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Tissue-resident mesenchymal stem/progenitor cells in skeletal muscle: collaborators or saboteurs?

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Abstract

Although the regenerative potential of adult skeletal muscle is maintained by satellite cells, other stem/progenitor cell populations also reside in skeletal muscle. These heterogeneous cellular pools with mesenchymal lineage potentially play important roles in tissue homeostasis, with reciprocal collaborations between these cells and satellite cells appearing critical for effective regeneration. However, in disease settings, these mesenchymal stem/progenitors adopt a more sinister role – likely providing a major source of fibrosis, fatty tissue and extracellular matrix protein deposition in dystrophic tissue. Development of therapies for muscle degeneration therefore requires complete understanding of the multiple cell types involved and their complex interactions.

Keywords

fibro/adipogentic progenitors; mesenchymal stem cells; muscular dystrophy; myogenesis; regenerative medicine; satellite cells; skeletal muscle; stem cells; tissue regeneration

Introduction

Despite its post-mitotic nature, skeletal muscle maintains remarkable plasticity. Muscle fibres (myofibres) are capable of large alterations in size as well as an enormous ability to regenerate following injury. Like most postnatal tissues, the regenerative potential of skeletal muscle is maintained by a pool of resident adult stem cells. The principal stem cell responsible for this potential in adult skeletal muscle is the satellite cell – a quiescent bi-potent [1] tissue-specific cell population located between the basal lamina and sarcolemma of myofibres and most reliably identified by expression of paired box transcription factor Pax7 [2,3]. Serial transplantation and lineage tracing studies have conclusively demonstrated that, as well as acting as a progenitor, primed to enter myogenic differentiation to fuse into and repair damaged myofibres following injury, satellite cells (or at least a subset of satellite cells [4,5]) are also capable of replenishing the existing stem cell pool via self-renewal [5–9]. Genetic ablation of $Pax7^{+}$ satellite cells in adult mice has been shown to entirely block regenerative myogenesis, demonstrating an absolute requirement for these cells in myogenic

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repair and providing evidence that satellite cells are an exclusive source of stem cells in skeletal muscle regeneration [10–12].

However, in addition to satellite cells, a variety of other stem/progenitor and immune cell populations are also found in skeletal muscle (Fig. 1) and play important and increasingly appreciated roles in maintaining tissue homeostasis. Indeed, evidence suggests that collaborative interactions between multiple heterogeneous cell types are required for effective repair processes. This collective 'society' of cells in skeletal muscle contributes to the regenerative potential by locally providing the correct environmental settings for stem cell function. This ensures that the biological processes taking place in the multiple types of progenitors are temporally coordinated during regeneration. As discussed in this review, 'social unrest' in the skeletal muscle niche may have deleterious consequences.

This review discusses the current literature on identification and characterization of skeletal muscle-resident stem/progenitor cell populations involved in regeneration that are distinct from satellite cells. These heterogeneous cell populations possess the capacity to differentiate in multiple lineages (Table 1) and may hold great therapeutic promise for the treatment of debilitating degenerative diseases such as muscular dystrophies, which are characterized by both impaired myogenic stem cell function and excessive deposition of extracellular matrix proteins (fibrosis).

