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Review article

Human embryonic stem cell-derived vascular smooth muscle cells in therapeutic neovascularisation

Christine Cheung, Sanjay Sinha *

The Anne McLaren Laboratory for Regenerative Medicine, West Forvie Building, Forvie Site, University of Cambridge, Robinson Way, Cambridge CB2 0SZ, UK
Division of Cardiovascular Medicine, University of Cambridge, ACCI Level 6, Box 110, Addenbrooke's Hospital, Hills Road, Cambridge CB2 0QQ, UK

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ABSTRACT

Ischemic diseases remain one of the major causes of morbidity and mortality throughout the world. In recent clinical trials on cell-based therapies, the use of adult stem and progenitor cells only elicited marginal benefits. Therapeutic neovascularisation is the Holy Grail for ischemic tissue recovery. There is compelling evidence from animal transplantation studies that the inclusion of mural cells in addition to endothelial cells (ECs) can enhance the formation of functional blood vessels. Vascular smooth muscle cells (SMCs) and pericytes are essential for the stabilisation of nascent immature endothelial tubes. Despite the intense interest in the utility of human embryonic stem cells (ESCs) for vascular regenerative medicine, ESC-derived vascular SMCs have received much less attention than ECs. This review begins with developmental insights into a range of smooth muscle progenitors from studies on embryos and ESC differentiation systems. We then summarise the methods of derivation of smooth muscle progenitors and cells from human ESCs. The primary emphasis is on the inherent heterogeneity of smooth muscle progenitors and cells and the limitations of current in vitro characterisation. Essential transplantation issues such as the type and source of therapeutic cells, mode of cell delivery, measures to enhance cell viability, putative mechanisms of benefit and long-term tracking of cell fate are also discussed. Finally, we highlight the challenges of clinical compatibility and scaling up for medical use in order to eventually realise the goal of human ESC-based vascular regenerative medicine.

1. Introduction

Vascular regeneration is a promising strategy for treating ischemic diseases such as myocardial infarction, chronic ischemic cardiomyopathy, stroke and critical limb ischemia. Earlier approaches to promote revascularisation of ischemic tissues have included exogenous administration of pro-angiogenic growth factors and angiogenic gene therapy [1]. In the past decade, the focus has been on autologous cell-based therapies using bone marrow- and peripheral blood-derived endothelial progenitor cells [2,3]. However, all these strategies yielded mixed outcomes in various clinical trials [1–3].

Replacement of endothelial cells (ECs) alone is apparently inadequate. Mural cells, commonly referred to as vascular smooth muscle cells (SMCs) in larger vessels and pericytes, in capillary beds are indispensable for the development of a mature and durable vasculature. The importance of these cells has been underscored in gene mutation studies of mouse models where the absence of mural cells resulted in severe vascular defects [4,5]. Newly formed endothelial vessels require the recruitment of pericytes and/or SMCs, which then regulate vessel remodelling by secreting extracellular matrix components [6]. Angiopoietin 1 is produced by mural cells to aid endothelial viability. Heterotypic EC-mural cell contacts and reciprocal signalling promote further morphological stabilisation [6,7]. Mural cells play a role in vascular homeostasis, and their contractile capability helps to mediate haemodynamics. As such, ECs and mural cells are both unequivocally necessary for the formation of a functional vasculature.

As a consequence of their distinct anatomical locations, SMCs and pericytes differ in their relationship with ECs, with pericytes having direct physical contact with the endothelium as well as sharing the basement membrane. Thus, SMCs and pericytes have functionally distinct roles, with the latter involved in microvascular homeostasis [8]. Importantly, diseases involving the microvasculature such as diabetic retinopathy or tumour angiogenesis involve pericyte defects in contrast to SMCs which are involved in larger vessel disorders such as atherosclerosis. Despite functional and pathological differences, it is unclear whether SMCs and pericytes represent different cell types since to some extent they share structural and functional properties such as the presence of myofilaments and contractile ability and co-express markers, such as smooth muscle α-actin (SMαA) and desmin [9]. While a variety of pericyte markers have been proposed, such as NG2, PDGFRβ and RGS5, these have variable expression and poor specificity since they may also be expressed by SMCs or other cell

* Corresponding author at: The Anne McLaren Laboratory for Regenerative Medicine, West Forvie Building, Forvie Site, University of Cambridge, Robinson Way, Cambridge CB2 0SZ, UK. Tel.: +44 1223 747479; fax: +44 1223 763350.
E-mail address: ss661@cam.ac.uk (S. Sinha).

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types. Indeed, there exists a range of phenotypes such that it is difficult to precisely demarcate SMCs and pericytes. However, compared to SMCs, information on the embryological origins of pericytes is relatively lacking. Where these data are available, for example in the face and forebrain [10], in the coronary vessels [11], and in the dorsal aorta [12], it appears that pericytes and SMCs in the same vascular region have common embryonic origins. Consequently, for the purpose of this review—generation of mural cells for regenerative medicine, we focus principally on vascular SMCs. Understanding vascular SMC development may also provide insights into pericyte developmental pathways although it should be borne in mind that there may be important differences between pericyte and SMC ontogeny that are not yet understood in detail. Given that regenerating a functional microvasculature in ischaemic tissues or building one in tissue-engineered organs are key translational aims, then further studies that identify more specific pericyte markers and a greater understanding of the development of these microvascular mural cells are essential.

Increasing evidence demonstrates the benefits of incorporating an additional source of supportive cells to aid ECs in blood vessel formation. Mesenchymal stem cells (MSCs) and fibroblasts are believed to be able to differentiate into pericytes/SMCs. These cells were shown to complement ECs in creating vascularised bone [13] and skeletal muscle [14] tissue patches in vivo. Co-transplantation of human endothelial cells with smooth muscle progenitors or with differentiated SMCs into mouse models promoted higher vessel densities than transplantation of either cell type alone [15,16]. Bioengineered vascular structures made of human endothelial progenitors or cells and MSCs demonstrated relatively long-term patency in vivo [17,18]. Clinical trials also reported that tissue-engineered blood vessels made from autologous skin fibroblasts and superficial venous ECs were functional for a minimum of 5 months after implantation into patients with end-stage renal disease [19,20]. Recently, human allogeneic SMCs were used to produce extracellular matrix components, forming the vascular tissue for making vascular grafts [21]. Therefore, obtaining clinically compatible and functionally competent cell sources for SMCs in addition to ECs may be essential for effective vascular regeneration.

Therapeutic cells used in current clinical trials include skeletal myoblasts, adipose-derived stem cells, bone marrow- and peripheral blood-derived mononuclear cells, endothelial progenitor cells and mesenchymal stem cells [3,22,23]. Despite the encouraging results observed in preclinical trials, patients suffering from acute myocardial infarction only showed marginal improvement in cardiac function after receiving autologous bone marrow cells or peripheral mononuclear cells [24]. Other sources of endothelial and/or smooth muscle progenitor cells identified in myocardium [25–27], hair follicles [28], vascular adventitia [29] and cord blood [16,30] have shown efficacy in animal studies but are not yet tested in clinical settings. Moreover, since endothelial progenitor cells constitute less than 1% of bone marrow or peripheral blood, substantial amounts of bone marrow or blood have to be harvested. Considerable time is also required for ex vivo expansion to clinically relevant quantities. Age and disease-related decline in number of circulating progenitor cells accentuates the shortage of therapeutic cell supply [31]. Stem and progenitor cells, especially from patients complicated with diabetes [32–34], ischemic heart disease [35,36] or old age [37,38], exhibit greater senescence, limited proliferative capacity and impaired ability to carry out hypoxia-induced vascular repair. Patients with genetic diseases are also poor candidates for autologous cell therapy due to their inherent genetic defects. Since adult stem and progenitor cells demonstrate limited efficacy, an alternate source of therapeutic cells may be crucial to advancing cell-based therapy.

