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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.

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 (1). The astonishing translational potential of SCs continues to fascinate, and the ever expanding knowledge of SCs and their microenvironment paves the 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 (2), blood vessels (3), bioengineering (4), SC function from a cell-intrinsic perspective (5) and an extensive review on SC biology (6).

Skeletal muscle regeneration and muscle stem cells

Comprising approximately 40% of body weight, skeletal muscle can be considered as the largest organ in the human body (7). Muscle not only supports breathing and movement, but is also a very important metabolic and endocrine organ. It comes as no surprise that skeletal

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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 within 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 (8–12). 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) (1), in close proximity to blood vessels (3) and the neuromuscular junction (13). 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 (14) as well as single cell (15) implantation in irradiated muscles of immunodeficient mice.

The classical cascade of regeneration resembles that of prenatal skeletal muscle development (16). In response to injury or other stimuli, SCs become activated, increase in size and begin proliferation. 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 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 cells, muscle-derived stem cells, bone marrow stem cells, $PW1^+$ interstitial cells, $CD133^+$ cells, mesoangioblasts and pericytes, can successfully regenerate muscles and some can even reconstitute the niche upon transplantation into damaged muscle (17). 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 (18–21).

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 (22), 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.

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 (23). 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 (24). 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 (25). 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 p38α/β MAPK activation and MyoD expression only in the committed daughter (26). 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.

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 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 (α2β1γ1) and non-fibrillar collagen IV (27). 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. 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 α7 and β1, which bind to laminin-2 on the BL side (28). 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 β3 integrin isoform, which probably binds to fibronectin in a complex with the αv chain (29). 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 (30).

Expression profiles of quiescent and activated SCs suggest that SCs actively contribute to maintaining niche quiescence while remaining highly sensitive to activating stimuli (31). Quiescent SCs express the protease inhibitors Serpin and Tfpi2 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 (32). MMPs are major enzymes responsible for ECM degradation.

Activated SCs also produce fibronectin (FN), an ECM glycoprotein whose role in SC maintenance by enabling their attachment to the niche has recently been demonstrated (33). 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 (34). 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 (35).

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 (36).

SCs are furthermore affected by the Notch and Wnt signaling pathways in regard to quiescence, activation, proliferation and differentiation (6). Proof-of-concept was provided in different studies, e.g. by ablation of RBP-Jκ, 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 (37, 38). 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 (39).

In regeneration, myofibers secrete stromal cell-derived factor-1 (SDF-1), which binds to the receptor CXCR4 on SCs and induces SC chemoattraction (40). 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 (41). 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 (42). 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 (43). 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 (44).

Much attention has been given to metabolic reprograming of SCs, that is, the effects of the metabolism of a SC on its fate (45). Some research proposes that in quiescence, SCs primarily rely on fatty acid oxidation (46), whereas upon activation, they increase substrate utilization through glycolysis, and finally switch to oxidative phosphorylation during differentiation (47). Other studies suggest that activated SCs depend more on oxidative phosphorylation (45, 48, 49). 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

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in this context probably induce SC activation through increased oxidative phosphorylation (49).

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 (50). Moreover, a similar difference in SC numbers can be achieved by endurance exercise, which promotes a switch from glycolytic to oxidative fibers (51, 52). 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 (3). However, this simple view has recently been challenged. Namely, mice with myofiber-specific overexpression of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), a nodal modulator of oxidative metabolism, exhibit both a switch to oxidative fibers and increased capillarity (53), but nevertheless have fewer SCs, albeit with an increased myogenic capacity (54). In fact, in regard to most metabolic and contractile traits, PGC-1α transgenic, *bona fide* oxidative and endurance-trained muscles are indistinguishable. Interestingly, the muscle fiber PGC-1α transgene affects expression of BM components FN and tenascin-C (54), 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 (11, 55). 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 (56). However, the link between SC number and fiber type-specific ECM composition is still poorly understood and thus awaits further research.

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 (57, 58). This is not surprising given the fact that a myriad of factors and cells that modify the satellite cell 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 (59). Conversely, differentiating myogenic cells also secrete VEGF, thereby stimulating angiogenesis (60). 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 (61), 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 (62). Myoblasts cultured under hypoxic conditions show increased quiescence and higher self-renewal efficiency upon transplantation *in vivo* (63).

