Review

Asymmetric Cell Division and Satellite Cell Fate Regulation in Skeletal Muscle Aging and Disease

Shenghe Wang [1,2], Guangchuang Yu [3] and Liwei Xie [1,4,5,*]

[1] State Key Laboratory of Applied Microbiology Southern China, Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Open Laboratory of Applied Microbiology, Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou 510070, China
[2] Key Laboratory of Fertility Preservation and Maintenance of Ministry of Education, School of Basic Medical Sciences, Ningxia Medical university, Yinchuan 750004, China
[3] Department of Bioinformatics, School of Basic Medical Sciences, Southern Medical University, Guangzhou 510515, China
[4] School of Life and Health Sciences, Fuyao University of Science and Technology, Fuzhou 350109, China
[5] Department of Endocrinology and Metabolism, Zhujiang Hospital, Southern Medical University, Guangzhou 510280, China
* Correspondence: xielw@gdim.cn
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Abstract: Satellite cells, the resident muscle stem cells, play a crucial role in skeletal muscle regeneration, growth, and repair. Asymmetric cell division is a critical process regulating satellite cell self-renewal and differentiation, and is governed by various intrinsic and extrinsic factors. Key biomarkers of satellite cell characteristics, such as Pax7, MRFs, and Sprouty1, are essential in maintaining satellite cell homeostasis. Signaling pathways, including Notch, Wnt, TGF-β, FGF2, and the PAR complex, intricately regulate satellite cell division and fate determination. Asymmetric division is orchestrated through the establishment of cell polarity and differential distribution of fate determinants. Aging and diseases like Duchenne muscular dystrophy disrupt asymmetric division, leading to impaired satellite cell function and muscle regeneration. Potential therapeutic strategies aim to rejuvenate satellite cells and promote muscle regeneration by targeting the gut microbiome, utilizing gene editing technologies, and harnessing the power of exercise-induced factors. Understanding the molecular mechanisms governing satellite cell behavior and Keywords should be in lowercase and separated by semicolons. Developing innovative therapies hold promise for combating age-related muscle deterioration and pathological conditions characterized due to impaired muscle regeneration. Future research should focus on unraveling the complex regulatory networks and translating findings into effective clinical applications to restore muscle function.

Keywords: Satellite Cells; Asymmetric Division; Skeletal Muscle Regeneration; Muscle Aging

1. Background

Skeletal muscle accounts for 30% to 40% of the total mass of the human body and is a highly organized tissue. It is composed of numerous syncytial cells known as muscle fibers, which are formed by the fusion of myogenic progenitor cells [1], a process crucial for muscle structure and function. Both under normal and injury conditions, skeletal muscle regeneration is key to maintaining the normal functioning of skeletal muscles. Failure in skeletal muscle regeneration can lead to movement defects, metabolic deficiencies, and fatality [2]. The efficiency of this regeneration process relies on resident muscle stem cells (MuSCs), also called "satellite cells (SCs)" because of their unique anatomical position at the periphery of the myofibers. Notably, these satellite cells, 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.

Muscle stem cells are characterized by their inherent capacity for self-renewal and the generation of differentiated cells, achieved through two primary strategies: symmetric and asymmetric cell division. Symmetric cell division serves the purpose of either replenishing the stem cell pool or producing terminal differentiated cells, while asymmetric division differentially segregates cell fate determinants, including proteins, organelles and

RNAs, into the two daughter cells. This intricate process is meticulously orchestrated and controlled by intrinsic and extrinsic factors. Identifying and utilizing human satellite cells for muscle regeneration represents a significant challenge in regenerative medicine. In this review, we delve into the molecular mechanisms of asymmetric division in muscle stem cells and explore its relationship with cell fate determination with summary and discussion of the latest advances in muscle regeneration.

2. MuSCs and Skeletal Muscle Regeneration

Skeletal muscle stem cells (MuSCs) are key cell types essential for maintaining muscle tissue integrity and regenerative capacity. Positioned on the surface of muscle fibers in their quiescent state, they are activated upon muscle injury to proliferate and differentiate into mature muscle cells, thus repairing damaged skeletal muscle (Figure 1). Understanding the behavior and regulatory mechanisms of MuSCs is pivotal in finding ways to enhance muscle repair and regeneration, crucial for treating muscle degenerative diseases and injuries. Upon tissue damage, satellite cells are vigorously activated, initiating symmetric divisions that not only renew the stem cell pool but also give rise to proliferative myoblasts [3]. These myoblasts eventually mature into myocytes, contributing to the restoration of muscle fibers and aiding in the repair of skeletal muscle. It has been recently uncovered that satellite cells exhibit significant population diversity, with their fate during regeneration being steered by both inherent properties and external influences. These external signals primarily stem from interactions with a variety of stromal cell types present in their surrounding microenvironment, fostering a dynamic interplay that influences their behavior and function [3]. Demonstrating remarkable regenerative capabilities, MuSCs have been the focus of recent research. One significant finding is the identification of Gli1 as a marker for a critical subset of muscle stem cells (MuSCs) that are instrumental in muscle regeneration. These Gli1+ cells are sentinel stem cells, essential for effective tissue repair [4]. Additionally, research on AMPKα2 have demonstrated its intrinsic regulatory role in MuSCs, specifically in controlling myonuclear accretion, a crucial process for muscle fiber growth and repair. These findings offer new insights into the cellular and molecular mechanisms driving skeletal muscle regeneration, highlighting potential targets for enhancing muscle repair and treating muscle-related diseases [5]. Furthermore, skeletal muscle regeneration depends on the expansion of resident quiescent SCs, a process that becomes less efficient with aging. Recent research show that mitochondrial dynamics are essential for the successful regenerative capacity of satellite cells. Innovatively, regenerative functions can be restored in fission-impaired or aged satellite cells by the re-establishment of mitochondrial dynamics (by activating fission or preventing fusion), OXPHOS, or mitophagy [6].

In the context of skeletal muscle repair, fibro-adipogenic progenitors (FAPs), are critical because they create an environment conducive to the repair process, aiding satellite cells in effective regeneration. Following a muscle injury, there is a notable surge in the number of FAPs present at the site of damage, a phenomenon largely ascribed to the replication of these cells within the muscle tissue itself [7]. Additionally, the absence of FAPs even under normal, uninjured conditions can result in muscle atrophy and a decrease in the population of MuSCs, indicating that FAPs are essential not only for recovery after injury but also for the ongoing maintenance and health of skeletal muscle and the MuSC pool [8]. The study finds that a key signal from FAPs, WISP1, which influences MuSC expansion and commitment via Akt signaling, declines with age. Replenishing old muscles with young FAPs or treating with WISP1 can restore MuSC function and improve muscle regeneration in aged mice [9].

Macrophages play a crucial role in muscle regeneration by metabolically supporting satellite cells. When muscles are injured or affected by aging, macrophages adapt by increasing glutamine synthetase activity, leading to enhanced glutamine secretion [10]. The research identifies that the response to interferon-gamma (IFN-γ) is significantly reduced in the muscle regeneration process of aged mice. A novel subset of macrophages, termed IFN-responsive macrophages (IFNRMs), is discovered to express IFN-responsive genes and is less prevalent in aged muscles. These macrophages secrete CXCL10, which enhances satellite cell proliferation and differentiation via the CXCR3 receptor. Enhancing CXCL10 in aged mice boosts satellite cell activity and muscle regeneration, suggesting that targeting IFN-γ responses in macrophages could rejuvenate aged muscle repair by supporting satellite cell function [11]. Chronic limb-threatening ischemia (CLTI) is a severe manifestation of peripheral arterial disease (PAD) that impairs blood flow to the limbs, resulting in compromised skeletal muscle regeneration and increased risk of limb amputation. In CLTI, pro-inflammatory macrophages trigger premature differentiation of muscle satellite cells, thereby undermining the muscle's regenerative capacity under ischemic stress, highlighting the critical role of macrophage-MuSC signaling interactions in muscle repair [12].

Treg cells, particularly $Foxp3^{+}CD4^{+}$ Treg cells play a crucial role in muscle regeneration by modulating the inflammatory environment essential for repair and reducing muscle damage through mechanisms like the secretion of growth factors such as Amphiregulin, which directly stimulate muscle satellite cells [13]. In IL6Ra TKO mice,

the lack of a specific interleukin receptor on T cells results in reduced Treg cell functionality, negatively affecting muscle regeneration and highlighting the critical importance of Treg-mediated pathways in maintaining muscle health [14].

