

Chapter 8

Plasticity of the Muscle Stem Cell Microenvironment

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Abstract Satellite cells (SCs) are adult muscle stem cells capable of repairing damaged and creating new muscle tissue throughout life. Their functionality is tightly controlled by a microenvironment composed of a wide variety of factors, such as numerous secreted molecules and different cell types, including blood vessels, oxygen, hormones, motor neurons, immune cells, cytokines, fibroblasts, growth factors, myofibers, myofiber metabolism, the extracellular matrix and tissue stiffness. This complex niche controls SC biology—quiescence, activation, proliferation, differentiation or renewal and return to quiescence. In this review, we attempt to give a brief overview of the most important players in the niche and their mutual interaction with SCs. We address the importance of the niche to SC behavior under physiological and pathological conditions, and finally survey the significance of an artificial niche both for basic and translational research purposes.

Keywords Skeletal muscle • Muscle regeneration • Satellite cells • Stem cell-niche • Muscular dystrophies • Extracellular matrix

8.1 Satellite Cells

Over the past half a century, the focus of research on muscle regeneration has shifted from other myogenic cells of muscle tissue to satellite cells (SCs), from developmental myogenesis to adult muscle regeneration, from cell-intrinsic properties of SCs to the relevance of extrinsic factors delivered by their niche. SCs, small, inactive cells wedged between the myofiber and the surrounding extracellular matrix (ECM), have attracted the attention of scientists since their discovery 56 years ago (Mauro 1961). The astonishing translational potential of SCs continues to fascinate, and the ever expanding knowledge of SCs and their microenvironment paves the

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way for the development of novel cell and gene therapies, in vitro disease models and preclinical drug testing paradigms. Here, we discuss different aspects of SC biology and the niche in health and disease. For a more detailed assessment of the particularities of SCs and the SC niche, we direct readers to several recent reviews focusing on the extracellular matrix (Thomas et al. 2015), blood vessels (Mounier et al. 2011), bioengineering (Bursac et al. 2015), SC function from a cell-intrinsic perspective (Almada and Wagers 2016) and an extensive review on SC biology (Yin et al. 2013).

8.1.1 Skeletal Muscle Regeneration and Muscle Stem Cells

Comprising approximately 40% of body weight, skeletal muscle can be considered the largest organ in the human body (Janssen et al. 2000). Muscle not only supports breathing and movement, but is also a very important metabolic and endocrine organ. It comes as no surprise that skeletal muscle has a remarkable capability to repair damage caused by injuries or simple everyday wear-and-tear. As numerous animal studies demonstrate, skeletal muscle is able to regain near original morphology and functionality after several weeks of serious damage caused by injection of myotoxic agents (e.g. cardiotoxin, bupivacaine, barium chloride or notexin), freezing, crushing, or complete mincing and re-transplantation (Rosenblatt 1992; Carlson and Gutmann 1972; Fink et al. 2003; Dinulovic et al. 2016a; Warren et al. 2004). However, aging, traumatic injuries in humans resulting in volumetric muscle loss and various myopathies result in impaired functionality and inability of the tissue to regain homeostatic conditions.

SCs are the main cells responsible for sustaining skeletal muscle morphology and functionality throughout the lifetime of an individual. They are largely lineage-committed adult stem cells located at the periphery of muscle fibers, situated between the sarcolemma (the myofiber membrane) and basal lamina (BL) (Mauro 1961), in close proximity to blood vessels (Mounier et al. 2011) and the neuromuscular junction (Kelly 1978). This specific environment surrounding SCs is known as the SC niche.

Under resting conditions, SCs are in the G0 phase (non-cycling state) and quiescent, with a heterochromatic nucleus and a thin rim of cytoplasm containing scarce organelles. These cells are most commonly distinguished by the expression of the paired box transcription factor Pax7. SCs have a tremendous myogenic potential and self-renewal capabilities, as demonstrated by single-fiber (Collins et al. 2005) as well as single cell (Sacco et al. 2008) implantation in irradiated muscles of immunodeficient mice.

The classical cascade of regeneration resembles that of prenatal skeletal muscle development (Bentzinger et al. 2012). In response to injury or other stimuli, SCs become activated, increase in size and begin to proliferate. The majority of the progeny reduces Pax7 and induces MyoD expression. After several rounds of proliferation, these myoblasts start to express myogenin and exit the cell cycle as myocytes.

The myocytes subsequently fuse in order to form new or repair existing myofibers (depending on the severity of injury). The myofibers then express myogenic regulatory factor 4 (MRF-4) and grow, supported by hypertrophy, until reaching their pre-injury size. At the same time, a part of the SC progeny reacquires high Pax7 levels and returns to quiescence, thereby replenishing the SC pool and maintaining sufficient reserves for future rounds of regeneration.

Besides SCs, several other cell types, such as muscle side-population (SP) cells, muscle-derived stem cells (MDSCs), bone marrow stem cells, PW1⁺ interstitial cells, CD133⁺ cells, mesoangioblasts (MABs) and pericytes, can successfully regenerate muscles and some can even reconstitute the niche upon transplantation into damaged muscle (Péault et al. 2007). However, the contribution of these cells seems to be very low under physiological conditions and dependent on SCs, which are essential for skeletal muscle regeneration and therefore represent the true stem cells of muscle tissue (Lepper et al. 2011; Sambasivan et al. 2011; McCarthy et al. 2011; Murphy et al. 2011).

According to their gene expression profiles and their characteristics in vitro SCs stemming from different muscle groups (e.g. head vs. limb muscles) are heterogeneous. Nevertheless, SCs from the masseter muscle (head) are able to regenerate the extensor digitorum longus (EDL) muscle (limb) as efficiently as SCs from the EDL muscle (Ono et al. 2010), attesting to the enormous influence of the in vivo microenvironment on the behavior and functionality of SCs, which in some cases can overcome the intrinsic differences between SCs.

8.1.2 The Heterogeneity of Satellite Cells and Its Dependence on the Niche

Several studies have addressed the heterogeneity of SC populations in regard to their renewal potential. Interestingly, SC heterogeneity was not only reported between different muscle beds, but also observed between SCs on the same muscle fibers, thereby implicating additional factors besides ontogeny and composition of the fiber type as possible causes. According to these studies, only a small proportion of SCs are *bona fide* stem cells, whereas the vast majority are committed progenitors with limited stemness. For example, Chakkalakal et al. discovered heterogeneity among SCs based on their proliferative history, suggesting that cells that cycle less frequently have higher self-renewal potential (Chakkalakal et al. 2012). On a related note, Rocheteau et al. evaluated differential DNA strand segregation, where one daughter cell retains the template strands, stays in the niche and returns to quiescence, while the other daughter cell receives newly synthesized DNA strands, continues to proliferate and finally differentiates (Rocheteau et al. 2012). It was suggested that such DNA strand segregation would prevent accumulation of proliferation-associated mutations in the stem cell, and therefore provide a lifelong supply of progenitors. Similarly, in a lineage tracing experiment with Myf5-Cre/

ROSA-YFP mice, Kuang et al. found that the majority of SCs are Pax7⁺/Myf5⁺, and only small subset are Pax7⁺/Myf5⁻ cells (Kuang et al. 2007). Upon isolation and transplantation, both cell populations are capable of proliferating and differentiating, but only Myf5⁻ SCs occupy the niche in the transplanted muscle. In addition, after in vivo activation, Pax7⁺/Myf5⁺ (committed progenitors) are exclusively prone to symmetrical division, giving rise to more committed progenitors, whereas Pax7⁺/Myf5⁻ (true stem cells) on the other hand can divide both symmetrically and asymmetrically, producing uncommitted and committed daughter cells. Mechanistically, the asymmetrical distribution of the Par complex results in p38 α / β MAPK activation and MyoD expression only in the committed daughter (Troy et al. 2012). Importantly, the capability to control the orientation of the cell division is tightly coupled to the SC niche. Following asymmetric division, the uncommitted progenitor remains in the niche in contact with the BL, whereas the committed progenitor is pushed towards the muscle fiber, thus losing contact with the niche. In contrast, both daughter cells retain contact with the BL and the myofiber during a stem cell pool expansion through symmetric division of Pax7⁺/Myf5⁻ cells.