Human pluripotent embryonic stem cells (ESCs) present an attractive option for regenerative medicine as their high proliferation and differentiation abilities confer on them the capacity to generate clinically useful quantities of desired cell types. Transplantation of human ESC-derived vascular cells resulted in significantly greater perfusion in mouse ischemic hindlimbs as compared to the use of human adult endothelial progenitors or cells [39,40]. Human ESCs can potentially serve as a source of disease-free and 'off-the-shelf' products for acute-phase patients. Furthermore, the use of patient-derived induced pluripotent stem cells (iPSCs) can circumvent potential immune rejection [41]. While the potential for vascularisation using human ESC-derived endothelial cells has been extensively reviewed [42,43], the role of SMCs is relatively neglected. In this review, we provide a unifying perspective of studies on the developmental origins of vascular SMCs, derivation of SMCs from human ESCs and iPSCs (taken together, human pluripotent stem cells), as well as their supportive role in therapeutic neovascularisation.

2. Developmental insights from embryonic vascular SMC precursors

An understanding of the embryonic development of vascular SMCs could facilitate their derivation from human pluripotent stem cells. Vascular SMCs arise from diverse embryonic origins and this topic has been reviewed in detail by Majesky [44]. SMCs in the ascending and arch of the aorta, the septation of the cardiac outflow tract, the pulmonary trunk, the ductus arteriosus, the innominate and right subclavian and both carotids including their branches to the face and forebrain arise from the neural crest [10,45]. Other vascular SMCs and pericytes are largely derived from various mesodermal lineages such as the secondary heart field, proepicardium, splanchnic mesoderm, paraxial or somitic mesoderm, mesoangioblasts and even transdifferentiation from endothelium [44,46]. Importantly, understanding this heterogeneity in VSMC origins may explain the presentation and localisation of congenital vascular defects such as cono-truncal defects with mutations affecting neural crest cells [47]. It is also possible that the location and presentation of acquired vascular diseases such as abdominal aortic aneurysms or atherosclerosis may be related, in part, to the heterogeneous responses of different SMC populations to disease mediators, although robust in vitro and in vivo models of the different types of SMCs will be required to test this hypothesis. This section focuses on fate-mapping studies in embryos with primary emphasis on the equivalent smooth muscle precursors which have been identified in mouse and human ESC differentiation systems.

2.1. Cardiovascular progenitors

Various common progenitors that give rise to SMCs and/or ECs in addition to other cell types have been identified. Flk1, a gene encoding vascular endothelial growth factor receptor 2, is expressed by a broad spectrum of mesodermal tissues in mouse embryos [48]. LacZ-expressing Flk1± cells from mouse ESCs were injected into avian embryos and subsequently differentiated into both mural cells and endothelial cells [49]. Moreover, isolated Flk1± cells from mouse embryos were shown to give rise to cells expressing smooth muscle markers [50,51]. Based on the temporal appearance of Flk1 expression during mouse embryoid body (EB) differentiation, a cardiovascular progenitor, which has cardiomyocyte, smooth muscle and endothelial potential and a haemangioblast, a common precursor for haematopoietic and vascular cells, could be identified [51]. In human EBs, two KDR (a human homolog of Flk1)-expressing populations can be distinguished as KDR^{low}/C-KIT− and KDR^{high}/C-KIT+ [52]. The former gives rise to cardiovascular lineages while the latter displays gene expression patterns that suggest predominance of haematopoietic and vascular progenitors. While these progenitor populations have considerable potential for dissecting out the mechanisms regulating cardiovascular tissue generation and understanding the sequence of developmental events, it should be remembered that in

vitro systems are highly reliant on culture conditions and exogenous growth factors that may not accurately reflect the in vivo situation.

Islet-1 (Isl1) is a LIM homeodomain transcription factor that marks the secondary heart field [53]. Lineage tracking in avian embryos and Isl1-cre mice provide strong evidence that SMCs at the base of the aorta and pulmonary trunk are derived from the secondary heart field [54,55]. The co-staining of ISL1 and smooth muscle myosin heavy chain (SMMHC) observed in the human foetal heart also suggests that this subset of SMCs originates from the secondary heart field in man [56]. Since vascular lineages are closely associated with cardiac and haematopoietic development, it is unclear whether a bona fide bipotential vascular progenitor exists that gives rise to SMCs and ECs only. Some studies suggest the early separation of smooth muscle and endothelial lineages. It has been shown in mouse EBs that myocardial and smooth muscle lineages can emerge from cells expressing Nkx2-5, one of the earliest acting transcription factors in cardiac development [57,58]. One of the Nkx2-5+ populations is Flk1− [57] and only gives rise to cardiomyocytes and SMCs while the other is Flk1+ [58] and can also differentiate into ECs. This latter Nkx2-5+/Flk1+ population has similarities to a multipotent Isl1+/Nkx2-5+/Flk1+ cardiovascular progenitor from another study [55], which develops before the diversification of endothelial and muscle lineages (Fig. 1).

In avian embryos, retroviral tagging of the proepicardium demonstrated that coronary SMCs emerged from a source of progenitors residing in the proepicardium [59]. Targeting of reporter genes into the endogenous loci of Tbx18 [60] and Wt1 [61] marked proepicardial progenitors which were able to give rise to coronary SMCs in the mouse knock-in models. Further lineage tracing revealed that Tbx18±/Wt1± proepicardial cells were actually derived from Nkx2-5±/Isl1± cardiovascular progenitors and hence they share a common embryonic origin [62] (Fig. 1). Coronary ECs were previously believed to also arise from the proepicardium. However, the recent discovery that coronary ECs arise from angiogenic sprouts of the sinus venosus [63] again supports the early separation of SMCs and ECs in cardiovascular development.

2.2. Haemangioblasts

The concept of a common progenitor for ECs and haematopoietic cells, commonly known as a haemangioblast, was first supported by the identification of a clonal blast colony-forming cell (BL-CFC) during mouse ESC differentiation [64]. Almost a decade later, single-cell fate mapping in zebrafish gastrula has successfully provided in vivo evidence of a haemangioblast that gives rise only to ECs and blood, and not other cell types [65]. However, emerging studies suggest that mouse and human ESC-derived BL-CFCs are tripotent, generating SMCs in addition to ECs and haematopoietic cells [50,66–68]. Therefore, in accordance with the notion of a bipotential haemangioblast, an ESC-derived BL-CFC may not be a true in vitro counterpart. Tal1 or Scl, a transcription factor found to be indispensable for the formation of haemogenic endothelium from BL-CFC [69], is antagonistic to SMC differentiation [50,67,70]. Hence, the separation between SMCs and endothelial/haematopoietic progenitors precedes that of ECs and haematopoietic cells from the haemogenic endothelium intermediate. This stepwise separation is

