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JB Special Review—Skeletal muscle cell biology and disease

The roles of muscle stem cells in muscle injury, atrophy and hypertrophy

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Skeletal muscle is composed of multinuclear cells called myofibers. Muscular dystrophy (a genetic muscle disorder) induces instability in the cell membrane of myofibers and eventually causes myofibre damage. Non-genetic muscle disorders, including sarcopenia, diabetes, bedridden immobility and cancer cachexia, lead to atrophy of myofibres. In contrast, resistance training induces myofibre hypertrophy. Thus, myofibres exhibit a plasticity that is strongly affected by both intrinsic and extrinsic factors. There is no doubt that muscle stem cells (MuSCs, also known as muscle satellite cells) are indispensable for muscle repair/regeneration, but their contributions to atrophy and hypertrophy are still controversial. The present review focuses on the relevance of MuSCs to (i) muscle diseases and (ii) hypertrophy. Further, this review addresses fundamental questions about MuSCs to clarify the onset or progression of these diseases and which might lead to development of a MuSC-based therapy.

Keywords: atrophy; hypertrophy; muscle stem cells; regeneration; skeletal muscle.

Abbreviations: CalcR, calcitonin receptor; MuSCs, muscle stem cells; Pax7, paired box 7.

Muscle Satellite Cells

Skeletal muscle is composed of giant multinuclear cells called myofibres. In adult newts and jellyfish, damaged myofibres are replaced by trans-differentiation of intact myofibres (*1*, *2*). In mammals, myofibres do not have this ability. Instead, muscle stem cells (MuSCs) called muscle *satellite* cells replenish myogenic progenitors to build new myofibres (*3*, *4*).

MuSCs are located between the basal lamina and plasma membrane of myofibres (sarcolemma) (*5*). The most commonly used marker of MuSCs is paired box 7 (Pax7), a functionally important transcriptional factor that is expressed only in MuSCs in adult muscle (*6*). During the past two decades, various studies of muscle stem-like cells that neither defined the 'satellite cell position' nor examined the expression of Pax7 have been reported (*7*–*9*). Thus, it is possible that these reported cells are a different cell population from muscle satellite cells. Several studies successfully depleted MuSCs from adult muscle of Pax7-CreERT2 and Rosa-DTA mice. These studies showed that Pax7-positive cells (MuSCs) are indispensable for muscle regeneration because they revealed no sign of regeneration when the mice were administered a treatment that typically induces muscle regeneration (*3*, *4*). The following sections first summarize recent new findings in MuSC biology.

Quiescence

Sacco *et al*. (*10*) demonstrated that a single MuSC has both self-renewal and differentiation abilities and that these two capacities meet the criteria for classification as a stem cell. Like stem cells found in other tissue types, MuSCs are maintained in a quiescent and undifferentiated state. Recent studies have revealed the molecular mechanisms that actively keep MuSCs in the quiescent and undifferentiated state (*11*). Canonical Notch signaling is a representative pathway for actively maintaining MuSCs in the undifferentiated state. Notch signaling directly induces the target genes including Hey1, HeyL and Hes1 through Rbp-J-NICD (a Notch intracellular domain). MuSC-specific Rbp-J-depleted mice showed a reduced MuSC pool and increased expression of MyoD and myogenin in their adult MuSCs (*12*, *13*). We previously reported that Hey1/HeyL double KO mice lose the ability to produce undifferentiated (MyoD-) MuSCs during postnatal development and do not maintain a MuSC pool (*14*). These studies suggest that the Notch-RbpJ-Hey1/L axis functions to keep MuSCs in the undifferentiated state in adult skeletal muscle (*15*).

Calcitonin receptor (CalcR) is a G protein-coupled receptor that regulates the quiescent state of MuSCs. The expression of CalcR is well-characterized in osteoclasts, but its expression and roles in skeletal muscle had not been investigated. Our microarray analyses showed that CalcR is exclusively expressed in the quiescent state of MuSCs in adult skeletal muscle (*16*). Using MuSC-specific CalcR-depleted mice, we showed an increase in the number of Ki67+ cells (non-quiescent state of MuSCs) compared to control MuSCs (*17*). CalcR expression has been used to identify quiescent or adult-type MuSCs (Fig. 1) (*18*, *19*). Remaining unknowns are the ligand of CalcR and its downstream target. Also, to reveal the mechanisms of self-renewal and the cell-fate decision of MuSCs, further investigation of the regulation of *Calcr* gene expression is of great interest.