8.2 The Satellite Cell Niche in Quiescence and Regeneration

SC quiescence, activation, proliferation, differentiation and renewal are intricately connected to the niche. There is a plethora of cell-cell and cell-matrix interactions, numerous paracrine and endocrine molecules (e.g. growth factors and cytokines), as well as biophysical properties of muscle that have a direct effect on the SC. However, this communication is bidirectional, as the SCs themselves also influence their local environment. The most important factors governing the niche in quiescence and activation are depicted in Figs. 8.1 and 8.2, respectively.

8.2.1 *The Extracellular Matrix*

In homeostatic conditions, SCs are situated just outside the muscle fiber, in direct contact with the sarcolemma and the ECM. The ECM surrounding muscle fibers is called the basal membrane (BM) and it consists of two parts—the reticular lamina (RL) and the BL, the latter being in direct contact with the fiber. The BM is a mesh composed of various glycoproteins and proteoglycans with sequestered growth factors. The main components of the RL are fibrillar collagens, whereas the main components of the BL are laminin-2 (α 2 β 1 γ 1) and non-fibrillar collagen IV (Sanes 2003). The laminins and collagen of the BL self-assemble into networks that are cross-linked by the glycoprotein nidogen. This network provides binding sites for components of the RL on one, and the sarcolemma and SC membrane on the other side. In addition, proteoglycans such as perlecan are anchored to the main BL mesh and bind polypeptidic growth factors with their glycosaminoglycan chains.

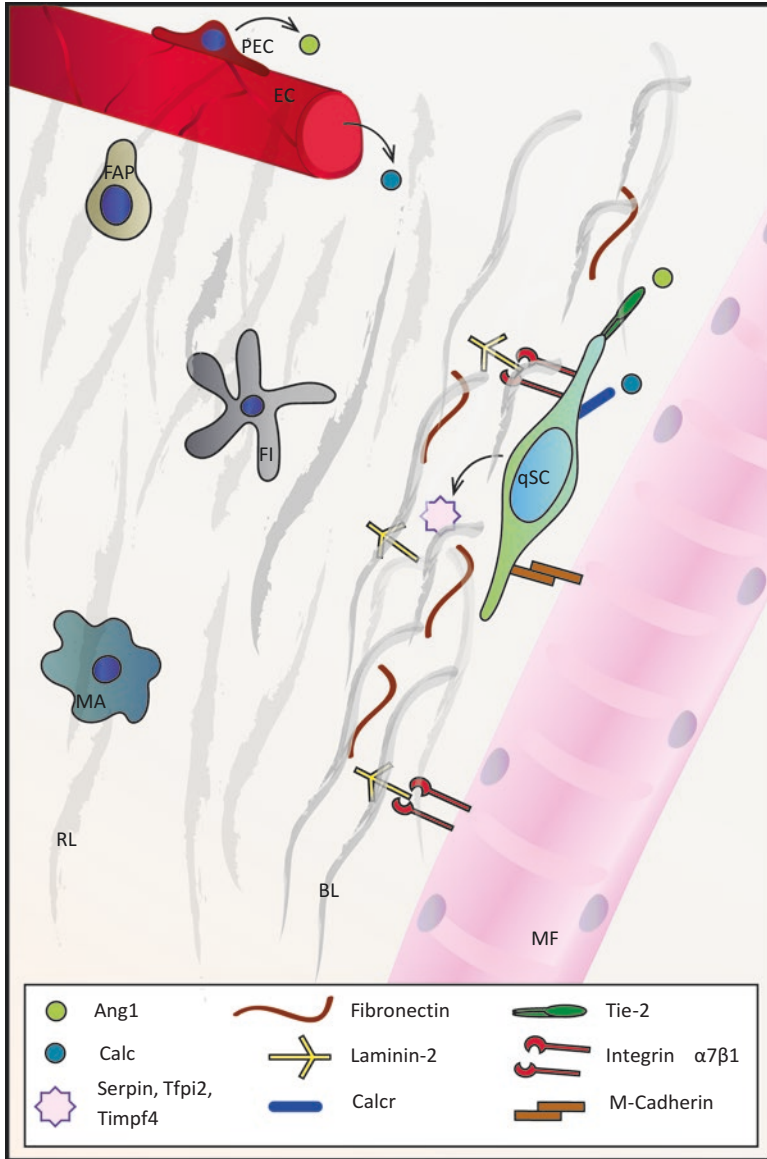


Fig. 8.1 The quiescent niche. Under homeostatic conditions, the SC and its niche are in a quiescent (q) state. The qSC receives signals that keep it dormant, such as angiopoietin 1 (Ang1) from periendothelial cells (PECs) and calcitonin (Calc) from the bloodstream, but at the same time actively contributes to the niche state by secreting protease inhibitors (Serpins, Tfpis, Timpfs). The SC is attached with M-cadherin to the myofiber (MF) and with integrin to the basal lamina (BL). Fibro/adipogenic progenitors (FAPs) and macrophages (MAs) are present in low numbers and are mainly inactive. Further abbreviations: *Calcr* calcitonin receptor; *EC* endothelial cell; *FI* fibroblast; *RL* reticular lamina; *Tie-2* receptor for Ang1

