Review

De novo vasculogenesis in the heart

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Abstract

The formation of the embryonic heart vasculature is a complex process and is the result of vasculogenic, angiogenic and arteriogenic mechanisms, involving cells from distinct origins. In the neonate and the adult, several sources of endothelial precursor cells (EPCs) have been identified that contribute to physiological and pathological vascularization, consistent with the concept of de novo vasculogenesis after birth, including in the heart. The existence of EPCs in the adult has offered the possibility to use these cells for revascularization of ischemic tissues. An important challenge for vascular regeneration in ischemic and post-infarction patients is now to identify the most adequate cell source and cell dose for sufficient revascularization. This review gives an overview of the cellular and molecular cues involved in the formation of the heart vasculature before and after birth and discusses some of the recent insights and outstanding questions on EPCs and other vascular progenitors—both from a biological and therapeutic perspective.

Keywords: Angiogenesis; Stem cells; Heart failure; Infarction; Ischemia

1. The developing heart and its vasculature

The heart is one of the first organs to develop in the embryo. In the mouse, heart tube formation is initiated around embryonic day (E) 8 (Fig. 1). This primitive avascular structure consists of a few layers of cardiomyocytes, lined by endocardial cells and receives nutrients and oxygen through diffusion across the endocardial endothelium. As the myocardial wall becomes thicker, the endocardial surface area increases by progressive trabeculation (Fig. 1), allowing maximal diffusion. However, as the myocardium grows, diffusion becomes insufficient. Shortly after onset of contraction, a primitive vascular plexus starts to develop. Around E9, the proepicardial organ attaches to the heart and angioblasts (endothelial precursors) start to migrate across the heart within the subepicardial matrix along with the epicardium (see below; Fig. 1). The angioblasts derive mostly from the sinus venosus-liver region, but an epicardial origin for some coronary endothelial cells cannot be ruled out [1]. Subsequently, these precursors differentiate in situ into endothelial cells and assemble into a primitive capillary network (coronary vasculogenesis; Fig. 1) that is not yet connected to the systemic circulation. This primary vascular plexus subsequently expands in an epi-to-endocardial direction and towards the heart base by endothelial sprouting from pre-existing capillaries (coronary angiogenesis; Fig. 1), eventually connecting to the aorta. Upon perfusion, these myocardial capillaries become remodeled into larger vessels ramifying into smaller branches and these vessels become enveloped with peri-endothelial cells [smooth muscle cells (SMCs) in large vessels, pericytes in smaller vessels] and an extracellular matrix (Fig. 1).

At birth, the myocardium has attained only a fraction of the thickness it will attain during adulthood. Myocardial thickness increases at least fourfold in postnatal life, due to
cardiomyocyte hypertrophy. The increased metabolic demands of hypertrophying cardiomyocytes are compensated by a significant expansion of the myocardial vasculature. In the mouse, capillary density increases three- to fourfold and the number of SMC-covered coronaries at least 10-fold during the first 3 weeks [2] (Fig. 1). These vessels undergo significant remodeling in order to assume adult branching characteristics and acquire specific properties of coronary arteries or veins. Until now, it has been postulated that postnatal expansion of the myocardial vascular bed only proceeds through angiogenesis. However, recent findings suggest that bone marrow (BM)-derived endothelial precursor cells (EPCs) contribute to (physiologic) neovascularization in the heart and the liver during the neonatal period, consistent with a vasculogenic paradigm [3].

2. Origins and heterogeneity of vascular cells in the developing heart

Much of what is known about the origin of vascular cells in the heart is derived from retroviral labeling and quail-chick chimera experiments. An important cell source, the proepicardial organ, is located between the sinus venosus and the primordial liver. Around E8.5–9 in the mouse, proepicardial cells migrate and form patches of epithelial-like cells that fuse to provide a continuous sheath enveloping the heart, the epicardium (Fig. 1). A subpopulation of epicardial cells, the epicardium-derived cells (EPDCs) undergoes an epithelial-mesenchymal transition, delaminates from the growing epicardium and migrates into the myocardium giving rise to at least three different cell types: SMCs, coronary and intermyocardial fibroblasts.
Endocardial cells have embryonic origins distinct from coronary vessels. These cells arise from within the myocardial plate, adjacent to the foregut endoderm, where they assemble into a loose vascular plexus that eventually forms a single endocardial tube [5].

