Adult Stem Cells: Adult Skeletal Muscle Stem Cells

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Abstract Maintenance, repair and regeneration of adult skeletal muscle are mediated by stem or precursor cells within the muscle. In addition to the satellite cell, which is the archetypal muscle stem cell, there are other stem cells within skeletal muscle that can contribute to muscle regeneration under experimental conditions. We describe these different cells within skeletal muscle and evaluate the experimental evidence for them being skeletal muscle stem cells. Further studies will be needed to determine the roles of different skeletal muscle resident cells to repair, maintain and regenerate skeletal muscle.

Keywords Adult skeletal muscle • Satellite cells • Blood vessel-associated stem cells • Muscular dystrophy

Abbreviations

MAPCs	Multipotent adult progenitor cells
MDSCs	Muscle-derived stem cells
MECs	Myoendothelial cells
PICs	PW1(+)/Pax7(−) interstitial cells
SP cells	Side population cells
VSELs	Very small embryonic-like stem cells

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1 Introduction

Skeletal muscle is the largest organ within the human body, comprising 30–40 % of the body mass [1] and is essential for movement and posture. Muscle fibres that contain the contractile elements are formed during development by the fusion of myoblasts to form multinucleated muscle fibres, in which the nuclei (myonuclei) are postmitotic. Postnatal growth, repair and maintenance of skeletal muscle are mediated by satellite cells; however, there are other stem cells within skeletal muscle that are also capable of differentiation into skeletal muscle. In this chapter, we review the different stem cells present within adult skeletal muscle and their contribution to skeletal muscle regeneration.

2 Satellite Cells

The classical stem cells within adult skeletal muscle are satellite cells, which were first identified by Mauro [2] and defined by their position between the basal lamina and sarcolemma of the muscle fibre. Early work provided evidence that satellite cells are the source of new myofibre nuclei during muscle growth [3, 4] and regeneration [5]. Only recently, with the availability of reliable antibodies [6–14] and genetically modified mice [15–17], have satellite cells been established as muscle stem cells, able to both contribute to muscle growth [18, 19], regeneration [20, 21] and to functionally reconstitute the satellite cell niche [22, 23].

Studies of satellite cells in mice have been facilitated by the relative ease by which they may be separated from other cells present within skeletal muscle. Isolated muscle fibres, bearing their complement of satellite cells under the basal lamina [24] enable studies of satellite cells in their niche [25–27]; satellite cells may also be physically [22, 23, 28] or enzymatically [29] removed from their niche on the fibre for in vitro or in vivo studies. There are also protocols for satellite cell purification from enzymatically disaggregated skeletal muscle either on the basis of size and granularity [30] or using cell-surface satellite cell-specific antibodies combined with antibodies against other cell types to enrich for satellite cells [31–33]. However, there are caveats in using cell-sorting techniques—the sub-population isolated may not be 100 % pure and satellite cells may be activated during the procedure and thus not express particular markers. In addition, some antibodies used for cell sorting are not ideal, e.g. the monoclonal antibody SM/C-2.6 [9], which is frequently used for satellite cell purification [10, 34, 35], is not commercially available, nor is the antigen that it recognises known.

The term ‘satellite cell’ is often used incorrectly in the literature. By definition, a satellite cell is a quiescent cell underneath the basal lamina of muscle fibres. When it is no longer under the basal lamina, it is therefore no longer a satellite cell, but some studies refer to cells in tissue culture as satellite cells [36, 37]. If the cell is

under the basal lamina of the fibre but has divided and is expressing myogenic regulatory factors such as MyoD, this cell is the progeny of a satellite cell (a myoblast), not a satellite cell [27].

An important caveat is that not all satellite cells are capable of contributing to muscle regeneration [23, 38], suggesting the existence of a 'stem' satellite cell sub-population [33] that survives into old age [39, 40]. Whether the 'stem' satellite cell sub-population really exists, or if satellite cell functional characteristics are stochastic, is still not clear. Despite age-related decrease in satellite cell number [40–42] and changes in signalling factors, hormones, cytokines and growth factors that modulate their function [43], efficient muscle regeneration in old age can occur, provided the local or systemic environment is modulated appropriately [39, 40, 44]. The extent to which the aged or dystrophic environment [45] and the satellite cell niche itself [39, 46] affect satellite cell function is the focus of much current research (reviewed [21, 47–49]).

