Chapter

Skeletal Muscle Stem Cell Niche from Birth to Old Age

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Abstract

Stem cells are defined as undifferentiated cells that are able to unlimitedly renew themselves within controlled conditions and to differentiate into a multitude of mature cell types. Skeletal muscle stem cells, represented predominantly by satellite cells, show a variable capability of self-renewal and myogenic differentiation. They were found to be involved not only in the growth of myofibers during neonatal and juvenile life but also in the regeneration of skeletal muscles after an injury. The microenvironment in which stem cells are nourished and maintained dormant preceding division and differentiation is known as "niche." The niche consists of myofibers, which are believed to modulate the active/inactive state of the stem cells, extracellular matrix, neural networks, blood vessels, and a multitude of soluble molecules. It was observed that changes in the composition of the niche have an impact on the stem cell functions and hierarchy. Furthermore, it seems that its layout is variable throughout the entire life, translating into a decrease in the regenerative capacity of satellite cells in aged tissues. The scope of this chapter is to provide a detailed view of the changes that occur in the skeletal stem cell niche during life and to analyze their implications on tissue regeneration. Future studies should focus on developing new therapeutic tools for diseases involving muscle atrophy.

Keywords: stem cells, niche, skeletal, aging, regeneration, muscle fibers

1. Introduction

Being crucial for the survival, the striated muscle tissue that forms skeletal muscles takes up to 40% of the human body weight and is responsible for locomotion, maintaining the posture of the body, breathing, swallowing, micturition, and defecation [1, 2]. Furthermore, skeletal muscles were found to present endocrine and paracrine functions through the secretion of myokines, as well as thermogenesis abilities [3]. Each muscle comprises a multitude of myofibers that organize themselves into fascicles by wrapping with a layer of connective tissue known as perimysium [4]. Myofibers are long, cylindrical multinucleated cells that are individually enveloped in another layer of connected tissue called endomysium [4]. The myofibers provide skeletal muscles with contractile abilities and are formed in the prenatal life by the fusion of a number of cell progenitors known as myoblasts [2].

While the myofibers enable the muscle to contract and exert its functions, there are other types of cells, known as skeletal muscle stem cells that were proved to be responsible for muscle regeneration after injury [5]. Stem cells were defined as undifferentiated cells that present self-renewal abilities when proper stimuli exist and can generate various mature cell types through differentiation [6]. The environment in which stem cells are found is known as "niche" and its changes in composition were found to consequently influence their behavior [1]. Previous research regarding the characteristics of the niche found that its composition is highly heterogenic, varying not only with age, but also with the demands of the body [7]. In general, the muscular niche comprises an extracellular matrix known as the basal lamina, various interstitial cells such as fibroblasts and adipocytes, blood vessels, neural fibers, and a multitude of growth factors and signaling molecules [5].

Satellite cells, which are the most frequent stem cells found in the skeletal muscles, were first observed on the electron microscope by Alexander Mauro over 50 years ago [8]. They were given this name due to their sublaminar position and their close connection to myofibers [2]. Following their discovery, numerous studies were conducted in order to uncover the role they play in muscle repair and regeneration and how the stem cell niche is modulating their behavior [2]. In addition to their involvement in muscle repair, recent studies suggest that the skeletal muscle stem cells might even play a secondary role in bone regeneration [9]. Although satellite cells are the most frequent and easiest to study, other stem cell populations residing either in the skeletal muscle, or in other tissues, were found to possess variable muscle regenerative abilities [10]. Satellite cell properties as well as the different types of muscle progenitors will be described in detail in this chapter.

Studies showed that the number of myofibers does not change during the first stages of life and that the growth of the muscular system is obtained through the fusion of satellite cells with myofibers, resulting in an increase in size of the latter [2]. After the physiological growth of the organism stops, the skeletal stem cells are maintained in an inactivated state by various factors in the stem cell niche until they are needed for muscle repair or to participate in the daily muscle turnover [11].

The satellite cells are activated by growth stimuli or by the physical trauma located in the muscle, leading them to enter the mitotic phase and start to divide into myoblasts, which through differentiation will be able to fuse among themselves and with other myofibers and repair the damaged muscle [12, 13]. In addition, satellite cells can expand their stem cell pool through asymmetric division, thus demonstrating their self-renewal abilities and ensuring the continuance of the muscle regeneration process [12]. However, with aging and also in various degenerative muscle diseases, the regenerative abilities of satellite cells diminish, leading to muscle atrophy and the replacement of muscle fibers with connective tissue [7, 14]. These changes were attributed to a multitude of changes in the composition of the stem cell niche that occur during life, which will be further described in this chapter [7, 14].

