REVIEW

Fate choice of post-natal mesoderm progenitors: skeletal versus cardiac muscle plasticity

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Abstract Regenerative medicine for skeletal and cardiac muscles still constitutes a fascinating and ambitious frontier. In this perspective, understanding the possibilities of intrinsic cell plasticity, present in post-natal muscles, is vital to define and improve novel therapeutic strategies for acute and chronic diseases. In addition, many somatic stem cells are now crossing the boundaries of basic/translational research to enter the first clinical trials. However, it is still an open question whether a lineage switch between skeletal and cardiac adult myogenesis is possible. Therefore, this review focuses on resident somatic stem cells of post-natal skeletal and cardiac muscles and their plastic potential toward the two lineages. Furthermore, examples of myogenic lineage switch in adult stem cells are also reported and discussed.

Keywords Skeletal muscle · Cardiac muscle · Resident stem cells · Myogenic regeneration · Muscular dystrophy · Lineage switch

From embryo to adult: skeletal versus cardiac myogenesis

During embryonic development, skeletal muscle precursors arise from the paraxial mesoderm. During the process

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of somitogenesis, somites develop in a cranial-to-caudal sequence from the segmental plate of the paraxial mesoderm and flank the neural tube [1, 2]. Subsequently, proliferative progenitor cells are recruited by delamination from the central dermomyotome, which will form the basis for the growth of the myotome and derived populations such as the satellite cells (SCs) during fetal and postnatal development [3].

Differently from the paraxial mesoderm, the splanchnic mesoderm (visceral or cardiogenic mesoderm) gives rise to the heart. The initial heart tube is orientated along the craniocaudal axis. Then the cardiac looping starts, the heart tube grows rapidly, and primary and secondary heart fields are specified. Progenitors from the primary heart field give rise to both ventricles and atria, and to the atrium-ventricular canal, while secondary heart field progenitors contribute to the formation of the outflow tract and the other cardiac regions, except the left ventricle. After looping, the heart is finally articulated in the four contractile chambers and the myocardium is composed of bi- or tri-nucleated myocytes, electrophysiologically coupled to each other [4].

During development, progression through skeletal or cardiac lineage relies on different gene networks, often recapitulated during post-natal regeneration (Fig. 1).
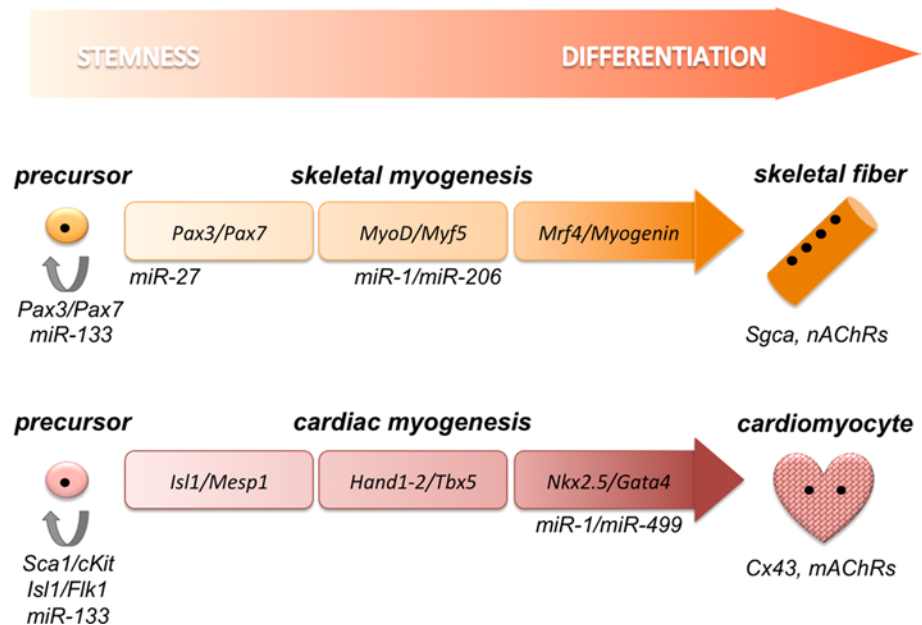
Skeletal muscle progenitors in the myotome express the paired-domain and homeodomain-containing transcription factors *Pax3* and *Pax7* [5–7]. These activate the muscle regulatory factors (MRFs), namely *Myf5*, *MyoD*, *Mrf4*, and *Myogenin*, which subsequently drive the skeletal myogenesis [8, 9].

In contrast to the skeletal muscle, cardiac commitment relies on less hierarchical gene networks. The earliest cardiac specification marker can be traced to mesoderm posterior protein 1 and 2 (*Mesp1*, *Mesp2*), markers of primitive heart tube formation and required for cardiac progenitor migration. *Mesp1* is able to promote transcription