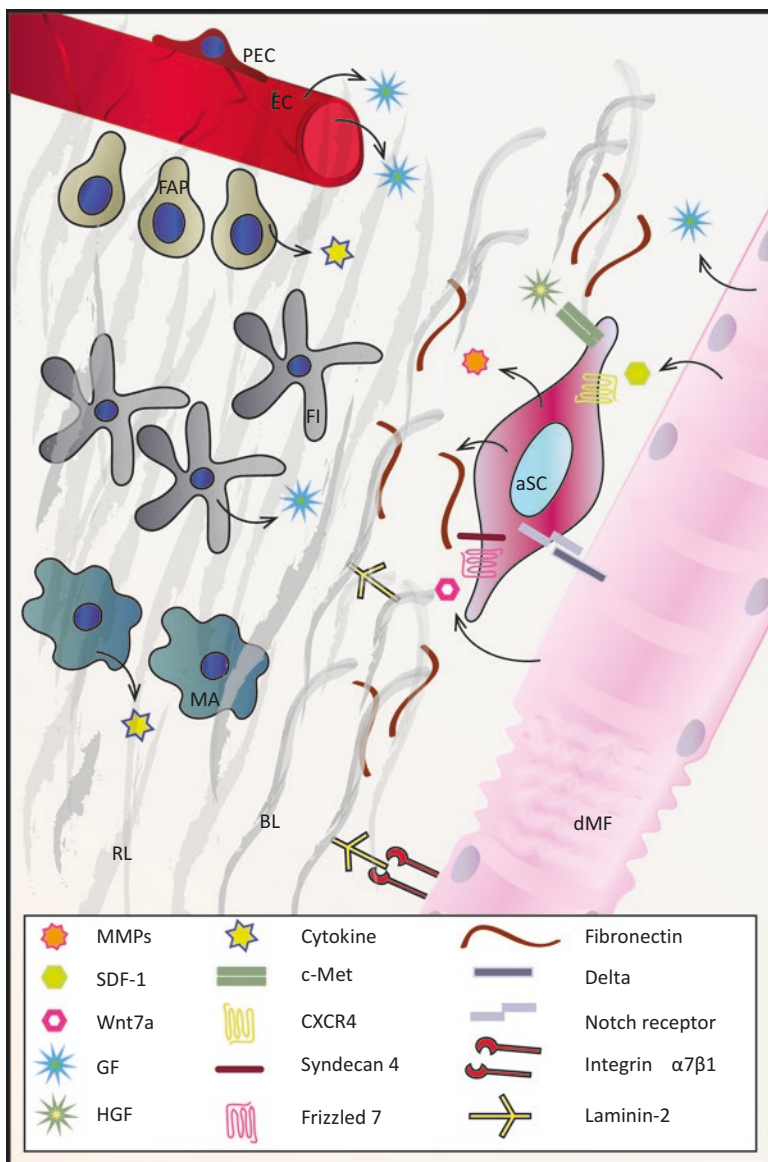


Fig. 8.2 The activated niche. The damaged myofiber (dMF) changes the signaling balance in the niche and causes activation (a) of the SC by liberating hepatocyte growth factor (HGF). The aSC increases in size and begin secreting matrix metalloproteases (MMPs), as well as fibronectin, which together with Wnt7a supports SC proliferation through the Frizzled 7 and syndecan 4 receptors. The dMF expresses Delta and secretes Wnt7a, stromal cell-derived factor 1 (SDF-1) and growth factors (GFs) that further regulate SC behavior. GFs are also secreted by fibroblasts (FIs), endothelial cells (ECs) and are delivered by the bloodstream to the niche. In addition, cytokine secreting fibro/adipogenic progenitors (FAPs) and macrophages (MAs) increase in number. The result is higher density of the basal lamina (BL) and reticular lamina (RL). Further abbreviations: *c-Met* receptor for HGF; *CXCR4* receptor for SDF-1; *Notch* receptor for Delta; *PEC* periendothelial cell

These growth factors, including fibroblast growth factors (FGFs), epidermal growth factor (EGF), insulin-like growth factors (IGFs) and hepatocyte growth factor (HGF), are secreted by various components of the niche, such as muscle fibers, interstitial cells and SCs, or can be delivered to the niche by blood vessels.

Integrins on the SC membrane and the sarcolemma bind to laminins in the BL, forming focal adhesions and contributing to mechanical stability between the ECM and intracellular cytoskeleton. However, these interactions also have important signaling functions. The main integrin isoforms on SCs are $\alpha 7$ and $\beta 1$, which bind to laminin-2 on the BL side (Blanco-Bose et al. 2001). After SC activation, the expression of integrins on the SC membrane changes, along with the preference for binding partners in the BL. For example, activated, but not quiescent SCs express the $\beta 3$ integrin isoform, which probably binds to fibronectin (FN) in a complex with the αv chain (Liu et al. 2011). Both quiescent and activated SCs also express the transmembrane heparin sulfate proteoglycans syndecan-3 and syndecan-4. These proteins form complexes with different tyrosine kinases such as c-Met and FGF receptor (FGFR) on the SC membrane and are consequently important not only for cell adhesion to the BL, but also for SC activation (Cornelison et al. 2001).

Expression profiles of quiescent and activated SCs suggest that SCs actively contribute to maintaining niche quiescence while remaining highly sensitive to activating stimuli (Pallafachina et al. 2010). Quiescent SCs express the protease inhibitors Serpin and Tfp12 as well as metalloprotease inhibitor Timpf4. Upon activation, however, these genes become downregulated, and instead, SCs start expressing the matrix metalloproteases MMP-2 and MMP-9 (Guérin and Holland 1995). MMPs are major enzymes responsible for ECM degradation.

Activated SCs also produce FN, an ECM glycoprotein whose role in SC maintenance by enabling their attachment to the niche has recently been demonstrated (Lukjanenko et al. 2016). SC-produced FN potentiates Wnt7a signaling through the receptor complex syndecan-4/Frizzled-7, thereby supporting symmetric division of SCs and expansion of the stem cell pool (Bentzinger et al. 2013). Specific knock-down of FN in SCs leads to a drastic reduction in symmetric division, in particular in the Pax7⁺/Myf5⁻ population, leading to a drop in SC numbers during regeneration.

Collagen VI is another BL component essential for preserving the SC pool. Fibroblasts are the prime producers of this protein as well as many other BL components. Collagen VI knock-out mice exhibit reduced regeneration and an inability to maintain SC numbers following injury. This defect is, however, rescued by transplanting wild-type fibroblasts, demonstrating the critical importance of non-SC-autonomous ECM factors in SC maintenance (Urciuolo et al. 2013).

8.2.2 *The Muscle Fiber*

On the apical side, SCs are bound to a muscle fiber, and M-cadherin is the main adhesion protein supporting the connection between these two cell types. Myofibers are important regulators of SC state: for example, myofiber damage or stretch induces nitric oxide (NO) synthesis in the BL, which is able to activate MMPs, and

through that action liberate ECM-bound HGF, allowing its binding to the c-Met receptor on SCs. This HGF signaling through c-Met has been proposed as an initial activation signal for SCs (Tatsumi et al. 2006).

SCs are furthermore affected by the Notch and Wnt signaling pathways in regard to quiescence, activation, proliferation and differentiation (Yin et al. 2013). Proof-of-concept was provided in different studies, e.g. by ablation of RBP-J κ , a downstream mediator of Notch. This ablation leads to spontaneous activation and differentiation of SCs without a proliferative phase, precipitating depletion of the SC pool and thus indicating that Notch signaling is essential for SC quiescence (Bjornson et al. 2012; Mourikis et al. 2012). Upon injury, damaged fibers express Delta, a ligand of the Notch receptor, which stimulates SC proliferation. In addition, regenerating fibers synthesize Wnt7a, which induces SC symmetrical cell division by binding to the Frizzled7 receptor (Polesskaya et al. 2003).

In regeneration, myofibers secrete stromal cell-derived factor-1 (SDF-1), which binds to the C-X-C motif chemokine receptor 4 (CXCR4) on SCs and induces SC chemoattraction (Ratajczak et al. 2003). Injured fibers and other cells of the niche also secrete FGFs, EGF and IGFs, which further regulate SC proliferation and differentiation. For instance, FGF-2 induces proliferation and represses differentiation of progenitor cells by binding to the tyrosine kinase FGFR and activating the Ras/MAPK pathway (Fedorov et al. 2001). Likewise, IGF-II supports proliferation, while the pleiotropic functions of IGF-I include stimulation of SC proliferation, differentiation, migration and anti-inflammatory effects on the niche (reviewed in (Philippou et al. 2007)). These effects of IGF-I are mediated through several signal transduction pathways, all initiated by IGF-I binding to the tyrosine receptor kinase IGF1R. The situation is further complicated by the existence of multiple IGF-I isoforms, as well as IGF binding proteins (IGFBPs) secreted by activated SCs, whose function is to transport IGFs and modulate their half-life (reviewed in (Jones and Clemmons 1995)). On the other hand, myofibers also secrete myostatin (Mstn), a member of the transforming growth factor β (TGF- β) family and negative regulator of muscle growth that has been implicated in reducing SC activation and self-renewal (McCroskery et al. 2003).

Much attention has been given to metabolic reprogramming of SCs, that is, the effects of the metabolism of a SC on its fate (Tang and Rando 2014). Some research proposes that in quiescence, SCs primarily rely on fatty acid oxidation (Ryall et al. 2015), whereas upon activation, they increase substrate utilization through glycolysis, and finally switch to oxidative phosphorylation during differentiation (Wagatsuma and Sakuma 2013). Other studies suggest that activated SCs depend more on oxidative phosphorylation (Tang and Rando 2014; Rodgers et al. 2014; Cerletti et al. 2012). It also remains unclear how metabolic substrate utilization in skeletal fibers (the SC niche) influences the SC state. Experiments with caloric restriction have suggested that the increased fatty acid oxidation and mitochondrial activity in the fiber in this context probably induce SC activation through increased oxidative phosphorylation (Cerletti et al. 2012).