The alteration of the skeletal stem cell niche and thus of satellite cell functions can be seen not only in aged muscle but also in a multitude of degenerative diseases. One example is Duchenne muscular dystrophy (DMD), a genetic disorder with no existing curative treatment in which a specific gene mutation causes the synthesis of an altered protein known as dystrophin, thus leading to progressive muscle degeneration and fibrosis which will result in loss of ambulation and cardiorespiratory insufficiency [15]. Dystrophin is known to be responsible for the basal lamina-myofibers connection; however, recent studies showed that it is also involved in the modulation of muscle stem cell division [13]. Additional research is needed in order to fully understand how satellite cells and their niches are affected by DMD.

It is crucial to understand all the pathways that are involved in the functioning of the skeletal stem cell niche and the way they are altered with the aging of the human body in order to be able to develop new treatment strategies for muscle degenerative diseases and maybe delay the effects that time has on the muscular system. Extensive research has been made in the field of regenerative medicine, making the idea of bioengineered muscle regeneration increasingly plausible. However, there are still many unanswered questions that prevent the applications of satellite cell's regenerative and self-renewal abilities to reach their full potential.

2. The skeletal muscle stem cell niche: structure and roles

The stem cell niche concept was first described in 1978 by Schofield, as an explanation to a series of experimental findings focusing on hematopoiesis and the bone marrow cells, which outlined notions concerning the anatomic site of reproduction, sustenance, and differentiation of the stem cells [16–18]. According to this theory, the niche represents a versatile environment, where the states change cyclically, in order to either support the quiescence of the stem cells or to activate them, according to the local or systemic stimuli [14]. Each type of tissue has a specific support system characterized by distinct cellular components; some of the most studied ones belonging to sites which present a high turnover rate such as the skin, with the matrix stem cells and the dermal papilla, the gut with the crypt stem cells and the mesenchymal and Paneth cells, or the hematopoietic stem cell niche and osteoblasts [14, 19, 20].

The skeletal muscle stem cell niche is also an example of a highly designated niche, consisting not only of specialized stem cells such as the satellite cells, but also of a complex milieu of elements ranging from the neural-vascular framework and surrounding cells to the extracellular matrix and diverse soluble molecules [2, 21]. In this chapter, we discuss in detail the cellular structure of the niche and the various roles that every type of constituent plays in the muscle behavior in regards to growth, maintenance, and regeneration [22].

2.1 Satellite cells and other muscle progenitors

During embryogenesis, the paraxial segmental mesoderm gives rise to the somites, which subsequently divide into the dermomyotome, which further generates the skeletal muscle of the body and limbs as well as the overlying derma, and the sclerotome, which contributes to the cartilage and bone formation of the spine and rib cage [10, 23, 24]. In the first stages of muscle development, a primary myotome is formed by delamination of muscle progenitor cells, expressing MYf5 and Mrf4, from the epithelial dermomyotome [25]. Subsequently, another subtype of muscle progenitors that express Pax7 and Pax3 migrate from the central dermomyotome toward the primary myotome, where some contribute to the further differentiation and growth and of the muscle, while others maintain a continuous pool of muscle progenitors that represent the largest reservoir of adult satellite cells for the muscles of the trunk and limbs [26, 27]. During the last decades, extensive research has been conducted in order to determine other types of non-somitic muscle stem progenitors, concluding that the embryonic dorsal aorta [28] can also serve as origin for the stem cells, along with various cells that exhibit myogenic potential such as the bone marrow stem cells [29, 30], pericytes [31], mesangioblasts [32], specific side population cells [33], and interstitial and mesenchymal cells [34, 35].

