muscle fibers contain considerably reduced fibronectin (FN) levels, resulting in a loss of SCs. An in vivo study on young mice demonstrated that a deletion of the gene encoding FN from regenerating muscles replicated the aging phenotype and led to a loss of SC numbers [76]. FN was identified as a preferred adhesion substrate for SCs. When it is an insufficient attachment of SCs to the niche in aged mice, some signaling pathways are strongly deregulated, such as integrin-mediated signaling through focal adhesion kinase and the p38 mitogen-activated protein kinase pathway. Restoring FN levels in the aged niche reestablished the normal state of SCs and youth-like muscle regeneration.

The function of fibro-adipogenic progenitors (FAPs), impaired by aging, indirectly affects the myogenic potential of SCs [77]. WNT1 inducible signaling pathway (WISP1) was identified as a FAP-derived matricellular signal lost during aging. WISP1, necessary for efficient muscle regeneration, controls the expansion and asymmetric commitment of SCs through Akt signaling. An experimental study on aged mice showed restoration of the myogenic capacity of SCs after transplantation of young FAPs or treatment with WISP1 [77] and demonstrated that this mechanism can be chosen for myogenesis rejuvenation.

The decline of Notch activators in the aged niche can also affect the SCs, causing their death by mitotic catastrophe and resulting in an impaired proliferative expansion of SCs [78].

7. Genetics of SC Asymmetric Divisions in Senescent Muscle

It was observed that DNA strands are asymmetrically segregated during the divisions of some SCs. Thus, one daughter cell receives older template strands, whereas the other inherits newly replicated DNA strands [79]. So, the Pax7high subpopulation is more susceptible to this asymmetric segregation of DNA, receiving the old DNA strand and expressing the dormant adult SC status, but this is reversible, giving rise to both Paxhigh and Paxlow SCs after several transplants. Chakkalakal et al. results confirmed this study, and they postulated that this subpopulation of SCs could be the true muscle stem cells controlling the maintenance of SC populations long-term throughout life [42].

Franco et al. showed that somatic mutations in aging muscle accumulate as an intrinsic factor contributing to impaired muscle function [80]. The loss of genome integrity could be produced by single-base changes, deletions or insertions of a few bases to chromosomal rearrangements. The somatic variants are propagated to a subpopulation of cells resulting in mosaicism in human tissues with genetically different cells. DNA damage occurs when these errors accumulate (Figure 1). Analyzing the whole genome of SC single clones of vastus lateralis muscle in healthy individuals, 13 somatic mutations were identified per genome per year, consistent with SC proliferation. In aging muscle, an increase in mutations in exons and promoters of the SC genes was found, changing their activity and finally the function of the muscle [80].

8. Epigenetics Mechanisms of SC Divisions in Aging

The study of epigenetic mechanisms of each step of myogenesis revealed many aspects of DNA methylation and histone post-transcriptional regulation in muscle aging in a spatio-temporal manner [81]. Thus, the dysregulation of each of these phases by alterations occurring in SC epigenome results in abnormal myogenic progress and incapacity to properly regenerate muscle. The epigenetic modifications include several aspects (Figure 1): DNA methylation, especially CpG methylation, DNA demethylation, histone proteins methylation, acetylation, phosphorylation, ubiquitination, citrullination, and gene regulation by noncoding RNAs. These modifications occur both in normal muscle, in disease, and aging. Signals from an altered muscle environment could trigger these epigenetic mechanisms, and an altered epigenome decreases the efficiency of tissue repair. The chromatin organization is modified by altering the transcription machinery to access gene sequences, starting with DNA promoters or enhancer modifications, sites for transcription factors, and continuing with modulation of chromatin conformation produced by reversible changes of DNA itself or histones [81]. The loss of canonical histones in quiescent SCs, produced by reduced biosynthesis or by lysosomal-mediated processing, is a common feature of aging [82]. Some histone post-translational mechanisms allow chromatin compaction or de-compaction. So trimethylation of lysine 9 and 27 of histone 3, or lysine 20 of histone 4 is responsible for local chromatin compaction leading to repression of gene expression. Contrastingly, acetylation of lysine 9 of histone 3 and lysine 20 of histone 4 and trimethylation of lysine 4 of histone 3 result in a relaxation of the chromatin state, increasing gene expression at that site [81,83]. Massenet et al. have described very well, in a review article, the epigenetic regulation of SC fate during muscle regeneration, also implying muscle disease and aging [81].

The canonical histones could be replaced with histone variants, modifying DNA replication and transcription in aging (Figure 1), such as the upregulation of macro H2A (mH2A), H2A.Z and H3.3, or downregulation of H2A.1 and H3.1 [82].