Activation, proliferation and differentiation

Skeletal muscle regeneration involves a well-regulated integration of the complicated biological and

Featured Article

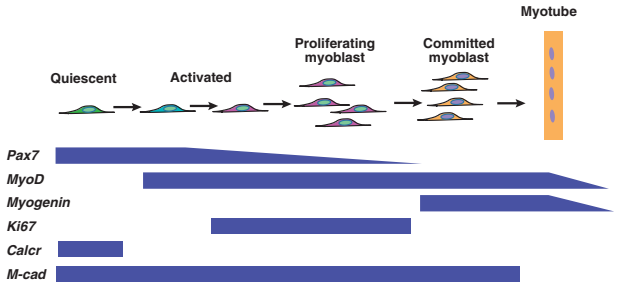

Fig. 1 A model of myogenic differentiation. Quiescent MuSCs are positive for Pax7, CalcR and M-cad. Activated MuSCs lose CalcR expression and start to express MyoD. Then, the MuSCs express Ki67 and start cell division. Committed myoblasts lose Pax7 expression and express myogenin. Myogenin-positive cells fuse with one another to make multinucleated myotubes.

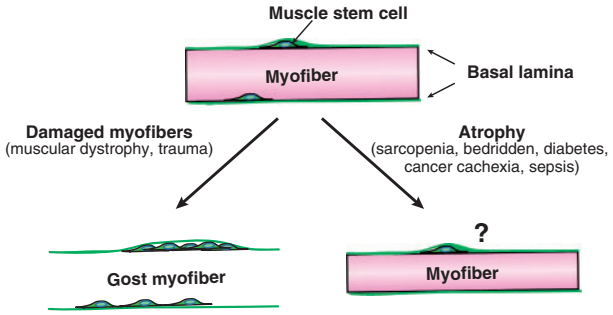

Fig. 2 Two types of muscle diseases. In muscular dystrophies or trauma, damaged myofibres are removed by infiltrating macrophages, and MuSCs proliferate beneath the basal lamina remnants. In sarcopenia and disused muscles including being bedridden, whether the MuSCs alter is still controversial.

molecular processes of cell activation, proliferation, differentiation and cell fusion. In response to muscle damage, MuSCs start to express MyoD and then Ki67. MyoD + Ki67 + MuSCs start to proliferate to produce large numbers of myoblasts (Fig. 1). A committed myoblast is defined by the expression of myogenin and loss of Pax7 expression. Myogenin expression allows myoblasts to fuse with each other to form multinuclear myotubes. The molecular mechanism underlying cell fusion had not been revealed, but the Olson group has identified myomaker (Tmem8c) and myomixer as critical for cell–cell fusion (*20*, *21*). Intriguingly, myomixer together with myomaker allow fibroblast to fuse with fibroblast. Similar to myogenin, myomixer (Gm7325) expression is regulated by MyoD (*22*).

Typically, stem cells self-renew to maintain the stem cell pool. Asymmetric division in the first cell division is a representative mechanism to maintain stem cell numbers. In MuSCs, apical-basal oriented asymmetric division was proposed to maintain the MuSC pool (*23*), but apical-basal oriented divisions were not observed *in vivo*, and planar division was found to be the major division pattern (*24*). In addition, we detected atypical myogenic cells in the middle stage of muscle regeneration (*25*). The events that take place during the early (2–3 days after injury) and middle phases (4–7 days after injury) of muscle regeneration are different, and there is a possibility that MuSCs divide asymmetrically during the middle phase of muscle regeneration.