The first description of a satellite cell was made in 1961, when Katz and Mauro discovered a mononucleated cell positioned at the outer edge of the muscle fiber, while studying the muscle tissue in frogs and rats with the help of electron

microscopy [8, 36]. Using the same imaging technique, it was established not only the cell's location between the basal lamina and the exterior plasma membrane of the myocyte, but also the morphological features: a small nucleus with elevated levels of heterochromatin, an abundant cytoplasm, and scarce organelles [37]. Since their discovery, extensive efforts have been made in order to demonstrate the stem cell characteristics and to identify the role they play in muscle growth and regeneration. In this regard, [3H]thymidine labeling and tracing experiments in regenerating or growing muscle proved that satellite cells contribute to this process by yielding myonuclei to emerging myofibers [38, 39]. To strengthen this evidence, in vitro cultures of isolated myofibers and their adjacent satellite cells showed that renewed myotubes arise from the satellite cell-derived myoblasts clonal expansion and fusion, demonstrating thus the stem cell's regenerative capacity [40–44].

Regeneration of the muscle tissue is a complex process that can be induced by either disease, injury, or exercise, involving a series of events like cellular degeneration, inflammation, further stem cell activation, and differentiation, followed by maturation and remodeling of the new fibers and the surrounding environment [45–47]. Activation of the satellite cells implies transitioning from the quiescent phase to a mitotic phase, event in which a series of signaling pathways and molecular elements, such as notch signaling pathway and map kinase phosphorylation process by the hepatocyte growth factor activation (HGF) and fibroblast growth factor 2 (FGF2), among others, participate [48–52]. Upon activation, satellite cells start expressing MyoD, a transcription factor promoting genes involved in the progression of the cell cycle, and along with preexisting expression of Pax7, M-cadherin, and Myf5, they start dividing [53, 54]. The differentiation process of the newly created myoblasts is governed by the Wnt signaling pathway, FGF, myostatin, an important regulator of muscle stem cell proliferation [55–57], which works together with myogenin and MyoD to generate multinucleated myofibers [58–60].

Apart from the regenerative capacity, satellite cells possess the ability to renew themselves, generating thus a continuous pool of stem cells. This theory of self-renewal was first stipulated in the pulse-chase experiments of Moss and Leblond, being further supported by the studies of other lineages such as the skin and gut that showed similarities between the transit amplifying cells and satellite cells [38, 61–63]. Another study focusing on transplanted myofibers in a myopathic mouse model found that a new population of satellite cells was generated after the resident muscle stem cells were inactivated by radiation, demonstrating thus the self-regenerating ability of the satellite cells [64]. As mentioned before, in restoring muscle tissue, satellite cells undergo a transition from a quiescent state to an activated state. Recent studies have demonstrated that the reverse process can also take place, as the activated satellite cells can exit the cell cycle and reenter the quiescent state, replenishing thus the progenitor pool [65–67], still, further research is required in order to elucidate the exact mechanisms of the self-renewal process.

Extensive research concluded that the satellite cells do not represent the only type of cell capable of muscle regeneration; several other cells exhibiting similar characteristics of which bone marrow stem cells [29, 30], pericytes [31], mesangioblasts [32] and specific side population cells [33] are some of the most studied ones. In this regard, strong evidence coming from lineage experiments indicated that bone marrow-derived stem cells, when administered intravenously or intramuscularly in irradiated mice, have the capacity to generate myofibers and to restore the satellite cell pool [68]. Following a study regarding the GFP-labeled bone marrow transplantation into mice, LaBarge and Blau et al. also concluded that bone marrow stem cells display myogenic potential by reconstructing the stem cell niche [68]. Recent studies suggest that pericytes, the contractile cells responsible for the regulation of capillary blood flow, exhibit a multipotent trait, allowing them to differentiate not only

toward the skeletal bone and adipose tissue precursors but also into skeletal stem cells [69–71]. Prototype experiments involving pericyte transplantation in mice with dystrophic muscles proved that pericytes may represent a promising candidate for future treatments for similar affliction in humans due to their myogenic potential [31, 72].

2.2 Satellite cell cellular and acellular environment

The skeletal muscle stem cell niche is the biologic environment of the satellite cells and other muscle progenitor cells where biochemical and biophysical factors sustain cellular processes such as quiescence, self-renewal, multiplication and differentiation, necessary for maintenance, and repair of the muscle. Apart from stem cells and myofibers, the niche is a home to a variety of other cellular and acellular components ranging from the basal lamina, connective tissue, nerves, vessels, extracellular matrix, or immune cells that together design the optimal conditions to assist the transition through the various processes of the niche.