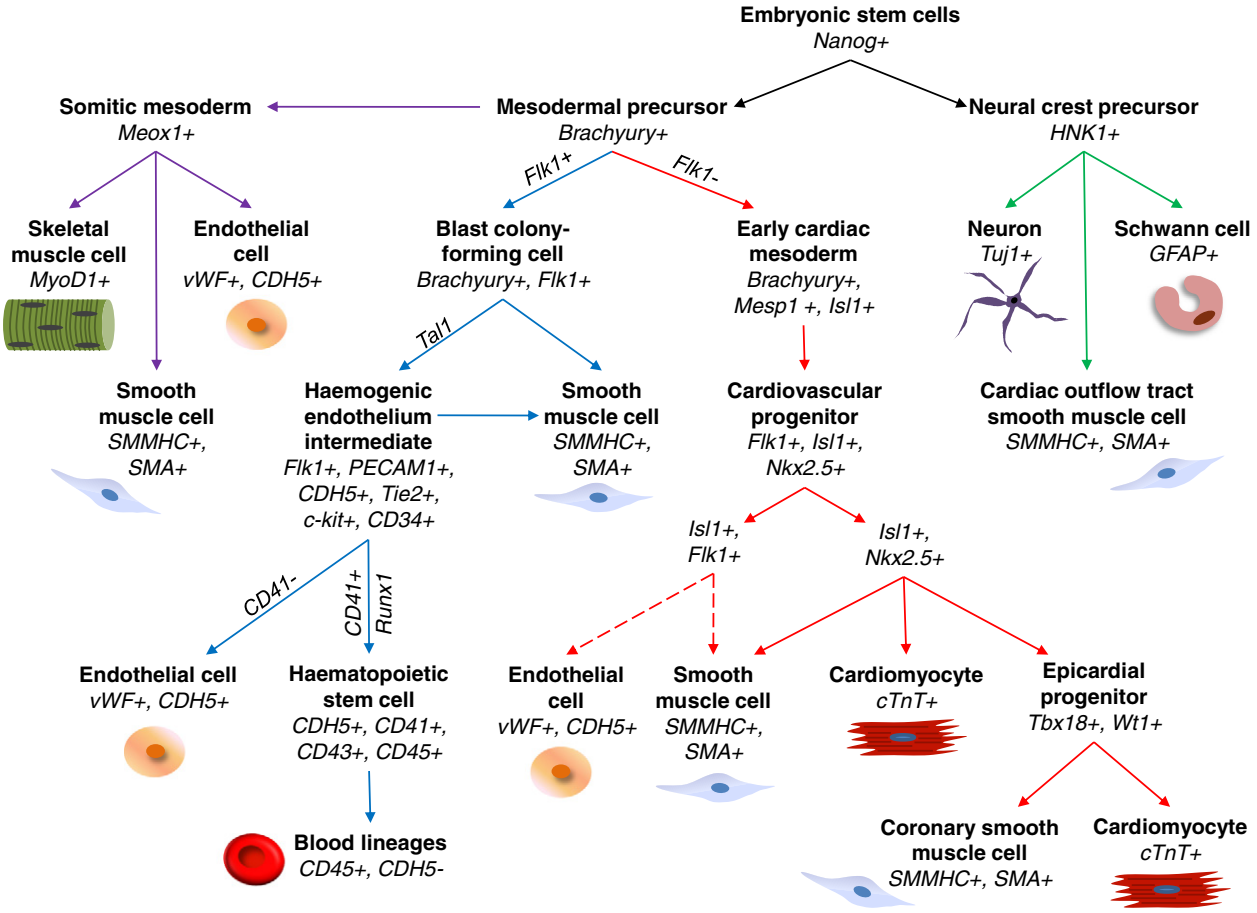

Fig. 1. Schematic diagram of SMC development from embryological and ESC differentiation studies. Positive and/or negative expression of phenotypic markers for the particular cell types are represented in italic. Derivatives of cardiac mesoderm, haemangioblast, somitic mesoderm and neural crest are indicated by red, blue, purple and green arrows respectively. Dotted arrows denote speculative differentiation pathways where there is still insufficient evidence at present.

mediated by the Notch pathway during specification of the ventral mesoderm into the SMC or EC/blood fate (Fig. 2) [70]. Thus, a BL-CFC may represent a multipotent progenitor in the ventral mesoderm before the emergence of a haemangioblast. Fig. 1 illustrates a schematic of the developmental diversification of smooth muscle, endothelial, haematopoietic and myocardial lineages. The existence of a bona fide vascular progenitor that gives rise only to ECs and SMCs still remains unproven.

2.3. Endothelial–mesenchymal transition

Endocardial cells are one of the subtypes of heart cells that arise during cardiac mesoderm differentiation. They express endothelial markers and are involved in endothelial–mesenchymal transition (EndMT), a process that is crucial for heart valve formation [71]. The regulation of EndMT by an interplay of signalling pathways such as notch and BMP/TGF-β has been reviewed in detail elsewhere [72]. During EndMT, these endocardial cells lose endothelial characteristics and acquire mesenchymal or smooth muscle-like phenotypes. This phenomenon has also been observed during aortic development in quail embryos, where the endothelium transdifferentiates into smooth muscle-like cells expressing SMA [73,74]. Likewise in prenatal human pulmonary arteries, SMCs may be generated from the endothelium and adopt smooth muscle-specific cytoskeletal proteins as they mature [75].

ESCs-derived ECs have also shown the ability to transdifferentiate into SMCs in vitro under the influence of transforming growth factor (TGF)-β1 and/or platelet derived growth factor (PDGF)-BB [76,77], as well as in vivo after transplantation into mouse models [76,78]. Human ESC-derived CD34+ progenitors have both smooth muscle and endothelial differentiation potential but this has not been studied at a clonal level [77,79–81]. In fact, the majority of the isolated CD34+ cells that show smooth muscle potential also co-express PECAM1, a marker associated with more mature ECs. It is suggested that CD34+ cells actually undergo EndMT via SNAI1-mediated TGF-β signalling to give rise to mural cells [82]. In addition to its role in vascular development, EndMT is becoming increasingly implicated in cardiovascular pathologies such as pulmonary hypertension and atherosclerosis and interested readers can refer to an existing review for the contribution of EndMT to vascular disease [83]. Further studies in this exciting area, including the molecular regulation of EndMT, are warranted in order to shed new light onto a wide variety of disease and developmental processes.

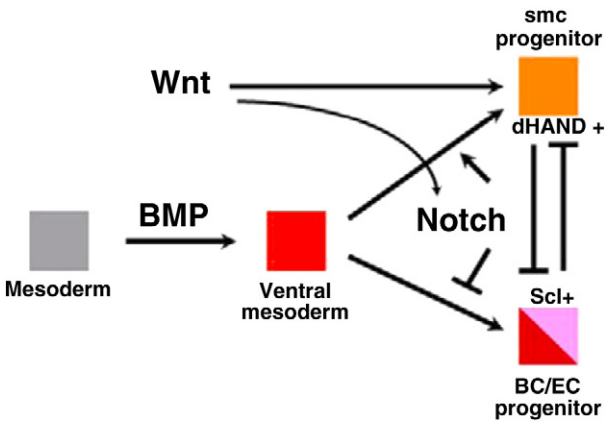

Fig. 2. Signalling pathways involved in the early separation of the SMC progenitor and blood/endothelial (BC/EC) progenitor during ventral mesoderm differentiation. Wnt activates SMC induction while notch mediates the specification towards SMC and BC/EC lineages. Mutual antagonism between dHAND-positive SMC and Scl-positive BC/EC progenitors strengthens their distinct lineage commitment.
Modified from Shin et al. 2009 [70] with permission.

2.4. Mesoangioblasts

Lineage tracing in mouse and avian embryos indicated that SMCs in the wall of the aorta are somite-derived [84,85] with an initial transient contribution to the ventral wall from lateral plate mesoderm [46]. These findings are difficult to reconcile with the proposed transition of ECs to aortic SMCs [73], although they do not entirely preclude a minor contribution through this mechanism. A follow-up study demonstrated that aortic vascular cells come from two independent somitic compartments—mural cells from the sclerotome; ECs from the dermomyotome [12]. However, conflicting evidence suggests that SMCs may in fact come from the dermomyotome [84]. In keeping with this, individually labelled epithelial progenitors in the dermomyotome give rise to either endothelial or mural cells but not both [86]. Currently, the role of different somitic compartments in generating aortic SMCs remains unclear.