Multi-differentiation abilities

Under some disease conditions, ectopic fat or bone formation is observed in skeletal muscle. It had been believed that MuSCs differentiate into other mesenchymal lineage cells (such as adipocytes and osteoblasts). In 2010, Uezumi *et al.* (*26*) identified mesenchymal progenitors in skeletal muscle that serve as a physiological cellular source for adipocytes, osteoblasts and fibroblasts (*27*, *28*). In addition, Myf5 and MyoD, myogenic regulatory factors, are transcriptionally active in MuSCs, and their translations are suppressed by miR-31 and an RNA-binding protein, Staufen1, respectively (*29*, *30*). These results also support the notion that MuSCs are not multipotent progenitors, but rather they are primed myogenic cells.

Human muscle stem (satellite) cells

The characterization of human MuSCs lags behind the current literature on mouse MuSCs. CD56 (NCAM) was the only traditional marker for identifying human MuSCs (*31*). Recently, Uezumi and Gussoni groups identified a human MuSC marker, CD82 (*32*, *33*). Human myoblasts also express Hey1, HeyL and Hes1 in response to Notch ligands (*34*). The reasons and mechanisms are not clear, but HeyL is remarkably increased in mouse MuSCs, and Hes1 is significantly upregulated in human MuSCs. Like murine MuSCs, human MuSCs do not differentiate into adipogenic cells (*28*).

Types of Skeletal Muscle Diseases: Muscle and Non-muscle Damage Related

Skeletal muscle disease can be divided roughly into two categories (Fig. 2): one that follows muscle damage or an injury and another that does not. Not all, but most muscular dystrophies show signs of muscle damage and that the ability of MuSCs directly contributes to the progression of dystrophies.

Muscle weakness may be a result of reduced myofibre size, known as muscle atrophy. Several disorders, including sarcopenia, diabetes, cancer cachexia and sepsis, lead to a reduction of myofibre size. During such disorders, a decrease in the number and proliferation of MuSCs has been reported, resulting in impaired or delayed regeneration. However, myofibre atrophy also occurs independently of MuSCs. Therefore, the roles of MuSCs in muscular atrophy are controversial (Fig. 2). The following section discusses the roles of MuSCs in damaged and undamaged muscle.

Muscle damage and MuSCs

As described earlier, the absolute requirement of MuSCs for muscle regeneration is widely accepted. In an acute injury model, basal lamina remnants from injured muscle govern MuSCs and their progenitors; Webster *et al.* (*24*) called the basal lamina remnants 'ghost fibres'. The remarkable ability of muscle to regenerate is well-known and well described, but the ability seems to depend strongly on the 'ghost fibres'. One basis for this belief is that the numbers of central myonuclei differ between the *mdx* (a model

of Duchenne muscular dystrophy) and dy^{2J}/dy^{2J} (a model of congenital muscular dystrophy type 1 A) mice. *Mdx* lacks dystrophin, a part of the dystrophin-associated protein complex located in the sarcolemma (plasma membrane of myofibres). The basal lamina remains similar to that of the acute injury model. The dy^{2J}/dy^{2J} has a mutation in laminin α2, a component of the basal lamina. *Mdx* mice have 50–70% centrally nucleated myofibres from the age of 2 to 18 months in the tibialis anterior and gastrocnemius muscles (*35*). On the other hand, dy^{2J}/dy^{2J} mice showed approximately 10% centrally nucleated myofibres from 8 to 18 weeks post-natal in the tibialis anterior and gastrocnemius muscles (*36*). Dy^{3k}/dy^{3k} mouse that exhibits severer form of dystrophy than dy^{2J}/dy^{2J}, has approximately 30% centrally nucleated myofibres (*37*). In mouse models, regenerated myofibres retain centrally located myonuclei for longer periods. Therefore, the fewer number of central myonuclei observed in dy^{2J}/dy^{2J} and dy^{3k}/dy^{3k} mice indicate the lower regenerative ability of MuSCs of laminin α2-deficient mice in an incomplete basal lamina environment.