In conclusion, the dynamic interplay between various cell types and molecular signals within the muscle microenvironment is pivotal for the regulation and enhancement of muscle regeneration. From a clinical perspective, understanding these cellular and molecular mechanisms opens up new avenues for targeted therapies in muscle degenerative diseases and injuries. Additionally, exploring the translational potential of these findings to human muscle repair could significantly impact the treatment of muscular dystrophies, ischemic diseases, and age-related muscle atrophy, offering hope for improved quality of life and enhanced muscle function in affected individuals.

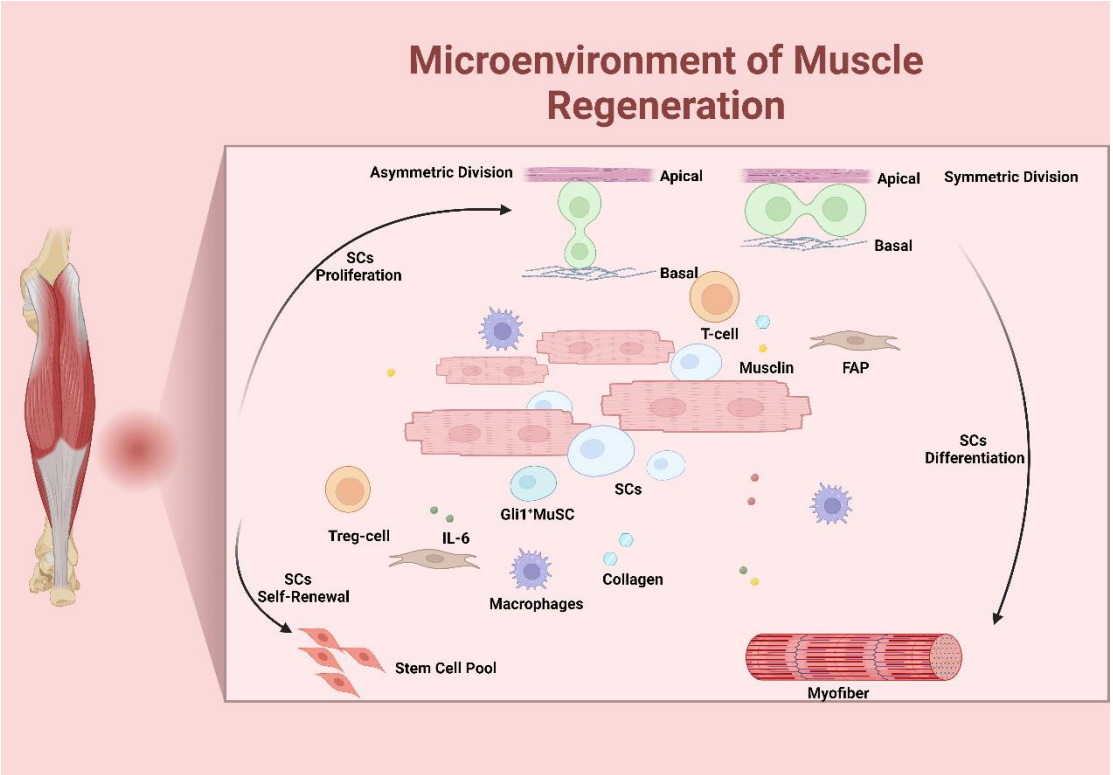

Figure 1. Microenvironment of muscle regeneration. Skeletal muscle stem cells (MuSCs), critical for muscle tissue repair and regeneration, are influenced by various cell types and molecular signals within their microenvironment. MuSCs, along with satellite cells, fibro-adipogenic progenitors (FAPs), macrophages, and Treg cells, play essential roles in muscle regeneration. These cells interact dynamically, responding to and influencing each other through specific pathways and signals. Created with BioRender.com.

3. Biomarkers of SC Characteristics

Satellite cells (SCs) are crucial for maintaining skeletal muscle homeostasis at rest, preserving a reservoir of muscle stem cells throughout an individual's life. Following injury, SCs become activated, yet only a subset contributes to muscle regeneration. This selective contribution underscores the functional diversity within the SC population. The remaining population returns to a quiescent state, ensuring the balance of the SC pool. Consequently, in these varying conditions, SCs can be characterized, to a greater or lesser extent, by several markers, such as Paired Box Protein 7 (Pax7), transcription factors, and protein homologs of Sprouty1.

The transcription factor Pax7 plays a pivotal role in the biology of satellite cells, which are essential for muscle regeneration. Identified initially in satellite cell-derived myoblasts, Pax7's expression is crucial for satellite cell specification and muscle tissue regeneration. Studies utilizing Pax7(-/-) mice revealed a profound impact on satellite cell presence and functionality, demonstrating that these cells are indispensable for skeletal muscle regeneration. Despite the absence of satellite cells in Pax7(-/-) muscle, the proportion of muscle-derived stem cells remained unchanged, suggesting distinct cellular populations within muscle tissue. Stem cells from Pax7(-/-) muscle showed an increased propensity to form hematopoietic colonies, indicating a potential shift in cell fate due to the absence of Pax7 [15]. Further research employing a model for the conditional depletion of satellite cells via

the Pax7 locus highlighted the irreplaceable role of $Pax7^+$ satellite cells in muscle regeneration. Following injury or stress, the absence of these cells led to significant muscle tissue loss and infiltration by inflammatory cells and adipocytes, underscoring the satellite cells' unique [16]. Additionally, the enzyme CARM1 has been shown to interact with Pax7, influencing myogenesis and satellite cell function. By methylating PAX7, CARM1 leading to the expression of Myf5 and is involved in various aspects of muscle cell differentiation and autophagy, highlighting a complex regulatory network that influences muscle regeneration and stem cell function [17].

Muscle Regulatory Factors (MRFs) are a group of transcription factors critical for myogenesis, the process of muscle development and differentiation (Figure 2). These factors, including MyoD, Myf5, Myogenin, and MRF4, play pivotal roles in determining muscle cell fate, regulating the transition from cell proliferation to differentiation, and facilitating the formation of multinucleated muscle fibers. MRFs are essential for muscle tissue development, repair, and maintaining proper muscle function, orchestrating the intricate processes that lead to the formation of functional muscle.

MyoD1, identified as a pivotal gene in myoblast determination, plays a crucial role in converting fibroblasts to myoblasts, as demonstrated by a study where its expression in fibroblast-like cells induced stable myoblast formation. The expression of this gene is specific to normal skeletal muscle, indicating its fundamental role in activating myogenesis, a process thought to originate from a common precursor for fibroblasts and myoblasts [18]. Further research established MyoD as a master regulator in myogenesis across various cell types. Experiments showed that MyoD expression could induce muscle-specific protein production in diverse cell lines, indicating its potential to activate terminal muscle differentiation independently or by activating other necessary factors [19]. In the context of muscle regeneration, MyoD's significance was underscored in studies involving MyoD mutant mice, which exhibited exacerbated myopathy symptoms when crossed with mdx mice, a muscular dystrophy model. The findings suggested that MyoD is essential for effective muscle regeneration, not due to a shortage of satellite cells but due to a disrupted progression through the myogenic program [20]. Further investigations into MyoD-/- myogenic cells highlighted their unique characteristics, including altered morphology and gene expression profiles, which suggested an intermediate stage in myogenic differentiation. Transfection with MyoD corrected these anomalies, reinforcing MyoD's role in the progression from a quiescent satellite cell to a myogenic precursor and eventually to a differentiated muscle cell [21].

Myf5 is a key protein regulating muscle differentiation, especially in skeletal muscle development, though it's not absolutely essential due to redundancy with other muscle regulatory factors like MyoD and MRF4. In zebrafish, Myf5 is the first muscle regulatory factor expressed during embryonic muscle development and is critical for adult viability [22]. Studies also reveal the interaction of Myf5 with various signaling pathways. For example, Salidroside can inhibit myogenic differentiation by modulating p-Smad3-induced Myf5 transcription [23]. Additionally, a regulatory cascade involving Pax3/Dmrt2/Myf5 plays a role at the onset of myogenesis, highlighting the complex gene regulatory network impacting Myf5 expression [24]. Moreover, research has reported a novel homozygous mutation in MYF5 due to paternal uniparental disomy, associated with a rare disease characterized by congenital external ophthalmoplegia, scoliosis, and vertebral and rib anomalies [25]. At the cellular level, Myf5 is regulated through multiple phosphorylation events during mitosis, with its degradation linked to changes in phosphorylation status [26]. Furthermore, investigations have found that the regulation of Myf5 involves long-range regulatory regions, revealing unexpected heterogeneity in skeletal muscles of mouse embryos [27].