The mesoangioblast, a multipotent progenitor, was first discovered in the mouse embryonic dorsal aorta [87]. When quail or mouse embryonic aorta was grafted into chick embryos, donor mesoangioblasts were incorporated into host blood vessels and also found to be able to generate a number of mesodermal tissues including smooth and skeletal muscles [88]. Since aortic SMCs may share a clonal origin with skeletal muscle cells in the dermomyotome, it was postulated that mesoangioblasts correspond to a progenitor cell from the hypaxial dermomyotome [84]. An equivalent mesoangioblast from ESC differentiation has not been reported so far.

2.5. Neural crest precursors

Using cre/lox technology to mark neural crest derivatives during mouse embryonic development, labelled cells were found distributed within the smooth muscle layers surrounding the ascending aorta and arch arteries [45]. SMCs with contractile properties were shown to be generated in vitro by treatment of neural crest stem cells with TGF-β [89]. In comparison, human ESC-derived SMCs of neural crest origin are not as well characterised as those derived from mesodermal progenitors. A subset of the mesenchymal precursors that were obtained from human ESC-derived neural crest was shown to have smooth muscle differentiation potential [90]. Cells expressing SMαA were also shown to be generated directly from human ESC-derived neural crest precursors [91,92]. SMCs derived from various embryonic origins manifest similar phenotypes and express the same set of SMC marker genes. However, the unique requirement for myocardin-related transcription factor-B only in neural crest-derived cells [93,94] suggests that the differentiation into origin-specific SMCs is differentially regulated. Based on the knowledge gained from the developmental studies in embryos and ESC differentiation, the next section discusses the derivation and transplantation of human ESC-derived smooth muscle progenitors and cells.

3. Neovascularisation and vascular remodelling: lessons from adult progenitors

An important question when considering the potential for human ESC-derived progenitors for therapeutic revascularisation is to consider the mechanisms underlying any putative benefit. Do progenitor cells give rise to vascular cells that physically integrate into newly developing vessels or do they promote new vessel formation through alternative means? In this regard, it is instructive to briefly review the role of adult stem/progenitor cells in neovascularisation and cardiovascular disease with a particular focus on progenitor cell contribution to SMCs.

Asahara et al. [95] originally reported that circulating 'endothelial progenitor cells' (EPCs) contributed to collateral vessel growth in ischaemic hind limb models in vivo. Further studies then proposed a major role for haematopoietic stem cells in transdifferentiating into a

variety of cardiovascular tissues on injection into the infarcted heart [96]. With respect to SMC origins, early studies reported that the neointimal cells in graft vasculopathy were of recipient origin rather than from the local donor vessel [97,98]. These findings were extended by Sata et al. [99] when they reported that in a variety of murine vascular disease models, namely vascular injury, allograft vasculopathy and atherosclerosis, a large proportion of SMCs that remodelled the vessel wall were of a bone marrow origin. Moreover, a study using sex mismatched bone marrow transplant subjects suggested that SMCs in atherosclerotic lesions in humans were also of donor bone marrow origin [100]. However, the paradigm that bone marrow contributed significantly to the SMCs remodelling vessels in vascular diseases or to other cardiovascular tissues has been reconsidered in light of strong evidence from further studies that challenged the bone marrow hypothesis in graft vasculopathy [101] and have raised major questions about the ability of bone marrow derived cells to transdifferentiate into other tissue types [102–105]. Moreover, the original so-called EPCs were found to represent a myeloid cell that did not differentiate into new endothelium although did have an angiogenic effect by releasing pro-angiogenic growth factors [105,106]. More recently, meticulous confocal microscopy with single cell resolution clearly showed that neointimal as well as endothelial cells in mouse models of atherosclerosis were derived from the local vessel wall [107,108]. It is likely that the earlier reports by Sata et al. [99] and others of a bone marrow origin for these cells were due to methodological issues leading to an inability to distinguish cells of a bone marrow lineage from SMCs within the neointimal lesions.

However, the situation remains unclear and there are many unresolved questions that are hotly debated. For example, circulating progenitor cells are being characterised in greater detail using functional properties rather than cell surface markers. Rare populations such as the endothelial colony forming cells (ECFCs), that are clonally distinct from the originally described bone marrow-derived EPCs, have been identified and appear to have true vasculogenic properties [109]. Importantly, it should be noted that most of these studies investigating the origin of cells involved in vascular diseases and SMCs in particular have been performed in murine models and it is unclear whether the proposed bone marrow origin of SMCs in sex-mismatched bone marrow transplant patients [100] is a result of inadequate methodology or whether SMC origins differ between mice and humans. Moreover, the majority of published studies have investigated adult or neonatal progenitor cells and there has been little investigation of the potential of ESC-derived progenitors to contribute to the mural cell component of the new vessel wall. Nevertheless, the lessons from adult progenitor cells can be usefully applied when considering the role of ESC-derived progenitors in therapeutic revascularisation and studies in this field will need to rigorously characterise the donor cell type, track progenitor cell location in vivo and use high resolution imaging to quantify their precise contribution to new vessels.

4. Derivation and transplantation of embryonic stem cell-derived smooth muscle progenitors/cells

Most in vivo studies have focused on the transplantation of ESC-derived ECs [40,42,78,110–117] or ESC-derived endothelial progenitors [118,119] into animal models of myocardial infarction or hindlimb ischemia. In this context, hybrid cellular approaches have demonstrated greater success than using single cell types. Co-implantation of mouse mesenchymal precursor cells and human ESC-derived ECs sustained long-term durability of engineered vascular conduits while implantation of ECs alone led to vessel regression within a week [120]. Inclusion of mouse embryonic fibroblasts in addition to human ESC-derived ECs and cardiomyocytes enhanced the viability of vascularised cardiac muscle patches which integrated stably onto murine myocardia [121–123]. Hence, human ESC- and iPSC-derived vascular SMCs could serve as a source of supportive perivascular cells of human origin to promote vascular regeneration.

4.1. Derivation of progenitors with smooth muscle differentiation potential

Induction of pluripotent stem cell vascular differentiation usually involves single or combinations of the four established methods: (1) treatment with soluble growth factors and cytokines in culture media supplemented with serum; (2) spontaneous differentiation in EB giving rise to all three germ layers; (3) culture on stromal feeder cells, which secrete essential factors to aid differentiation; and (4) culture on synthetic or natural polymeric coatings, the best known of which, type IV collagen, is efficient in promoting mesoderm specification [49,124]. Detailed derivation methods for progenitors with smooth muscle potential are outlined in Table 1. The temporal window when the inducers act on SMC differentiation is difficult to determine because of the heterogeneity in EB differentiation, varying cell seeding densities in monolayer cultures and undefined animal-derived components. Although differentiation of human ESCs under serum-free and feeder-free systems has been possible [125], rigorous fine-tuning of chemically defined conditions will be required to achieve equal or improved differentiation efficiency. The kinetics of SMC differentiation requires further elucidation in order to attain controllable and reproducible differentiation systems.

Isolation of progenitors typically employs fluorescence activated cell sorting (FACS) or magnetic activated cell sorting (MACS) based on phenotypic markers. Sorting for cell surface markers is relatively straightforward. In the case of intracellular markers, transgenic or knock-in reporter cell lines have to be generated. Using this approach, expression of the fluorescence reporters is controlled by the regulatory elements of the genes of interest, allowing detection and isolation of desired progenitors. Of the multiple potential markers (Table 1), three primary markers—KDR, ISL1 and SSEA-1 have been used most extensively to identify cardiovascular progenitors from human ESCs. One question is how different these progenitors are from one another. Fig. 1 gives some indication of the developmental pathways of Flk1, Nkx2-5 and Isl1 expressing progenitors towards cardiovascular specification in mouse ESCs. SSEA-1 is a more recently identified marker of vascular progenitors [126]. Freshly sorted SSEA-1+ cells expressed NKX2-5 and ISL1 but only a minority (5%–7%) were KDR+. Since SSEA-1+ progenitors can give rise to all three cardiovascular lineages, they should exist before the diversification of endothelial and muscle lineages. SSEA-1+ progenitors were isolated at a relatively early developmental stage but whether they are the human equivalent of the multipotent Isl1+/Nkx2-5+/Flk1+ progenitor has not been determined.