Muscle regeneration depends on the mouse strain. The DBA/2 strain shows lower MuSCs ability than C57BL/6 (B6) and C57BL/10 (B10) inbred strains. *Mdx* and γ-sarcoglycan-null (a mouse model of limb-girdle muscular dystrophy 2 F) having a DBA/2 background show severe phenotypes, also indicating the importance of MuSCs in the progression of muscular diseases (*38–40*). If the capacities of human MuSCs are similar to those found in the DBA/2 strain, finding the factor responsible for the excellent regenerative ability of B6 or B10 strains will lead to a 'regeneration-promoting therapy' for muscular dystrophies.

Atrophy and MuSCs

Muscle atrophy is defined as a decrease in the size of myofibres. The essential roles of the E3 ubiquitin ligases MuRF1 and Atrogin1 in myofibre atrophy have been established, but roles and behaviours of MuSCs are controversial. There are several models used for induction of muscle atrophy in rodents. Using a mouse hind limb suspension model, Mitchell *et al.* (*41*) reported that the number of MuSCs was reduced after 2 weeks of hind limb suspension. They also showed low proliferation of MuSCs 2 weeks after hind limb suspension compared to control MuSCs. The mechanical stimulation generated by daily life might be important for sustaining MuSCs, but in a human study, 2 weeks immobilization of skeletal muscle led to a considerable loss in skeletal muscle mass and strength, but it was not accompanied by a decline in MuSC numbers (*42*). Further studies are necessary to conclude whether atrophic conditions affect MuSC numbers or behaviour, which could indicate the importance of daily mechanical stress for maintaining MuSCs in a quiescent state.

Age-related loss of skeletal muscle is referred to as 'sarcopenia'. In addition to a decrease in the size of myofibres (atrophy), the number declines with aging. MuSCs are the only cells producing myofibres; thus, their relevance in sarcopenia is implicated. Using MuSC-depleted mice, Fry *et al.* (*43*) showed that MuSCs are dispensable for the onset and progress of sarcopenia. Using a similar strategy, Kardon group argued that MuSCs contribute to maintaining the myofibre size of fast-type myofibres in extensor digitorum longus and soleus muscles (*44*). They pointed out the low frequency of MuSC depletion in the study by Fry *et al.* Contrary to other types of atrophy, sarcopenia develops through a decrease in the number of myofibres, but the number of myofibres in these murine models was not changed. Lushaj *et al.* (*45*) investigated the quadriceps muscles of healthy aging Fischer brown Norway rats from five to 39 months of age and reported that the number of myofibres decreased with age. In a human study, Verdijk *et al.* (*46*) reported that type II myofibre-specific atrophy was accompanied by a decline in the number of both MuSCs and myonuclei in the vastus lateralis muscle (a part of the quadriceps) in aging. These alternations were not observed in type I myofibres. Taken together, the contribution of MuSCs to the onset and progress of sarcopenia appeared to depend on the tissues analyzed and the animal model used. Further studies will be necessary to achieve conclusive results on the roles of MuSCs in aging.

Hypertrophy and MuSCs

Resistance training induces hypertrophy, an increase in myofibre size. The Akt-mTOR pathway in myofibres plays critical roles in this process (*47*). In addition, many studies have independently shown evidence of activation and proliferation of MuSCs during muscle hypertrophy. The proliferated MuSCs are finally incorporated into myofibres, and eventually the number of myonuclei increases. Using MuSCs-depleted mice and a synergistic ablation (SA) model, McCarthy *et al.* (*48*) showed that the increase in the myonuclei number completely depends on MuSCs. On the other hand, an increment in myofibre size normally occurred in MuSC-depleted mice, suggesting that myofibre