Although there is good evidence that satellite cells are required for postnatal skeletal muscle regeneration [20], they lose their regenerative capacity following culture [30], only have a very local effect after intra-muscular injection and do not seem to be systemically deliverable, so much work has focussed on other skeletal muscle stem cells that might be more appropriate for treating conditions such as muscular dystrophies (reviewed [50, 51]). However, other stem cells within skeletal muscle have been less intensively studied, largely due to challenges in identifying and purifying them. To complicate matters, the same, or similar, stem cells are often given different names or acronyms, e.g. pericytes [52] and muscle-derived cells (mdcs) [53], satellite cells and their putative stem cell sub-population, muscle stem cells (MuSCs) [33]. An additional problem in studying different cell types is that, if cells have to be expanded in culture, they may change their phenotype, so it is always best to study them either in vivo or immediately following their direct isolation.

3 Other Stem Cells Within Skeletal Muscle

Stem cells other than satellite cells that have been shown to contribute to skeletal muscle regeneration include blood vessel-associated stem cells, such as muscle side population (SP) cells, myoendothelial cells (MECs) and pericytes/mesoangioblasts; stem cells of unknown origin, such as muscle-derived stem cells (MDSCs), multipotent adult progenitor cells (MAPCs), CD133+ cells, PW1(+)/Pax7(−) interstitial cells (PICs) and very small embryonic-like stem cells (VSELs). But the extent to which these cells contribute to muscle regeneration is often slight [54–56] and whether these cells participate in muscle growth, maintenance, repair and regeneration in non-experimental conditions often remains unclear (Table 1).

Table 1 Stem cells within skeletal muscle—location, identification and potential

Cell type	Location	Markers	Differentiation potential (in vitro and in vivo)	Participation in muscle growth and maintenance	Muscle formation after transplantation	Satellite cell formation after transplantation	Systemically deliverable
Satellite cell	Underneath basal lamina of myofibre	Pax7+, CD34+, Myf5+, c-Met+, M-Cad+, α7 integrin+, syndecan3 and 4+, Caveolin1+	Skeletal muscle	Yes	Robust	Yes	No
Pericyte	Outside endothelium of the blood vessel	ALP+, NG2+, PDGFRβ+, CD146+	Skeletal and smooth muscle, osteoblasts, adipocytes	Yes	Yes	Yes	Yes
PIC	Interstitial	Pax7−/PW1+	Skeletal and smooth muscle	Not determined	Equivalent to satellite cells	Yes	Not determined
MEC	Blood vessel associated	CD56+/CD34+ /CD144+	Skeletal and cardiac muscle	Not determined	Robust, better than endothelial cells and myoblasts	Not determined	Not determined
MDSC	Not known	Desmin+, MyoD+, CD34+, Sca-1+ Bcl-1+, CD45− and c-kit−	Skeletal and cardiac muscle, haematopoietic, osteogenic, endothelial and neuronal	Not determined	Robust	Not determined	Not determined
MAPC	Not known	Cd13+, CD44−, CD45−, MHC I− and II−, c-kit−	Endothelium, neurons, glia, hepatocytes	Not determined	Limited	Not determined	Not determined
SP cell	Not known	Hoechst low	Muscle and haematopoietic	Not determined	Limited	Not determined	Yes
CD133+ cell	Not known	CD133+	Muscle	Not determined	Robust, better than myoblasts	Yes	Yes
VSEL	Not known	Oct-4+, SSEA-4+, Nanog+, Sox-2+, Rex-1+, Tert+, CD133+, CXCR4+	Haematopoietic, cardiomyocytes	Not determined	Not determined	Not determined	Not determined