Fig. 1 Schematic comparison of factors regulating skeletal and cardiac differentiation, as mentioned in the text. Skeletal fibers and cardiomyocytes are distinguished by αSarcoglycan (Sgca) and nicotinic acetylcholine receptors (nAChRs) in the former, and by Connexin43 (Cx43) and muscarinic acetylcholine receptors (mAChRs) in the latter

of several other cardiomyogenic markers and, accordingly, in *Mesp1-null* mice cardiac formation is abolished since the early stage [10]. Recently, *Mesp1* has been proposed as a context-dependent determination factor to specify different lineage outcomes, including cardiac cells [11]. In addition, MADS-box factor myocyte enhancer factor-2 (*Mef2*), in conjunction with other transcription factors, directly activates the expression of genes encoding myofibrillar proteins [12]. Similarly, *serum response factor* (*Srf*), a related MADS-box factor, associates with an array of transcription factors including *NK2 transcription factor related locus 5* (*Nkx2.5*), *GATA binding protein 4* (*Gata4*) and *Myocardin* to control the expression of the contractile apparatus genes, e.g., actin, myosin, and troponins [13]. Furthermore, Mef2C physically interacts with T-box 5 (Tbx5) to regulate early stages of heart development and expression of cardiac myosin, as reported in zebrafish [14]. Intriguingly, overexpression of *Gata4*, *Tbx5* and *Mef2C* converts murine fibroblasts into functional cardiomyocytes [15]. Finally, *Hand1* and *Hand2* can be considered to exert the analogous function of *Pax3/Pax7*, since they control the entry into the cardiac differentiation program. Loss of *Hand1* results in increased cardiomyocyte differentiation, whereas *Hand1* gain-of-function supports cardiomyocyte proliferation [16].

Stem cell plasticity in the post-natal skeletal muscle

The intrinsic regenerative potential of the post-natal skeletal muscle has been linked to resident precursors since the first observation of quiescent SCs residing under the basal lamina of adult fibers [17]. Later, many somatic stem cells

have been isolated with diverse molecular signatures and plasticity grades [18]. Understanding the in vitro/in vivo properties of resident myogenic cell pools, whose subset is reviewed here (Table 1; Fig. 2), is fundamental for advancing our knowledge of post-natal plasticity of the skeletal muscle and for promoting novel therapeutic strategies for cell-mediated myogenic regeneration.

Satellite cells are resident stem cells, expressing the surface antigens *Syndecan-4*, *M-Cadherin*, *CD34*, *Cxcr4*, *β1-Integrin* and the myogenic factors *Pax3* and *Pax7* [19]. After injury, SCs enter the cell cycle, rapidly upregulate the expression of MRFs that induce the terminal differentiation to novel myofibers. A small percentage of activated SCs does not undergo myogenic differentiation but instead self-renews, restoring the quiescent pool [20].

Albeit strongly primed to the skeletal myogenesis, it has been reported that SCs can transdifferentiate at little extent toward the adipogenic and the osteogenic lineages in vitro, although contaminations with non-myogenic cells can easily occur [21–23]. In this view, it has been recently reported that SCs can be stimulated to store lipids, but fail in undergoing terminal adipogenic differentiation in vitro [24]. Furthermore, exposure to bone morphogenetic protein (BMP) ligands is not per se sufficient to obtain robust commitment to the osteogenic lineage [25].

SC intrinsic commitment relies on a balanced expression of MRFs. Murine strains carrying ablation of *Myf5*, *MyoD* or *Mrf4* show only mild myogenic abnormalities, while triple knockout mice completely lack any skeletal muscle [26–29]. Analogously, adult triple knockout murine SCs are unable to differentiate in vitro and in vivo [30]. *MyoD-null* SCs express low levels of *Myogenin* and show a dramatic differentiation

Table 1 Main characteristics of resident post-natal stem cells in skeletal muscle, with emphasis on species, antigen profile, lineage marker, and in vivo plasticity

Cell type	Species	Surface antigens		Lineage markers	In vivo plasticity	References
		Positive	Negative			
SCs	Mouse	M-cadherin, c-Met, CD34	NA	Pax7	Skeletal fibers	[19]
SCs	Mouse	Desmin	NA	Myf5, MyoD, Pax7	Skeletal fibers	[21]
SCs	Mouse	NA	NA	MRFs	Skeletal fibers	[22]
MABs	Dog	CD44, CD13	CD34, CD45, CD117, CD31	NA	Skeletal fibers	[41]
MABs	Mouse, human	AP, NG2, CD13, CD44, CD49b, CD63, CD90, CD105, CD140b, CD146	CD31, CD34, CD45, CD56, CD62L, CD71, CD106, CD117, CD133	NA	Skeletal fibers	[45]
MABs	Mouse, human	AP, NG2, Sca1, CD13, CD44, CD49f, CD90, CD140a, CD140b	CD31, CD45, CD56, CD133	NA	NA	[48]
PICs	Mouse	Sca1, CD34	Pax7	Pw1	Skeletal fibers	[50]
FAPs	Mouse	CD34, Sca1	Lin, CD31, CD45, α7integrin	NA	Supportive role for SCs	[51]
FAPs	Mouse	Sca1, CD140a	α7integrin	NA	Supportive role for SCs	[52]

Each entry relates to one reference and the entry order is referred to the review

NA information not available in the cited reference, *SCs* satellite ells, *MABs* mesoangioblasts, *PICs* Pw1^{+} interstitial cells, *FAPs* fibroadipogenic progenitors

deficit, demonstrating the importance of the sequential MRF hierarchy to achieve terminal specification [31].