Another mode of chromatin remodeling is related to nucleosome assembly or repositioning and the exchange of histone variants by ATP-dependent chromatin remodelers, described in Yi et al. in a review [82].

However, what about how epigenetic mechanisms are transmitted in daughter cells after symmetric vs. asymmetric divisions? A study on SNAP-tagged histone H3-reporter mice showed the inheritance pattern of parental H3.1/3.3 histone in dividing SCs and concluded that it is a global inheritance in this case but a limited local asymmetry at the replication fork cannot be excluded [72]. Future studies need to be conducted regarding DNA segregation dynamics, transcription factors, and histone mechanisms during skeletal muscle regeneration and aging.

Regarding the micro-RNAs, it was observed that microRNA-708 is highly expressed in quiescent SCs and downregulated in activated SCs, controlling the quiescence by regulating the SC migration [84]. It is modulated by Notch signaling and plays an essential role in SC maintenance and migration in the niche targeting *Tensin3 (Tns3)* mRNA that encodes a fibrillary adhesion protein. This protein links integrins and actin.

Another microRNA regulating SC quiescence and self-renewal in the same way is miR-489 [85]. In aged SCs, a loss of control of asymmetric division occurs [12], thus involving, among other mechanisms, modifying the activity of these micro-RNAs (Figure 1).

9. Exercise Effect on Aged SCs

In aging, there are more prolonged periods of muscle disuse due to illness, injury or muscle disuse, leading to rapid atrophy of skeletal muscle fibers and muscle weakness (Figure 3). As a consequence, decreased mobility occurred. Some studies relate to a compromising recovery of atrophied muscle in aging, the previous size and functionality being delayed or never fully achieved [86,87]. Altered SC function and collagen accumulation contribute, at least in part, to poor muscle recovery in the elderly. One possible cause might be a dysfunctional immune system in aging, a poor pro-inflammatory macrophage

response following injury, or muscle disuse [88]. It is known that macrophages are very important to promote SC function [89], and without this infiltration at the site of injury, muscle recovery and myogenic function are compromised, resulting in smaller fibers and collagen deposition. Muscle atrophy is implied in many morbidities, and SCs are responsible for muscle regeneration/degeneration.

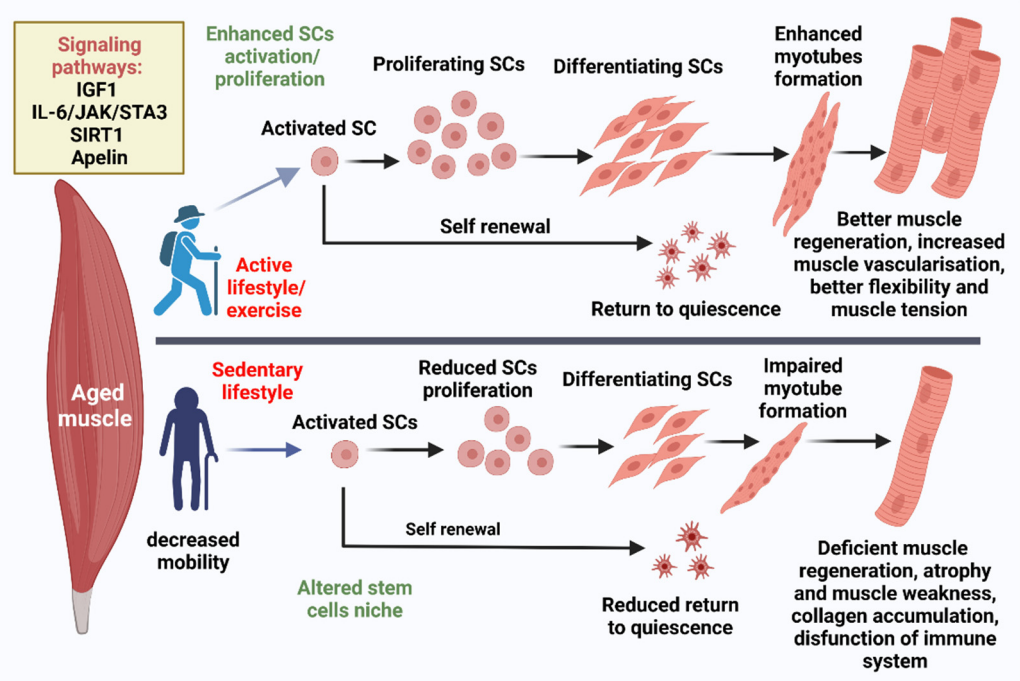

Figure 3. Exercise effect on aged skeletal muscle: enhanced recruitment of muscle fibers by a stimulation of SC activation and proliferation with a subsequent return to quiescence, contrary to the state of muscular immobility and sedentarism. The signaling pathways that intervene in SC activation include IGF1, IL-6/JAK/STAT3, SIRT1, and Apelin, stimulating the regenerative capacity of SCs. During aging, SCs suffer a decline in their functions and number, following defects in self-renewal activity, maintaining quiescence or regenerative capacity, which are amplified by muscle inactivity. Created with BioRender.com, (access on 22 November 2022).