3.1 Blood Vessel-Associated Stem Cells

3.1.1 Side Population Cells

Side population (SP) cells were identified by a low Hoechst staining 'tail' in their FACS profile [57]. The 'tail' disappears in the presence of a calcium channel blocker, verapamil. SP cells have been found in a wide variety of mammalian tissues and in many cases this cell population has been shown to contain multipotent stem cells [58]. Skeletal muscle SP cells express the stem cell marker Sca-1, but no myogenic markers and are located outside the basal lamina of muscle fibres, apparently associated with the vasculature [59]. The fact that they are present in mice in which Pax7, expressed in satellite cells, is knocked out [14] is compelling evidence that they are not derived from satellite cells.

Murine muscle SP cells do not differentiate into skeletal muscle in vitro, but after co-culture with myogenic cells or on intra-muscular transplantation, they do give rise to skeletal muscle [54, 59]. They are also capable of differentiating into haematopoietic cells in vitro [59] and can reconstitute the haematopoietic system of lethally irradiated mice [60]. Skeletal muscle SP cells are systemically deliverable to skeletal muscle [61–63], but not to any therapeutically significant levels [63, 64].

3.1.2 Endothelial/Myoendothelial Cells

Myoendothelial cells (MECs), co-expressing both myogenic and endothelial markers (CD56, CD34 and CD144) have been derived from human (but not mouse) skeletal muscle by flow cytometry [65]. However, it has been suggested that mouse MDSCs and human MECs are in fact the same, as they have a similar phenotype and ability to contribute to muscle regeneration [66]. Human MECs gave rise to significantly more skeletal muscle regeneration following intra-muscular grafting in mice than either endothelial cells (CD56−CD34+CD144+) or myoblasts (CD56+). When MECs cells were transplanted into infarcted myocardium, they stimulated angiogenesis, attenuated scar tissue, and promoted proliferation and survival of endogenous cardiomyocytes more effectively than either myoblasts or endothelial cells [67]. However, although blood vessel associated, there is no evidence that myoendothelial cells can transmigrate to skeletal muscle if transplanted systemically.

3.1.3 Pericytes/Mesoangioblasts

Myogenic cells derived from the mouse embryonic dorsal aorta, which co-expressed endothelial and myogenic markers, were shown to contribute to skeletal muscle growth and regeneration [68]. These cells, termed mesoangioblasts, are multipotent stem cells [69], able to differentiate into several mesodermal tissues and might be the origin of postnatal mesodermal stem cells. Mesoangioblasts have been shown to contribute to muscle regeneration and improve the muscle function after intra-arterial transplantation into either dystrophic mice [70] or dogs [71].

Pericytes are ALP+ cells located along the blood vessels and may be the adult counterpart of embryonic mesoangioblasts. However, unlike mesoangioblasts that express endothelial markers, they express pericyte markers such as alkaline phosphatase (ALP), NG2 and PDGFR-β [52]. Pericytes may also be isolated from skeletal muscle and other tissues by direct sorting of CD146+ CD34− CD45− CD56− cells [72, 73]. Like mesoangioblasts, skeletal muscle-derived pericytes are myogenic and can contribute extensively to skeletal muscle regeneration after intra-arterial [52] and intra-muscular [53] transplantation into dystrophin-deficient immunodeficient mice. In addition to myogenic differentiation, pericytes can also give rise to many other mesenchymal lineages, suggesting a close relationship with mesenchymal stem cells (MSCs) [52, 73].

Recent work using genetically modified mice has provided evidence that pericytes, but not endothelial cells, contribute to muscle fibres and to satellite cells during normal postnatal development [74].