Non-satellite skeletal muscle-resident stem/progenitors with myogenic potential

Although the satellite cell is the critical stem cell in regenerative myogenesis, there are additional muscle-resident cell populations that are also believed to hold myogenic potential. Gussoni *et al.* [13] first demonstrated that 'side population' cells found in skeletal muscle, identifiable by the preferential exclusion of fluorescent dye Hoechst 33342, give rise to dystrophin-positive myofibres when intravenously injected into mdx mice. However, defining progenitor pools based on Hoechst 33342 dye exclusion is not a particularly stringent parameter in terms of population homogeneity compared to presentation of defined cell-surface antigens [14]. Indeed, there still appears to be a great deal of inconsistency in the gating strategies used to define populations that are Hoechst-positive or -negative. Nevertheless, muscle-resident 'side population' cells have been described and characterized by several groups. These studies have found that 'side population' cells reside in the muscle interstitium, share close proximity to the endothelium, and contain subsets positive for Sca-1 and CD34 and also for satellite cell markers such as Pax7 [14–20]. Not surprisingly given its heterogeneity, this cell population holds some myogenic potential when exposed to appropriate environmental cues, such as stimulation *in vitro* [16,18], co-culturing with myoblasts [15], injection into regenerating muscle [13,17,18,20] or over-expression of MyoD [15]. However, whether, to what extent and which 'side population' cells participate in regenerative myogenesis following injury *in vivo*, or even whether muscle 'side population' cells are functionally different from their antigenically similar counterparts found in the so-called 'main population' (a term that comprises all the remaining cells) with regard to anything other than their ability to efflux dye remains under investigation.

Recently, Mitchell *et al.* [21] made progress in identifying and characterizing a non-satellite cell myogenic progenitor in the neonate. Here, a cell population in the muscle interstitium identifiable by the expression of stress mediator PW1, but negative for Pax7 ($PW1^+/Pax7^-$ interstitial cells, PICs), is described. PICs were isolated based on lineage-negative $CD45^-$/$Ter119^-$ and $Sca1^+/CD34^+$ cell-surface antigen presentation using fluorescence-activated cell sorting. These cells showed some capacity to spontaneously enter myogenic differentiation *in vitro* in a Pax7-dependent manner, and this was greatly enhanced when PICs were co-cultured with myoblasts [21]. Most interestingly, when transplanted into a regenerating muscle environment *in vivo*, PICs were able to contribute to new myofibre formation with great efficiency, as well as generating satellite cells and PICs [21]. However, as the majority of data collected in this study were from the neonate, it remains unclear whether a phenotypically comparable cell population is present in adult skeletal muscle, and whether this progenitor functions in normal regenerative myogenesis. In addition, as endothelial markers were not used when prospectively isolating PICs, it remains to be seen whether these cells phenotypically resemble myoendothelial cells [22] or mesoangioblasts [23–25] (endothelial-like mesodermal progenitors with pericytic features and myogenic potential found in several tissues). Having said this, $PW1^+$ cells were negative for CD31 when analysed immunohistochemically [21].

Muscle-resident pericytes have also been shown to possess myogenic potential, and efforts have been made to understand the phenotypic links between them and mesoangioblasts. By dissecting, plating and culturing human muscle interstitium tissue under specific culture conditions, Dellavalle *et al.* [26] isolated a cell population consistent with pericytes. *In vivo*, this cell population was negative for myogenic markers (Myf5, MyoD and MyoG) and showed heterogeneous expressed of the pericyte markers neuro-glial 2 proteoglycan, platelet-derived growth factor receptor β (PDGFRβ) and α smooth muscle actin (αSMA) but was most reliably identified by expression of alkaline phosphatase (AP). A proportion of these cells were capable of myogenic differentiation when stimulated *in vitro*. Most interestingly, these cells were able to produce enough dystrophin-positive muscle fibres to yield a measurable improvement in muscle function when intravenously injected into *SCID*-mdx mice after expansion in culture [26]. Based on these findings, the same group recently developed a mouse strain in which tamoxifen-inducible Cre recombinase (Cre-ERT2) is expressed under the control of the AP locus, allowing, when crossed with a reporter line, inducible lineage tracing of AP^+ pericytes in skeletal muscle development and regeneration [27]. Interestingly, they demonstrate that AP^+ pericytes enter myogenesis and contribute to maturing myofibres during development as well as in response to injury during the early postnatal period. They also provide evidence that a small percentage of adult satellite cells are descendants of AP^+ progeny; however, AP^+ cells appear to have a minimal role in adult regenerative myogenesis [27]. Although such elegant lineage tracing strategies have become an important tool in stem cell biology, their reliability remains dependent on the efficiency of Cre-mediated recombination, on the assumption that Cre expression mimics that of the endogenous locus despite the fact that transgenes often do not include sequences involved in post-translational regulation, and last but not least, on the assumption that a given promoter is only expressed in a specific cell type. The exact expression pattern of AP may be dynamically and heterogeneously regulated during muscle development, and it remains

unclear whether myofibres themselves are capable of expressing AP during these events. Nevertheless, these studies provided strong evidence for the involvement of pericytic cells in myofibre formation during specific developmental stages.