Another question is what causes the diversity observed among the progenitors identified with the same markers. There are large variations in marker profiles of these progenitors, which could be due to their isolation at different stages of differentiation. One view is that multipotent cardiovascular progenitors become progressively more lineage-restricted in a step-wise manner analogous to haemopoiesis [127]. Identification of appropriate cell surface markers may further clarify the hierarchy and enable selection of more specific sub-populations within a heterogeneous progenitor population. For example, Bai et al. discovered that a doubly positive CD34+/PECAM1+ population exhibited greater propensity towards smooth muscle differentiation than a CD34+/PECAM1− population [80]. No progenitor has been found to give rise only to SMCs. Identification of specific phenotypic markers for a smooth muscle precursor would enable its isolation and the generation of a homogeneous population of SMCs which may significantly facilitate regenerative therapies.

4.2. Smooth muscle cell differentiation, maturation and heterogeneity

Reported studies on direct SMC differentiation bypassing a defined progenitor stage are detailed in Table 2. The growth factors and

Table 1
Derivation of smooth muscle progenitors and differentiated cells from human ESCs and iPSCs.

Progenitor-identifying markers	Cell source	Inducers for derivation of progenitors	Marker profile of progenitor	Inducers for SMC differentiation from progenitors	Other differentiation potential	References
KDR	ESC and iPSC	OP9 feeders	Flt1+, PDGFRα+, PDGFRβ+, AC133+, CD34−, VE-cadherin−, CD45−, CD11b−, CD14−	Collagen IV; PDGF-BB	ECs	[39,144,183,184]
	ESC	EB formation; BMP4, bFGF, Activin A, VEGF and DKK1	KDR^{low}, $NKX2.5^{low}$, ISL1+, C-KIT−	Gelatin; VEGF and DKK1	ECs, cardiomyocytes	[52]
ISL1	ISL1-cre DsRed ESC	Initial EB formation. Isolated ISL1−DsRed+ cells were cultured on mouse embryonic feeders (MEF).	KDR−	Fibronectin	ECs, cardiomyocytes	[56]
	iPSC	MEF feeders; BMP2 and FGFR inhibitor	NKX2.5+, KDR+	VEGF and ascorbic acid	ECs, cardiomyocytes	[185]
SSEA-1	ESC	BMP2 and FGFR inhibitor	OCT4+, Brachyury+, MESP+, 5–7% KDR+, Myocardin+, NKX2.5+, ISL1+	MEF; PDGF	ECs, cardiomyocytes	[126]
BL-CFC	ESC	Initial EB formation; BMP4, VEGF, stem cell factor, thrombopoietin and Flt3. Dissociated EBs were cultured in blast-colony expansion medium.	Calponin+, SM22α+, SMA+	Fibronectin; SMC medium	ECs, haematopoietic cells	[68]
CD34	ESC	EB formation	55% PECAM1+, 45% SMA+, 43% SSEA-4+, 16% KDR+, 1% CD45+	Gelatin; PDGF-BB	ECs	[79,186]
	ESC	M2-10B4 stromal cells	74.1% PECAM1+, 56% KDR+	Fibronectin; initial treatment with VEGF, hFGF, EGF, followed by TGF-β1 and PDGF-BB.	ECs	[77,177]
	ESC and iPSC	Initial treatment with PD98059, followed by BMP4, VEGF and bFGF	77.7% CD34/CD31+, SMαA+, VE-cadherin+, CD31+, RUNX1+, LMO2+, β-, ε- and ζ-GLOBIN+	bFGF and PDGF-BB	ECs, haematopoietic cells	[81]
	ESC	Hs27 (human foreskin fibroblast feeders); BMP4, VEGF and bFGF.	74% PECAM1+	Collagen I; SMC growth medium	ECs	[80]
p75	ESC-derived neural rosettes	Polyornithine-laminin; FGF2, AA and BDNF	AP2+	αMEM with 10% FBS. Isolated NCAM+ cells were expanded and differentiation was induced in N2 medium.	Neurons, Schwann cells, adipocytes, chondrocytes, osteocytes	[90]
Frizzled-3+/ cadherin-11+	ESC	EB formation	Not shown	IGF-1 and TGF-β1	Chondrocytes, glia, neurons, osteoblasts	[92]
Neural crest precursor	ESC-derived neural rosettes	Matrigel; bFGF and EGF	Nestin+, p75+, Slug+, Sox10+, FoxD3+	FBS	Neuron, cartilage, pigmented cell	[91]
CD105+/ CD24−	iPSC	bFGF, PDGF-AB, EGF	CD44+, CD49a/e+, CD73+, CD166+, CD34−, CD45−, CD133−	Sphingosylphosphorylcholine	Adipocytes, osteocytes, chondrocytes, ECs	[187]

signalling pathways involved in SMC differentiation from ESCs have been recently reviewed [128]. Smooth muscle differentiation stage is commonly confirmed by gene and protein expressions of SMαA (early), calponin, SM22α and desmin (intermediate), and SMMHC and smoothelin (late). SM1 and SM2, isoforms of SMMHC, have been used to indicate the degree of maturation of mouse ESC-derived SMCs [129]. Day-15 and day-28 EBs expressed SM1 and SM2 respectively [129], consistent with their sequential expression during vascular development [130]. However, characterising ESC-derived SMCs based just on the putative SMC markers has several drawbacks. Firstly, expression of most of the SMC markers is downregulated in vitro. This can be caused by a wide range of factors including the absence of heterotypic cell–cell interactions and biomechanical forces normally present in physiological settings. Under serum-containing culture conditions, SMCs tend to undergo phenotypic switching from a non-dividing contractile phenotype to a proliferative synthetic phenotype [131], resulting in a decrease of protein and mRNA levels of SMC markers.

Secondly, no marker is definitive for SMCs. The aforementioned SMC markers are also expressed in other cell types such as the myofibroblasts, skeletal and cardiac muscle during development [131]. Finally, markers to distinguish between vascular SMCs and visceral SMCs are limited and demonstrate poor specificity. So far, only splice variants of myosin heavy chain [132], smoothelin [133] and telokin [134] have been used to demarcate vascular and visceral SMCs. In one study, the spontaneous contractile regions on mouse EBs gave rise predominantly to SMCs expressing a vascular SMC-specific MHC isoform [132]. In another study, the contractile regions in mouse EBs contained gut-like SMCs [135,136]. One of the challenges of ESC-derived vascular research is to efficiently deliver a homogeneous population of vascular SMCs. The use of a panel of SMC markers coupled with functional assessments is needed to reliably characterise ESC-derived SMCs.

Contractility is a hallmark of mature SMCs. Classical contractility tests include agonist-induced intracellular Ca^{2+} transients and voltage clamp to confirm expression of functional ion channels and contractile force generation [132,137,138]. Vascular and visceral SMCs differ in their contractile nature. The former exhibits tonic (slow and persistent) contraction while the latter exhibits phasic contraction (fast followed by relaxation). In addition to intrinsic SMC function, it is important to assess the ability of ESC-derived SMCs to complement ECs in neovascularisation. One way to study EC–SMC interactions is

Table 2
Derivation and in vitro characterisations of SMCs from human ESCs unless otherwise stated. All studies reported here have used serum-containing media for differentiation.