Remarkably, SC self-renewal and commitment are also strongly influenced by post-translational regulations and epigenetic cues. *Sirt1* is a NAD$^+$-dependent protein deacetylase [32], which induces premature differentiation of SCs when downregulated [33]. Similarly, primary SCs derived from *Sirt1$^{+/null}$* differentiate precociously and are resistant to anti-differentiation stimuli, such as glucose restriction [34]. Conversely, SC proliferation increases when *Sirt1* overexpression inhibits *MyoD* activity [35]. Furthermore, tumor necrosis factor alpha (TNFα) signaling has been linked to the chromatin remodeling Polycomb Repressive Complex 2 (PRC2) and to p38α kinase, during SC differentiation [36]. During post-injury inflammation, TNFα activates p38α, which favors PRC2 relocation from muscle structural genes to *Pax7* regulatory regions, repressing its expression and stimulating differentiation. Accordingly, genetic or pharmacological interference with TNFα, p38α, or PRC2 results in sustained *Pax7* expression and SC self-renewal, and this effect is reversible [36].

In addition, regulatory RNAs, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) modulate the balance between quiescence and commitment in SCs. Skeletal muscle-specific *miR-206* is expressed under the control of *MyoD* and *Mef2C*, and, together with *miR-133* and *miR-181*, regulates SC fate [37]. *miR-27b* tunes *Pax3* expression and promotes a rapid and robust entry into the myogenic program [38], whereas *miR-489* is highly expressed during quiescence and is quickly downregulated during SC activation [39]. In addition, lncRNAs are also emerging as regulators of the myogenic program [40]. In particular, low or high levels of *lnc-MD1* correlate with, respectively, delayed or early onset of SC specification by modulating *Mef2C* levels, and *lnc-MD1* is strongly reduced in SCs from Duchenne muscular dystrophy (DMD) patients [41].

Besides the potential of the SC pool, multiple studies suggest that also other resident cells of different origins and not localized in the SC niche are able to transit from the interstitial compartment and to contribute to skeletal muscle plasticity [42]. SCs cannot migrate through the circulation and easily undergo senescence after ex vivo expansion, thus novel progenitors, featuring myogenic, proliferative, and migratory properties, are particularly attractive for translating somatic cell plasticity into putative treatments.

A potential source of muscle progenitors consists of vessel-associated stem cells, i.e., mesoangioblasts (MABs), which reside around the microvasculature bedewing the skeletal muscle [43]. MABs express pericytic markers, e.g., *stem cell antigen 1* (*Sca1*) (although restricted to murine cells), *NG2*, *Alkaline Phosphatase* (*AP*), *CD140a*, and *CD140b*, and can be easily isolated from adult muscles of mice, dogs, and humans [44–47]. Interestingly, MABs are proliferative and multipotent, due to their capability to differentiate toward myogenic, osteogenic, chondrogenic, and adipogenic lineages [48]. MABs have been demonstrated to undergo skeletal myogenesis in vitro and in vivo. When intra-arterially injected in *αSarcoglycan-null*

Fig. 2 Schematic representation of resident (*within upper colored lobes*) and non-resident stem cells for post-natal plasticity in skeletal and cardiac muscles, as reviewed in the text. *Within the lower lobe*, resident stem cells undergoing lineage switch are reported
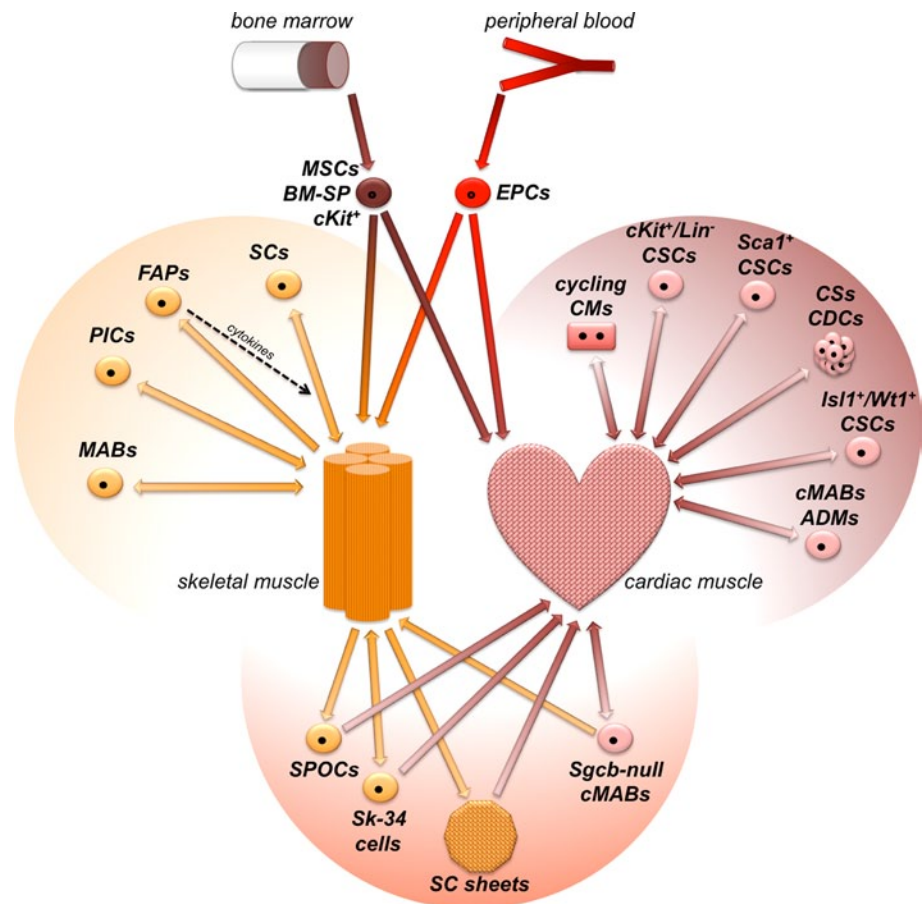
(*Sgca-null*) mice or in Golden Retriever dogs exhibiting DMD, MABs are able to fuse and generate sarcoglycan+ or dystrophin+ fibers, improving electrophysiological properties of injected muscles [43, 44]. Moreover, when engineered to express human *minidystrophin* and transplanted into *scid/mdx* immunodeficient dystrophic mice, DMD MABs give rise to fibers expressing a mature pattern of the transgene [45]. Based on these observations and on the suitability of MABs to systemic delivery, a phase-I/II clinical trial is currently ongoing on DMD patients (EudraCT No. 2011-000176-33).