Muscle loss can be attenuated with exercise, which increases vascularization, and leads to enhanced recruitment of muscle fibers, better flexibility, and muscle tension [90]. Exercise stimulates the activation/proliferation of SCs, and unlike chronic SC activation in ageing, acute activation after physical activity leads to SC regeneration with a subsequent return to quiescence. Signaling pathways being responsible for SC activation after exercise may include **IGF1** (insulin growth factor 1), **IL-6/JAK/STAT3**, **SIRT1** (sirtuin 1) [90].

Abreu et al. showed that endurance exercise has beneficial effects resulting in SC metabolic reprogramming, for example, a **reduced mitochondrial respiration** [91]. They observed in vitro that inhibition of mitochondrial O2 consumption enhanced SC self-renewal. These cells, or SCs from exercised mice, transplanted to healthy mice promoted the reduction in inflammation. However, the regenerative potential of SCs observed in exercised mice, depending on cell characteristics and their particular niche, was no longer found in animals upon transplantation [91].

Petkov et al. showed very recently that the physical activity is critical for developing muscle mass and its maintenance and functionality [92], using a mouse model DUhTP (non-inbred Dummerstorf marathon model—Dummerstorf high Treadmill Performance) and its associated control line. The number of SCs was considered the main determinant of muscle growth and homeostasis by replenishment of aging myonuclei, and this is higher in DUhTP mice. Muscle stem cells were isolated from sedentary male DUhTP and control after voluntary wheel running, and the results revealed a faster activation and a higher

proliferation rate, with a lower proportion of $Pax7^+$ cells in DUhTP mice. This means a quicker transition of SCs to differentiation (high proportion of $Pax7^+/MyoD^+$ cells) and a reduced reserve cell formation. The **higher fusion index** led to a very large myotubes formation. Thus, a lower activation threshold and a faster transition to SC differentiation could cause negative consequences by the exhaustion of the SC pool.

A critical protein induced by muscle contraction after exercise, the endogenous peptide **apelin**, was reduced in aged rodents and humans [93]. Mice deficient in this peptide or its receptor altered muscle function, especially in older ones. It was demonstrated that apelin triggers mitochondriogenesis, autophagy, and anti-inflammatory signaling pathways in myocytes and targets SC enhancing their regenerative capacity. Thus, apelin could be a marker for early sarcopenia and a target for therapeutic strategy in aging muscle.

The exercise results in aging muscle need more studies to better understand the cellular and molecular mechanisms governing muscle repair and regeneration in sarcopenia prevention.

10. Conclusions

Healthy skeletal muscles largely support the quality of life for good independent movement and locomotion. It is known that a sedentary lifestyle and muscle aging are the basis for the appearance of very serious pathologies, such as obesity, and cardiovascular diseases. In this context, satellite cells play a key role in maintaining muscle mass until an advanced age. Many SC mechanisms decline in ageing, but not all are known yet; some remain to be discovered. Given the complexity of the intrinsic and extrinsic biological processes that govern the symmetric vs. asymmetric division of SCs, mechanisms reviewed in this evaluation, it is essential to constantly update the latest studies in this field. It would be interesting to study in the future the behavior of satellite cells in older subjects, by age groups, and their evolution over time.

A promise for some interventional therapies in regenerative medicine for sarcopenia may approach the factors influencing the asymmetric cell division of satellite cells. Understanding the mechanism of asymmetric division is essential to advancing the control of stem cell self-renewal versus differentiation. Such control is necessary for the use of stem cells in regenerative medicine.

Author Contributions: Conceptualization, E.M., L.C.C. and M.E.H.; writing—original draft preparation, E.M., L.C.C. and G.G.; figures, E.M. and L.C.C.; writing—review and editing, L.C.C. and M.E.H.; supervision, E.M. and M.E.H.; funding acquisition, L.C.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by Ministry of Research, Innovation and Digitalization in Romania, under Program 1—The Improvement of the National System of Research and Development, Subprogram 1.2—Institutional Excellence-Projects of Excellence Funding in RDI, Contract No. 31PFE/30.12.2021 and the National Program 1N/2019/PN19.29.01.03 and PN 19.29.02.01.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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