3.2 Other Skeletal Muscle Stem Cells

3.2.1 Multipotent Adult Progenitor Cells

MAPCs were first isolated from human and mouse adult bone marrow (BM) [75], then from other postnatal tissues such as brain and muscle [55] and have the potential to differentiate into cells of all the three germ layers, including skeletal muscle. Skeletal muscle-derived MAPCs can be expanded up to 75 population doublings in vitro and similar to mouse- and human BM-derived MAPC, muscle MAPCs are CD13+, Flk1dim, c-kit−, CD44−, CD45−, MHC class I− and MHC class II−. Human and mouse MAPCs were reported to improve ischemic limb function after transplantation intramuscularly to C57BL/6 mice or BALB/c-nu/nu mice after artery ligation [76]. Although they did give rise to donor-derived muscle fibres, the percentage was low, suggesting the positive effects of these cells were most likely via their immunomodulatory or trophic effects, e.g. by increasing angiogenesis and endogenous stem cell proliferation, than by making a direct contribution to skeletal muscle fibres.

3.2.2 Muscle-Derived Stem Cells

Cells with stem cell capabilities have been isolated from mouse skeletal muscle on the basis of their adhesion and proliferative capabilities. MDSCs or long-term proliferating cells [77] were purified as a multipotent stem cell from neonatal mouse muscle by serial pre-plating, the less adherent cells being MDSCs. These cells derived from both mouse [77, 78] and human [56] contribute to muscle regeneration after transplantation into dystrophin-deficient mdx [the mouse homologue of Duchenne muscular dystrophy (DMD)] muscles. However, MDSCs derived from human muscle were phenotypically different from mouse MDSCs and gave rise to fewer donor-derived dystrophin+ fibres than did mouse MDSCs.

Mouse MDSCs are non-tumorigenic and can be expanded in vitro up to 300 population doublings without entering senescence [79]. Clones of MDSCs express myogenic markers (desmin and MyoD) and some stem cell markers such as CD34, Sca-1 and Bcl-1 and lack the haematopoietic stem cell marker CD45, c-kit and blood lineage markers [80]. These cells can reconstitute the haematopoietic system [78, 81] and elicited significant improvement in cardiac function in comparison to myoblasts following transplantation in a mouse cardiac injury model [82, 83]. MDSCs exist only in vitro and the cell within skeletal muscle from which they are derived is not known.

3.2.3 PW1(+)/Pax7(–) Interstitial Cells

PW1, also known as paternally expressed gene 3 (Peg3), a zinc finger protein which regulates two key cell-stress pathways, TNF and p53 signalling [84], is a key regulator of muscle atrophy. PW1 expression initiates in the early embryonic mesoderm and is down-regulated in tissues as they differentiate. It was recently suggested that PW1 might represent a pan-marker for multiple adult stem cells within mammalian tissue [85]. In mouse skeletal muscle immediately after birth, PW1 expression was detected not only on satellite cells but also on some Pax7– interstitial cells, termed PICs [86]. PICs are bipotent in vitro, generating both smooth and skeletal muscle and were able contribute to muscle regeneration in vivo within injured host mouse muscle [86]. However, PICs do not seem to be present within adult mouse muscle and their human counterparts have not yet been identified.

3.2.4 CD133+ Cells

CD133 is a pentaspan transmembrane glycoprotein (5-transmembrane, 5-TM), which specifically localises to cellular protrusions. The function of CD133 (also known as prominin-1 and AC133) is currently unknown. However, there is great interest in this marker, as it is expressed on many different types of stem cell, including haematopoietic stem cells [87], neural stem cells [88], endothelial progenitor cells [89, 90] and very small embryonic-like stem cells (VSELs) [91, 92]. CD133+ cells isolated from human skeletal muscle are able to contribute to muscle regeneration after both intra-muscular delivery to injured immunodeficient mouse muscle [93] and systemic administration to dystrophic immunodeficient mice [94]. But, as human muscle CD133+ cells were isolated by enzymatic disaggregation, the origin of these cells is unclear. As skeletal muscle is heavily vascularised, it is possible that the skeletal muscle-derived AC133+ cells isolated by Benchaouir et al. were blood borne and the same, or similar to, blood-derived AC133+ cells that can contribute to muscle regeneration [95]. A limitation to the study of these cells is that, although there are antibodies that can be used for FACS or MACS isolation [94], the anatomical location of these cells within skeletal muscle remains unknown. Skeletal muscle-derived CD133+ cells share some cell surface markers, such as CXCR4 and CD34, with satellite cells [93, 94] and cultured CD133+ cells express not only myoblast