Coronary SMCs have different origins, depending on their location in the heart and their function. While SMCs of the proximal coronary arteries (as well as the large arteries in the thorax, head and neck) originate from the neural crest, the rest of the coronary arteries derive their smooth muscle coat from the proepicardial organ [6] (Fig. 1). In contrast, coronary vein medial cells are from atrial cardiomyocyte origin [7]. It has been shown that the first layers of SMCs in the dorsal aorta transdifferentiate from the endothelium, but there is no evidence as yet that such a transdifferentiation mechanism contributes to the development of the coronary vasculature [6]. SMC markers, like SMC α-actin, become only expressed in the coronary vasculature upon connection of the coronary plexus with the circulation, indicating that—unlike vasculogenesis—‘muscularization’ of coronary vessels is flow-dependent (Fig. 1). Coronary veins develop before arteries, however, the coronary veins become enveloped with a media only at later time points than coronary arteries. Presumably, the pressure increase and blood flow alteration after connection to the aorta, is a trigger for SMC differentiation in coronary arteries, while differentiation is delayed in the low-pressure environment in coronary veins [7].

Endothelial cells in vascular beds of different organs acquire specialized characteristics to allow optimal function in that specific organ. For example, endothelial cells in the brain are tightly linked to each other and are surrounded by numerous peri-endothelial cells, which constitute a barrier that protects brain cells from potentially toxic blood-derived molecules. In contrast, vessels in endocrine glands are leaky and their endothelial cells have fenestrations, allowing hormone trafficking. In addition to endothelial cell heterogeneity between distinct organs, endothelial cells within the same organ can be heterogeneous. In the heart, the heterogeneity of endothelial cells in distinct locations of the heart vascular tree has been suggested by differences in expression patterns for several molecules, like the endothelial constitutive nitric oxide synthase (NOS) isoform (ecNOS) [8], brain derived neurotrophic factor (BDNF) [9] or adhesion molecules [10,11]. In addition, functional differences have been observed between micro- and macrovascular endothelial cells, isolated from human hearts [12]. This heterogeneity within cardiac endothelial cells was shown to play a critical role in inducing conduction cell differentiation in the embryonic myocardium [13]. In the chick embryo, cells from the distal Purkinje fibers differentiate in close association with coronary arteries, through a neighboring vascular signal, likely endothelin [14]. Like cardiac endothelial cells, heart SMCs seem heterogeneous. Culture studies revealed that neural crest-derived SMCs behave differently from mesoderm-derived cells, as evidenced by their distinct reaction pattern to transforming growth factor (TGF)-β [6].

3. Molecular control of vessel formation in the developing heart

In addition to identifying the cellular origins of vascular cells, defining the molecules that determine cell fate and function is of invaluable importance for vascular therapy. Studies in the chick, the zebrafish and gene-deficient mice have significantly contributed to our current understanding of molecular signaling during vessel development. The general molecular cues involved in vessel formation have been reviewed previously [15] and many of these molecules may also drive vessel growth and remodeling in the heart. In addition, the formation of a specialized vascular network fine-tuned to a complex organ such as the heart may require specific angiogenic signals. Recently, EGF-VEGF (endocrine gland-derived vascular endothelial growth factor) was described, an angiogenic factor only affecting endothelial cells in endocrine glands [16]. This was the first organ-specific angiogenic factor to be described and it is likely that others exist, for instance in the heart.