Myogenin (Myog) is a crucial transcription factor in muscle development, playing a vital role in the fusion of myocytes during development and influencing muscle stem cell dynamics in adulthood. Research in myog-/- zebrafish revealed that the absence of Myog results in hypotrophic muscles, with adult myofibres exhibiting a severely reduced nuclear number and increased myonuclear domain size. The expression of fusogenic genes is diminished, and there's an upregulation of Pax7, leading to a significant increase in the number and misplacement of muscle stem cells (MuSCs) along the myofibres. Additionally, the loss of Myog affects mTORC1 signaling, placing MuSCs in an 'alerted' state characterized by precocious activation and rapid cell cycle entry, alongside the upregulation of myod [28]. Furthermore, in mouse models, the use of a knock-in line expressing the tdTOMATO fluorescent protein under the Myogenin locus has shed light on Myogenin's expression patterns during both embryonic muscle formation and adult regeneration. This approach has enabled the tracking and isolation of $MYOGENIN^+$ cell populations, providing insights into the cell division dynamics of differentiating myoblasts. Interestingly, $MYOGENIN^+$ myoblasts have shown the capability for cell division, although at a much lower frequency compared to MYOGENIN- cells [29].

Myf6 (or *MRF4*), a key transcription factor, plays a vital role in the interaction between muscle stem cells (MuSCs) and skeletal myofibers, which are crucial components of the MuSC niche. Myf6 is responsible for regulating the expression of various myokines and muscle-secreted proteins, including EGF, within skeletal

myofibers. This regulation is crucial as EGF signaling, through its receptor EGFR, can inhibit the differentiation of muscle stem cells by blocking *p38* MAP kinase activity. When *Myf6* is absent, due to a homozygous deletion, there is a notable decrease in EGF production in muscle, which leads to disrupted EGFR signaling. This disruption causes the muscle stem cells to exit their quiescent state prematurely and, over time, leads to a decrease in the MuSC pool during postnatal development. This newly identified role of *Myf6* underscores its significance in maintaining muscle stem cell homeostasis by regulating key signaling pathways that prevent premature differentiation and potential stem cell exhaustion [30].

Sprouty1 (Spry1) is pivotal in regulating the function and maintenance of $Pax7^+$ satellite cells, the stem cells essential for skeletal muscle regeneration. In the undisturbed state, Spry1 is expressed in quiescent satellite cells. Its expression fluctuates during muscle injury and repair: decreasing as satellite cells proliferate and contribute to muscle regeneration, and increasing as some cells return to a quiescent state, ensuring the preservation of the satellite cell pool [31].In the context of aging, Spry1′s role becomes increasingly critical. It helps maintain the quiescence and self-renewal capacity of aged satellite cells, counteracting the negative effects of elevated Fgf2 expression that drives these cells out of quiescence [32]. The aging process is associated with increased SPRY1 gene methylation, affecting stem cell self-renewal and, consequently, muscle regeneration [33]. Additionally, *Spry1* is a key downstream target of HIF2A, influencing satellite cell behavior by promoting stemness and inhibiting premature myogenic differentiation [34].Recent studies also highlight the influence of external factors, such as Lycium barbarum extract (LBE) and its component *LBP1C-2*, on satellite cell function. These substances have been shown to LBP1C-2 might bind to FGFR1 to activate SCs and promote SC self-renewal through Spry1 upregulation, showcasing a potential therapeutic avenue for muscle regeneration, particularly in aging [35].

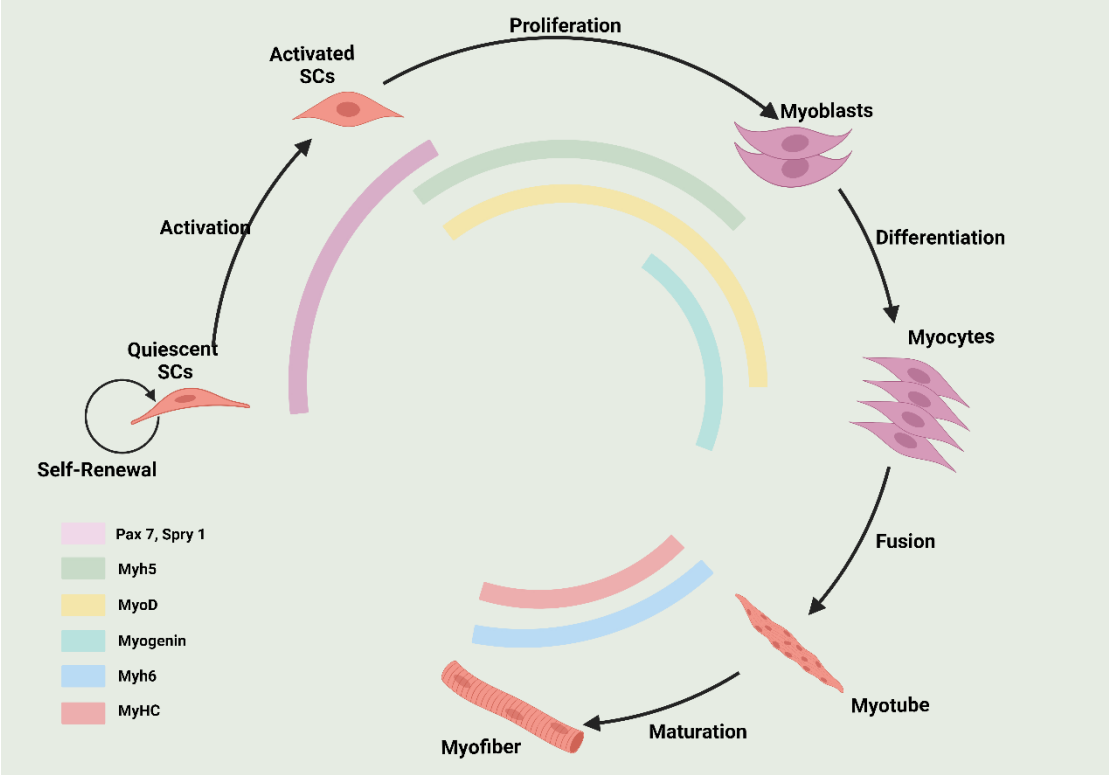

Figure 2. Specific muscle related transcription factors at different stages of myogenesis. The regulation of myogenesis at various stages involves both myogenic transcription factors and protein kinases. Initially, embryonic precursors or dormant satellite cells divide to form myoblasts. These myoblasts then differentiate into myocytes, which subsequently fuse to create multinucleated myotubes.

4. Regulation of Satellite Cell Division

Satellite cells, typically in a state of mitotic dormancy, are primed to become active and proceed through the cell cycle when provoked by stress from weight load or injury. When activated, these cells give rise to myogenic progenitor cells—transiently amplifying cells that divide several times before differentiating, thereby repairing and replenishing injured myofibers. Additionally, these activated satellite cells can self-renew, sustaining the pool of dormant satellite cell essential for ongoing muscle maintenance and repair. Recent studies elucidate how satellite cells manage the equilibrium between self-renewal and differentiation, specifically through the regulation of

asymmetric and symmetric cell division. During muscle regeneration, for instance, the asymmetric partitioning of intrinsic fate determinants before cell division can lead to the emergence of daughter cells with distinct destinies. A satellite cell that divides asymmetrically can produce another satellite cell and a committed myogenic progenitor, both of which may proliferate stochastically to aid in effective muscle repair. Therefore, maintaining a precise balance between self-renewal and differentiation is crucial for sustaining the satellite stem cell population and for generating an adequate number of transient amplifying progenitors to support the robust growth and renewal of muscle tissue [36]. The division of satellite cells is subject to complex regulation, and here we summarize some important signaling pathways and molecules.