Effects of fiber metabolism on SCs are furthermore implied by the observation that resting SC numbers are considerably higher in oxidative slow-twitch compared

to glycolytic fast-twitch myofibers (Gibson and Schultz 1982). Moreover, a similar difference in SC numbers can be achieved by endurance exercise, which promotes a switch from glycolytic to oxidative fibers (Shefer et al. 2010; Wilson et al. 2012). Although a conclusive explanation for the correlation between SC numbers and the oxidative fiber type remains elusive, the metabolic properties and the vascularization have been linked to this observation. The existence of a denser blood vessel network in slow fibers is of particular interest given close vicinity of the majority of SCs to blood vessels (Mounier et al. 2011). However, this simple view has recently been challenged. Namely, mice with myofiber-specific overexpression of peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 α), a nodal modulator of oxidative metabolism, exhibit both a switch to oxidative fibers and increased capillarity (Lin et al. 2002), but nevertheless have fewer SCs, albeit with an increased myogenic capacity (Dinulovic et al. 2016b). In fact, in regard to most metabolic and contractile traits, PGC-1 α transgenic, *bona fide* oxidative and endurance-trained muscles are indistinguishable. Interestingly, the muscle fiber PGC-1 α transgene affects expression of BM components FN and tenascin-C (Dinulovic et al. 2016b), which might account for the increased myogenic potential of the SCs. However, a possible influence of other differences in the microenvironment, for instance the increased percentage of M2 macrophages in resting conditions in these animals, should not be overlooked (Dinulovic et al. 2016a; Furrer et al. 2017). Therefore, an alternative explanation for the correlation between SC number and fiber type could be a difference in ECM organization. For example, the slow soleus muscle has double the amount of collagen IV and half the amount of laminin-2 compared to the fast rectus femoris in rats (Kovanen et al. 1988). However, the link between SC number and fiber type-specific ECM composition is still poorly understood and thus awaits further research.

8.2.3 Blood Vessels, Oxygen, (Peri)Endothelial Cells and Secreted Systemic Factors

The close proximity of SCs and capillaries suggests that blood vessels are an important part of the niche. Indeed, the close correlation between a well-developed capillary network and successful skeletal muscle regeneration has been demonstrated (Arsic et al. 2004; Ochoa et al. 2007). This is not surprising given the fact that a myriad of factors and cells that modify the SC niche, such as hormones and monocytes, are delivered by blood vessels. In addition, endothelial cells can secrete growth factors (EGF, IGF-I, bFGF) including vascular endothelial growth factor (VEGF) and platelet-derived growth factor BB (PDGF-BB), which promote SC proliferation (Christov et al. 2007). Conversely, differentiating myogenic cells also secrete VEGF, thereby stimulating angiogenesis (Rhoads et al. 2009). Interestingly, peri-endothelial cells, such as smooth muscle cells, secrete angiopoietin 1 (Ang1), which regulates the SC state by binding to the Tie-2 receptor that is highly expressed

in resting SCs. This interaction in turn induces the expression of quiescence markers and blocks the expression of differentiation markers in SCs through ERK1/2 signaling (Abou-Khalil et al. 2009), resulting in a return to quiescence at the end of regeneration.

A reduction in partial oxygen pressure has also emerged as an essential factor in SC biology. Hypoxia is a critical factor for many stem cells, with a strong link between low oxygen levels and the undifferentiated cell state (Mohyeldin et al. 2010). Myoblasts cultured under hypoxic conditions show increased quiescence and higher self-renewal efficiency upon transplantation in vivo (Liu et al. 2012).

Finally, systemic, circulating factors facilitate the adjustment of SCs to distal processes away from the niche. For example, calcitonin, a thyroid hormone that is secreted in response to high blood calcium levels, is important for SC dormancy and sub-laminar location. It exerts its effects by binding to the calcitonin receptor (Calcr), which is expressed by resting, but not by activated SCs (Fukada et al. 2007). A specific knock-out of Calcr in SCs results in their relocation from the niche and loss by apoptosis (Yamaguchi et al. 2015). Likewise, SC-specific knock-out of the androgen receptor, which is expressed in this cell population (Sinha-Hikim et al. 2004), leads to induction of Mstn expression, a fiber-type switch and a reduction in muscle mass and strength (Dubois et al. 2014).

8.2.4 Motor Neurons, Fibroblasts, Fibro/Adipogenic Progenitors and Immune Cells

In slow-twitch muscles, SCs are located in close proximity to the neuromuscular junction (NMJ) (Kelly 1978), and the difference in SC numbers between slow- and fast-twitch fibers is correlated with the pattern of neuron firing (Gibson and Schultz 1982). When denervated, skeletal muscle fibers undergo atrophy, to which SCs initially respond with activation and proliferation similar to what is observed in damaged muscle, but after several weeks of denervation, SC number declines due to loss of proliferative capacity and apoptosis (Kuschel et al. 1999; Jejurikar et al. 2002). Conversely, it has been shown that developing muscle produces neurotrophins, which function as retrograde survival factors for the motor neuron (Griesbeck et al. 1995), and SCs secrete the axonal guidance factor semaphorin 3A with possible implications in muscle regeneration (Tatsumi et al. 2009). Although initially found to have a role in neuron survival, neurotrophins are emerging as important modulatory factors for various cell populations and tissues including skeletal muscle. For example, nerve growth factor (NGF) is expressed by regenerating fibers, which implies its involvement in muscle regeneration. Similarly, SC expression of brain-derived neurotrophic factor (BDNF) is important for SC maintenance, and consequently affects muscle regeneration (Clow and Jasmin 2010; Menetrey et al. 2000).

Fibroblasts contribute to the niche by secreting growth factors and structural components of the BL. Temporary thickening of the ECM coupled with an increase

in the number of muscle tissue fibroblasts is a hallmark of muscle regeneration (Serrano et al. 2011). Furthermore, interactions between Tcf4⁺ fibroblasts and SCs are necessary for successful regeneration. Selective, conditional ablation of SCs in Pax7^{CreERT2/+};R26R^{DTA/+} mice leads to insufficient proliferation of fibroblasts in the initial phases of regeneration and fibrosis at the later stages, whereas the partial ablation of fibroblasts in Tcf4^{CreERT2/+};R26R^{DTA/+} mice causes reduced proliferation and precocious differentiation of SCs, resulting in a decreased diameter of regenerated muscles and depletion of the SC pool (Murphy et al. 2011).

Skeletal muscle-resident mesenchymal progenitors expressing PDGFR α are known as fibro/adipogenic progenitors (FAPs) due to their ability to differentiate into adipocytes and fibroblasts (Uezumi et al. 2010). In homeostatic conditions, these cells are in close proximity to blood vessels (Pretheeban et al. 2012), and their number quickly rises in the event of muscle damage. FAPs facilitate myofiber formation and myoblast differentiation by secreting specific ECM components and cytokines, respectively (Joe et al. 2010). These cells also display the ability to remove necrotic tissue (Heredia et al. 2013), thereby supporting muscle regeneration. Interestingly, proper signaling from myotubes and eosinophils prevents FAP differentiation into adipocytes (Uezumi et al. 2010).