Interstitial stem cells are another population of Pax7−/Sca1+/CD34+ cells and are characterized by expression of the stress mediator *Pw1*, a zinc-finger-containing protein expressed by C2C12 myoblasts but absent in fibroblasts [49]. Pw1+ interstitial cells (PICs) are self-renewing and have been identified in mouse as myogenic progenitors able to differentiate both to skeletal and smooth muscle cells in vitro [50]. Further studies will be necessary to identify the human counterpart of these cells and to evaluate their translational potential.

Recently, other stromal cells have been suggested to play a supportive role in myogenic differentiation. Fibro/adipocyte progenitors (FAPs) are as abundant as SCs and show a strong predisposition toward the generation of adipocytes and myofibroblasts. Two independent groups have isolated FAPs as CD34+/Sca1+/Lin−/CD31−/CD45−/α7integrin− or as Sca1+/CD140a+/α7integrin− populations respectively [51, 52]. In resting muscles, the interaction with intact myofibers prevents FAP differentiation into fibro-adipocytes [53]. However, muscle injury stimulates these cells to produce paracrine factors such as IL-6 and IGF-1 that positively influence myogenic differentiation [51]. In a recent study, FAPs from young *mdx* mice have been shown to promote in vitro SC-mediated formation of myotubes. Moreover, histone de-acetylase (HDAC) inhibitor enhances FAP ability to promote differentiation of adjacent SCs, through upregulation of the soluble factor *follistatin*, while inhibiting FAP adipogenic potential [54]. Because the human counterpart of Sca1 antigen is currently unknown and FAPs are isolated as Sca1+ cells in mice, future efforts should be directed towards the identification of reliable markers that can allow the isolation of FAPs from human biopsies.

Thus, enhancing the basic knowledge and the translational application of novel myogenic candidates, and their relations with SCs, will likely corroborate current regenerative strategies for the skeletal muscle, through either cell transplantation, either support of the endogenous potential.

Table 2 Species, antigen profile, lineage marker, and in vivo plasticity of post-natal stem cells located in the heart of different origins

Cell type	Species	Surface antigens Positive	Surface antigens Negative	Lineage markers	In vivo plasticity	References
Lin⁻ CSCs	Mouse	cKit, MDR, Sca1	Lin, CD45	Ets1, Gata4, Mef2C	CMs	[60]
cKit⁺ CSCs	Rat	cKit	Lin, CD31, CD8, CD20, CD34, CD45, CD45RO, TER-119	Nkx2.5, Gata4, Gata4, Gata5, Mef2	CMs, SMCs, ECs	[61]
cKit⁺ CSCs	Mouse, human	cKit	CD31, CD90, CD146	NA	CMs, ECs, fibroblasts	[62]
cKit⁺ CSCs	Human	cKIT	LIN	NKX2.5, GATA4, MEF2C	NA	[63]
Lin⁻ CSCs	Rat	cKit, MDR, Sca1	Lin	Nkx2.5, Gata4, Mef2C	CMs, SMCs, ECs	[64]
Lin⁻ CSCs	Human	cKIT, MDR	LIN	MEF2C	CMs, SMCs, ECs	[67]
Sca1⁺ CSCs	Mouse	Sca1, CD31, CD38	Lin, cKit, Flk1, CD34, CD45	Gata4, Mef2C, Tef1	CMs	[69]
Sca1⁺ CSCs	Mouse	Sca1, CD29, CD44, CD34	cKit, CD31, CD45	Nkx2.5, Gata4, Mef2C	CMs	[70]
Sca1⁺ CSCs	Mouse	Sca1	cKit, CD31, CD45	Nkx2.5, Gata4, Mef2C	NA	[71]
Sca1⁺ CSCs	Mouse	Sca1	CD31, CD45	Nkx2.5, Gata4	CMs, ECs	[72]
CSs	Mouse, human	Sca1, cKit, Flk1, CD31, CD34,	NA	NA	CMs, SMCs, ECs	[73]
CDCs	Pig, human	cKIT, CD31, CD34, CD90, CD105	LIN, MDR, CD45, CD133,	NKX2.5	CMs, ECs	[74]
CDCs	Rat, human	cKit, Ddr2, MHC-II, CD31, CD90, CD105, CD140b	MHC-I, CD45, CD80, CD86	NA	CMs, ECs	[75]
CDCs	Human	CD105	CD45	NA	NA	[76]
Isl1⁺ CSCs	Mouse	NA	cKit, Sca1, CD31	Isl1, Nkx2.5, Gata4	NA	[79]
Wt1⁺ CSCs	Mouse	Sca1	cKit	Wt1, Isl1, Nkx2.5, Tbx18	CMs	[82]
cMABs	Human	AP, NG2, cKIT, CD31, CD34, CD44, CD146	CD45, CD133	NKX2.5, GATA4, MEF2A, TBX2, TBX5	CMs	[83]
cMABs	Mouse	AP, NG2, Sca1, cKit, CD31, CD34, CD44, CD140	CD45	Isl1, Nkx2.5, Gata4, Gata6, Mef2A, Mef2C	CMs	[84]
cMABs	Dog	AP, NG2, cKit, Flk1, CD140a, CD140b	CD31, CD73, CD105	Gata4, Gata6, Mef2A, Mef2C, Mesp1, Tbx2, Hand2	CMs	[85]
ADMs	Mouse	Sca1, CD34, CD90.1	CD13, CD31, CD45, CD146	Nkx2.5	CMs	[86]