Notch signaling plays a crucial role in the regulation of satellite cell division and muscle regeneration. Activation of Notch promotes the proliferation of myogenic precursor cells, maintaining them in a proliferative state by expressing the premyoblast marker *Pax3*. Conversely, an increase in *Numb* expression, an antagonist of *Notch*, leads satellite cells towards differentiation by enhancing the expression of myogenic regulatory factors and initiating myoblast commitment. This balance between Notch and Numb is essential for maintaining cellular homeostasis and determining cell fate during postnatal myogenesis [37]. In aging, a reduction in Notch signaling is associated with reduced regenerative capacity in muscle tissue. However, this decline can be partially reversed by environmental factors; old mice exposed to young serum show restored Notch signaling and improved satellite cell function, suggesting that environment factors influence Notch activity and satellite cell regeneration [38]. Further evidence of Notch's role in muscle pathology is seen in cases of muscular dystrophy, where mutations affecting Notch signaling pathways result in decreased satellite cell pools and impaired muscle regeneration [39]. Notch signaling is also implicated in maintaining satellite cell quiescence, with specific microRNAs like miR-708 regulates quiescence and selfrenewal by antagonizing cell migration through targeting the transcripts of the focal-adhesionassociated protein Tensin3 [40]. Moreover, Notch signaling interacts with other cellular pathways, such as the Ras/Mek/Erk pathway, particularly in the context of neurofibromatosis type 1, where it plays a role in coordinating muscle growth and establishing the muscle stem cell pool [41].

The Wnt signaling pathway plays a pivotal role in regulating the behavior and functionality of satellite cells, which is central to influencing satellite cell proliferation, differentiation, and self-renewal. In aged mice, satellite cells tend to shift from a myogenic to a fibrogenic lineage during the initial phase of proliferation. This conversion is mediated by factors present in the systemic environment of aged animals and is associated with the activation of the canonical Wnt signaling pathway in aged myogenic progenitors. This suggests that Wnt signaling is instrumental in governing tissue-specific stem cell aging and the associated increase in tissue fibrosis as age progresses [42]. The transition from progenitor cell proliferation to differentiation is vital for effective adult tissue repair. Research indicates that this switch is driven by a transition from Notch to Wnt signaling in myogenic progenitors, accompanied by an increase in Wnt expression in the tissue and heightened progenitor responsiveness to Wnt. The interplay between Notch and Wnt signaling, mediated by GSK3b, which is activated by Notch but inhibited by Wnt, underscores the importance of the temporal balance between these pathways in guiding the progression of muscle precursor cells along the myogenic lineage pathway [43]. Quiescent satellite cells express the Wnt receptor Fzd7, with its potential ligand Wnt7a being upregulated during regeneration. Wnt7a significantly promotes the symmetric expansion of satellite stem cells without affecting the growth or differentiation of myoblasts. Through the planar cell polarity pathway, Wnt7a signaling controls the homeostatic level of satellite stem cells, thus regulating the regenerative potential of muscle. The modulation of Wnt7a activity impacts muscle regeneration, with an overexpression enhancing regeneration and a lack thereof leading to a notable decrease in satellite cell numbers post-regeneration [44].

TGF-β plays a pivotal role in regulating satellite cell division and muscle regeneration, particularly in aging muscle. Elevated TGF-β levels in aged muscle increase *pSmad3*, which upregulates CDK inhibitors, hindering satellite cell proliferation and muscle repair. *Notch* signaling serves as a counterbalance, negating TGF-β/pSmad3′s adverse effects and fostering satellite cell division and regeneration [45]. Smad3′s absence in mice results in muscle atrophy and satellite cell dysfunction, illustrating its necessity for muscle maintenance and regeneration [46]. Additionally, Podocan, by interacting with TGF-β1, shows potential in enhancing muscle regeneration, suggesting a complex interplay between *TGF-β* signaling and extracellular matrix components in satellite cell function and muscle repair [47].

Fibroblast Growth Factor 2 (FGF2) plays a significant role in regulating satellite cell division and function. In aged muscle stem cell niches, FGF2 is expressed under homeostatic conditions, leading to the activation of a subset of satellite cells. This activation causes them to exit quiescence and lose their capacity for self-renewal. Specifically, aged satellite cells express *Sprouty 1 (Spry1),* an inhibitor of FGF signaling, which helps maintain their dormancy. However, when FGF signaling is increased by removing Spry1, these cells lose their quiescence, leading to satellite cell depletion and reduced regenerative capacity. On the other hand, reducing niche-derived

FGF activity either by inhibiting Fgfr1 signaling or overexpressing *Spry1* in satellite cells helps prevent their depletion [32]. Furthermore, interactions between satellite cells and the niche involve β1 integrin, which, along with FGF2, maintains SC homeostasis and supports their expansion and self-renewal during regeneration. In aged satellite cells, altered β1 integrin activity and reduced sensitivity to FGF2 are observed. Enhancing β1 integrin activity can restore FGF2 sensitivity and improve muscle regeneration. This synergy between β1 integrin and FGF2 is crucial for activating common downstream pathways like the MAP kinase Erk and protein kinase B (Akt), essential for satellite cell function and muscle regeneration [48].

The PAR complex plays a critical role in satellite cell function, which is essential for the regeneration and maintenance of skeletal muscle. This complex's spatial arrangement influences satellite cell fate, with the phosphorylation of PAR3 (*Pard3* in mammalian cells) by the kinase PAR1 (Par1b or Mark2 in mammals) being a key regulatory event. This phosphorylation disrupts the PAR complex's symmetry, impacting crucial cellular processes such as asymmetric division and progenitor cell generation in satellite cells. Pard3 is located on the apical surface of satellite cells, and its knockdown results in loss of p38 mitogen-activated protein kinase (MAPK) regulation, reduced numbers of asymmetric divisions, and reduced capacity to form progenitors [49].

Upon muscle injury, satellite cells are activated, exiting quiescence to proliferate and repair damaged muscle tissue. The p38a/b MAPK pathway is central to this process, promoting cell cycle entry and differentiation in a subset of these cells. The asymmetric localization of the PAR complex leads to selective activation of this pathway, enabling satellite cells to divide asymmetrically, thus replenishing the satellite cell pool and supporting muscle regeneration [50].

Aging adversely affects skeletal muscle, leading to a decrease in muscle mass, function, and regenerative capacity, contributing to conditions like sarcopenia. Alterations in key signaling pathways, including those involving the PAR complex and p38/MAPK, are implicated in the age-related decline in satellite cell function and self-renewal. Pharmacological interventions targeting these pathways show promise in mitigating these age-associated defects, offering potential therapeutic strategies for age-related muscle wasting [51].

Furthermore, the hormone unacylated ghrelin (UnAG) has been shown to enhance satellite cell activity and promote their asymmetric division via the Par polarity complex and p38/MAPK signaling, aiding in muscle regeneration. This finding underscores the therapeutic potential of UnAG in treating muscle dystrophies, where it can support satellite cell function and combat muscle degeneration [52].

Upon sustaining injury, muscle stem cells assimilate signals from their surroundings to kickstart the healing process. This intricate signaling ensures that the muscle stem cells not only multiply to mend the torn muscle fibers but also replenish the reservoir of stem cells. The destiny of these cells is molded by variations in gene activity, spurred by the cellular communications within the muscle milieu. The amalgamation of these signals prompts alterations in gene expression via epigenetic routes. Such routes encompass the post-translational adjustment of chromatin and the strategic repositioning of nucleosomes, which modifies the accessibility of certain gene regions to the transcriptional apparatus, thereby influencing gene expression [53]. Epigenetic mechanisms play a pivotal role in the regulation of satellite cell activity and cell division, essential for muscle maintenance and regeneration [54]. Chromatin modifications, including histone methylation and DNA methylation, orchestrated by enzymes like Dnmt3a, significantly influence the quiescence, proliferation, and differentiation of satellite cells [55]. In quiescent satellite cells, a permissive chromatin state allows for the potential activation of genes necessary for muscle repair. Age-related epigenetic changes can impair these processes, affecting muscle regeneration capacity. Additionally, the modulation of specific epigenetic markers, such as those mediated by HDAC4, impacts satellite cell proliferation and commitment to differentiation, highlighting the importance of epigenetic regulation in maintaining muscle stem cell functionality and promoting effective muscle repair [56,57].

Various types of non-coding RNAs, including microRNAs (miRs), long non-coding RNAs (LncRNAs), and circular RNAs (circRNAs), play crucial roles in the epigenetic regulation of muscle stem cell (MuSC) growth and development. Among these, a specific group of microRNAs known as myomiRs, which are predominantly found in skeletal muscle, are regulated by myogenic regulatory factors (MRFs) [58]. This group includes several key miRs such as miR-1, miR-133a/b, miR-206, miR-208b, miR-486, and miR-499, all of which are integral to the myogenic process [59,60].