Immune cells are additional important players in defining the SC niche in regeneration. Some of these cells, like tissue macrophages and mast cells, are permanent members of the niche, but their importance in modulating the SC microenvironment in quiescence is likely limited. However, they take on an active role upon sterile injury, which induces muscle fiber damage and necrosis. Resident immune cells react by secreting cytokines and chemokines including tumor necrosis factor α (TNF- α), interleukin 6 (IL-6) and macrophage inflammatory protein 2 (MIP-2), which primarily drive the extravasation of neutrophils (Wang and Thorlaciuc 2005; Brigitte et al. 2010). Next, neutrophils secrete MIP-1 α , monocyte chemoattractant protein-1 (MCP-1) and other cytokines attracting monocytes from blood vessels, which rapidly become the most abundant inflammatory cell type in the damaged tissue (Scapini et al. 2000). Depending on the milieu of inflammatory signals and immune cells present in the niche, the macrophages derived from the monocytes can acquire the M1 or M2 type. M1 macrophages secrete proinflammatory cytokines (TNF- α , IL-1 β) and are characteristic of the early post-injury stages. They are essential for the removal of necrotic tissue and promote SC proliferation. Upon clearance of cellular debris, the altered conditions in the niche promote an increase in the number of M2 macrophages, which secrete anti-inflammatory cytokines (IL-4, IGF-I, TGF- β) and support the differentiation stages of regeneration (Ceafalan et al. 2014; Arnold et al. 2007). Temporal regulation of the inflammatory cascade is crucial in the process. For example, suppression of M1 macrophages leads to reduced SC proliferation, persistence of necrosis and results in fat and fibrotic tissue accumulation. Likewise, suppression of the switch from the M1 to the M2 type negatively affects myogenesis and myofiber growth (Segawa et al. 2008; Deng et al. 2012; Summan et al. 2006). In addition to paracrine signaling, macrophages establish direct contact with myoblasts and myotubes through cell adhesion interactions (e.g. via VCAM-1-VLA-4, ICAM-1-LFA-1, PECAM-1-PECAM-1 and CX3CL1-

CX3CR1), which prevent apoptosis of myogenic cells (Sonnet et al. 2006). Apart from innate immunity, cells of the adaptive immune system are also central to regulating SC behavior during sterile injury. An instrumental role of T regulatory cells in proper SC expansion and muscle regeneration, as well as in the M1 to M2 macrophage switch after injury has been described (Castiglioni et al. 2015; Burzyn et al. 2013).

8.2.5 *Other Stem Cells with Myogenic Capacity*

Apart from the true stem cells of muscle tissue and the battalion of auxiliary cells that participate in skeletal muscle regeneration, additional multipotent progenitors from muscle and other organs can contribute to this process. The function and ontogeny of many of these heterogeneous groups of cells are unclear, but interestingly, they are all found in close proximity to blood vessels (Péault et al. 2007). In addition, they have not been fully characterized and therefore, their interaction remains obscure.

Some of those myogenic progenitors, such as SP cells, CD133⁺ cells and MDSCs, can be found in skeletal muscle tissue, while others originate from the blood vessel wall, for example MABs, pericytes, endothelial as well as myo-endothelial cells (Péault et al. 2007). Interestingly, these blood vessel-derived progenitors have a myogenic potential even when isolated from organs other than skeletal muscle (e.g. pericytes and endothelial cells isolated from adult human pancreas or adipose tissue) (Péault et al. 2007).

Unlike SCs, all these myogenic progenitors are able to cross the blood wall and home in on muscle tissue when administered via the bloodstream. They can engraft skeletal muscle, albeit often to a low extent, as demonstrated for SP cells (Péault et al. 2007). Although systemic delivery represents an enormous advantage in cell therapy for muscle disorders, some problems do exist. For example, the majority of intravenously injected cells end up trapped in filter organs (liver, lung, spleen) instead of muscle. In addition, systemic delivery of cells can cause blood flow obstruction, e.g. pulmonary embolism and myocardial infarction, resulting in ischemia and tissue damage (Berry 2015). And finally, their potential to form several cell populations poses a danger of e.g. ectopic formation of bone tissue in muscle (Birbrair et al. 2014).

Due to lack of specific markers, these progenitors are heterogeneous in nature and possibly consist of several groups of cells with different functions. For instance, in skeletal muscle tissue, type 2 pericytes (nestin⁺) have myogenic and angiogenic potential, while type 1 pericytes (nestin⁻) have adipogenic and fibrogenic potential. Importantly, other resulting cell populations depend on the pericyte microenvironment, which is perturbed in dystrophic conditions and aging (Birbrair et al. 2015).

Some of these multipotent progenitors offer several potential advantages over SCs in terms of systemic delivery, better survival and proliferation potential, leading to increased regenerative capacity, as demonstrated for MDSCs (Qu-Petersen et al.

2002). Intra-arterial delivery of MABs in a mouse model for limb-girdle muscular dystrophy (LGMD) and a dog model of Duchenne muscular dystrophy (DMD) also resulted in amelioration of the dystrophic phenotype (Sampaolesi et al. 2003, 2006). However, the outcome of transplantation can largely depend on the local microenvironment, e.g. caused by tissue damage, or the presence or absence of other cell populations and signaling molecules (Birbrair et al. 2014, 2015). Importantly, the significance of these cell populations to contribute to tissue maintenance upon skeletal muscle damage or in skeletal muscle formation under physiological conditions is largely undetermined. In any case however, even if the contribution of these populations to skeletal muscle formation independent of cell therapy is minor or non-existent, these cells could still significantly contribute to regeneration by secreting paracrine factors, such as growth factors, as suggested for pericytes and SPs (Birbrair et al. 2014; Péault et al. 2007).

8.2.6 The Biophysical Properties of Muscle

Aside from other factors of the niche, rigidity of the microenvironment can profoundly affect SC behavior. The elastic stiffness of uninjured skeletal muscle is ~12 kPa, and ECM deposition during regeneration increases this value (Engler et al. 2004). SCs can sense and react to this biophysical property of the environment through focal adhesions (Geiger et al. 2009). When cultured on rigid plastic dishes (~10⁶kPa), SCs quickly lose their quiescence and stemness. Myoblasts cultured on hydrogels prefer a substrate stiffness of ~21 kPa, while softer (~ 3 kPa) and stiffer (~ 80 kPa) gels reduce their proliferative rate (Boonen et al. 2009). In line, SCs cultured on soft hydrogels that mimic the stiffness of natural muscle (12 kPa) are able to self-renew and significantly improve their contribution to muscle regeneration upon transplantation (Gilbert et al. 2010).

8.3 The Satellite Cell Niche in Pathological Contexts

Aging, muscle dystrophies and related pathologies invariably lead to perturbed conditions of the SC niche. These changes can cause a reduction or an expansion in the SC pool, irresponsiveness to stimuli and therefore a reduced SC activation rate, aberrant proliferation and precocious or reduced differentiation, or SC senescence and apoptosis upon activation. For example, a disproportion of symmetric and asymmetric SC division might tip the balance towards SC loss in aging and a pathological SC expansion with a reduced number of myogenic progenitors in dystrophic conditions (Chang et al. 2016). Irrespective of the dysregulation, the outcome is diminished SC regenerative capacity in both contexts.

Although some of the pathological changes are SC intrinsic, altering the niche can alleviate the underlying condition in many cases. Nevertheless, it is difficult to

precisely discriminate between intrinsic and extrinsic origins of the SC pathology due to the bidirectional signaling between SCs and their microenvironment. Importantly, the niche can induce modifications in SC properties that can persist even after removal of SCs from the niche, and are hence perceived as “intrinsic”.