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 (64). A specific knock-out of Calcr in SCs results in their relocation from the niche and loss by apoptosis (65). Likewise, SC-specific knock-out of the androgen receptor, which is expressed in this cell population (66), leads to induction of Mstn expression, a fiber-type switch and a reduction in muscle mass and strength (67).

Motor neurons, fibroblasts, fibro/adipogenic progenitors and immune cells

In slow-twitch muscles, SCs are located in close proximity to the neuromuscular junction (NMJ) (13), and the difference in SC numbers between slow and fast-twitch fibers is correlated with the pattern of neuron firing (50). 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 (68, 69). Conversely, it has been shown that developing muscle produces neurotrophins, which function as retrograde survival factors for the motor neuron (70), and SCs secrete the axonal guidance factor semaphorin 3A with possible implications in muscle regeneration (71). 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 (72, 73).

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 (74). 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 (21).

Skeletal muscle-resident mesenchymal progenitors expressing PDGFRα are known as fibro/adipogenic progenitors (FAPs) due to their ability to differentiate into adipocytes and fibroblasts (75). In homeostatic conditions, these cells are in close proximity to blood vessels (76), 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 (77). These cells also display the ability to remove necrotic tissue (78), thereby supporting muscle regeneration. Interestingly, proper signaling from myotubes and eosinophils prevents FAP differentiation into adipocytes (75).

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 (79, 80). Next, neutrophils secrete MIP-1α, 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 (81). 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-1β) 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 (82, 83). 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 (84–86). 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 (87). 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 (88, 89).

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 ~12kPa, and ECM deposition during regeneration increases this value (90). SCs can sense and react to this biophysical property of the environment through focal adhesions (91). When cultured on rigid plastic dishes (~10^6kPa), SCs quickly lose their quiescence and stemness. Myoblasts cultured on hydrogels prefer a substrate stiffness of ~21kPa, while softer (~ 3kPa) and stiffer (~ 80kPa) gels reduce their proliferative rate (92). In line, SCs cultured on soft hydrogels that mimic the stiffness of natural muscle (12kPa) are able to self-renew and significantly improve their contribution to muscle regeneration upon transplantation (93).

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 (94). 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”.

The satellite cell niche in aging

With advanced age, skeletal muscle mass and neuromuscular performance diminishes, a condition termed sacropenia. 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 (95, 96). The reduction of the SC pool has been proposed as an explanation for the underlying condition (51). However, based on conflicting results in different studies (97), the prevailing opinion is that a drop in the myogenic potential of SCs might be the causative factor of the impaired regenerative capacity.

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 (97). 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 (98). The subsequent search for rejuvenating humoral factors led to the implication of the hormone oxytocin (99) and growth factor GDF11 (100, 101) 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 (102–104). 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 (105). Increased NF-κB and TGF-β signaling in aged muscles are additional examples of how the immediate niche can negatively impact the regenerative potential of SCs (106, 107).

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 collagen IV while fast muscles elevate the levels of laminin with aging (56). 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 regular of SC proliferation (107), and FGF-2. FGF2 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 (23). The p38α/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 p38α/β MAPK pathway in SCs (108, 109). 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 (110, 111). 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 (112). Intriguingly, blocking autophagy in young SCs causes senescence, while its restoration in old age reestablishes the regenerative potential of SCs (113). 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 (33). In addition, mislocalization of integrin β1 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 β1-integrin reverts the impairment of SC function (114).

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 (115), e.g. by boosting the number and myogenic capacity of SCs (51, 116). Although an SC pathology is most likely not the only driving force for development of sacropenia, SC dysfunction contributes to impaired muscle regeneration and increased fibrosis (105). 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.

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 (117). 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 Duchenne muscular dystrophy (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 (118).

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 (119). For instance, in acute injury, a wave of TNFα-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 (120). 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 (121).

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-β2 secretion, which in turn induces pro-fibrotic gene expression in SCs, thereby limiting their myogenic potential (122). Besides progressive fibrosis, the SC niche in DMD is affected by other events, such as alterations in the BL with differential expression of laminin α2, laminin β1 and collagen IV, which are implicated in the direct interactions with SCs (123), as well as that of decorin and biglycan, proteoglycans linked to TGF-β sequestration (124). 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 (75). Of note, these alterations to the SC niche can be extrapolated to other dystrophies and muscle pathologies with prominent fibrosis and fat accumulation, even diseases such as type 2 diabetes (125, 126).