5. Asymmetric Division and Cell Fate Determination

Satellite cells, located in a niche beneath the basal lamina and closely juxtaposed to the muscle fiber, play a critical role in the growth, maintenance, and repair of postnatal skeletal muscle. Observations using *Myf5*-Cre and *ROSA26*-YFP Cre-reporter alleles reveal that 10% of sublaminar *Pax7*-expressing satellite cells have never expressed *Myf5* in vivo. It has also been discovered that $Pax7^{+}/Myf5^{-}$ satellite cells can produce $Pax7^{+}/Myf5^{+}$

satellite cells through apical-basal oriented divisions, asymmetrically generating a basal *Pax7*$^+$/*Myf5*$^-$ cell and an apical *Pax7*$^+$/*Myf5*$^+$ cell. Upon prospective isolation and transplantation into muscle, it was found that while *Pax7*$^+$/*Myf5*$^+$ cells showed precocious differentiation, *Pax7*$^+$/*Myf5*$^-$ cells significantly contributed to the satellite cell reservoir across the injected muscle [61]. Asymmetric self-renewal hypothesizes that cell fate and mitosis are linked, supporting the idea that stem cell spatial localization and its environmental interaction dictate inheritance of fate determinants [59].

5.1. The Establishment of Satellite Cell Polarity

The establishment of satellite cell polarity is a critical component in skeletal muscle regeneration, involving a complex network of molecular interactions and signaling pathways. Coactivator-associated arginine methyltransferase 1 (CARM1) plays a vital role in this process by epigenetically regulating the expression of myogenic genes like Myog and MEF2C, essential for muscle differentiation. CARM1′s influence extends across various stages of myogenesis, from embryonic development to adult muscle repair, underscoring its importance in muscle stem cell function [17]. CARM1 methylates PAX7, facilitating the activation of Myf5, a gene crucial for the determination of muscle cell fate during asymmetric satellite cell division. This methylation event is pivotal for the recruitment of a specific histone methyltransferase complex to Myf5's promoter, initiating a cascade of gene expression essential for satellite cell differentiation. The phosphorylation of CARM1 by p38γ/MAPK12, which is influenced by its interaction with the dystrophin-glycoprotein complex, highlights the interplay between CARM1 and cellular architecture. In the absence of dystrophin, such as in Duchenne muscular dystrophy (DMD), this interaction is disrupted, leading to altered CARM1 activity, impaired satellite cell function, and compromised muscle regeneration [62].

Dystrophin's role extends beyond structural support to regulating satellite cell polarity and asymmetric division, crucial for maintaining the satellite cell pool and muscle regenerative capacity [63]. Its association with Mark2 and Pard3 is essential for proper satellite cell function, and its deficiency in DMD results in a loss of polarity and abnormal cell division patterns, exacerbating muscle degeneration [36].

Moreover, the EGFR and Aurora kinase A (Aurka) pathway has been identified as a regulator of satellite cell division symmetry, offering potential therapeutic targets for enhancing muscle repair, particularly in conditions like DMD where satellite cell function is compromised [64]. The transcriptional co-activator with PDZ-binding motif (TAZ) stimulates muscle regeneration via satellite cell activation, the polarity protein Pard3 induces TAZ through p38 MAPK [65].

5.2. Satellite Cell Asymmetric Division

The ability to dictate cell fate decisions is critical during animal development. Evolutionarily conserved protein complexes control cell fate decisions across diverse tissues. Maintaining proper daughter cell inheritance patterns of these determinants during mitosis is therefore a fundamental step of the cell fate decision-making process [66]. Asymmetric cell division (ACD) is a fundamental process that has evolved to create cellular diversity. A central aspect of ACD is the formation of two distinct sibling cells with different fates, influenced by mitotic events. Cells can achieve asymmetric outcomes either by responding to external signals or through the unequal distribution of cell-intrinsic factors like proteins or RNAs, which guide distinct fate decisions. This concept was illustrated over a century ago by Edwin Conklin, who observed that in the early cell divisions of ascidian embryos, the uneven partitioning of yellow cytoplasm plays a crucial role in determining muscle cell destiny [67].

When stimulated appropriately, satellite cells undergo proliferation, leading to either self-renewal to maintain their population or differentiation to aid in muscle repair. Over the last two decades, two theories regarding the division pattern of satellite cells in vivo have been debated. Firstly, the stochastic model suggests that the division of satellite cells and their subsequent fate decision are independent processes. According to this theory, satellite stem cells execute symmetric divisions, with the resulting daughter cells randomly selecting their destinies. Secondly, the hypothesis of asymmetric self-renewal proposes a direct connection between cell fate and division, emphasizing that the position of the stem cell and its interactions with the surrounding environment influence the distribution of fate determinants during division [59]. Through BrdU pulse-chase labeling to trace the potential stem cell niche, a distinct subpopulation of satellite cells was identified, some of these cells exhibit selective segregation of template DNA strands during mitosis. The study found that the cell fate determinant Numb is asymmetrically distributed to one daughter cell during cell division, preceding differentiation, which aligns with its role in self-renewal [68].

Satellite cells are a heterogeneous mix of stem cells and more differentiated progenitors, with the asymmetric division playing a key role in maintaining this diversity. Using Myf5Cre and ROSA26-YFP Cre-reporter alleles,

it was observed that 10% of Pax7-expressing satellite cells under the basal lamina never activated Myf5, indicating a subset of satellite cells that might represent true stem cells. These $Pax7^+/Myf5^-$ satellite cells were shown to give rise to $Pax7^+/Myf5^+$ cells through divisions oriented apically-basally, resulting in asymmetric division that produces one $Pax7^+/Myf5^-$ cell at the base and one $Pax7^+/Myf5^+$ cell at the apex. Isolation and transplantation experiments demonstrated that while $Pax7^+/Myf5^+$ cells rapidly differentiated, $Pax7^+/Myf5^-$ cells significantly replenished the satellite cell pool within the transplanted muscle [62].

Using transgenic Tg: Pax7-nGFP mice, cells with high levels of Pax7-nGFP (Pax7-$nGFP^{Hi}$) exhibit traits indicative of a less committed state, possessing lower metabolic activity and experiencing a delayed entry into mitosis compared to their Pax7-$nGFP^{Lo}$ counterparts. During proliferation, Pax7-$nGFP^{Hi}$ cells maintain low metabolic rates and predominantly undergo asymmetric DNA segregation during cell division, with the daughter cells retaining the original template DNA strands displaying stem cell markers. Utilizing chromosome orientation-fluorescence in situ hybridization, it was revealed that Pax7-$nGFP^{Hi}$ cells consistently segregate all chromatids asymmetrically, in contrast to the random DNA segregation observed in Pax7-$nGFP^{Lo}$ cells [69].

Upon muscle injury, satellite cells activate the p38a/b MAPK pathway, initiating a transition from quiescence to proliferation for muscle repair and self-renewal. A subset of these cells undergoes asymmetric division, where the Par complex's asymmetric localization activates p38a/b MAPK and MyoD in only one daughter cell, driving its proliferation as a myoblast. The other daughter cell, without p38a/b MAPK activation, remains quiescent, ensuring the renewal of the satellite cell pool. This mechanism allows satellite cells to produce distinct daughter cells, linking injury response to self-renewal [51].

Dystrophin, known for its role in maintaining myofiber integrity, is crucial in Duchenne muscular dystrophy (DMD) pathogenesis. The study reveals that dystrophin is also vital in activated muscle stem cells, or satellite cells, where it interacts with Mark2, a key cell polarity regulator. The absence of dystrophin leads to reduced Mark2 expression, disrupting Pard3 localization and consequently diminishing asymmetric satellite cell division. This results in satellite cells with lost polarity, abnormal division patterns, and impaired progenitor generation, highlighting an additional mechanism by which dystrophin deficiency exacerbates muscle degeneration in DMD. The study underscores dystrophin's significant role in satellite cell polarity and division [36]. Administering EGF in vivo significantly stimulates asymmetric cell division in satellite cells lacking dystrophin in mdx mice, boosting the count of progenitor cells, improving regeneration, and augmenting muscle strength. Consequently, triggering a polarity pathway dependent on EGFR facilitates the functional recovery of satellite cells deficient in dystrophin and improves muscle power production [68].