8.3.1 The Satellite Cell Niche in Aging

With advanced age, skeletal muscle mass and neuromuscular performance diminishes, a condition termed sarcopenia. Decreased fiber and motor neuron numbers, reduced fiber size, a myofiber switch towards the oxidative type and loss of myonuclei resulting in an increase in myonuclear domain size are all common observations in aging, collectively resulting in a marked decrease of the efficiency of muscle regeneration (Larsson and Ansved 1995; Faulkner et al. 2007). The reduction of the SC pool has been proposed as an explanation for the underlying condition (Shefer et al. 2010). However, based on conflicting results in different studies (Conboy et al. 2003), the prevailing opinion is that a drop in the myogenic potential of SCs might be the causative factor of the impaired regenerative capacity. Some characteristics of the aged niche that lead to reduced potency of SCs are illustrated in Fig. 8.3.

Some changes in the aged niche are precipitated by aberrant signaling. For instance, lack of Delta upregulation by injured aged muscles leads to reduced Notch signaling in SCs and hence reduced SC proliferation — a phenotype that can be overcome by alternative Notch activation (Conboy et al. 2003). Interestingly, experiments with heterochronic, parabiotic pairings (a shared circulatory system between a young and an old animal) demonstrated that systemic factors at least partially account for the perturbed SC biology, as the exposure to young blood restored otherwise reduced Notch signaling and improved SC proliferation in old mice (Conboy et al. 2005). The subsequent search for rejuvenating humoral factors led to the implication of the hormone oxytocin (Elabd et al. 2014) and growth factor GDF11 (Sinha et al. 2014; Walker et al. 2016) as systemic factors that decline with age and whose induction is able to revert aging-related SC pathology. However, the function of GDF11 in promoting muscle and cardiac health in aging has been largely discredited in more recent studies (Schafer et al. 2016; Egerman et al. 2015; Harper et al. 2016). Exacerbated canonical Wnt signaling due to elevated circulating Wnt activators in aged mice was also suggested as being responsible for aging-related tissue fibrosis and conversion of myoblasts into fibroblasts, a process that can be curbed by Wnt inhibitors (Brack et al. 2007). Increased NF- κ B and TGF- β signaling in aged muscles are additional examples of how the immediate niche can negatively impact the regenerative potential of SCs (Oh et al. 2016; Carlson et al. 2008).

ECM deposition in the aged niche in general is thought to act as a damper and therefore exert a negative influence on the activation potential of SCs, e.g. by increasing tissue stiffness. For example, slow muscles boost the expression of

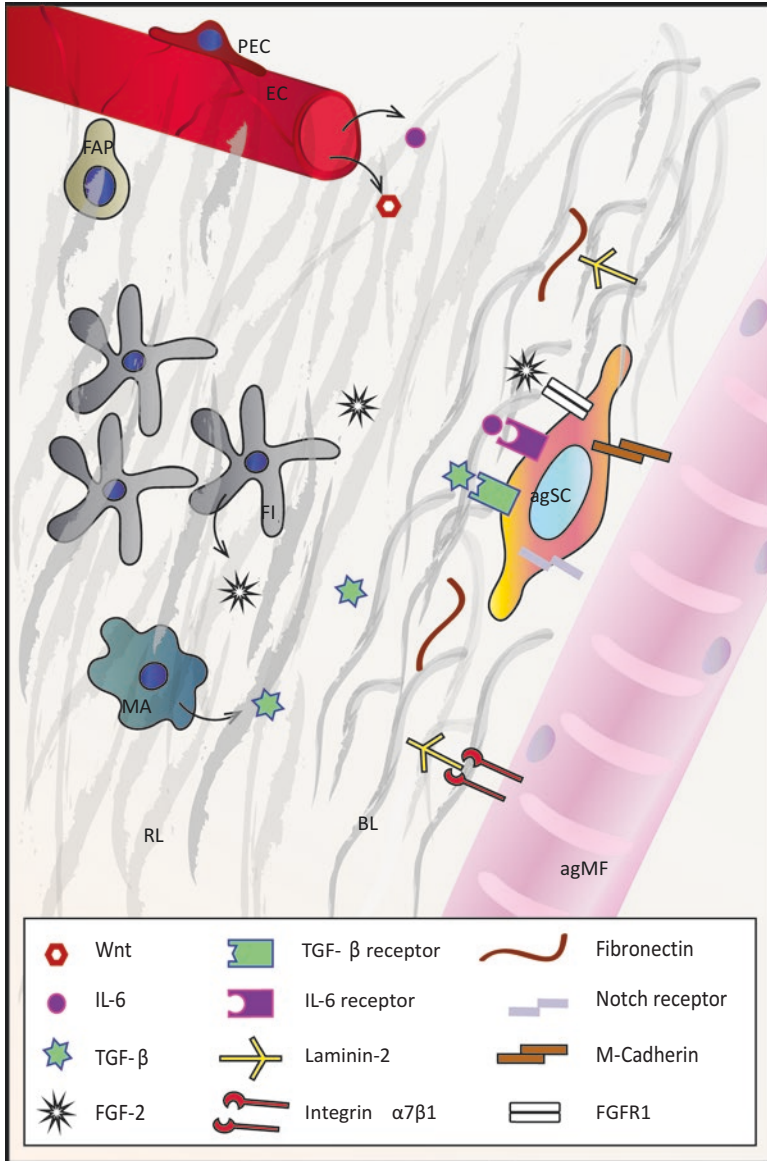


Fig. 8.3 The aged niche. The aged myofiber (agMF) has a smaller diameter, fewer myonuclei and reduced levels of Delta, a ligand of Notch, which is crucial to SC proliferation. Further changes to the niche include increased fibroblast (FI) numbers and a denser basal and reticular lamina (BL, RL) with reduced fibronectin levels, active macrophages (MAs) secreting TGF- β , as well as increased levels of FGF-2, IL-6 and Wnt signaling molecules. Further abbreviations: *EC* endothelial cell; *FAP*, fibro/adipogenic progenitor; *PEC*, periendothelial cell

collagen IV while fast muscles elevate the levels of laminin with aging (Kovanen et al. 1988). The ensuing imbalance in the components of the BL in old muscle disturbs the signal transduction pathways that govern SCs in the niche, such as those triggered by higher levels of TGF- β , a negative regulator of SC proliferation (Carlson et al. 2008), and FGF-2. FGF-2 signaling through FGFR1 results in SC loss based on unmitigated cycling. Importantly, this effect can be reverted by increasing Spry1 in SCs, an inhibitor of FGF signaling and preserver of SC quiescence (Chakkalakal et al. 2012). The p38 α / β MAPK pathway, downstream of FGF signaling, is consequently overactivated in aged SCs, leading to reduced asymmetric division and higher numbers of committed daughter cells, hence resulting in diminished self-renewal. Improving the SC environment by transplanting old SCs into a young host could not revert this condition, in contrast to the successful pharmacological inhibition of the p38 α / β MAPK pathway in SCs (Cosgrove et al. 2014; Bernet et al. 2014). Most likely triggered by increased IL-6 blood levels, the JAK/STAT signaling pathway is also overactivated in aged SCs and results in a reduction of symmetric division and self-renewal, which can be reverted with pharmacological inhibitors (Price et al. 2014; Tierney et al. 2014). In geriatric mice (30 months of age), SCs lose their ability for reversible quiescence by switching to pre-senescence. At that age, the respective stimuli fail to induce SC activation and proliferation, but instead prompt senescence in a process termed geroconversion. Silencing of p16^{INK4a}, a cell cycle inhibitor that triggers the switch to pre-senescence, is able to restore the activation and proliferation potential of SCs (Sousa-Victor et al. 2014). Intriguingly, blocking autophagy in young SCs causes senescence, while its restoration in old age reestablishes the regenerative potential of SCs (Garcia-Prat et al. 2016). Furthermore, loss of FN from the aged BL prevents sufficient attachment of SCs to the niche and thus disturbs signaling through focal adhesion kinase, thereby precipitating SC loss (Lukjanenko et al. 2016). In addition, mislocalization of integrin β 1 on aged and dystrophic SCs leads to impaired sensitivity to FGF-2, consequently causing reduced SC proliferation and ultimately SC depletion, resulting in impaired regeneration. In both models, activation of β 1-integrin reverts the impairment of SC function (Roza et al. 2016).