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 suggests 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 (127). 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 (128, 129). Given the reciprocal regulation between SCs and fibroblasts (21), 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 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 (130).

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 (131). 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 (132–135).

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 (136), poor migration when delivered intramuscularly (137), 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 (138). 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-1α, can help to lead to enhanced early muscle tissue formation after transplantation (139). 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 (140). From cylindrically shaped, collagen I-based gels to various natural hydrogels and finally fibrin gels, conditions conductive to increasing cell survival, fusion and maturation are constantly improving (4). 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 (141, 142). 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 satellite cell 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 satellite cell self-renewal *in vitro* and engraftment *in vivo* (93). This substrate, in combination with pharmacological inhibition of the p38α/β MAPK pathway, was also able to reverse the age-related SC pathology (108).

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-1α, IL-13, TNF-α and INF-γ that is sufficient and necessary to maintain SC potency *in vitro* (143). 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 (143). 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 microscaffold with an elasticity between 1-2kPa based on collagen, recombinant laminin and α4β1 integrin provided optimal stiffness and enabled signaling pathways to keep the cells in reversible quiescence (144). 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* (144).

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 (145, 146). Together with novel imaging and cell tracking techniques (147), 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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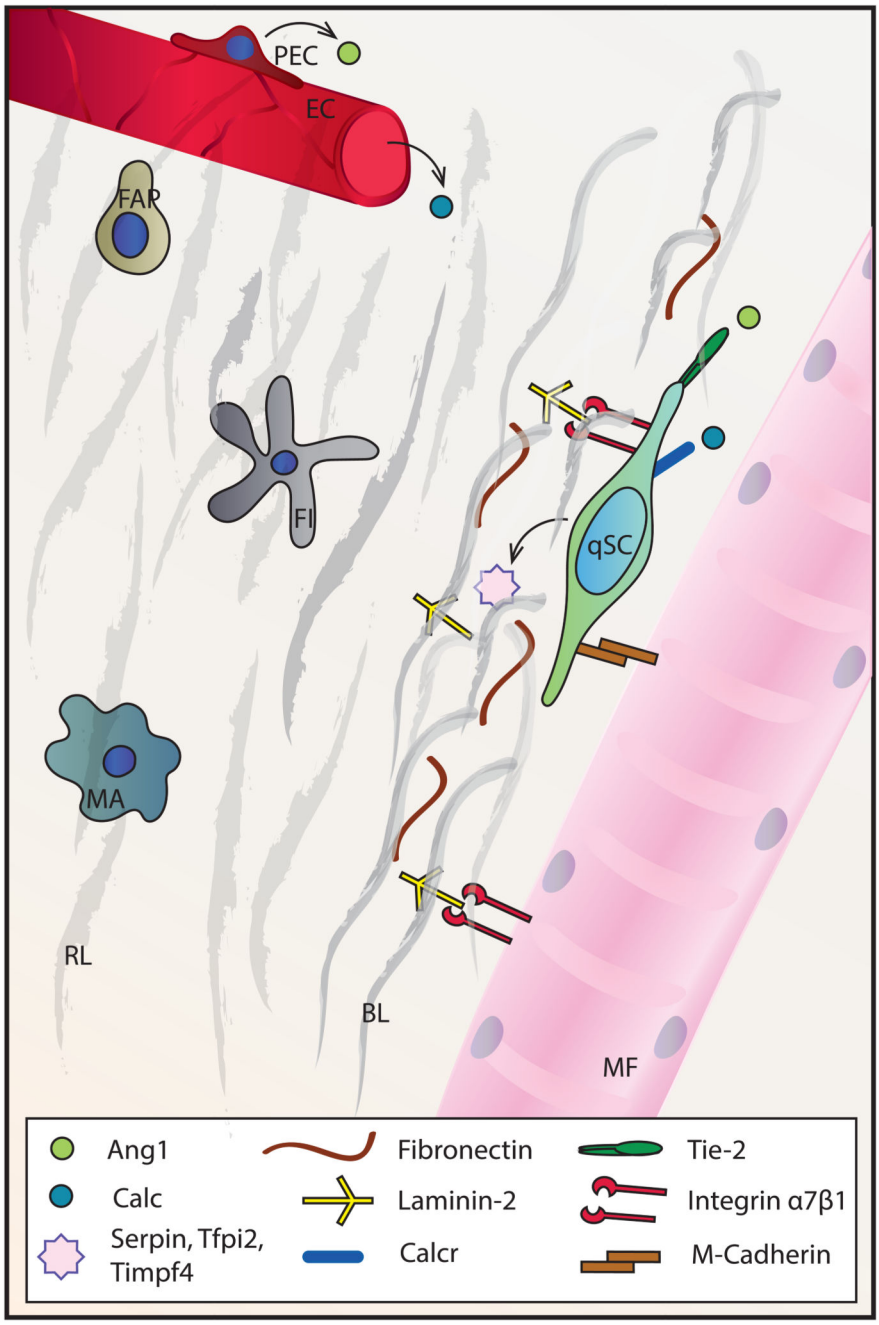