The formation of the endocardial tube is one of the first steps in cardiac development. Endocardial cells first aggregate into a loose vascular network that subsequently coalesces into progressively large tubes, eventually generating a single endocardial tube lining the avascular myocardium [5]. VEGF secreted from the adjacent endoderm appears to play a role in directing endocardial development [17]. Subsequent trabeculation of the ventricular myocardium expands the endocardial endothelial surface area in order to increase diffusion capacity for oxygen and nutrients prior to development of the coronaries. This process requires reciprocal interactions between the endocardium and the myocardium. Neuregulins, a family of secreted and cell-membrane-associated factors generated by alternative splicing of a single gene, have been implicated as potential endocardial-derived signals in trabeculation. Neuregulins interact with erbB receptors of the epidermal growth factor family, some of which are expressed in the myocardium (erbB2 and erbB4). Targeted deletion of neuregulin, erbB2 or erbB4 all block trabeculation and deficient embryos die early in gestation [18–20]. Deficiency of RXR-α, a co-receptor for retinoid acid receptors, results in defective ventricular maturation and trabeculation [21,22]. Formation of trabeculae was also impaired in mice lacking VEGF [23,24], angiopoietin-1 (Ang-1) [25] or the basic helix-loop-helix transcription factor dHAND (deciduum, heart, autonomic nervous system, neural crest derived) [26].
As mentioned above, the generation of coronary vessels is integrally associated with the development of the epicardium. Migration of epicardial epithelium is blocked in vascular cellular adhesion molecule (VCAM)-1 deficient mice and these mice therefore do not develop coronary vessels [27]. In addition, adhesion between epicardium and myocardium is severely disrupted in α₄ integrin-deficient mice, resulting in the lack of proper coronary circulation. A recent knock-in study revealed that α₃β₁ integrin-mediated adhesion is necessary for migration of progenitor cells to form the epicardium (Ref. [28] and references therein). Coronary vessel development in the embryo not only depends on the epicardium but is now known to be coordinated by signals derived from at least four distinct cell types: endothelial, myocardial, (pro)epicardial and neural crest cells [29].

An important family of regulators is the GATA family of transcription factors. The members of the GATA-4/5/6 subfamily are expressed principally in the heart and gastrointestinal tract with overlapping expression patterns. Their transcriptional activities are regulated by physical association with other transcription factors. One of these factors is FOG-2 (friend of GATA-2). Mice deficient in FOG-2 have an intact epicardium, but completely lack coronary vessels [30]. Since mice harboring a single amino acid mutation in GATA-4 preventing the FOG/GATA-4 interaction also feature lack of a coronary vasculature, this interaction is likely involved in coronary vessel development [31]. However, unlike FOG-2/−/− mice, the GATA-4 mutant mice show impaired outflow tract septation, resulting in mice with double-outlet right ventricle. This suggests that for the latter event distinct mechanisms or interactions other than with FOG-2 are involved. Fibroblast growth factors (FGFs) have been associated with embryonic vasculogenesis by determining angioblast specification from the mesoderm (reviewed in Ref. [32]). In addition, the spatiotemporal expression pattern of FGF-1 and its receptors during cardiac morphogenesis correlate with proliferation and differentiation of endothelial cells and SMCs of the coronary vessels, as well as of cardiomyocytes [33]. In culture studies, FGFs, particularly FGF-1, -2 and -7, positively regulate the epicardial-mesenchymal transformation that precedes formation of coronary vessels. FGF-2 was shown to act both in an autocrine (from the epicardium) and a paracrine (from the myocardium) way. Myocardium-derived TGF-β3 inhibits this transformation process [29]. A role for FGF-1 later in life was indicated in mice overexpressing this factor in the heart. The myocardium of these mice showed an increased density of SMC α-actin-positive coronary arteries and an increased number of coronary branches [34].