Each entry relates to one reference and the entry order is referred to the review

NA information not available in the cited reference, *CSCs* cardiac stem cells, *CSs* cardiospheres, *CDCs* cardiosphere-derived cells, *cMABs* cardiac mesoangioblasts, *ADMs* aorta-derived mesoangioblasts, *CMs* cardiomyocytes, *SMCs* smooth muscle cells, *ECs* endothelial cells

Stem cell plasticity in the post-natal cardiac muscle

During the last 15 years, the intrinsic possibility of heart renewal has been suggested through numerous findings, including the discovery of cycling cardiomyocytes after myocardial infarction in human hearts [55] and the measurement of ^{14}C incorporation by cardiomyocytes in humans from 1955 onwards [56]. Although controversies persist on whether the primary contribution to heart renewal in homeostatic and pathological conditions relies on pre-existing cardiomyocytes (CMs) [57] or resident progenitors [58], it is certain that the booming field has fostered the isolation of many types of resident cardiac stem cells (CSCs) from the post-natal heart, with diverse characteristics and plastic potential in vitro and in vivo. Assessment of the regenerative potential of resident CSCs, whose subset is reviewed here (Table 2; Fig. 2), will certainly help in shedding light on post-natal cardiac plasticity and on novel therapeutic strategies.

Somatic CSCs can be prospectively isolated according to specific combinations of surface antigens and culture conditions. A possible surface marker of CSCs is cKit (CD117) [59], the tyrosine kinase receptor of the stem cell factor (SCF) ligand. cKit$^+$/Lin$^-$ CSCs form rare clusters, preferentially embedded in atria and apexes, express cardiomyogenic transcription factors, e.g., *Nkx2.5*, *Gata4*, and *Mef2C*, and contact the surrounding myocardium through N- and E-cadherins [60]. Once clonally expanded ex vivo and intramyocardially injected at the borders of the infarcted myocardium in rats, EGFP$^+$ cKit$^+$ CSCs extensively engraft in the ischemic region, differentiating into EGFP$^+$ CMs, smooth muscle cells (SMCs) and endothelial cells (ECs) and improving ventricular functionality [61]. Similar observations of clonal plasticity in vitro and in vivo have also been reported with murine and human c-Kit (CD117)$^+$ CSCs [62]. Moreover, a phase-1 clinical trial is currently assessing the safety and efficacy of intracoronary injection of autologous cKit$^+$ CSCs in patients with ischemic cardiomyopathy, with preliminary beneficial effects on the left ventricle ejection fraction [63]. Interestingly, the regenerative potential of these CSCs apparently parallels the decreasing adaptation potential of the ageing heart. As compared to younger individuals, the pool of cKit$^+$ CSCs of aged rats presents shortened telomeres, accumulation of the senescence marker p16^{INK4a} and decreased expression levels of *Igf1* and *Hgf* [64], ligands that promote CSC proliferation [65] and homing [66], respectively. Accordingly, in human hearts affected by acute or chronic infarcts, when compared to healthy controls, CSCs present significantly higher levels of not only commitment toward CMs, SMCs, and ECs, but also senescence and apoptosis [67].

Another surface marker to isolate putative CSCs in rodents is the Sca1 [68], a member of Ly-6 antigen family.

Once isolated from the adult murine myocardium, Sca1$^+$ CSCs are CD31$^+$/CD38$^+$ and Lin$^-$/cKit$^-$/CD4$^-$/CD8$^-$/CD34$^-$/CD45$^-$/Flk1$^-$, and express *Gata4* and *Mef2C* but not *Nkx2.5*. After intravenous delivery after ischemia/reperfusion injury, Sca1$^+$ CSCs home at the borders of the infarcted region and differentiate into mature grafts, probably through both de novo CM production and fusion with pre-existing CMs [69]. Moreover, clonal Sca1$^+$ CSC pools can form monolayer sheets that, once transplanted in ischemic hearts, graft in the myocardium and stimulate EC mobilization, by secreting soluble VCAM1 [70]. Interestingly, Sca1$^+$ CSCs present features of in vitro plasticity, as they can differentiate either into beating CMs, when stimulated with oxytocin, either into osteocytes and adipocytes, in the presence of appropriate inducing conditions [71]. In contrast, intramyocardial injection of Sca1$^+$/CD31$^-$ cells alleviates functional decline and adverse remodeling of the ischemic left ventricle, primarily triggering angiogenesis and CM function through paracrine signals, rather than directly differentiating into CMs or ECs [72].