Skeletal muscle-resident mesenchymal progenitors

In 2010, two groups characterized a population of skeletal muscle-resident progenitors with bipotent fibro/adipogenic potential [28,29]. Using fluorescence-activated cell sorting on digested mouse muscle preparations, Joe *et al.* [28] isolated fibro/adipogenic progenitors (FAPs) based on $CD45^{-}/CD31^{-}$ (lineage-negative), $\alpha 7$ integrin^{-}, $Sca1^{+}$ and $CD34^{+}$ cell-surface antigen presentation. Similarly, Uezumi *et al.* [29] isolated a functionally and phenotypically equivalent population of mesenchymal progenitors based on $CD45^{-}/CD31^{-}$, $SM/C2.6^{-}$ and $PDGFR\alpha^{+}$ expression. FAPs [28] and mesenchymal progenitors [29] readily entered adipocyte and fibroblast differentiation spontaneously *in vitro* in bulk cultures as well as in clonal assays, producing both αSMA-expressing fibroblasts and perilipin/peroxisome proliferator-activated receptor γ-positive adipocytes. Both groups demonstrated that this cell population was capable of *in vivo* adipogenic differentiation when transplanted into glycerol-injected skeletal muscle (a fatty degeneration model) [28,29]. The fibrogenic potential of this $PDGFR\alpha^{+}$ population has also been verified *in vivo* following transplantation of genetically labelled cells (PDGFRα–GFP) into γ-irradiated skeletal muscle after cardiotoxin injury [30]. Here, GFP-labelled cells accumulated in areas of fibrosis within the muscle interstitium, presumably consistent with differentiation into collagen type I-producing cells. Recent work by Wosczyna *et al.* [31] revealed further developmental potency of this progenitor population *in vivo*. Lineage tracing of muscle-resident cells based on a Tie2-driven Cre-dependent GFP reporter revealed a significant contribution of this cell type to cartilage and bone formation in a model of heterotopic ossification. Analysis of cell-surface antigen expression in lineage-negative, $Tie2–GFP^{+}$ cells revealed that ~ 90% were $PDGFR\alpha^{+}$ $Sca1^{+}$, while the $PDGFR\alpha^{-}$ $Sca1^{-}$ fraction did not contribute to bone or cartilage formation [31]. These results provide evidence that an equivalent or closely related skeletal muscle progenitor as described by Joe *et al.* [28] and Uezumi *et al.* [29] is also capable of osteogenic and chrondogenic differentiation *in vivo*.

Immunohistochemistry demonstrates that these progenitors are localized to the muscle interstitium and adjacent to myofibre-associated blood vessels [28–31], although they do not express markers such as neuroglial 2 proteoglycan [31], defining a cell population distinct from pericytes. Although provisionally labelled as FAPs, evidence highlighting the perivascular localization and capacity to differentiate down multiple skeletal lineages *in vivo* (as determined by the microenvironment they are transplanted into) suggests these cells may be best recognized as skeletal muscle-resident mesenchymal progenitors/stromal cells (MSCs).