Adult mammalian stem cells have the unique ability to remain dormant for extended periods, the microRNA (miRNA) pathway is crucial for maintaining this quiescent state. MiR-489, regulates satellite cell quiescence by inhibiting the expression of the oncogene Dek, which is allocated to the more differentiated progeny during satellite cell asymmetric division, influencing their proliferative expansion. This research underscores the significance of the miRNA pathway, especially miR-489, in preserving the quiescent state of satellite cells and impacting their asymmetric division and differentiation [70].

During asymmetric satellite cell divisions, the satellite cell integrates signals from the niche to maintain cell polarity. Satellite cells respond to specific signals from their niche microenvironment, enabling the asymmetric distribution of fate determinants during cell division (Figure 3). The asymmetric division is supported by the polarized allocation of p38A-B MAPK and chromatin alterations driven by Carm1, which encourages myogenic development in one of the daughter cells. Through this process of asymmetric division, satellite cells maintain the stem cell reservoir by generating one progenitor capable of random expansion, contributing to muscle repair.

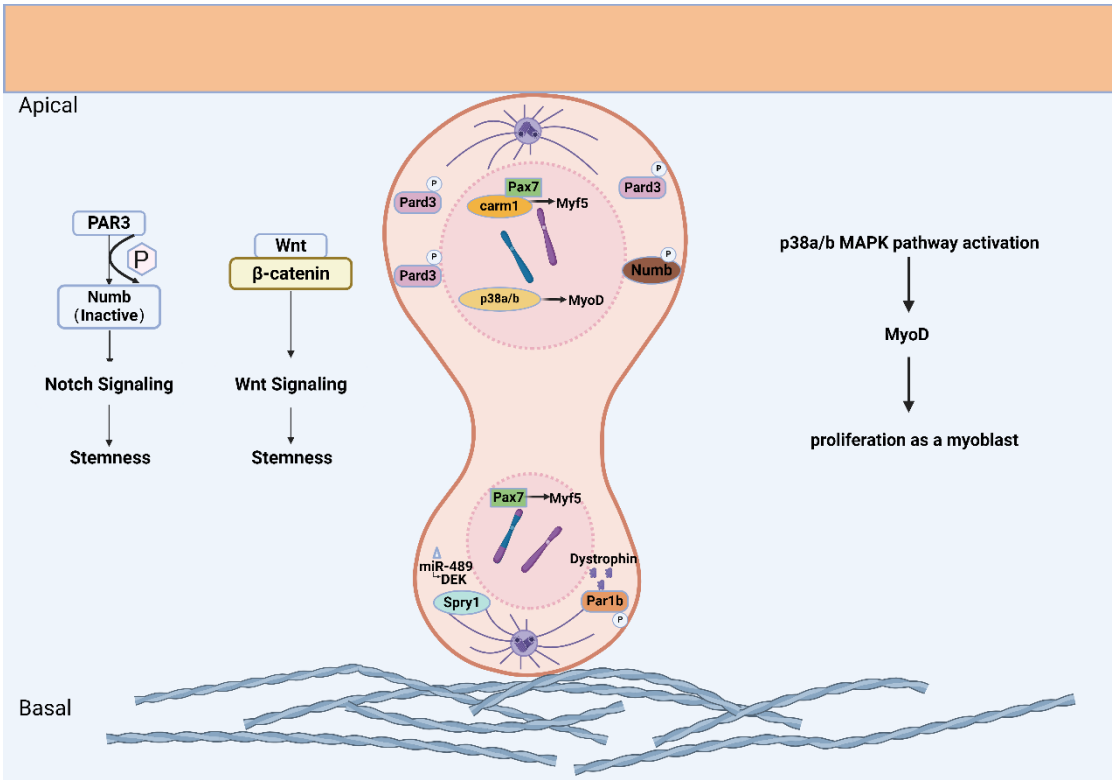

Figure 3. The signaling pathways and molecules involved in asymmetric division of muscle satellite cells. These processes are regulated by intricate signaling pathways including Notch, Wnt, and TGF-β, as well as by interactions with proteins such as dystrophin and components of the PAR complex. By removing Numb, Notch signaling is active to maintain the stemness of the daughter cell at the apical side. Wnt signaling also participates in promoting self-renewal in the daughter cell. Created with BioRender.com.

6. Asymmetric SC Division in Aging and Disease

In the event of acute muscle damage, symmetric division of satellite cells fosters the expansion of the satellite stem cell population, ensuring the equilibrium of the stem cell niche. Conversely, asymmetric division results in the creation of a stem cell alongside a transient-amplifying progenitor, which can undergo multiple rounds of division to produce a group of myogenic progenitor cells. These cells then either merge with existing myofibers or contribute to the formation of new ones. While myogenic progenitors can proliferate in response to injury, their self-renewal capacity is likely limited to the short term. Thus, the regenerative potential of muscle tissue hinges on a finely tuned balance between self-renewal and commitment to myogenesis, a balance that is meticulously regulated.

Skeletal muscles possess a prominent regenerative capacity reliant on MuSCs, but this post-injury regenerative ability declines with aging [71]. Compared to young and adult animals, satellite cells from aged animals exhibit a significantly higher percentage of activated caspase and TUNEL-positive cells, along with significantly lower levels of Bcl-2. These data indicate an increased susceptibility to apoptosis in aged satellite cells [72]. In elderly muscle tissue, there's a gradual reduction in the count of satellite cells, accompanied by a decrease in their ability to self-renew and regenerate [73]. As stem cells age, particularly muscle stem cells or satellite cells, they undergo changes in their niche that affect their ability to divide asymmetrically. The aged muscle stem cell niche begins to express Fgf2, prompting some satellite cells to exit quiescence and lose their capacity for self-renewal. A key observation in aged mice is that dormant satellite cells highly express Spry1, an inhibitor that modulates fibroblast growth factor (FGF) signaling. Modifying FGF signaling in these cells, either by removing Spry1 or altering niche-derived FGF activity, impacts their quiescence and regenerative potential [32]. In aged mice, approximately two-thirds of MuSCs exhibit intrinsic functional decline compared to those from younger mice, demonstrating a reduced ability to repair muscle fibers and replenish the stem cell pool upon transplantation. This diminished capacity is associated with a higher prevalence of cells expressing senescence markers, attributed to increased activation of the p38α and p38β mitogen-activated protein kinase pathway. Interestingly, these deficits in aged MuSCs cannot be rectified by transplanting them into the younger muscle microenvironment [52]. However, a significant rejuvenation of MuSC function is observed when cells from aged

mice are subjected to temporary inhibition of p38a and p38b pathways and cultured on soft hydrogel substrates [74]. In aged muscle, there is a noted decline in Notch activation alongside an overproduction of TGF-β, leading to elevated levels of TGF-β pSmad3 in satellite cells, which hampers their ability to regenerate muscle tissue. Notch and pSmad3 signaling exhibit a reciprocal regulatory relationship, where Notch activation can inhibit the TGF-β-driven increase of cell cycle inhibitors (p15, p16, p21, p27), whereas Notch inhibition promotes their upregulation. The balance between Notch and pSmad3 signals leads to impaired regeneration due to their disruption in aging muscles [45].

In satellite cells, aging or genetic defects result in reduced mitochondrial fission cause disruption of the mitochondrial electron transport chain (ETC), which in turn leads to less effective oxidative phosphorylation (OXPHOS) metabolism and mitophagy, alongside an uptick in oxidative stress [6]. A multiomic analysis comparing muscle stem cells (MuSCs) between young and aged mice reveal that aged MuSCs are predisposed to respond to oxidative stress and are geared towards glutathione (GSH) production. This observation aligns with the recognized escalation of pro-oxidant events with aging and the critical role of the GSH system in neutralizing and eliminating oxygen radicals [75]. These results illuminate potential strategies for developing therapies intended to restore or bolster MuSC functions for their regenerative capacity, not just in aging muscle but also in pathological situations where oxy-inflammatory alterations lead to muscle deterioration [76].