CSCs can be also isolated according to distinctive morphological features, such as cardiospheres (CSs). CSs spontaneously arise after subculture of heart biopsies of rodents, pigs, and humans, and contain a heterogeneous mix of immature cells, partially positive for *cKit*, *CD34*, or *Flk1* expression, and committed cells, presenting expression of *cTnI*, *ANP*, and *MyHC*. Murine CSs spontaneously beat in vitro, whereas porcine and human CSs display beating potential in co-culture with neonatal rat cardiomyocytes [73]. Moreover, CS-derived cells (CDCs) can be expanded from porcine and human CSs as cKit$^+$/CD31$^+$/CD34$^+$/CD90$^+$/CD105$^+$ heterogeneous pool and, when injected in infarcted ventricles, differentiate into CMs and ECs, leading to increased ejection fraction [74]. Notably, long-term beneficial effects on the infarcted myocardium can also be achieved with injection of allogeneic CDCs, transiently engrafting and stimulating resident CSCs and angiogenesis [75]. Notwithstanding CS innate heterogeneity, intracoronary injection of autologous CDCs has been proven safe and partially efficacious in a phase 1 clinical trial on patients with myocardial infarction [76].

CSC pools can also be defined by specific transcription factors, such as *Islet1* (*Isl1*) or *Wilm's tumor 1* (*Wt1*), although the CSC sources are then confined to transgenic animal or cellular systems. *Isl1* is a homeodomain-containing transcription factor, expressed during secondary heart field specification and identifying a plastic progenitor pool, differentiating into CMs, SMCs, and ECs during murine [77] and human [78] development. After birth, *Isl1* is largely repressed and its expression is confined to a rare subset (≈500 cells per rat heart) of resident CSCs, scattered within ventricular myocardium or arranged in clusters in the atria. *Isl1*$^+$ CSCs strongly upregulate *Nkx2.5* and *Gata4*

expression after isolation and differentiate in vitro into electrically competent CMs without cell fusion [79]. *Wt1* is a transcription factor identifying a pool of epicardial progenitors, which contribute to the murine fetal cardiomyogenesis [80] and, in the presence of thymosin β4, form vascular precursors [81]. Intriguingly, pre-treatment of murine adult hearts with thymosin β4 reactivates *Wt1*^{+} resident CSCs, expressing *Sca1*, *Isl1*, and *Nkx2.5*, differentiating into electrically coupled CMs in the ischemic myocardium and ameliorating functional outcome after infarction [82].

An alternative source of cardiac regeneration potential relies on cardiac pericytes and, particularly, cardiac mesoangioblasts (cMABs). cMABs can be isolated from murine, canine, and human cardiac explants and display pericytic markers, e.g., NG2 and AP, in combination with cardiomyogenic transcription factors, e.g., *Nkx2.5* and *Gata4* [83]. Low-passage murine cMABs differentiate in vitro into beating cardiomyocytes, presenting sarcomeric structures and Cx43 junctions and, after intraventricular delivery in ischemic hearts, mainly home at the periphery of the necrotic area and differentiate into CMs, participating in the myocardial regeneration [84]. Remarkably, when isolated from samples of canine [85] and human [83] cardiomyopathic hearts, cMABs present impairment of several markers of proliferation and plasticity. Furthermore, aorta-derived MABs (ADMs) present plasticity toward mesodermal and ectodermal derivatives. When injected intramyocardially, ADMs engraft the myocardium of dystrophic mice, differentiating into CMs and ECs and preventing the onset of dilated cardiomyopathy [86], whereas, under appropriate inducing conditions, ADMs can also transdifferentiate in vitro and in vivo toward myelinating glial cells [87]. Although prone to senescence and less efficient in vivo than other resident CSC types, cardiomyogenic MABs constitute an interesting reservoir of cell plasticity, given their relative abundance around the rich microvasculature network bedewing the cardiac muscle.

Non-resident stem cells for skeletal and cardiac muscle regeneration

Besides the intrinsic stem cell pools, other non-resident adult stem cells have been reported to positively contribute to the regeneration of skeletal and cardiac muscle. Because already extensively treated in dedicated reviews [88, 89] and considering that this review preferentially deals with the resident populations, we will briefly report here several interesting studies involving progenitors isolated from the bone marrow (BM) and the peripheral blood (Fig. 2). Because they are relatively easy to isolate and expand, BM- or blood-derived progenitors are still potentially attractive for regenerative studies of the post-natal muscles.