In response to muscle injury, skeletal muscle mesenchymal progenitors become activated and expand rapidly [28,32]. However, unlike satellite cells, which enter myogenic differentiation and repair damaged myofibres, mesenchymal progenitors do not contribute directly to regenerative myogenesis. Transplantation of genetically labelled FAPs (lineage-negative, $\alpha 7$ intrigin^{-}, $Sca1^{+}$, $CD34^{+}$) or mesenchymal progenitors (lineage-negative, SM/

C2.6^{-}, PDGFRα^{+}) into regenerating muscle made little or no contribution to the regenerating myofibres [28]. Similarly, no myosin heavy chain-positive (MyHC^{+}) myotubes were observed in clonal assays or following stimulation by low serum conditions *in vitro*, indicating minimal myogenic capacity of these cells [28,29]. Instead, Joe *et al.* [28] demonstrated that mesenchymal progenitors play an important non-cell autonomous role in facilitating myogenesis. Using co-culture assays *in vitro*, mesenchymal progenitors were shown to promote myotube formation and differentiation of muscle progenitors [28]. Although the precise 'pro-myogenic' signals/factors released from mesenchymal cells during muscle injury remain under investigation, interleukin-6 was significantly up regulated and remains an obvious candidate. The important role of non-myogenic mesenchymal cells was further highlighted by Murphy *et al.* [11] and Mathew *et al.* [33]. Here, creation of Tcf4-$^{Cre/CreERT2}$ alleles used in combination with the Cre-responsive ablator allele R26-^{DTA} allowed the authors to investigate the consequences of ablation of Tcf4^{+} (transcription factor 7-like 2, Tcf7L2) connective tissue fibroblasts on developmental and regenerative myogenesis *in vivo*. During development, muscle-resident fibroblasts were shown to be important for slow MyHC expression and the maturation of fetal myofibres [33]. In the adult, despite effective deletion of only 40% of Tcf4^{+} fibroblasts, skeletal muscle regeneration was significantly impaired following injury [11]. Loss of skeletal muscle fibroblasts altered the proliferative kinetics of satellite cells and induced premature differentiation as determined by the early appearance of embryonic MyHC^{+} myofibres only 3 days after injury. Although regeneration still proceeded with a depleted fibroblast population in Tcf4-^{CreERT2}/R26-^{DTA} mice, regenerated myofibres were smaller in diameter and cross-sectional area. Using the same genetic strategy, Murphy *et al.* [11] also ablated satellite cells under the Pax7 locus (Pax7^{CreERT}), and found that Tcf4^{+} fibroblasts failed to expand effectively in response to injury and did not decrease to pre-injury levels 28 days after injury. These results demonstrate nicely the important reciprocal interactions that occur between satellite cells and other progenitor populations during skeletal muscle regeneration. Interestingly, premature myogenic differentiation in the absence of fibrogenic cells differs from the results obtained by co-culture experiments by Joe *et al.* [28], who showed that mesenchymal cells provide a micro-environment that supports myotube formation *in vitro*. Such differences in findings probably reflect the differing models, cellular systems and precise cellular populations examined. Indeed, precise phenotypic and functional distinctions, if any, between 'fibroblasts' and MSCs in skeletal muscle and other tissues remain largely uncharacterized. In a classical model, a fibroblast perhaps represents a more lineage-committed cell type capable of producing extracellular matrix proteins such as collagen and other connective tissue components. However, an MSC may be distinguished by its broadened potency, being capable of differentiating into bone or fat as well as connective tissues, and perhaps, although yet to be shown, capable of self-renewal. Such controversies and conceptual ideas are the subject of an excellent recent review [34].

The above evidence highlights that skeletal muscle comprises, in addition to satellite cells, populations of tissue-resident mesenchymal progenitors with multipotent lineage potential *in vivo*. Although not directly contributing to regenerative myogenesis in normal settings, such progenitors represent a critical component of the cellular niche required for effective satellite cell-mediated regeneration. Indeed, the paracrine effect of MSCs in assisting tissue

regeneration has provided the underlining rationale for a plethora of clinical trials investigating stem cell transplantation therapies in various tissues, such as the heart [35,36]. However, it seems unlikely that such progenitor populations exist solely to provide the correct environmental cues to facilitate myogenesis. Little is know about the contribution of mesenchymal progenitors to normal muscle repair processes. Do these progenitors differentiate into collagen-producing cells to restore damaged extracellular matrix and repair muscle architecture needed for myofibre/myofibril support? Are these cells capable of self-renewal? What are the molecular networks involved in their regulation? Answering of such questions will provide a more complete picture of the mechanisms and processes involved in muscle regeneration in the future.