VEGF is a well-studied angiogenic factor involved in vasculogenesis as well as angiogenesis (reviewed in Ref. [15]). The mammalian VEGF family comprises at least five homologues (VEGF-A, -B, -C, -D, and placental growth factor or PI GF) with different receptor specificities. Deletion of a single allele of VEGF-A results in early embryonic lethality due to severe vascular defects including defective trabeculation (see above), precluding study of its role at later stages, like during coronary vessel development [23,24]. In addition, different splice variants (isoforms) of VEGF-A exist (VEGF₁₂₀, VEGF₁₆₄ and VEGF₁₈₈ being the most prominent ones in the mouse), with possible differential functions due to their distinct chemotactic properties, receptor specificity, endothelial mitogenicity and tissue-specific expression (reviewed in Ref. [35]). Therefore, we generated mice expressing the VEGF₁₂₀ isoform alone (VEGF₁₂₀/₁₂₀ mice) using the Cre/loxP system to remove exons 6 and 7, encoding basic domains only present in VEGF₁₆₄ and/or VEGF₁₈₈ [2]. A fraction of homozygous VEGF₁₂₀ embryos died shortly after birth and the remainder gained less weight and died before day 14 after birth of cardiac failure, exhibiting depressed myocardial contractility and cardiac dilation. Analysis of VEGF₁₂₀/₁₂₀ hearts revealed severe angiogenic defects in the myocardium, suggesting that VEGF₁₂₀ by itself is insufficient for normal blood vessel growth. In contrast to wild type mice, capillary and coronary artery density did not increase during the first weeks of life. As mentioned above, the expansion of the myocardial vessel network is not only due to angiogenesis (involving mature endothelial cells), but also to vasculogenesis (involving EPCs) and VEGF was shown to modulate EPC engraftment in the neonatal heart [3]. The fact that the formation of SMC-surrounded coronary arteries was reduced in VEGF₁₂₀/₁₂₀ mice suggests a VEGF-mediated effect on SMCs, either directly [36,37] or indirectly via an effect on the production of recruitment factors such as platelet-derived growth factor-B (PDGF-B). Expression levels of PDGF-B and its receptor PDGF-receptor β were indeed reduced in VEGF₁₂₀/₁₂₀ hearts [2]. A recent study revealed that cardiomyocyte-derived VEGF is an important signal for normal development of the coronary microvasculature [38].

The role of VEGF-A homologues has generally been less extensively evaluated. PI GF-deficient mice survived beyond birth with no obvious vascular defects in the heart or other vital organs [39], suggesting that this factor is redundant for physiological vessel development. Although the most prominent expression of VEGF-B in the developing myocardium [40] suggested a role in vascularization of the embryonic heart, mice lacking VEGF-B were viable and had no gross abnormalities in the heart, except for an atrial conduction defect [41] and vascular dysfunction after coronary occlusion [42]. For VEGF-C and the closely related VEGF-D, no specific functions in the development of the murine heart vasculature have been described. Expression levels of VEGF-C were reduced in the hearts of VEGF₁₂₀/₁₂₀ mice, however, it is unknown whether this directly contributed to the coronary vascular defects in these mice. One recent report, using a quail heart explant
model, suggests a role for both VEGF-A, -B and -C in coronary vessel formation [43]. Only a few factors specifically important in heart vessel development have been described. This may be—in part—due to the fact that many studies in knock-out mice have not included an extensive investigation of the fetal heart phenotype in these animals. Bves (blood vessel/epicardial substance) is an adhesion molecule expressed in the proepicardial organ, the migrating epicardium, the delaminated vasculogenic mesenchyme and coronary SMCs [44]. It was suggested that this factor regulates coronary vessel development through a dynamic subcellular redistribution mechanism involved in cell migration and adhesion. Antibodies against Bves were able to block epicardial sheet migration in proepicardial organ explant cultures [45]. Secondly, capsulin (also known as epicardin or pod-1) is a basic helix–loop–helix factor highly expressed in the proepicardial organ and expression is apparent immediately before mature coronary vessels develop [46–48]. Based on this spatio-temporal expression pattern, this molecule has been proposed to predict coronary vascular SMC fate, however direct evidence for such a role from knock-out studies is lacking. Differentiation of coronary SMCs from proepicardial cells involves serum response factor (SRF), a member of the MADS box family of DNA binding proteins. Rather than affecting epicardial-mesenchymal transformation, this factor regulates transformation of mesenchyme to SMCs through RhoA-Rho kinase signaling, thereby mediating cytoskeletal changes [49]. BDNF, a member of the neurotrophin family, and its receptor trkB are selectively expressed by neonatal and adult heart vessels. BDNF was suggested as a specific survival factor for coronary endothelial cells. Deficiency of BDNF in the mouse impaired survival of endothelial cells in myocardial arteries and capillaries in the early postnatal period, resulting in intraventricular wall hemorrhage, decreased cardiac contractility and early postnatal death [9].