Only 0.0001–0.001 % of nucleated cells in adult BM are considered to be mesenchymal stem cells (MSCs) [90, 91]. Identified by the expression of CD73 and CD105, MSCs do not express other hematopoietic or endothelial markers such as CD14, CD31, CD34, CD45. Moreover, MSCs are CD29^{+}/CD44^{+}/CD71^{+}/CD90^{+}/CD106^{+}/CD166^{+} [92, 93]. BM-derived MSCs grow as adherent cells in vitro and differentiate under defined conditions into various tissues, including bone, cartilage, muscle, marrow stroma, tendon, ligament, adipous, and other connective tissues [94]. Since 20 years, the myogenic potential of MSCs and other BM-derived progenitors has been investigated. Progenitors from the BM are recruited and participate in muscle regeneration, and donor nuclei are traceable after many years at a very low frequency [95]. Multipotency and ease of isolation render adult MSCs an attractive candidate for stem cell therapy and several circulating BM-derived stem cells can participate in skeletal muscle regeneration, although further studies will be useful to pinpoint the cues eliciting recruitment and transdifferentiation [96–98]. In contrast, other reports suggest that MSC myogenic capability is per se scarce, and that beneficial effects are limited to local cell recruitment and paracrine effects [99–101]. Nevertheless, tuning key molecular pathways could in principle enhance MSC intrinsic commitment toward skeletal muscle, as reported with Notch signaling overactivation [102].

Regarding the regeneration of the cardiac muscle, BM-derived cKit[+]/Lin^{−} stem cells show dramatic engraftment in the infarcted myocardium and robust differentiation into CMs, SMCs, and ECs, when intramyocardially administered after coronary ligation [103]. However, systemic intravenous injection of Sca1^{+}/cKit^{+} cells, isolated as BM side population (BM-SP) according to Hoechst 33342 dye efflux, resulted in low rates of engraftment and negligible levels of regeneration in the chronically damaged striated muscle of dystrophic δ*Sarcoglycan-null* (*Sgcd-null*) mice [104].

Endothelial progenitor cells (EPCs) from the peripheral circulation constitute another attractive source of non-resident cells to favor muscle repair, because they are easily isolatable and highly angiogenic. CD34^{+} circulating EPCs promote extensive neovascularization of ischemic hindlimb muscles [105] and myocardium [106], after intramuscular and intravenous injection, respectively. Interestingly, when co-cultured with neonatal rat cardiomyocytes, human EPCs transdifferentiate in vitro toward cardiomyocyte-like cells, exhibiting calcium transients and functional gap junctions, without cell fusion [107].

Thus, a more refined knowledge of intrinsic mechanisms and extrinsic manipulation of non-resident somatic cells is still required to better coax them in acquiring myogenic commitment or, intriguingly, in supporting resident myogenic progenitors.

Table 3 Cell progenitors exhibiting potential lineage switch between skeletal and cardiac muscle

Cell type	Species	Surface antigens		Lineage markers	In vivo plasticity	References
		Positive	Negative			
SPOCs	Mouse	Sca1	cKit, CD34, CD45	Nkx2.5, Gata4	CMs	[110]
Sk-34 cells	Mouse	CD34	CD45	NA	Skeletal fibers, ECs, Schwann cells	[112]
Sk-34 cells	Mouse	CD34	CD45	Pax7, MyoD, Myf5, Isl1, Gata4, Mef2C, Hand2	CMs, ECs	[113]
Sgcb-null cMABs	Mouse	AP, NG2, Sca1, cKit, CD31, CD34, CD44, CD140b	CD13, CD45, CD56	Pax3, MyoD, Myf5, Myogenin, Isl1, Nkx2.5, Gata4, Mef2A	Skeletal fibers	[115]

Each entry relates to one reference and the entry order is referred to the review

NA information not available in the cited reference, *SPOCs* skeletal-based precursors of cardiomyocytes, *Sk-34 cells* skeletal-derived CD34$^+$ myoendothelial cells, *Sgcb-null cMABs* cardiac mesoangioblasts isolated from *βSarcoglycan-null* (*Sgcb-null*) mice, *CMs* cardiomyocytes, *ECs* endothelial cells

Is the switch between the two myogenic lineages possible?

Both skeletal and cardiac muscles originate from embryonic mesoderm and, during adulthood, present intrinsic regenerative capacity. However, while the regenerative machinery of the skeletal muscle is efficient, extensive damage in the heart results in scar tissue formation, compromising cardiac function. Despite profound differences in regenerative potential and specific stem cell properties, the two types of striated muscle share many molecular, structural, and functional features. In this perspective, the question whether a crossover between the two lineages is possible in vivo gathers challenging answers, particularly in terms of post-natal cell plasticity.

Hereafter some cell lines able to present these characteristics are reported (Table 3; Fig. 2). A paradigmatic case of lineage crossover is constituted by the cranial paraxial mesoderm (CPM) in chick embryos. The neural tube inhibits cardiomyogenesis in CPM by releasing Wnt agonists, whereas ectopic secretion of Wnt antagonists or Bmp4 conversely promote migration of CPM progenitors toward the developing heart [108]. Accordingly, CPM progenitors can also be traced in their migration and participation to the cardiac outflow tract [109]. In addition, in vitro CPM explants express *MyoD*, *Myf5*, and *Myogenin*, markers of skeletal myogenesis, and, in the presence of Bmp4, also activate the expression of *Nkx2.5*, *Gata4*, *Gata5*, *Gata6*, and *Isl1*, markers of early cardiomyogenesis [109].