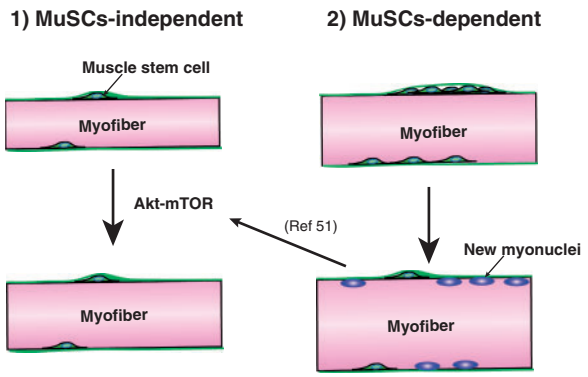

Fig. 3 Dependency and independency of MuSCs **in hypertrophy.** There are at least two events involved in the process of muscle hypertrophy: (i) activation of Akt-mTOR pathway in myofibres, which is MuSCc-independent and (ii) increased numbers of myonuclei through supply of MuSCs, which is MuSC-dependent. Egner *et al.* indicated that an inadequate supply of new MuSC-derived myonuclei failed to sufficiently activate the Akt-mTOR pathway. This, in turn, suggests a possibility that MuSCs are affected by an MuSC-independent mechanism.

hypertrophy does not require MuSCs (Fig. 3) (*48*). These are surprising results because it had been believed that myofibres maintain the myonuclei to cytoplasm ratio ('myonuclei domain theory') (*49*). According to this theory, in order to maintain the ratio of myonuclei to cytoplasm, a myofibre would require new MuSC-derived myonuclei when it becomes larger. There are two recent studies supporting this 'myonuclei domain theory'. Egner *et al*. (*50*) reported the necessity of MuSCs for an adequate increase in muscle weight using a similar SA mouse model. A study by Goh *et al*. also supports the MuSC requirement for hypertrophy. Using MuSC-specific myomaker-depleted mice, they reported that the hypertrophic response to SA in the mice was impaired through two mechanisms (*51*): (i) an insufficient supply of new MuSC-derived myonuclei due to a fusion defect and (ii) a failure to activate Akt/mTOR-mediated protein synthesis within the myofibres (Fig. 3). The relevance of cell fusion and the Akt/mTOR pathway is unknown, but these studies showed that myomaker made an essential contribution to MuSC-derived progenitor–myofibre fusion during muscle hypertrophy.

In addition, Fry *et al*. also indicated the necessity of MuSCs for long-term hypertrophy by suppressing fibrosis via MuSC-derived exosomes containing miR-206 (*52*, *53*). In contrast to the atrophy model, it seems possible to obtain consensus on the cell number changes of MuSCs in the hypertrophy model. However, the requirement for new MuSC-derived myonuclei is still controversial. If the addition of myonucleus numbers via increasing MuSCs enhances the function of skeletal muscle, a MuSC-targeted drug discovery might become a new therapeutic strategy.

Unsolved questions for MuSC-based therapy

In past decades, accumulating evidence has revealed some of the biology of MuSC. One important goal of MuSC research is to achieve a stem cell-based therapy. Improvement of cell culture conditions is a challenge because the traditional conditions often lead to a loss of the original capacity of MuSCs (*54*, *55*). Quarta *et al*. (*56*) succeeded in creating an artificial niche *in vitro* that enhanced the transplantation efficiency of MuSCs. Understanding the molecular mechanism underlying MuSC maintenance *in vivo* is essential for the achievement of the goal. Further study will be necessary to prepare more realistic, adequate numbers of MuSCs for cell therapy, which will also contribute to potential iPS-cell based therapy for muscular disorders.

Second, neither the involvement of MuSCs in the onset and progression of sarcopenia nor the primary reason for MuSC depletion during aging is known. As mentioned earlier, several molecules regulating the state and number of MuSCs in mice are known, but their relevance to the molecular signaling and aging process is unclear. Basic MuSC biology is also necessary to understand muscle aging and to apply treatments for sarcopenia.

Third, if the quality of myonuclei declines throughout the lifetime, then replenishment of new myonuclei by MuSCs is necessary to maintain the homeostasis of myofibres. A study comparing young and aged myonuclei might help to clarify the potential of MuSCs for replenishment of myonuclei in the treatment of sarcopenia. In addition, the activation and proliferation mechanisms of MuSCs in muscle hypertrophy remain to be elucidated. If regulating activation, proliferation, and differentiation of MuSCs becomes possible, it will advance the development of MuSCs-based therapy not only for sarcopenia but also for various currently intractable muscle disorders.

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Conflict of Interest

None declared.

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