Hormonal and pharmacological interventions, calorie restriction as well as cell therapy have been proposed for the prevention and treatment of sarcopenia. However, to date, physical activity remains the most efficacious approach to combating this disease (Jang et al. 2011), e.g. by boosting the number and myogenic capacity of SCs (Shefer et al. 2010; Snijders et al. 2009). Although an SC pathology is most likely not the only driving force for development of sarcopenia, SC dysfunction contributes to impaired muscle regeneration and increased fibrosis (Brack et al. 2007). Recent advances in understanding aberrant signal transduction pathways and communication between aged SCs and their niche will potentially offer new pharmacological avenues in the treatment of sarcopenia that could circumvent the inherent problems of exercise interventions in geriatric patients.

8.3.2 *The Satellite Cell Niche in Dystrophic Conditions*

Muscular dystrophies are a heterogeneous group of sporadic and inherited disorders that lead to progressive muscle wasting and weakness. Fiber size variation, fiber necrosis followed by inflammation, and muscle tissue replacement by fat and scar tissue are often hallmarks of these pathologies, depending on the severity of the dystrophy in question (Emery 2002). Many dystrophies are caused by a mutation in structural proteins of the cytoskeleton, membrane or ECM, which comprise a part of the SC niche.

One of the most common and extensively studied dystrophy is DMD, which arises due to a genetic mutation in the structural protein dystrophin. Lack of dystrophin, a member of the membrane-bound protein complex, leads to the improper connection of the cytoskeleton to the ECM, rendering fibers more prone to mechanical damage. As a consequence, recurring rounds of degeneration and regeneration form a vicious cycle and impose proliferative pressure on SCs. It has been proposed that progressive worsening of the disease over time is at least partially due to telomere shortening and ultimately loss of the regenerative potential of SCs (Sacco et al. 2010). The most important signaling molecules implicated in the dystrophic niche are presented in Fig. 8.4.

Infiltrating macrophages and T cells induce fibrosis through secretion of pro-fibrotic cytokines, which in chronic diseases such as muscular dystrophies result in fibrotic tissue formation at the expense of functional muscle tissue (Mann et al. 2011). For instance, in acute injury, a wave of TNF- α -secreting M1 macrophages induces a reduction of the preceding FAP expansion, thereby limiting ECM accumulation. Under chronic conditions, however, loss of proper control of macrophage polarization results in exacerbated TGF- β secretion that in turn causes FAP persistence and fibrosis (Lemos et al. 2015). Therefore, anti-inflammatory drugs like corticosteroids, despite their potential pro-atrophic side effects, are the current standard of care for DMD. A big portion of current DMD therapy-related research focuses on intercepting the pathways implicated in fibrotic tissue formation, namely those triggered by TGF- β and Mstn (Bentzinger et al. 2010).

Interestingly, SC fate conversion from the myogenic to the fibrogenic lineage can contribute to fibrosis development in DMD. Thus, increased Wnt signaling in dystrophic muscle triggers TGF- β 2 secretion, which in turn induces pro-fibrotic gene expression in SCs, thereby limiting their myogenic potential (Biressi et al. 2014). Besides progressive fibrosis, the SC niche in DMD is affected by other events, such as alterations in the BL with differential expression of laminin α 2, laminin β 1 and collagen IV, which are implicated in the direct interactions with SCs (Hayashi et al. 1993), as well as that of decorin and biglycan, proteoglycans linked to TGF- β sequestration (Fadic et al. 2006). These changes presumably also contribute to alterations in muscle stiffness, which further affects SC behavior. In addition, perturbed conditions can alter the differentiation of several multipotent progenitor populations in the muscle, including FAPs, resulting in extracellular fat accumulation (Uezumi et al. 2010). Of note, these alterations to the SC niche can be extrapolated

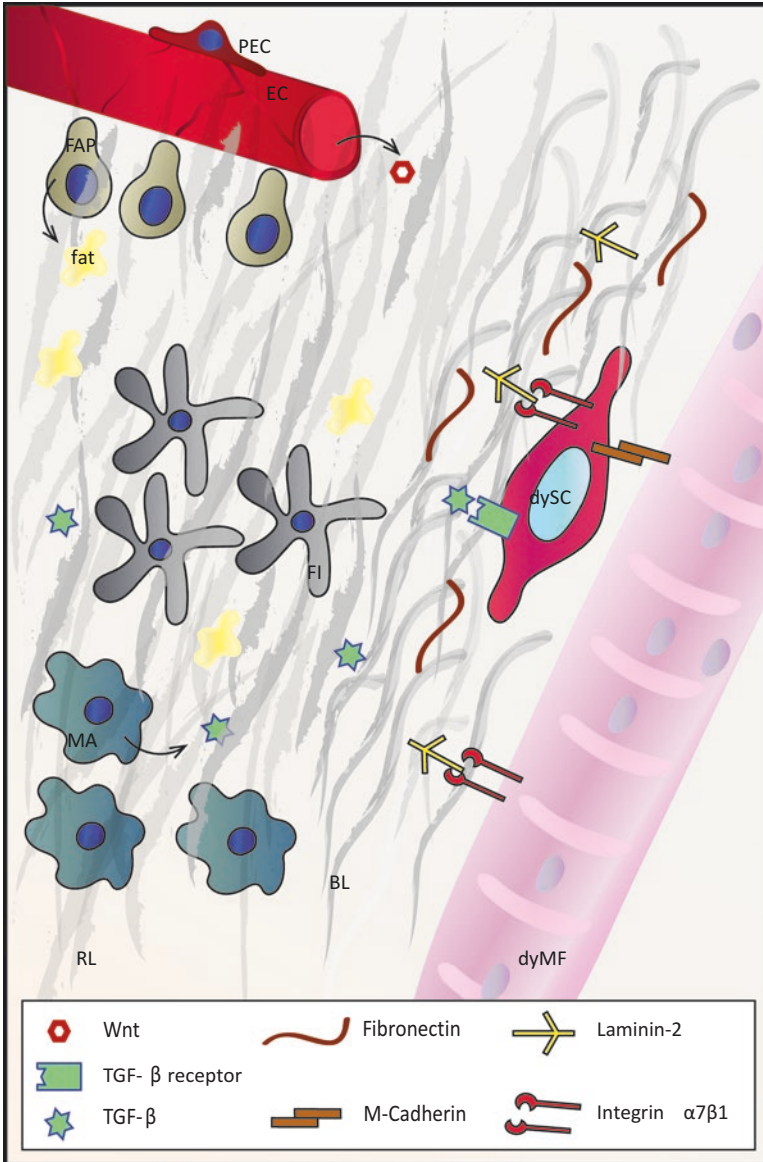


Fig. 8.4 The dystrophic niche. The dystrophic fiber (dyMF) is smaller in diameter with centrally located nuclei, a hallmark of ongoing damage and regeneration. As a result, dySCs are under high proliferative pressure. Similar to the aged niche, elevated levels of TGF-β and Wnt, as well as increased numbers of fibroblasts (FIs), are common features of the dystrophic niche. In addition, inflammation caused by high numbers of fibro/adipogenic progenitors (FAPs) and macrophages (MAs) contributes to the formation of fat and scar tissue (a denser basal and reticular lamina—BL, RL) at the expense of muscle tissue. Further abbreviations: *EC* endothelial cell; *PEC* periendothelial cell

to other dystrophies and muscle pathologies with prominent fibrosis and fat accumulation, even diseases such as type 2 diabetes (Berria et al. 2006; Goodpaster and Wolf 2004).