In this respect, one of the most intimate structures within the niche is the basal lamina, a network of extracellular matrix composed of collagen IV, laminin α2, fibronectin, and tenascin, linked together through a glycoprotein core of heparan sulfate [18, 73, 74]. This structure enables not only the anatomical sustenance of the myofibers through integrin linkage but also accumulations of growth factors such as FGF, HGF, VEGF, and TGFβ1 [75–77]. Several studies concluded that the loss or deficiency of laminin α2 impacts the muscle stem niche quiescence by reducing the number of stem cells during development, as well as increased myogenin expression, inhibiting proper differentiation [78, 79].

Another major component of the niche environment is represented by the interstitial cells, of which the most abundant types are the fibroblasts and the adipocytes. Both of these types of cells increase in number due to the transdifferentiating potential of the myoblasts and satellite cells showed by in vitro studies [80, 81], supporting the hypothesis that the muscle is able to sustain a balanced environment during regenerative processes. Nevertheless, surplus in number regarding adipocytes and excess connective tissue produced by the fibroblasts have been thoroughly linked to conditions, such as aging or muscular dystrophy [82–84].

The vascular network is one of the main nourishment suppliers for the stem cell muscle niche, playing an important role not only in angiogenesis but also in myogenesis. It has been shown that these two processes emerge simultaneously during muscle regeneration, the most important factors involved in this event being represented by VEGF, IGF-I, PDGF, and HGF [85]. VEGF has been observed to stimulate not only angiogenesis but also cell migration and differentiation, myofiber hypertrophy to prevent apoptosis [86–88].

Several studies have observed that stem cells tend to group around the neuromuscular junction, suggesting that the motor neurons interact with the niche during specific times. Denervation studies portrayed that the modifications in membrane potential, ion channel conductance, and distribution of acetylcholine receptors lead to the remodeling of the niche composition, following the activation of the muscle stem cells [89]. A combination between the absence of neurotrophic factors and a prolonged state of loss in neural communication has been also proved to lead to structural alterations, more specifically to myofiber atrophy [90].

This dynamic environment can be also influenced by a number of systemic factors, some of them being represented by immune cells and inflammation, androgens or nitric oxide [2]. Upon injury, satellite cells release the proinflammatory cytokines that promote immune cell migration to the muscle that in turn help the stem cells to detach from the basal lamina through a series of diffusible molecules, in order for them to further proliferate, differentiate, and repair the muscle in

regards to muscle [91]. Androgens seem to impact the satellite cell nice by stimulating the stem cell activation and proliferation, while nitric oxide has been shown to provide a protective effect against fibrosis [92, 93].

3. Alterations of the skeletal stem cell niche during aging

Satellite cells, known as muscle specific stem cells, take the responsibility of generating new muscle fibers as a response to injury in the adult human body. However, the regenerative abilities of an aged muscle are significantly reduced, while the susceptibility of developing age-related pathologies is increased [14]. In order to better understand the mechanisms that contribute to declining stem cell function with age, it is important to firstly identify the cell-extrinsic and cell-intrinsic factors that have an influence on stem cell activity. Conditions within the niche are extremely important in order to maintain stem cell activity, and they need to be conducive to maintaining stem cell quiescence in the absence of any external activating cues while also promoting proliferation, maturation, and ensuring the self-renewal of the stem cell pool. Thus, the niche represents an inherently dynamic environment, which switches between the quiescent and the activated niche as a response to local and systemic influences. Any perturbation between the cell resident in the immediate vicinity and in direct contact with the stem cell is predicted to alter stem cell function [94]. Some previous research was focused on describing the characteristics of satellite cells residing in aged muscle, thus providing critical information on the transformations that occur with the passing of time. One study conducted on old mice revealed that the nuclear-cytoplasmic ratio is significantly higher compared to other cytological features that are almost identical with the ones identified in younger mice [95]. During the aging process, satellite stem cells display a delayed response to activating stimuli and also have a reduced proliferative expansion due to the fact that some progenitors tend to adopt alternate lineages [80, 82, 96, 97]. Furthermore, satellite cells were described to have higher apoptosis rates in the aged muscles [98].