Inducers for direct SMC differentiation	Positively expressed markers	Functional assessment of derived SMCs	References
Collagen IV; PDGF-BB	SMA, calponin, SMMHC	–	[124]
Gelatin; retinoic acid	SMαA, SM22α, Desmin, Calponin, SMMHC	–	[188]
Initial EB formation. EB outgrowth cells were cultured on matrigel-coated plate, then switched to gelatin-coated plate	SMαA, SMMHC, caldesmon, myocardin, calponin, smoothelin, SM22α, telokin	Visible contractions when induced with carbachol or KCL.	[189]
Collagen IV; PDGF-BB and TGF-β1	SMαA, SMMHC, SM22α,calponin	Expression of ECM components; secretion of fibronectin; visible contractions when induced with carbachol; SMCs surrounded human cord blood-derived endothelial progenitor cells in capillary-like structures on matrigel.	[140]
EB formation from human iPSCs; SMC differentiation medium	SMαA, γ-SMA	Display of agonist-induced Ca^{2+} transients; visible contractions of collagen gel embedded with iPSC-derived SMCs when treated with carbachol or angiotensin II.	[190]

through a tubulogenesis assay. ESC-derived SMCs were observed to surround endothelial tubes, recapitulating their biological niches in the peri-endothelial locations [139,140]. Another well-known function of vascular SMCs is the synthesis of extracellular matrix (ECM) components such as collagen, fibronectin and laminin which provide structural support to the vessel and attachment surface for cell–matrix interactions [140].

Vascular SMCs display a variety of phenotypes even in the postnatal state. There is considerable heterogeneity in SMCs from blood vessels of different organs and within the same blood vessel. Consistent with their diversity being in part due to their different embryonic origins, vascular SMCs respond to environmental cues in a lineage-dependent manner [44]. For optimal outcomes in therapeutic neovascularisation, it may be important to generate lineage-, vessel- or organ-specific SMCs. The precise origins of SMCs derived from spontaneous EB differentiation are unclear and likely reflect multiple embryonic lineages. Better control over the type of SMCs which are being generated may be obtained by directing differentiation via a progenitor stage of known developmental origin. More definitive lineage-tracing experiments are also required to fully clarify the origins of some organ-specific vascular SMCs.

4.3. Transplantation studies of human ESC-derived vascular progenitors and cells

In vivo studies are critical for assessing the ability of human ESC-derived vascular progenitors and cells to promote recovery of ischemic tissues. Animal models of deficient vascularisation such as ischemic hindlimb, stroke and myocardial infarction are commonly used (Table 3). Cell enrichment before transplantation is a key step in eliminating undesired cell types or residual pluripotent cells that can lead to teratoma formation [137]. ECs and some vascular progenitors can be isolated with ease based on cell surface markers using FACS or MACS. On the other hand, SMC identity is not aptly established by cell surface markers. Genetic selection based on conferred antibiotic resistance or reporter gene expression driven by smooth muscle lineage-restricted promoters can facilitate SMC isolation [137,138,141]. However, genetic insertion of selection cassettes may disrupt endogenous genes or activate oncogenes, rendering transgenic cells unsuitable for therapy. Therefore, profiling for novel SMC-specific surface markers will be useful for enrichment.

Modes of cell delivery might influence the efficacy of cell-based therapy. Systemic intravenous delivery or direct organ injections are commonly used in transplantation studies of human ESC-derived cells (Table 3). Although systemic delivery is less invasive, it requires the homing of transplanted cells to the sites of ischemia. A problem with systemic delivery may be the lodging of transplanted cells in unintended locations [115]. A recent study has reported the use of magnetic resonance imaging to guide iron oxide-labelled cells through vascular conduits [142], holding much promise for localising therapeutic cells to target regions. Direct organ injection may be superior if the local shortage of blood supply impedes the homing of transplanted cells to the ischemic regions. However, the survival of donor cells could be adversely affected by local hypoxia. Additional measures to enhance cell survival will be discussed in Section 5.2.

In studies where the human ESC-derived progenitors were further differentiated into vascular cells before transplantation, co-administration of SMCs and ECs has synergistically resulted in better blood flow restoration than transplantation of ECs alone (Table 3). The benefits of SMCs as supportive perivascular cells are therefore indisputable. However, what is the most appropriate stage of human ESC differentiation for transplantation—vascular progenitors or committed vascular cells? It was demonstrated that Flk1+ or KDR+ progenitors may be too immature to be transplanted directly to promote neovascularisation [143,144]. On the other hand, maturity of transplanted cells seems to correlate with a decrease in proliferative power and extent of vascularisation in vivo [145]. Progenitors may retain a certain degree of structural and functional plasticity. However, there is still insufficient evidence showing that the transplanted progenitors can respond to local cues and adopt the fate of tissue-specific vascular cells preferentially. From histological examinations, it is frequently reported that the transplanted human ESC-derived progenitors can undergo differentiation into SMαA-expressing SMCs in vivo. These results have to be interpreted with care as SMαA is not definitive of SMCs. Moreover, SMαA is a relatively early SMC marker which may already be expressed by the progenitors before transplantation. The dilemma of transplanting progenitors or committed vascular cells can be resolved with more concrete evidence from comparative in vivo studies.

In clinical trials of adult cell therapy, it is recognised that a principal mode of action of the stem or progenitor cells is to secrete cytokines and growth factors, which induce angiogenesis of host vessels, activate resident stem cells and elicit regeneration of host tissues [146,147]. Transplantation of human ESC-derived vascular progenitors or differentiated cells has resulted in functional recovery of animal ischemic models (Table 3). However, the exact mechanisms mediating the observed improvements are largely unclear. Reminiscent of studies using adult stem cells (see Section 3), it is uncertain whether the transplanted cells restore target tissue function by direct contribution to neovascularisation, or through paracrine effects. Whilst it is likely that ESC-derived progenitors have a greater ability

Table 3
Assessment of the revascularisation potential of smooth muscle progenitors and differentiated cells derived from human ESCs and iPSCs unless otherwise stated.