Asymmetric Muscle Stem Cell Division in Disease

Duchenne muscular dystrophy (DMD) is a devastating X-linked muscular disease, caused by mutations in the DMD gene encoding Dystrophin and affecting 1:5000 boys worldwide. Lack of Dystrophin leads to progressive muscle wasting and degeneration resulting in cardiorespiratory failure [77]. Duchenne muscular dystrophy (DMD) arises from a mutation in the DMD gene, the human genome's largest gene spanning approximately 2.2 Mb, responsible for producing the dystrophin protein. In skeletal muscle, dystrophin is present in myofibers, attaching to the actin cytoskeleton through its N-terminal domain and connecting to the dystrophin-associated glycoprotein complex (DGC) at the cellular membrane via its C-terminal domain. The absence of dystrophin leads to myofibers becoming weak and susceptible to damage, resulting in the gradual deterioration of skeletal muscle [66]. In Duchenne muscular dystrophy (DMD), the lack of dystrophin not only compromises myofiber integrity but also significantly affects the behavior of muscle stem cells (satellite cells). Dystrophin is crucial in these cells for maintaining their polarity and supporting asymmetric division, as it interacts with the kinase Mark2, a key cell polarity regulator. Without dystrophin, Mark2 levels decrease, disrupting the localization of the polarity regulator Pard3, which in turn reduces the number of asymmetric cell divisions. This leads to satellite cells losing their polarity, exhibiting abnormal division patterns, issues with mitotic spindle orientation, and extended cell division times. These changes undermine the production of myogenic progenitors necessary for effective muscle regeneration, suggesting that DMD's muscle wasting is not only due to myofiber vulnerability but is also worsened by the intrinsic dysfunction of satellite cells in supporting muscle repair [36]. In skeletal muscle, asymmetric division of muscle stem cells is crucial for generating committed progenitor cells, a process regulated by the activation of Myf5 through Pax7, with Carm1 playing a pivotal role in this transcriptional activation by methylating Pax7. Carm1′s function is modulated by its phosphorylation by p38g/MAPK12, which influences its nuclear translocation. In muscle stem cells, the localization of the p38g/p-Carm1 complex is mediated through its interaction with the dystrophin-glycoprotein complex (DGC) via b1-syntrophin. However, in the context of Duchenne muscular dystrophy (DMD), where dystrophin is absent, this interaction is disrupted, leading to increased phosphorylation of Carm1. This alteration affects Carm1's interaction with Pax7, resulting in diminished histone methylation at the Myf5 promoter and reduced expression of Myf5 and other Pax7 target genes. These findings indicate that in DMD, the dysregulation of the p38g/Carm1 pathway impacts the epigenetic regulation of gene expression during the asymmetric division of muscle stem cells, contributing to the impaired muscle regeneration observed in the disease [65]. In dystrophin-lacking satellite cells of mdx mice, EGF treatment significantly promotes asymmetric divisions, increases progenitor cell numbers, and improves muscle regeneration and strength. Thus, stimulating the EGFR-Aurka axis offers a potential strategy for rescuing the regenerative capacity of dystrophin-deficient satellite cells, contributing to enhanced muscle function [68]. In muscle stem cells (MuSCs) from both mice and humans revealed that there is no variation in telomere length between young and old MuSCs in uninjured wild-type mice. However, MuSCs from young dystrophic mice displayed notably shorter telomeres. Furthermore, the research found that telomere shortening is also a characteristic of human dystrophic MuSCs, highlighting its significance in the compromised regenerative capacity associated with disease [78].

Potential therapeutic strategies for addressing muscle wasting due to aging and disease encompass cell-based treatments, gene editing, and enhancing inherent repair mechanisms. These approaches are enhanced by focusing

on satellite cells, which are essential for effective muscle regeneration. A myopathologic analysis was conducted on twenty-four muscle biopsies from DMD patients, focusing on regeneration, fibro-adipogenic progenitors, and muscle stem cell behavior. An increase in fibro-adipogenic progenitors, key drivers of fibrosis and lipid accumulation, was observed alongside a decrease in muscle regenerative capacity. This decline in regeneration is closely associated with impaired activation and proliferation of muscle stem cells [77]. A novel approach to enhance MuSC function and maintain their environment involves leveraging the regenerative capabilities of mesenchymal stromal cells from the amniotic membrane (hAMSCs), which are multipotent and known for their tissue repair roles in various disease contexts. It has been shown that the secretome from hAMSCs (CM hAMSC) and the extracellular vesicles (EVs) derived from it can directly promote the proliferation and differentiation of human myoblasts and mouse MuSCs from dystrophic tissue. These findings offer a foundation for creating new therapies to combat DMD by reducing fibrosis and promoting myogenesis in affected muscles [79]. Deletion of interleukin 34 (IL34) sustains expansion by sacrificing the differentiation of SCs and leads to significant muscle regeneration defects, deleting IL34 or interfering Akt in mdx mice ameliorates dystrophic muscles [80]. Moreover, TSHR activity plays a role in warding off senescence. It was discovered that forskolin, an activator of signaling pathways downstream of TSHR, decreases the senescence of skeletal muscle stem cells, enhances their regenerative capacity, and stimulates myogenesis, leading to improved muscle functionality in DMD rats [81].CRISPR editing of muscle stem cells (MuSCs) with adeno-associated virus serotype-9 (AAV9) holds promise for sustained gene repair therapy for muscular dystrophies [82].

7. Therapeutic Strategies

Enhanced insights into satellite cell regulation herald new strategies to augment their regenerative capacity, offering novel therapeutic avenues for age-related muscle deterioration. Such advancements also hold promise for facilitating muscle repair after injuries, combating muscle atrophy from prolonged inactivity, and restoring muscular function in conditions marked by satellite cell depletion or diminished regenerative ability, like Duchenne muscular dystrophy.

7.1. Gut microbiota Regimens as Strategies for Satellite Cell Rejuvenation

In recent decades, extensive research has revealed the significant influence of gut microbiota on host immunity, neural functions, and intestinal barrier integrity (Figure 4). The interplay between gut health and muscle properties has increasingly captured scientific interest. Evidence suggests a strong association between gut microbiota and various skeletal muscle attributes, such as muscle mass, functionality, and metabolic processes. This relationship is crucial for metabolic equilibrium, insulin responsiveness, and inflammation control within the organism. In studies with germ-free (GF) mice, the lack or imbalance of gut microbiota led to noticeable changes across different skeletal muscle types. These changes are partly linked to the interaction of the AMPK (AMP-activated protein kinase), FoxO3 (Forkhead box protein O3), and atrogin signaling pathways, underscoring the gut microbiota's essential role in skeletal muscle health [83].

A role for the gut microbiota in regulating skeletal muscle mass and function in mice has been identified. Transferring gut microbiota from pathogen-free mice to germ-free mice led to enhanced skeletal muscle mass, decreased markers of muscle atrophy, improved oxidative metabolism in the muscle, and increased levels of Rapsyn and Lrp4 genes, which are crucial for neuromuscular junction formation. Administering short-chain fatty acids, which are metabolites produced by microbes, to germ-free mice partially ameliorated the skeletal muscle deficiencies [84]. Our resent study provided further support on the link between gut microbiota and satellite cells. We demonstrated that butyrate can serve as an alternative selection strategy to enhance SC homeostasis and function during skeletal muscle aging [85].

Foxp3-expressing regulatory T (Treg) cells, a critical subset of $CD4^+$ T lymphocytes, play an essential role in sustaining immunological tolerance. The specialized subgroup known as "tissue-Treg cells" possesses unique transcriptomes and T cell receptor (TCR) repertoires and resides in non-lymphoid tissues, including visceral adipose tissue, skeletal muscle, skin, and the colonic lamina propria. Gut microbes influence the differentiation of $ROR\gamma^+$ regulatory T (Treg) cells, which are crucial for intestinal stability and also aid in the regeneration of injured tissues like skeletal muscle. These Treg cells, originating from the gut, manage inflammation and support the healing process in damaged muscles, highlighting the gut's significant role in tissue repair beyond the intestinal environment [86].

In dystrophin-deficient mdx mice, a model for Duchenne muscular dystrophy, there is a notable alteration in gut microbiota composition and a decrease in microbial diversity. These changes are associated with disrupted intestinal function, increased systemic inflammation, and altered metabolic signaling in skeletal muscles. The

findings highlight the intricate interplay between the gut microbiota and skeletal muscle health, suggesting that the gut microbiome may play a pivotal role in the pathophysiology of muscle dystrophy [87]. In the mdx mouse model of Duchenne muscular dystrophy (DMD), there are changes in the composition of fecal microbiota and variations in the circulating concentrations of short-chain fatty acids (SCFAs) and their associated metabolites when compared to healthy controls [88].