In aging muscles, due to the accumulation of toxic products derived from the degradation of connective tissue components, some essential functions of the basal lamina are compromised. Necrosis is the result of the cleavaged fibronectin and elastin products present in the connective tissue of aging mice [99]. Studies on aged muscle sections revealed the presence of extra lamina encroaching into the satellite cell-myofiber interspace and mononucleated cells completely enveloped by the basal lamina [95]. Although the functional consequences of this less intimate association of satellite cells with myofibers in aged muscles are still unknown, it is believed that this phenomenon can be correlated to the decreasing percent of satellite cells in the later stages of life [82].

Numerous studies were conducted focusing on the molecular mechanisms that underline satellite cell aging. Heterochronic satellite cells were transplanted from old mice into young specimens, indicating that the mechanisms that modulate the satellite cell regeneration potential may be cell-extrinsic. Furthermore, various changes were observed regarding the availability of Wnt, Notch, FGF, and TGF-β-superfamily ligands, and also in cytokine signaling through the JAK-STAT pathway. Moreover, the self-renewal defects may be cell-intrinsic, as satellite cell aging was associated with an increase in stress-induced p38-MAPK signaling and cellular senescence [100].

3.1 Niche composition and functions at birth and in the early life

Myogenesis is a well-controlled process in which the dermomyotome is formed from the dorsolateral side of the somite, and from there, the progenitor cells will

differentiate in order to form multinucleated myofibers [24, 101, 102]. Even if it was thought to be an interrelation between the existence of multipotent cells and tissue development, a group of somatic stem cells was discovered both in mature and early post-natal skeletal muscle. These are believed to have important contribution in regeneration, homeostasis, and muscle growth [103].

The first remarks about a stem cell population that originate in skeletal muscle were made by Mauro and Katz in 1961 [8, 104]. They analyzed the muscle samples from frog and rat, and using electron microscopy for identification, they postulated that satellite stem cells are located in a particular place (between the basal lamina and the sarcolemma), and it represents an exclusive niche which preserves and regulates the survival and behavior of the stem cell [105]. Satellite cells express specific markers: Pax7 and Pax3 (paired box transcription factors) [106, 107], M-cadherin [108], FoxK (Forkhead box protein K) [109], NCAM (neural cell adhesion molecule) [110] c-Met (tyrosine-protein kinase Met) [111], VCAM-1 [112], CD34 [113], Syndecan 3, Syndecan 4 [114], Sox 8 and Sox 15 [115, 116], Integrin α7, Integrin β1 [117], caveolin-1 [118], CTR (Calcitonin receptor) [119], Emerin, Lamin A/C [120], Hairy [121], and Dystrophin [122].

During post-natal life, satellite cells are responsible for muscle growth and tissue regeneration under the action of appropriate stimuli. This role was confirmed by a study which analyzed transgenic mice without satellite stem cells. The mice revealed a significant deficiency in skeletal muscle mass, lower body weight, and smaller myofiber size [106]. An important decrease in the number of cells was observed, from 30% at birth to 5% at 2 months old. In the adult life period, the cell number remained constant [123]. Even if the implication of satellite cells in muscle regeneration has been well documented and described, their role in muscle growth during adult life still needs further studies [124].

3.2 Changes in niche composition throughout the time

Discovering the link between stem cells and their niches presents a great interest for the biology field. Although previous reports debating the caring relationship between stem cells and signaling molecules deployed by niche cells were published, the role of extra-cellular matrix (ECM) into the niche is still unclear. Previous studies highlighted that at activation, satellite cells are responsible for establishing the local reshaping of the ECM, and for the accumulation of laminin-α1 and laminin-α5 right into the basal lamina of the satellite cell niche. Moreover, genetic modification of laminin-α1, integrin-α6 signaling, or blocking matrix metalloproteinase activity were shown to prevent the cell capacity of expansion and self-renewal. Remodeling of the ECM favors dissemination and self-renewal, and could justify the effect of laminin-α1 containing supports on stem cells [5].

Stem cells competence decreases with age, and it is associated with chronic diseases in mammals. In diseased or aged muscles, myofibers are replaced by fat and fibrous tissues, while the remaining myofibers decrease in mass. During aging, not only the percent of satellite cells decreases, but also their expression levels of Pax7, consequently leading to a decrease in myogenicity and an increase in apoptosis [125].