Intriguingly, cell pools switching from skeletal to cardiac lineage have also been isolated from adult murine skeletal muscles. On one hand, skeletal-based precursors of cardiomyocytes (SPOCs) are isolated as round, floating CD34$^-$/CD45$^-$/cKit$^-$ cells and express markers of cardiac, but not skeletal, myogenesis, when cultured in the presence of EGF and FGF. SPOCs differentiate in vitro into beating cardiomyocytes, derived from the Sca1$^-$ subfraction, and, after intravenous delivery, engraft in the ischemic ventricle and partially differentiate into CMs at the peripheral region of the infarct [110]. On the other hand, skeletal-derived CD34$^+$ (Sk-34) myoendothelial cells can be sorted from the interstitial tissue as CD34$^+$/CD45$^-$, are Sca1$^+$/cKit$^-$/CD31$^-$ and express, once in culture, skeletal myogenic markers [111]. After intramuscular injection into severely damaged skeletal muscles, freshly isolated Sk-34 cells extensively engraft and generate myogenic, endothelial, and Schwann cells, thus exhibiting broad in vivo plasticity toward mesodermal and ectodermal lineages [112]. Strikingly, Sk-34 cells undergo also cardiomyogenic transdifferentiation in co-culture with fetal cardiomyocytes in vitro and, once intramyocardially injected, participate in the regeneration of infarcted rat hearts, with significant improvement of left ventricle functionality [113].

Furthermore, post-natal stem cells crossing from cardiac to skeletal lineage have also been reported, shedding new light on the cardiomyopathic progression in a murine model of muscular dystrophy [114]. When isolated from *βSarcoglycan-null* (*Sgcb-null*) dystrophic mice, in fact, cMABs co-express cardiac and skeletal myogenic transcriptions factors. Moreover, cMABs spontaneously differentiate into skeletal myotubes in vitro and arrhythmogenic skeletal muscle patches in vivo, after intramyocardial injection into infarcted hearts. The robust lineage shift relies on downregulation, due to calcium leakage by the plasma membrane and knockout of the first *Sgcb* intron, respectively, of miRNAs *miR-669a/q*, normally repressing *MyoD* translation. Reintroduction of *miR-669a*, in fact, partially rescues the cardiomyogenic commitment of *Sgcb-null* both in vitro and in vivo [115] and results in alleviation of the cardiomyopathy in the long term.

However, translation of basic research on lineage plasticity into human patients is still facing prominent hurdles. After the first promising reports of feasibility and efficacy of SC therapy for cardiac repair in freeze-injured hearts of rats [116] and rabbits [117], subsequent clinical trials have yielded contrasting results. In patients undergoing coronary artery bypass graft, injection of autologous SCs (approximately 65 % CD56^{+}) in and around the post-infarction scar tissue increases regional contractility and viability of the scarred myocardium in both the first clinical case [118] and the first 1-year follow-up [119]. Nevertheless, when tested in a multi-centered randomized trial, autologous SC therapy fails to induce significant beneficial effects on regional or global ventricular function and increases the number of early post-operative arrhythmogenic events, as compared to placebo [120].

However, recent observations in infarcted rats suggest that application of SC sheets on the epicardium of damaged ventricles alleviates hypertrophy and ameliorates ventricular function, avoiding the events of arrhythmia and tachycardia observed after injection of SC suspension [121]. Moreover, when engineered to secrete an artificial angiogenic peptide, SC sheets further improve also novel vessel formation around the infarct area [122].

Therefore, new evidences suggest the need for further research in this provocative field, learning from and potentially bypassing hurdles and failures of past attempts.

Conclusions

In conclusion, many theoretical as well as applicative questions about the cell plasticity in post-natal skeletal and cardiac muscles still remain open.

With regards to the adult mammalian skeletal muscle, many types or subtypes of resident stem cells have been isolated and characterized. However, further translational studies are required to address the boundaries of the intrinsic plastic potential of each cell type. Moreover, it will be particularly intriguing to deepen our knowledge about the epigenetic signatures potentially regulating intrinsic fate choices and to define the patterns of cellular crosstalk among the different pools. This will be useful to enhance applicability of regenerative medicine to the skeletal muscle, through not only cell-based strategies but also stimulation of the endogenous potential.

Similarly, numerous CSC pools with different characteristics have been isolated from the adult cardiac muscle of mammals according to often-incomparable procedures of isolation and handling. Additionally, refined details about epigenetic control on CSC potency and commitment are still lacking. Moreover, it is still an open question whether the different CSC types derive from actual distinguishable pools in vivo or represent different states of endogenous CSC activation or ex vivo culture. Results from the first clinical trials and more refined translational studies will probably help in shedding light on this controversial issue.

Finally, with regards to the lineage switch between skeletal and cardiac myogenesis, the path is still long before achieving translational relevance, particularly within adult stem cell pools. Nonetheless, advancing our knowledge of myogenic lineage plasticity still constitutes an intriguing perspective not only for regenerative medicine but also for drug screening and disease modeling systems. In this view, fundamental insights will be gained by combining epigenetic, transcriptional, and signaling studies at the cellular or tissue level. Converting these complex data networks into refined in vitro/in vivo approaches will then constitute the necessary challenge to enhance the myogenic potential of adult stem cells and other post-natal reservoirs of potency, such as induced pluripotent stem cells.

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