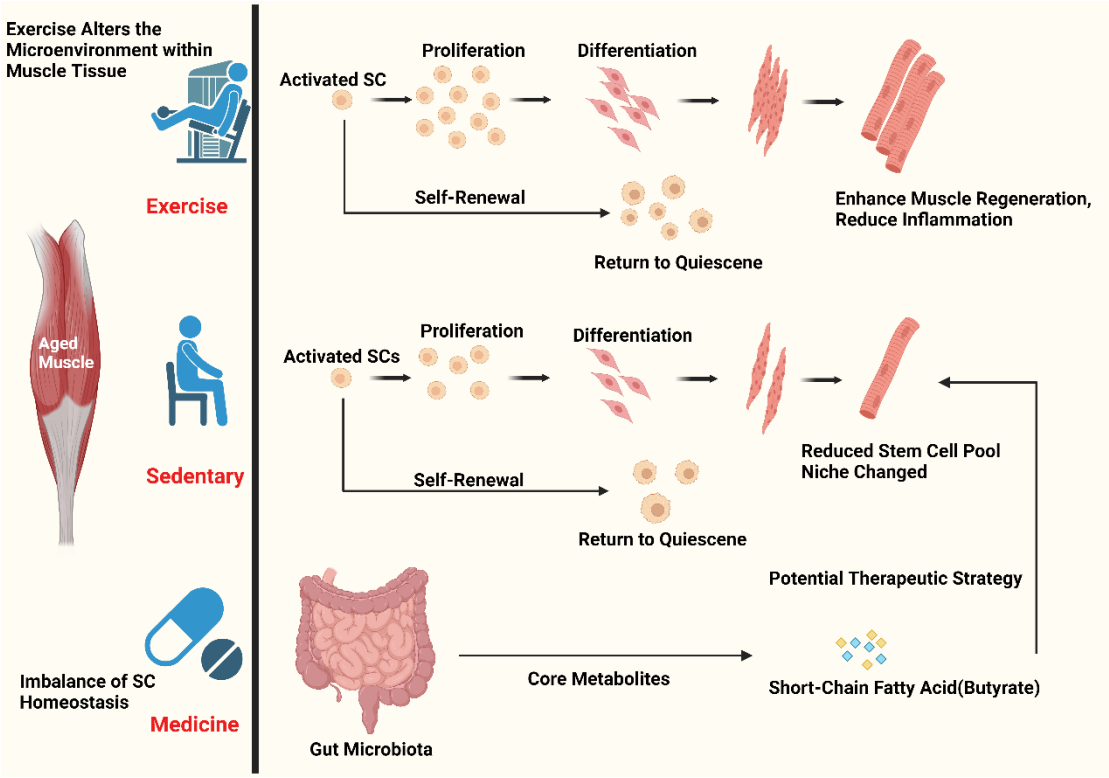

Figure 4. Gut microbiota and exercise regimen as strategies for satellite cell rejuvenation. Aging satellite cells show increased apoptosis, reduced self-renewal, and diminished muscle repair ability. Revitalizing satellite cells and promoting muscle regeneration through modulation of the gut microbiome and utilization of exercise-induced factors can serve as potential therapeutic strategies. Created with BioRender.com.

7.2. Exercise Regimens as Strategies for Satellite Cell Rejuvenation

Exercise is recognized for its beneficial impact on health, particularly in enhancing muscle growth and addressing muscle-related diseases like sarcopenia. Recent advancements have shown that physical activity prompts several body tissues, such as the liver, muscles, and fat, to release a variety of cytokines. These cytokines have been found to enhance or increase the numbers of adult stem cells, offering potential treatments for various illnesses. Satellite cells (SCs) play a vital role in the growth, upkeep, and restoration of skeletal muscle after birth. There's growing evidence that exercise affects muscle functionality largely through the release of cytokines induced by physical activity, which influence the potential of SCs, although the mechanisms involved are complex and still being unraveled [89].

Exercise positively influences muscle health by altering the microenvironment within the muscle tissue, specifically targeting FAPs. Through the secretion of the myokine Musclin during exercise, the frequency of FAPs is reduced, which in turn diminishes collagen deposition and fat formation in muscles, especially after injury or disuse. Musclin acts by suppressing FAP proliferation and encouraging their apoptosis, a process mediated by the upregulation of FILIP1L, with FoxO3a playing a pivotal role as the transcription factor. Furthermore, the Musclin/FILIP1L pathway enhances the clearance of apoptotic FAPs by macrophages, which is crucial for reducing fibrosis and fatty infiltration in muscles. These findings underscore the importance of regular physical activity in maintaining muscle integrity, providing a potential therapeutic strategy to counter muscle atrophy or accelerate recovery following acute muscle injury [90]. Exercise significantly benefits muscle health by enhancing the functionality of muscle-residing Tregs, which are vital for maintaining muscle integrity and promoting regeneration. Regular physical activity leads to an increase in the functionality of Tregs, characterized by elevated levels of important molecules like amphiregulin, EGFR, and ST2. This process is crucial for the repair and

maintenance of muscle tissue, particularly following injury or in conditions like sarcopenia [14]. The transcriptional co-activator with PDZ-binding motif (TAZ) activation in satellite cells post-exercise or muscle injury promotes muscle regeneration and may counteract muscle aging, suggesting its potential as a therapeutic target for conditions like sarcopenia [69].

Different types of exercise interventions, such as aerobic exercise and strength training, engage distinct muscle fiber types. Aerobic exercise primarily targets slow-twitch (type I) muscle fibers, enhancing endurance and oxidative capacity, while strength training predominantly engages fast-twitch (type II) muscle fibers, promoting hypertrophy and strength gains [91]. Previous research has shown that aging-related muscle deterioration, or sarcopenia, primarily affects fast-twitch fibers, highlighting the critical role of strength training in mitigating this loss. For example, studies have demonstrated that resistance training can significantly improve muscle mass and function in older adults by specifically targeting type II fibers, which are most susceptible to age-related atrophy [92].

In contrast, aerobic exercise interventions, such as walking or cycling, have been shown to improve cardiovascular health and metabolic function, but may have a limited impact on preserving fast-twitch fibers [93]. Understanding the specific types of exercise interventions and their effects on different muscle fiber types is essential for elucidating the mechanisms behind exercise-induced adaptations in aging muscles. Such knowledge will contribute to the development of precision exercise rehabilitation plans tailored to individual needs, particularly for preventing or reversing sarcopenia [94].

In addition to the existing methods, other potential treatments for satellite cell rejuvenation include the use of growth factors, such as insulin-like growth factor 1 (IGF-1), which has been shown to enhance satellite cell proliferation and differentiation. Furthermore, modulating the extracellular matrix (ECM) environment with matrix metalloproteinase inhibitors can support satellite cell function by promoting a more favorable regenerative niche. Pharmacological agents targeting mitochondrial function, such as resveratrol or nicotinamide riboside, may also play a role in improving satellite cell rejuvenation by enhancing cellular metabolism and reducing oxidative stress. These emerging strategies hold promise for enhancing muscle regeneration and combating age-related decline in satellite cell activity.

8. Conclusions and Perspectives

The review underscores the intricate and multifaceted relationships between satellite cells, their division patterns, and the broader physiological and pathological contexts of skeletal muscle. The crucial role of satellite cells in muscle regeneration, their response to aging, and their dysfunction in diseases like Duchenne muscular dystrophy highlight the complexity of muscle homeostasis and the potential for targeted therapeutic interventions. Emerging research on the interplay between the gut microbiota and skeletal muscle offers new insights into systemic factors influencing muscle health, suggesting that modulating the microbiome could provide novel strategies for enhancing muscle regeneration and function. Future research should continue to unravel the molecular mechanisms governing satellite cell behavior, explore the systemic influences on muscle health, and develop innovative therapies to combat muscle-related diseases and aging. The potential of manipulating gut microbiota and leveraging advanced gene editing technologies holds promise for not only understanding muscle biology in greater depth but also for offering new avenues for treatments aimed at restoring muscle function in various pathological conditions.

Lead Contact and Materials Availability

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Liwei Xie (xielw@gdim.cn). All unique/stable reagents generated in this study are available from the Lead Contact with a completed Materials Transfer Agreement.

Author Contributions: L.X. designed the frame, S.W. drafted the manuscript, G.Y. and L. X. edited the manuscript. L.X. provided financial support and resources. All authors have read and agreed to the published version of the manuscript.

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