Tissue-resident mesenchymal stem/progenitor cells in skeletal muscle degeneration

Although tissue-resident mesenchymal progenitors appear to be important contributors to the cellular environment required for muscle regeneration in healthy settings, evidence suggests these cells play a more sinister role in conditions of disease. Consistent with degenerative disease states in many tissues, failed skeletal muscle regeneration is associated with fibrosis, accumulation of extracellular matrix proteins and fat deposition. Such histopathological features are commonly observed in dystrophic and ageing muscle, and contribute to the impaired contractile and metabolic functions of this tissue [37]. Indeed, elevated levels of transforming growth factor β (TGFβ) consistent with a 'pro-fibrotic' environment, have been well described in dystrophic muscle [38,39].

Efforts have been made to identify the cellular source of fibro-adipogenic tissue formation in degenerative skeletal muscle. Initial studies provided evidence that satellite cells, when exposed to certain environmental cues, may trans-differentiate into a fibroblastic lineage. Brack *et al.* [40] showed that exposure of primary myoblasts to serum from aged mice induced a proportion of cells to adopt a 'non-myogenic' cell fate *in vitro* – a phenotype that is apparently dependent on activation of canonical Wnt signalling. Accordingly, pharmacological inhibition of Wnt signalling *in vivo* resulted in a small reduction in skeletal muscle fibrosis following acute injury in aged mice [40]. Other studies reported that isolated myoblasts from normal and dystrophic muscle are capable of conversion into fibroblastic collagen-producing cells [41–43], suggesting that fibrogenesis from satellite cells may contribute to degenerative processes.

However, more recent work has suggested that populations of tissue-resident mesenchymal progenitors are likely to be the major source of pro-fibrotic cells in skeletal muscle. Comparisons of the mRNA expression profiles of muscle-resident cell populations in dystrophic muscle have found that expression of the fibrosis-related genes encoding collagen type 1a (Col1a), Collagen type 3a1 (Col3a1), connective tissue growth factor and αSMA occurs almost exclusively in PDGFRα$^+$ cells [30]. Similarly, PDGFRα$^+$ cells treated with TGFβ show conversion into Col1a- and αSMA-producing cells with far greater sensitivity compared to muscle progenitors and muscle-resident endothelial/hematopoietic cell populations [30]. These findings have been extended by data obtained by Dulauroy *et al.*

[32]. Using an elegant lineage tracing strategy, Dulauroy *et al.* showed that a population of gp23$^+$, PDGFa$^+$, Sca-1$^+$ progenitors transiently expressing Adam12 are a major source of fibrotic tissue accumulation following muscle damage. The population of Adam12$^+$ cells rapidly expanded upon muscle injury *in vivo*, peaking 4 days after injury and then decreasing over time, resembling the proliferation kinetics of mesenchymal progenitors observed by Joe *et al.* [28]. *In vitro*, Adam12$^+$ cells readily differentiated into αSMA-expressing myofibroblasts upon TGFβ stimulation, but showed poor differentiation into adipocytes. These data suggest that Adam12 may represent a marker for a more committed 'fibrogenic' sub-population of mesenchymal progenitors in skeletal muscle. Interestingly, Adam12$^+$ cells displayed phenotypic features of pericytes, sharing close proximity to blood vessel walls and expressing molecular markers such as neuro-glial 2 proteoglycan. Reasoning that Adam12$^+$ cells are a major source of pro-fibrotic cells, Dulauroy *et al.* [32] next investigated whether selective ablation of Adam12$^+$ cells dampened the fibrotic response following acute muscle injury. Indeed, inducible expression of diphtheria toxin under the control of the Adam12 promoter led to depletion of Adam12$^+$ cells and a significant reduction in interstitial collagen formation 3 weeks following muscle injury. These findings have provided the most convincing demonstration to date of the contribution of mesenchymal progenitors to tissue fibrosis during skeletal muscle regeneration. In the future, it will be important to examine whether ablation or inhibition of similar progenitor populations affects the progression of fibrosis in longer-term degenerative models of muscle disease such as mdx mice.