The niche has been the primary focus of research on SC dysfunction in DMD, mainly due to a body of literature suggesting that dystrophin expression is limited to differentiated myofibers. However, recent findings suggest a direct role of SCs in the pathology based on the discovery that dystrophin is also expressed in activated SCs and is important for establishing cell polarity, thus enabling asymmetric SC division (Dumont et al. 2015). Lack of SC dystrophin therefore results in reduced numbers of committed progenitors and differentiated myocytes, as well as a higher numbers of Myf5⁻ progenitors. However, both increased and decreased SC numbers have been reported in DMD, a discrepancy that could be due to the difference in age of the subjects in the studies in question (Kottlors and Kirschner 2010; Jiang et al. 2014). Given the reciprocal regulation between SCs and fibroblasts (Murphy et al. 2011), it will be interesting to further explore the role of SC dystrophin in fibrotic tissue accumulation and other DMD symptoms.

Dysferlinopathy is another example of a muscular dystrophy (LGMD) with a complex etiology. In this disease, a mutation in the structural protein dysferlin primarily prevents myotubes from patching contraction-induced small ruptures in the sarcolemma. However, dysferlinopathy also affects proper muscle regeneration, where impairment in the release of cytokines upon injury results in reduced neutrophil recruitment and leads to a prolonged inflammatory phase, creating a suboptimal environment for successful regeneration by SCs (Chiu et al. 2009).

Despite extensive efforts, no treatment for most of these debilitating diseases has been found so far. Therapies are mainly symptomatic and palliative, relying on corticosteroids as well as pulmonary and cardiac management in the case of DMD (Wagner et al. 2007). Experimental treatments centered on stem cell therapy (e.g. SC transplantation), gene therapy (e.g. antisense oligonucleotide exon skipping, viral delivery of mini-dystrophin, CRISPR/Cas9-mediated deletion) and pharmacology (e.g. Mstn blockade) might, however, result in therapeutic breakthroughs in the future (Chakkalakal et al. 2005; Fairclough et al. 2013; Young et al. 2016; Mendell and Rodino-Klapac 2016).

8.4 Future Directions: An Artificial Niche

Autologous SC therapy represents one of the most promising treatments both for dystrophies and sacropenia. In sacropenia, enhancement of the myogenic potential of SCs and expansion of *bona fide* SCs in vitro prior to their transplantation in order to boost regeneration would most likely be sufficient, while in dystrophic conditions, the approach would comprise stem cell and gene therapy, including correction of a relevant genetic mutation in vitro. However, several hurdles impede the success of such trials. For example, the inability of SCs to home in on muscle with systemic delivery (Elster et al. 2013), poor migration when delivered intramuscularly

(Bentzinger et al. 2014), as well as reduced proliferation, immediate differentiation and apoptosis of injected cells have been reported. These effects are further compounded by the rapid and irreversible loss of SC stemness in culture, resulting in reduced myogenic potential upon transplantation (Montarras et al. 2005). Thus, as expanding the stem cell population is a necessary step prior to implantation, improving the intrinsic myogenic potential of SCs, e.g. by overexpressing PGC-1 α , can help to lead to enhanced early muscle tissue formation after transplantation (Haralampieva et al. 2017). Furthermore, attempts have been made to mimic the SC niche in vitro to circumvent some of the aforementioned problems.

Bioengineering efforts have made progress in creating 3D biomimetics as acellular or cellular scaffolds for use in regenerative therapy (Handschin et al. 2015). From cylindrically shaped, collagen I-based gels to various natural hydrogels and finally fibrin gels, conditions conducive to increasing cell survival, fusion and maturation are constantly improving (Bursac et al. 2015). For example, in the case of trauma-induced volumetric muscle loss, acellular biodegradable materials filled with anti-fibrotic and pro-myogenic factors on one, and angiogenic and neurotrophic factors on the other hand, would possibly provide optimal conditions to tip the balance towards functional muscle tissue instead of scar tissue formation when transplanted in a timely manner (Sicari et al. 2014; Shvartsman et al. 2014). These scaffolds would provide not only fast infiltration and proper activation of the myogenic cells of the host, but also support fast establishment of the vascular and neural network necessary to support the newly formed muscle tissue. Other conditions such as aging and dystrophies require, however, more intricate cellular approaches, with biomaterials that closely resemble the SC niche in terms of stiffness and composition, enabling the cell-matrix interactions that are crucial for proper SC function. In that regard, polyethylene glycol hydrogels cross-linked with laminin have been used successfully in improving SC self-renewal in vitro and engraftment in vivo (Gilbert et al. 2010). This substrate, in combination with pharmacological inhibition of the p38 α / β MAPK pathway, was also able to reverse the age-related SC pathology (Cosgrove et al. 2014).

Besides identification of ECM proteins as crucial components of an artificial niche, the search for extrinsic factors that would enable SC expansion in vitro without loss of cell stemness has led to the discovery of a cocktail of four cytokines. Intrigued by the role of CD4⁺ and CD8⁺ T cells in regeneration, Fu and colleagues identified T cell-derived factors that are responsible for increased SC proliferation. They defined a pro-inflammatory cytokine combination composed of IL-1 α , IL-13, TNF- α and INF- γ that is sufficient and necessary to maintain SC potency in vitro (Fu et al. 2015). This combination of cytokines promoted proliferation and limited differentiation of SCs for 20 passages. The gene expression profile of cells expanded in this way suggests that these cells retain at least some of the features of freshly isolated SCs, such as high Pax7 and low MyoD expression. SCs expanded under such conditions were not only able to engraft efficiently and occupy the niche upon transplantation into muscle, but also to respond to secondary injury by undergoing activation and self-renewal (Fu et al. 2015). In addition, the transplantation efficiency of such expanded cells in vitro was comparable to freshly isolated SCs. Since

the cocktail in question has been optimized for murine SCs, efforts will have to be made to find proper conditions and factors for human SCs.

Recently, Quarta and colleagues successfully mimicked the *in vivo* microenvironment of SCs by using a defined serum-free quiescence medium and artificial muscle fibers. A 3D micro scaffold with an elasticity between 1 and 2 kPa based on collagen, recombinant laminin and $\alpha 4\beta 1$ integrin provided optimal stiffness and enabled signaling pathways to keep the cells in reversible quiescence (Quarta et al. 2016). This method proved effective in keeping both murine and human SCs in a quiescent state for up to a week. With this system, the engraftment potential and self-renewal of cultured cells upon transplantation surpassed that of freshly isolated SCs and was comparable to SCs associated with their native fibers. These results confirm the importance of the niche and mimicking the *in vivo* microenvironment for maintaining SC stemness *in vitro* (Quarta et al. 2016).

These studies provide crucial insights into the optimal conditions for keeping SCs in a quiescent state *in vitro*, SC propagation, and preservation of the stemness for subsequent *in vivo* transplantation. Importantly, an artificial niche not only enables disease modeling and gene therapy, but also provides an amenable experimental system for toxicology screenings of novel drugs, thereby reducing the burden of animal studies (Madden et al. 2015; Huh et al. 2012). Together with novel imaging and cell tracking techniques (Haralampieva et al. 2016), the increasing knowledge about SC biology, the importance of the niche, and the interplay of SCs with myofibers and other cell types will hopefully result in novel therapeutic approaches to treating sarcopenia, muscular dystrophies and other skeletal muscle-associated pathologies.

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