Animal model	Cell source	Transplanted cell type	Cell delivery	Post-transplantation assessment	References
Nude mice	ESC	(1) CD34+ progenitor-derived SMCs (2) CD34+ progenitor-derived ECs (3) ECs and SMCs	Subcutaneous injection into the dorsal region with matrigel	Microvessels made up of the transplanted cells were found in matrigel implants of all transplanted groups. Approximately 5% of microvessels contained host blood cells, indicating integration with host vasculatures. In group (3), SMαA+ SMCs formed small tubules or surrounded PECAM1+ ECs in the microvessels.	[79]
	ESC	Blast cells which had been differentiated in SMC medium for 2 weeks	Subcutaneous injection into the dorsal region with matrigel mixed with VEGF and bFGF	SMαA+ cells and PECAM1+ cells formed tube-like structures.	[68]
Hindlimb ischemia in mice	ESC	(1) KDR+ progenitor-derived SMCs (2) KDR+ progenitor-derived ECs (3) SMCs and ECs	Intra-arterial injection through the right femoral artery with PBS	Substantial blood flow recovery in all transplanted groups compared to no-cell control. Ratio of ischemic/non-ischemic blood flow was significantly greater in group (3) than groups (1) and (2). Transplanted cells (group 3) incorporated into host vessels. Group (3) displayed significantly higher PECAM1+ capillary density compared to groups (1) and (2). Maturity of newly formed vessels was confirmed by significantly higher SMαA+ capillary density in all transplanted groups than no-cell control.	[39]
	ESC	Blast cells	Intramuscular injection into the areas of peri-ischemic muscles	SMαA+ cells were found incorporated into blood vessel structures.	[68]
	iPSC	CD105+/CD24− MSC	Intramuscular injection into the gracilis muscle of the medial thigh	20% of the mice suffered limb loss. Another 20% showed limb necrosis while the majority had their limbs salvaged. Improved blood perfusion, increased muscle regeneration and less fibrosis than bone marrow MSC control. SMαA+ cells which were found among muscle cells and in arterioles. Some transplanted cells differentiated into PECAM1+ cells.	[187]
	ESC and iPSC	(1) CD34+ progenitors (2) CD34− progenitors	Intramuscular injection into the ischemic hindlimbs with culture medium	Recovery of blood perfusion in the ischemic hindlimbs with group (1) but necrosis was observed in group (2). In group (1), PECAM1+ cells were found to incorporate into host vasculatures. Group (1) cells secreted bFGF, ANG-1, ANG-2, promoting neovasculogenesis.	[81]
Stroke in mice	ESC	(1) KDR+/VE-cadherin+ progenitor-derived ECs (2) KDR+/VE-cadherin− progenitor-derived SMCs (3) ECs and SMCs	Intra-arterial injection through the left common carotid artery with PBS	Recovery of cerebral blood flow was significantly greater in group (3) than groups (1) and (2). Transplanted ECs were found in the infarct area and incorporated into host capillaries. Transplanted SMCs surrounded capillaries in the infarct area. Group (3) induced significantly greater vascular density than groups (1) and (2). Group (3) significantly reduced the infarct size and number of apoptotic cells in the ischemic penumbral area compared to no-cell control. Group (3) significantly improved recovery of impaired motor function compared to no-cell control as confirmed by longer exercise time of mice on an accelerating rota-rod.	[183]
Myocardial infarction	ESC	Cells derived from KDR^{low} progenitor after 5–10 days of treatment with VEGF, bFGF and DKK1.	Direct injection into the hearts of mice after coronary ligation	There was 31% higher ventricular ejection fraction in mice with transplanted cells than no-cell control.	[52]
	ESC	ESC-derived ECs	Direction injection into the sites near the peri-infarct zones of mice myocardium	Transplanted cells demonstrated engraftment into host myocardium and SMαA+ cells were found. Massive donor cell death observed within the first 2 weeks post-transplantation. Significant improvement in fractional shortening and cardiac viability was observed in treated hearts compared to no-cell control. However, no significant difference in cardiac functions was observed more than 28 days post-transplantation.	[78]
	Rhesus ESC	SSEA-1+ progenitors	Direct injection into the myocardial infarcted areas of Rhesus monkeys	Transplanted cells differentiated into ventricular myocytes which were surrounded by SMαA+ cells and PECAM1+ cells. Transplanted cells engrafted in the infracted myocardium and reconstituted 20% of the scar tissue.	[126]
	ESC	(1) CD34+ progenitor-derived ECs (2) CD34+ progenitor-derived SMCs and ECs	Injection into the peri-infarct regions of mice myocardium with saline Fibrin gel mixed with cells was applied to infracted areas of swine hearts.	Group (2) significantly improved ejection fraction. Stable engraftment of group (1) and (2) cells was observed post-transplantation. Scar size of infracted hearts which received group (2) cells decreased significantly as compared to no-cell control. Group (2) significantly improved ventricular function, reduced wall stress and increased capillary density but not group (1). Stable engraftment of group (1) and (2) cells was observed post-transplantation.	[177]

to generate vascular cells than their adult counterparts, there are also indications that transplanted CD34± progenitors produce paracrine factors such as basic fibroblast growth factor, angiopoietin 1 and 2 to promote neovascularisation [81]. Although several groups have documented that human progenitors can express vascular markers and contribute to functional vessels in vivo [81,110], to date there has been no quantitative assessment at the single cell level of their ability to form new vessels. The recovery of ischemic tissues is commonly assessed by imaging techniques. Laser Doppler perfusion imaging monitors restoration of peripheral blood flow while magnetic resonance imaging, echocardiography and positron emission tomography (PET) are used extensively for assessing cardiac function. A better understanding of the mechanisms of benefit involved in ESC therapy may expedite its translation to clinical applications.

5. Moving one step closer to clinical reality

5.1. Clinical compatibility of human ESC-based therapy

To date, the FDA has approved three human ESC-based clinical trials to be led by Geron Corporation and Advanced Cell Technology [148,149]. Long-term immunosuppression is the current strategy to mitigate possible immunological rejection of allogeneic human ESC-derived cells. Recently, it was discovered that short-term inhibition of the co-stimulatory receptors on T cells could induce tolerance of transplanted pluripotent stem cells into mice [150]. Another potential strategy is to establish a bank of human ESC lines to offer matching of human leukocyte antigens for regional populations [151,152] which would allow a reduction in immunosuppressive regimes.

On the other hand, patient-derived iPSCs may avoid the potential allogeneic immune rejection and ethical concerns involved in human ESCs. While viral vectors and exogenous transgenes raise the risk of oncogenesis [153], various alternative reprogramming methods are under development [154–159]. However, many hurdles such as genomic instability, line-to-line variability, unsuitability for acute phase patients and possible residual immunogenicity remain to be overcome [160–164]. Recently, scientists have been able to bypass reprogramming to a pluripotent state by direct conversion of fibroblasts into cardiomyocytes, neurons or blood lineages [165–167]. However, the transdifferentiation efficiency is still relatively low and it remains to be seen how effective this approach will be for translational purposes.

Therapeutic use of human ESC-derived vascular progenitors and cells necessitates controllable differentiation conditions that are chemically defined. It has been reported that ECs can be generated from human ESCs using a method free of serum and feeder cells [40] and expanded to clinically useful quantities [168]. This has not yet been achieved for SMCs derived from human ESCs. Moreover, unlike committed vascular cells, serial passage to scale up progenitor cells before transplantation could prove problematical as it may alter the desired differentiation stage or impair clonogenicity. Thorough optimisation will be essential to ensure the derivation of vascular progenitors and cells in reliably scalable and good manufacturing practice-compliant processes.

5.2. Cell enhancement and biomaterials for transplantation

The elevated levels of inflammatory cytokines at ischemic sites often hamper the survival of transplanted cells. Cell enhancement approaches such as transduction with genes regulating anti-apoptotic, anti-senescence and pro-angiogenic effects or pretreatment with relevant small molecules have shown to improve survival and efficacy of donor cells [169]. Various biodegradable hydrogels made of synthetic or natural polymers have also been developed [170]. The advantage of using hydrogels for cell delivery is that they provide initial encapsulation of the donor cells and gradually precondition the cells to the local biological environment. Dual delivery of vascular progenitors/cells and bioactive hydrogels with controlled release of pro-angiogenic growth factors may enhance revascularisation [171–174]. Furthermore, biomaterials such as alginate-based microcapsules can play an immunoprotective role by isolating the donor cells from the host immune system and thus increase allogeneic cell transplantation tolerance [175].

Human ESC-derived vascular progenitors and differentiated cells can potentially be used to vascularise tissue patches or construct vascular grafts. The ideal scaffolds for engineered tissue patches and vascular grafts are usually composed of synthetic or natural ECM components. The presence of ECM can encourage the maturation of newly formed blood vessels. The spatial complexity of ECM creates defined topological cues that can regulate vascular specification and organise vascular cells into tube-like structures [176]. Physical contacts with the ECM also modulate biophysical signals between the dynamic biological environment and the donor cells, leading to better viability, engraftment and patency of vascularised tissue patches or vascular grafts after implantation.