4. Implications on muscular regeneration and disease

Skeletal muscles possess contractile properties that are crucial for vital functions of the body such as breathing, postural support, and movement while also participating in the systemic metabolism and thermogenesis due to their endocrine and paracrine functions [3]. Following actions that involve contraction and stretch, micro-lesions

can occur in the plasma membrane of muscular cells or in the T-tubule organization, leading to the organization of specific proteins and lipids which form a repair-patch and seal the injury. However, during trauma or surgery numerous contusions, strains and laceration can occur, and, in these circumstances, myoblasts fuse between themselves or with adjacent myofibers and repair the damaged muscle. One important fact is that myoblasts can only fuse with non-lethally damaged muscle cells [126–128].

It is widely known that skeletal muscle has a remarkable capacity for regeneration, which places it second after the bone marrow. The main type of stem cells in charge of muscle regeneration is represented by satellite cells. Satellite cells are able to remain in a non-dividing state in the unharmed muscle and can be recognized by their α7 integrin and Pax7 expression. This specific population of cells gets triggered when muscle trauma occurs, thus activating the expression of MYF5 and MYOD and becoming fusion-competent myoblasts which will further fuse in order to give rise to new muscle fibers [8, 129–132]. During muscle injury, there are satellite cells that do not differentiate, with downregulated MYF5 and MYOD expression levels, which were described to replace the satellite cell population, ensuring the ability to respond to future muscle damages [2, 67, 133, 134].

Studies showed that alongside with satellite cells, there exist various populations of non-satellite cells, such as side populations, CD133 + cells, pericytes, and mesangioblasts (Mabs) that have myogenic abilities, contributing to regeneration and homeostasis maintenance [31, 135–138]. Their involvement in muscle regeneration was not firmly demonstrated and future studies are needed. The regenerative capacity of this cell category was demonstrated following some experiments on mice [135]. Side population cells were transplanted into mice suffering from a form of Duchenne muscular dystrophy, leading to an improvement in muscle function and a restoration of dystrophin expression levels [135]. Similar results were obtained by intraarterial or intramuscular injecting CD 133+ cells into scid/mdx mice [136, 139]. Two other populations of non-satellite cells are pericytes and Mabs, the latter were described to derive from pericytes [31]. Pericytes are involved in the in situ regeneration and muscle growth in early life [140]. Studies revealed that Mabs can take part in muscular regeneration after being engrafted or intraarterial injected in dogs and mice [137, 138]. Researchers discovered that the behavior of satellite cells could be highly influenced by surrounding cells, growth factors such as the vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-1, fibroblast growth factor (FGF), cytokines, and neighboring cellular matrix [141]. For example, one study showed that satellite cells which have grown in vitro for a short period of time partially lost their myogenic capacity in contrast to freshly isolated satellite cells [129]. In order to sustain a faster and more adequate tissue regeneration, a positive feedback loop was described between the endothelial cells and satellite cells located near small blood vessels. Endothelial cells enable satellite cell proliferation through the secretion of growth factors, while differentiated myoblasts stimulate angiogenesis [85].

Lately, two studies, both conducted by injecting diphtheria toxin in mouse models, speculated that muscle repair is not possible without satellite cells, even under normal physiological conditions [142, 143]. During the experiments, neither non-satellite cells, nor the innervation and vasculature were altered. One of the studies developed mouse models in which only cells expressing Pax7 were killed by the toxin, while the second study crossed murine expressing an inducible diphtheria toxin with murine expressing under the control of Pax7 tamoxifen-inducible conditional recombinase [142, 143] . However, further studies are needed in order to undoubtably state that muscle restoration can only take place if satellite cells are present.

4.1 The regenerative muscle stem cell niche

In order to analyze the myogenic mechanism of the skeletal muscle, several injury models in mice were developed, including chemical injuries such as intramuscular injection of snake venoms notexin, cardiotoxin, and barium chloride, together with freeze injury and crash [144, 145]. The following regenerative response was found to comprise three phases: an inflammatory phase, a proliferative phase and, lastly, a differentiation phase.