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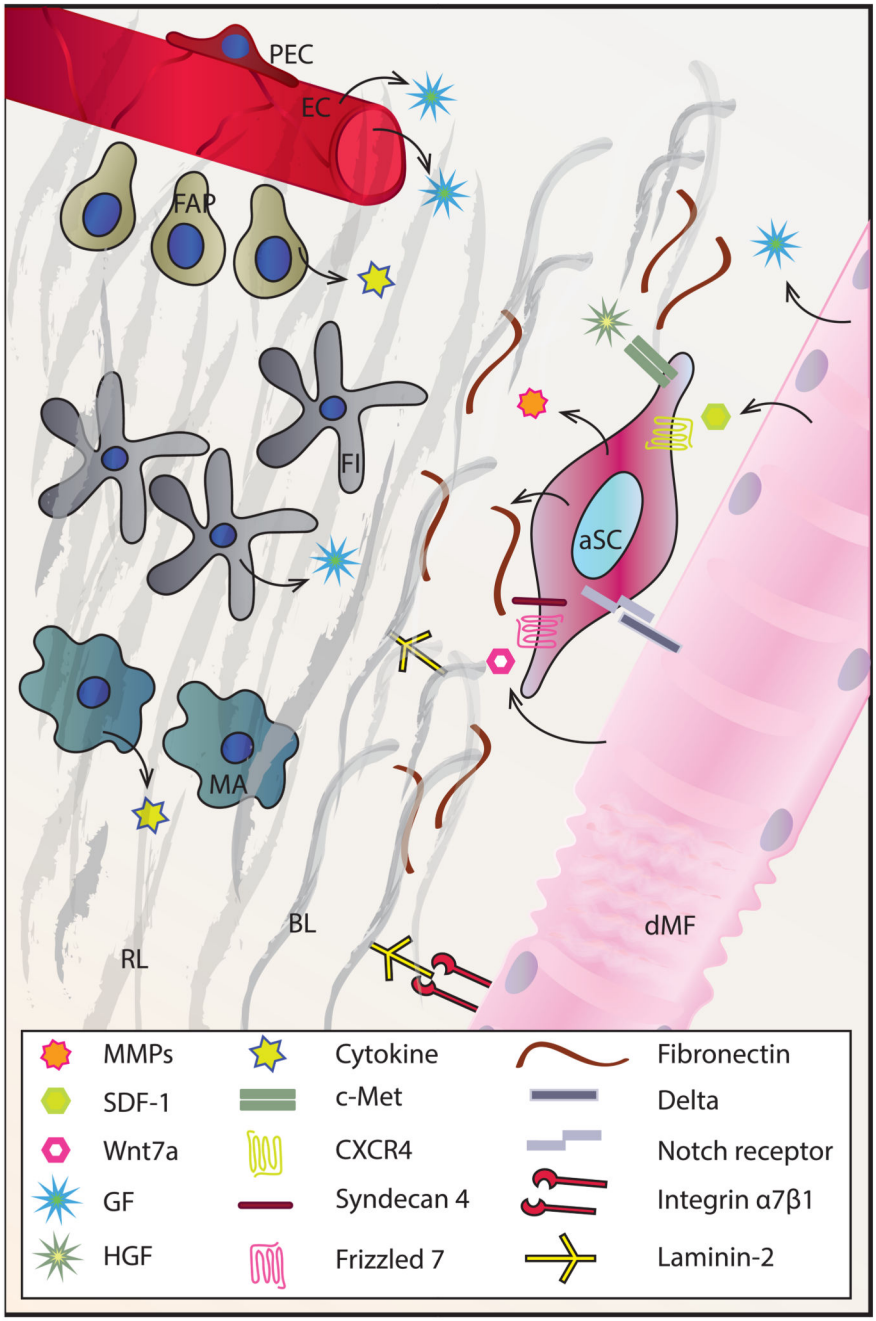