A recent study reported that the use of a porous fibrin biomatrix promoted significant engraftment of hESC-derived vascular cells in a swine model of myocardial infarction [177]. Nevertheless, there are only a few studies on biomaterial-assisted generation and transplantation of vascularised tissue patches or vascular grafts using hESC-derived vascular progenitors or cells. More comparative assessments of different biomaterials on cell delivery and development of functional vascularised tissues are required to further advance this field.

5.3. Tracking fate of transplanted cells

Non-invasive and longitudinal monitoring of cell fate after transplantation is imperative for assessing the therapeutic efficacy of cell-based therapy. In experimental animals, post-mortem histological examinations can only offer a semi-quantitative and single time-point analysis of the transplanted cells. Cell labelling with magnetic nanoparticles, radionuclide and quantum dots has enabled the visualisation of cell homing and localisation in live subjects by various imaging modalities [178,179]. However, dilution of the labelling particles due to subsequent cell division hinders long-term observations and accurate quantification of cell numbers. Furthermore, survival of the transplanted cells cannot be accurately determined as dead or phagocytic cells can retain the labelling particles and emit false positive signals.

Molecular imaging based on reporter gene expression is a reliable way to track cell survival and proliferation over time. Progenies of transplanted cells will inherit the reporter genes while dead cells will not express them. If the reporter gene expression is driven by the activity of a tissue-specific promoter, it is possible to monitor the differentiation of transplanted cells into the specific cell types [180]. As each imaging technique has unique advantages and limitations, multimodality imaging can be used to obtain the required resolution and sensitivity. Fusion reporter genes allowing small animal imaging by PET and bioluminescence have been applied to ESC transplantation studies in rats [181]. The intensity of the bioluminescence signal correlates linearly with the number of viable cells whereas PET imaging delineates the three-dimensional tomographic location of the transplanted ESCs. PET reporter gene imaging has also been used successfully to image the localisation of therapeutic cells in cancer patients [182]. Consequently, molecular imaging utilising PET and magnetic resonance could be extended to human ESC-based therapy to accelerate the progress of human trials.

6. Conclusions

Human ESC-derived vascular SMCs could have far-reaching implications in supplementing and modulating revascularisation by

endothelial progenitors and cells. Future studies will need to identify molecular markers of progenitors that are definitive for the subtypes of SMCs and pericytes and direct differentiation to obtain these specific cells. The ability of human ESC-derived mural cells to complement ECs in alleviating ischemic disorders and vascularising engineered tissues warrants further investigation. The ultimate success of human ESC-based therapy is governed by safety, efficacy and cost. Therapeutic cells have to be derived by clinically compliant methods that are devoid of animal components. Measures must be taken to eliminate residual pluripotent stem cells in order to avoid teratoma formation after transplantation. Since neovascularisation is the key to tissue repair, co-administration of vascular progenitors/cells, tissue-specific cell types and angiogenic stimulators might yield better outcomes than transplantation of a single cell type. An important consideration will be to understand the precise cellular mechanisms by which therapeutic benefit arises. Consequently, non-

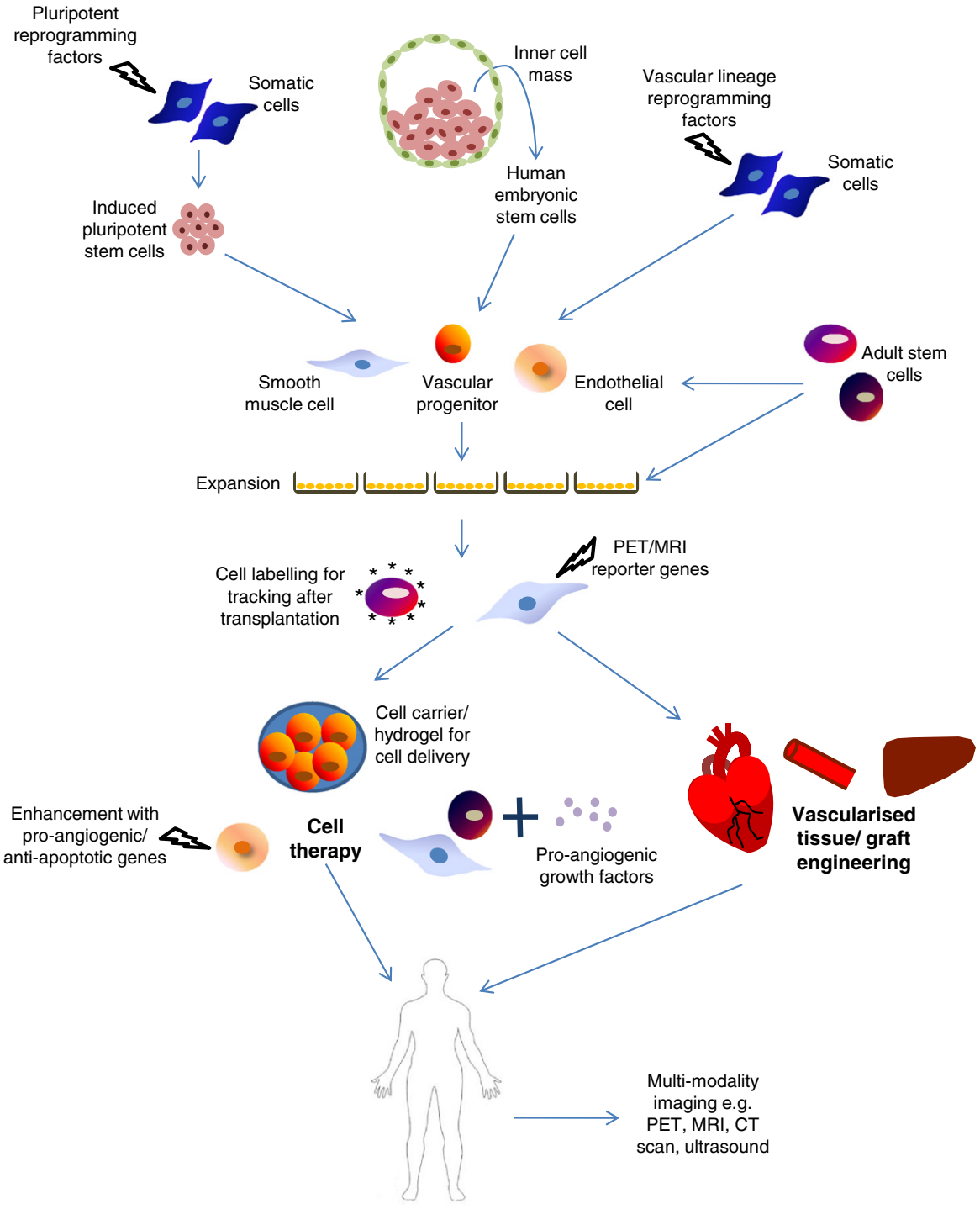

Fig. 3. Pathways to clinical translation of cardiovascular cell therapy. This figure outlines some of the key steps and methods being used or under consideration for implementing vascular cell therapy. Vascular progenitors or differentiated vascular cells may be derived from human pluripotent stem cells, adult stem cells or by direct conversion from somatic cells. Cells may then be expanded in vitro and labelled with reporters for subsequent tracking. Survival, homing and differentiation of injected cells in vivo may be augmented by a variety of approaches or alternatively cells may be used to generate intact tissues and organs in vitro before implantation in the patient. Non-invasive imaging techniques are likely to be essential for understanding the regenerative process in man and optimising the procedure.

invasive and long-term imaging is likely to be extremely valuable for tracking fates of transplanted cells and addressing questions regarding the mechanisms of benefits. Fig. 3 summarises the important implementations needed to facilitate clinical translation of cell therapy. If the current human clinical trials on ESC-based therapy go well, human ESCs and iPSCs and their descendants could be used in regenerative medicine in the foreseeable future.

Disclosures

None.

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