When the above studies are considered in combination, it is clear that skeletal muscle-resident MSCs adopt some menacing characteristics in reparative disorders. Instead of cooperating to exert a positive influence on muscle repair, failed regeneration triggers the accumulation of MSCs and deposition of fibrogenic, adipogenic and extracellular matrix proteins.

Targeting skeletal muscle-resident mesenchymal progenitors for the treatment of muscle disease

As skeletal muscle mesenchymal progenitors appear to possess dual roles, both influencing myogenesis and contributing to fibrosis, these cells represent an attractive target for treatment of chronic degenerative diseases such as muscular dystrophies. Indeed, muscular dystrophies are classically characterized by fatty and connective tissue accumulation, and therefore manipulation of the differentiation, survival, proliferation or lineage choices of MSCs represents a logical therapeutic strategy to impair fibrotic accumulation and enable other strategies to boost regeneration. Indeed, the results obtained by Dulauroy *et al.* [32] provide a conceptual framework for this idea by demonstrating that ablation of a sub-population of mesenchymal progenitors in skeletal muscle significantly reduced fibrosis in response to muscle damage.

From a pharmacological point of view, several groups have shown that the tyrosine receptor kinase inhibitor imatinib appears to be effective at inhibiting connective tissue accumulation in several models of fibrosis, including mdx mice [44–46]. Recent findings revealed that the

anti-tumoral kinase inhibitor imatinib appears to have a specific influence on PDGFRα-expressing mesenchymal progenitors in skeletal muscle. Ito *et al.* [46] demonstrated that, unlike satellite cells, isolated PDGFRα$^+$ cells from damaged muscle displayed decreased proliferation and reduced expression of 'pro-fibrotic' genes (αSMA, tissue inhibitor of metalloproteinases 1 and Col3a1) when treated with imatinib *in vitro*. These findings suggest that imatinib may represent a pharmacological means to target muscle-resident mesenchymal progenitors and prevent fibrosis; however, further research is required to understand which other cell types that imatinib affects and the precise molecular pathways upon which this tyrosine receptor kinase inhibitor acts. Other groups have examined the affects of blocking TGFβ – a secreted ligand known to stimulate fibrosis development. Promising initial results have revealed that systemic administration of a TGFB neutralising antibody reduces connective tissue accumulation in mdx mice; however, off-target effects were observed [47]. In summary, we consider that identifying drugs to target mesenchymal progenitors or the 'pro-fibrotic' molecules that trigger fibrosis is an important research goal in the treatment of muscular dystrophies.

In addition, further work is required to shed light onto the molecular regulation of MSCs in muscle. Understanding the signalling networks and mechanisms that regulate MSC differentiation, proliferation, gene expression and lineage choice is likely to open up further therapeutic avenues to target these cells in settings of acute/chronic fibrosis.

Conclusion

In summary, accumulating evidence indicates that tissue-resident mesenchymal progenitors play important roles in skeletal muscle repair. In healthy tissue, reciprocal interactions between MSCs and satellite cells exist to facilitate effective stem cell function and repair. However, in disease settings, MSCs appear to adopt a more sinister role – providing a major source of fibrosis, fatty tissue accumulation and deposition of extracellular matrix proteins. It is therefore clear that, in order to unlock the regenerative potential of skeletal muscle in therapeutic settings, a complete understanding of the multiple cell types involved and their complex interactions is required.