Instantly after muscular damage, necrotic fibers hyper contract inside their basal lamina layer [146]. The remnant basal lamina is reconditioned by matrix remodeling enzymes and serves as a pattern for the development of new muscle fibers, and also guides the growth cones of motor neurons for reinnervation at original synaptic spots [147–153]. The necrosis of muscular fibers releases into circulation damage-associated molecular patters (DAMPs) that are tracked by both macrophages and mastocytes and mobilize neutrophils which deliver trophic factors to call up the satellite cells within 2 hours of damage [10, 154–156]. In this early phase of muscle regeneration, muscle tissue is cleansed of necrotic fibers through phagocytosis by macrophages and lymphocytes during this high inflammatory response phase [1, 147]. The proliferative stage is characterized by the expansion of stem cell niche and the generation of numerous transiently amplifying myoblasts which are waiting to differentiate [1]. The structural configuration of skeletal stem cell niche is modified by the accumulation of diverse components of the regenerative matrix. One of the components is represented by fibronectin, secreted by fibroblasts, satellite cells, and many other cells in the muscular tissue [157, 158]. Attachment to fibronectin is crucial for the prevention of anchorage-dependent cell's death, the regulation of asymmetric division and satellite cells segregation [159, 160]. Another component of the ECM is collagen VI secreted by fibroblasts, which is upregulated during the peak of satellite cells expansion and has essential mechanical properties in the skeletal muscle stem cell niche [161]. The satellite cells show a considerable proliferative ability in day 2 and 3 after an injury [10, 147]. Following the activation of satellite cells, monocytes convert into macrophages. M1 macrophages also exist in the mitogenic niche and secret VEGF, TNFα, IL-6, factors that are responsible for the limitation of early differentiation of myoblasts, stimulating the proliferation of stem cells instead [141, 162, 163]. When M2 macrophages become predominant to M1 macrophages, the first myoblasts start to differentiate [141, 164]. During the differentiation phase, myoblasts fuse to form multinucleated muscle cells and resident satellite cells and start to transit into a non-dividing state (quiescent state) [1]. At this point in the process of muscle regeneration, the blood vessels that irrigate the new muscle fibers become denser and well organized; smooth muscles and pericytes are initiated to sustain their structure, while immune cells limit the inflammatory reaction and secret anti-inflammatory cytokines to sustain tissue repair, resulting in the restoration of muscular architecture within nearly 2 weeks [10, 144, 147, 165–167].

4.2 Muscular stem cell niche in disease

The muscular stem cell niche suffers significant changes in muscle diseases such as inflammatory maladies, primary myopathies, and metabolic disorders [1]. The most notable, highly studied muscle pathologies are muscular dystrophies, defined by progressive muscle weakness caused by mutations in nuclear or sarcolemmal proteins such as dysferlin, dystrophin, and sarcoglycans, or by alterations of extracellular proteins [156]. Of these, the most common is Duchenne muscular dystrophy, an X-linked recessive disorder, diagnosed in early childhood, which is characterized by a progressive muscle-wasting process that affects skeletal muscles

including diaphragm, limb, and heart muscles, in which death occurs in teenage years to 20s by cardiorespiratory failure [168]. In Duchenne disorder, the affected gene is dystrophin, which has an important structural function in anchoring the muscle fibers to the ECM in the muscular stem cell niche [13]. Moreover, dystrophin, which is expressed by satellite cells, is situated near the cell membrane and coordinates the flow of signaling molecules; therefore, a low level of dystrophin has a direct influence on the downstream cell-intrinsic signaling pathways of satellite cells, altering their functions [13, 169].

In most of the muscular dystrophies, the structural architecture of muscle cells is fragile, and fibers are doomed to get ruptured during repeated contractions; the stem cell niche is changing in such a way that the skeletal muscles get infiltrated with fat and fibrotic tissue [156, 170, 171]. Muscle ruptures are followed by protein leakage that activates inflammatory cells (lymphocytes, neutrophils, natural killer, macrophages) [172]. In muscular dystrophies, the inflammatory response is distinct than the one in trauma: there are many foci of injury developed in a continuous and asynchronous manner and the inflammatory process becomes chronic, and the ECM becomes thick and rigid, altering the muscular stem cell niche [173, 174]. In the extracellular environment, researchers discovered an accumulation of collagen I, III, IV, V, higher levels of various heparan sulfate proteoglycans and, moreover, a distinct regulation of the expression levels of MMPs and their endogenous inhibitor (TIMPs), together with various serine proteases and their endogenous inhibitors (serpins) [175–182]. Furthermore, the increased levels of matricellular proteins like fibrinogen, dermatopontin, asporin, and periostin were observed, together with a downregulation of fibrillin and nidogen [183–186]. The muscular stem cell niche is also enriched in signaling molecules during this inflammatory process, which influences the myoblast differentiation and fusion [155, 187]. For example, higher levels of prostaglandins, cytokines, and chemokines are described in muscular dystrophy, fact that supports the regenerative failure of dystrophic fibers [188–193]. This long-term inflammatory process changes the satellite cells in such manner that they can no longer compensate for the fiber degeneration, leading to an altered muscle functionality.