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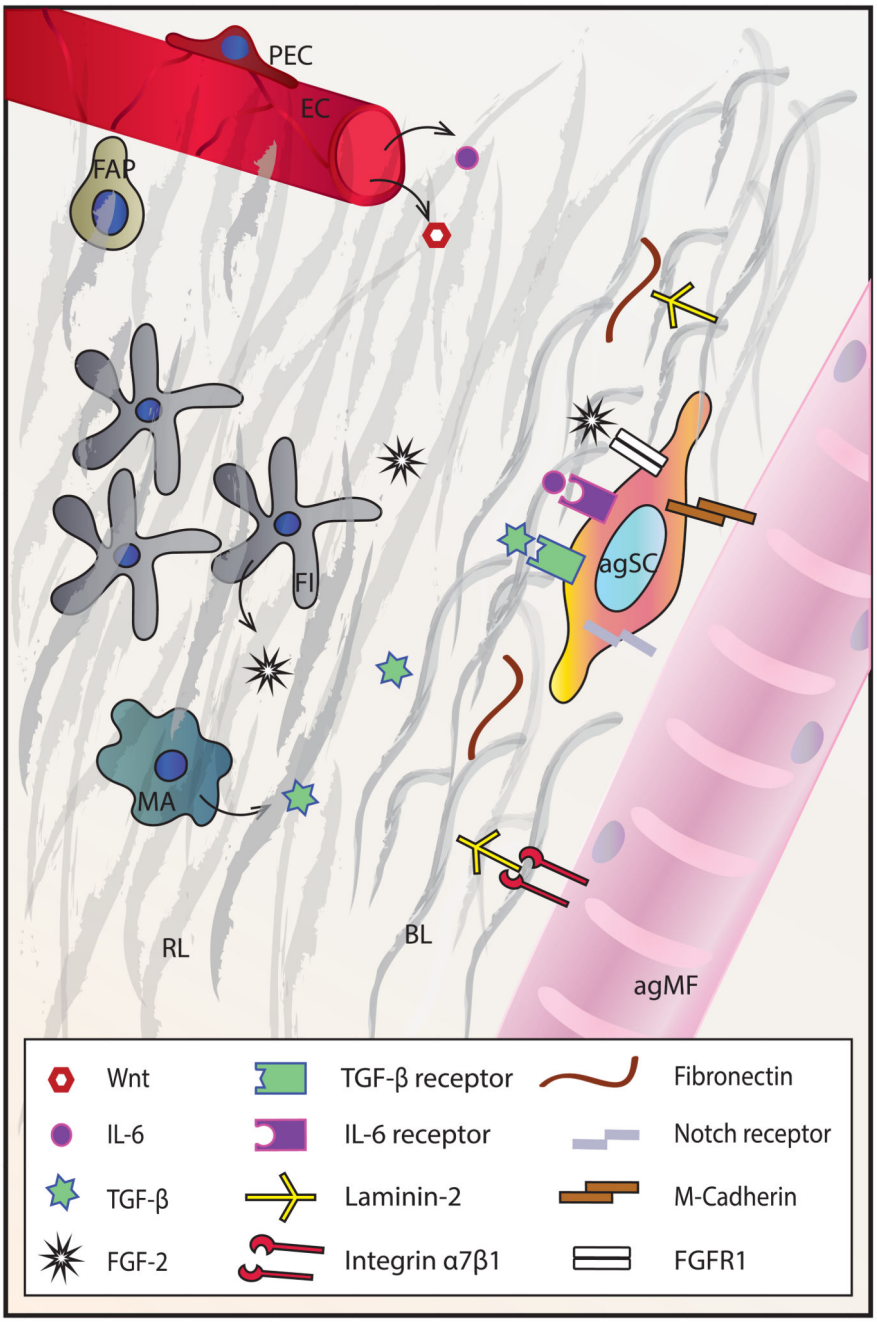

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