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Abbreviations

AP	alkaline phosphatase
Col1a	collagen type 1a
Col3a1	collagen type 3a1
CreERT2	tamoxifen-inducible Cre recombinase
FAPs	fibroadipogenic progenitors

MSCs	mesenchymal stem/progenitor cells
MyHC	myosin heavy chain
PDGFRα/β	platelet-derived growth factor receptor α/β
PICs	$PW1^{+}/Pax7^{-}$ interstitial cells
Tcf4	transcription factor 7-like 2 Tcf7L2
TGFβ	transforming growth factor β
αSMA	α smooth muscle actin

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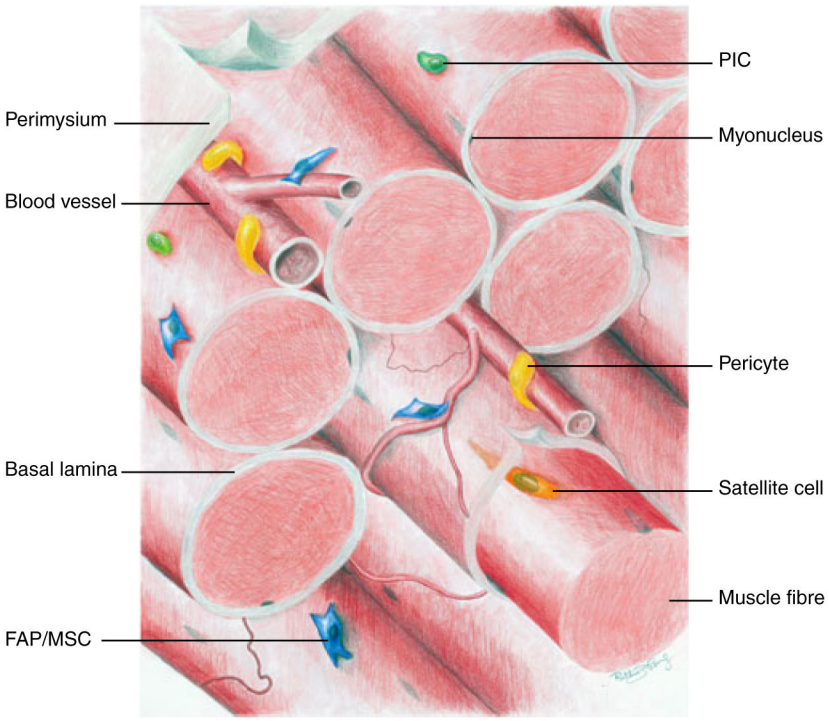

Fig. 1.

Illustration of the various populations of tissue-resident progenitors in skeletal muscle: PICs ($PW1^{+}/Pax7^{-}$ interstitial cells, green), FAPs/MSCs (fibroadipogenic progenitors/mesenchymal stem/progenitor cells, blue), vessel-associated pericytes (yellow) and satellite cells (orange) beneath the basal lamina.

Table 1

Summary of differing populations of skeletal muscle-resident progenitors. FAPs, fibro-adipogenic progenitors; MSCs, mesenchymal stem/progenitor cells; PICs, $PW1^{+}/Pax7^{-}$ interstitial cells; AP, alkaline phosphatise; NG2, neuro-glial 2 proteoglycan; PDGFRα/β, platelet-derived growth factor receptor α/β; Tcf4, transcription factor 7-like 2 (Tcf7L2). For most of these cell types, potentials other than myogenic have not been investigated thoroughly *in vivo.*

Described developmental potential in regenerating skeletal muscle				
Population	**Cellular markers**	***In vitro***	***In vivo***	**References**
Satellite cells	Pax7, CD34, Myf5, caveolin-1 and Vcam-1	Myogenic and fibrogenic (?)	Myogenic	[5–9,40,48,49]
FAPs/MSCs	Sca-1, CD34, PDGFRα, Adam12 and Tcf4 (?)	Adipogenic, fibrogenic, osteogenic and chondrogenic	Adipogenic, fibrogenic, osteogenic and chondrogenic	[28–33]
PICs	PW1, Sca-1 and CD34	Myogenic (smooth muscle)	Myogenic	[21]
Pericytes	AP, PDGFRβ and NG2	Myogenic, adipogenic (smooth muscle) and osteogenic	Myogenic	[26,27]
Side population cells	Hoechst-negative	Myogenic, adipogenic, fibrogenic, osteogenic and haematopoietic	Myogenic	[13,15,20]