markers but also the smooth muscle marker α-SMA and pericyte markers NG2 and PDGFRβ (Meng, unpublished data), suggesting the heterogeneity of this cell population. The majority of freshly isolated mouse CD133+ cells are very small in size—2–6 μm (Meng and Asfahani, unpublished observations)—a size very similar to those reported for VSELs [91, 92, 96]. In addition, their robust myogenic potential and ability to form Pax7+ cells in the satellite cell position [93] suggests a very close relationship of CD133+ with satellite cells, but whether they are a satellite cell sub-population, or a precursor of satellite cells, remains to be elucidated. Whether CD133+ cells derived from mouse skeletal muscle are equivalent to human CD133+ cells remains to be seen.

3.2.5 VSELs

Recently, a population of stem cells termed 'very small, embryonic-like stem cells' (VSELs) was discovered within many tissues, including skeletal muscle [91]. These cells are approximately 6.5 μm in diameter in the human [96] (i.e. smaller than red blood cells) and can be purified by flow cytometry [97] (reviewed [98]). VSELs express several markers of pluripotent stem cells, including Oct-4, cell surface protein SSEA-4, Nanog, Sox-2, Rex-1 and Tert [99] and form embryoid body-like spheres in vitro [97]. VSELs derived from the mouse bone marrow are radiation resistant and may be long-term repopulating haematopoietic stem cells [100] as well as differentiating to cardiomyocytes in vitro [101]. VSELs therefore show intriguing similarities to 'stem' satellite cells, which are also of small size [23, 30] and radiation resistant [39, 102, 103]. But their function and relationship to other cells within skeletal muscle is at present unknown.

4 The Relationships Between Stem Cells Resident in Skeletal Muscle

Some muscle stem cells clearly have close relationships (e.g. pericytes and satellite cells, PICs and satellite cells), whereas the hierarchy, if any, between other skeletal muscle resident stem cells is not clear. Even if one cell type does not directly give rise to another, they may affect each other's function. The close proximity of satellite cells to blood vessels [104] will facilitate satellite cell interactions with endothelial cells, pericytes and other blood vessel-associated cells [105, 106].

5 Why Are We Interested in Skeletal Muscle Stem Cells?

Interest in skeletal muscle stem cells was initiated because of the possibility of using them, or their progeny, to treat muscular dystrophies such as DMD [50, 107]. For this purpose, cells derived either from a normal donor or from the patient,

genetically modified to express the defective gene (e.g. dystrophin), could be used. Ideally, cells would be able to be systemically delivered to skeletal muscle and repair or replace dystrophic muscle fibres, thus restoring dystrophin expression within fibres that have donor-derived myonuclei. If the donor stem cells also reconstituted the skeletal muscle stem cell pool, they could contribute to muscle repair and regeneration and restoration of dystrophin protein throughout the lifetime of the individual.

Donor muscle stem cells might also be a therapeutic option for sarcopenia (the age-related loss of skeletal muscle mass and strength). However, the systemic or local environment as a result of age or dystrophy-related changes may prevent efficient stem cell function. There is therefore a pressing need to understand the effect of age or different muscular dystrophies on the satellite cells themselves and on their environment. Modification of pathways that promote muscle stem cell function could be an alternative means to alleviate the loss of muscle that occurs as a result of ageing or muscular dystrophies.

6 Conclusions

In this review, we have summarised the stem cell types within the skeletal muscle and the evidence for them being skeletal muscle stem cells. Skeletal muscle contains many stem or precursor cells that can contribute to muscle regeneration under experimental conditions, but, apart from satellite cells and pericytes, their contribution (if any) to 'normal' muscle growth, maintenance and repair is not known. Further studies will be needed to determine the roles of different skeletal muscle-resident cells within both normal and dystrophic muscles and how to augment their function to prevent or delay the loss of skeletal muscle fibres that occurs as a consequence of both age and muscular dystrophies.

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