Diabetes mellitus represents a category of metabolic diseases characterized by a deficiency in insulin generation and function, leading to hyperglycemia, a condition which decreases the antioxidant level and increases the levels of free radical species [194, 195]. Muscle renewal is altered in type 1 and 2 of diabetes mellitus, these patients having a poor lesion-healing capacity [194, 196–198]. There is a fibrotic disposition of collagen and atypical levels of TNFα, TGFβ and ILs in diabetic or obese rats and patients due to the high level of M1 macrophages [199–202]. A sustained exposure to glucose generates an accumulation of glycated lipids and proteins that have an unfavorable impact on myoblasts from both rats and humans [203].

Another dramatic muscular pathology is cachexia. This state occurs as a consequence of various disorders such as AIDS, COPD, cancer, and heart failure and consists in the heavy and accelerated loss of striate muscle mass [204]. Muscular fibers from mice with neoplasms or from cachectic patients present abnormalities in the architecture of the basal lamina and in the membrane of the muscle cells, rather than infiltration of immune cells like in dystrophies or diabetes mellitus [205, 206]. This affected niche together with circulating plasma factors contributes to a hyperactivation of satellite cells and other non-satellite cells including pericytes. Furthermore, satellite cells constantly express Pax-7 self-renewal factor, an action that abolishes the differentiation process, leading to regenerative failure of muscular fibers [1].

Collectively, the data reviewed above showed the importance of stem cell niche behavior in the muscle regenerative process; yet further studies are required to fully understand these complex mechanisms involved in the renewal of normal and pathological muscle.

5. Perspectives

Over the last three decades, researchers found that satellite cells are a heterogenous population of stem cells and dedicated progenitors for myogenesis in striate muscle. With the development of new technologies, like single cell sequencing, mass cytometry, or super resolution imaging, the detailed study of satellite cells during growth, differentiation, and quiescence state is continuously improving [1]. The progress in discovering personalized therapies is slow and full of challenges, especially in the field of rare muscle pathologies, yet the stimulation of endogenous repair as a prospective therapy for muscle diseases should be one of the key perspectives that should be further looked into [207, 208]. The stem cell niche changes in behavior and composition during a lifetime, having tree periods: juvenile, adult, and old age. It is known that there are difficulties in muscular stem cells isolation and preservation due to the fact that they lose their myogenic ability after growing in vitro even for a short period of time [129]. A question that is yet to be answered is whether the use of juvenile stem cells instead of adult ones would provide for more adequate cell cultures, increasing plasticity and improving muscle regenerative therapies. For this purpose, and for a better understanding of skeletal stem cell niche, future challenging studies are needed.

6. Conclusion

The muscular stem cell niche is a remarkable structure that enables satellite cells and other non-satellite myogenic cells to repair and regenerate skeletal muscles when needed. As previously stated, the niche componence is highly variable, depending not only on the age of the body, but also on its well-being since a multitude of degenerative muscle disorders can alter the stem cell environment, leading to a decrease in the regenerative abilities of satellite cells. One of the elements of the niche that was proved to change during aging is the basal lamina, a key structure that apparently tends to interpose between the myofibers and satellite cells in older muscles, thus altering their communication, a fact that is believed to be associated to the latter's decrease in number. Furthermore, it was observed that in aged skeletal muscles, myofibers were decreased in mass, in contrast to the number of fibroblasts and adipocytes, which tended to increase. Satellite cells displayed diminished myogenic abilities and an accelerated apoptosis, probably due to lower expression levels of Pax7. Similar changes were described in degenerative muscle disorders, one of the most studied and severe being Duchenne muscular dystrophy. The chronic inflammation that appears in these diseases is believed to thicken the basal lamina and overflow the satellite cells with signaling molecules, impairing their capacity to restore muscle fibers. Other chronic disorders like diabetes mellitus and cachexia were also associated with niche alterations. Research in the field of regenerative medicine promises to innovate the therapies in these pathologies; however, there is a long way ahead and additional studies are needed.

Conflict of interest

The authors declare no conflict of interest.

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