4. The adult heart and its vasculature

In contrast to the active vessel growth in the embryo and the newborn, the adult myocardial endothelium remains in a quiescent state. Only when provoked by stress or pathologic conditions, does the coronary vascular bed expand. Vascular expansion in the adult heart may encompass three different mechanisms, possibly driven by distinct signals. Until recently, it was generally accepted that vessels in adult ischemic tissues could only grow by angiogenic mechanisms (Fig. 2), i.e., the sprouting of mature endothelial cells from pre-existing vessels, likely in response to angiogenic growth factors. However, recent studies have revealed that EPCs also circulate postnatally in the peripheral blood and may be recruited from the BM and incorporated into sites of active neovascularization, like in the ischemic myocardium [50–52]—a process termed postnatal vasculogenesis (Fig. 2). In addition, the in situ development of large collateral vessels from pre-existing arteriolar anastomoses (collateral growth or ‘adaptive arteriogenesis’; Fig. 2) may not result from ischemia, but rather from shear-stress-induced upregulation of angiogenic and inflammatory factors (reviewed in Ref. [53]).

Although the myocardial vascular bed can expand, its expansion capacity is limited and soon the heart becomes ischemic due to an imbalance between oxygen supply and consumption. Initially, the myocardium develops a protective response—hibernation—in order to preserve high-energy metabolites at the expense of contractile dysfunction. The hibernating myocardium is still viable and able to restore its contractile function upon proper revascularization. However, when the ischemic insult becomes too severe, the hibernating myocardium may undergo irreversible structural changes and die to become replaced by fibrotic scar tissue. VEGF120/120 mice featuring impaired myocardial angiogenesis developed ischemia with signs of hibernation, that over time, progressed to cardiac failure [2]. This mouse model provided genetic evidence that insufficient availability of an angiogenic growth factor results in ischemic heart disease and constitutes a rationale for administering angiogenic factors or their corresponding genes (‘therapeutic angiogenesis’; Fig. 2) to rescue hibernating myocardium or to prevent myocardial necrosis and cardiac failure. Therapeutic angiogenesis might also be indicated for post-infarct patients to prevent ischemia in the remodeling, viable myocardium, remote to an infarcted region. In addition, neovascularization of the infarct area is essential for a normal healing process after myocardial infarction [54]. Therapeutic angiogenesis might be used alone or in combination with coronary bypass surgery.

However, insufficient availability of angiogenic growth factors might not be the only reason for patients to develop myocardial ischemia. Several conditions (aging, diabetes and hypercholesterolemia) in which the adult endothelium becomes less responsive to angiogenic stimuli have been identified in mice and these conditions are also present in a large group of patients that suffer from myocardial ischemia. The impaired endothelial function likely contributes to the increased severity of cardiovascular disease in the geriatric population [55]. In addition, dysfunction of EPCs has been suggested to occur in diabetic mice [56] and an inverse correlation between the number of cardiovascular risk factors (including diabetes, hypertension) and the number and migratory activity of EPCs has been measured in humans [57]. Dysfunction of mature endothelium and EPCs is a possible indication for vascular regeneration with EPCs (Fig. 2). EPC transplantation might complement current strategies of therapeutic angiogenesis for patients in whom endogenous endothelial (precursor) cells fail to sufficiently respond to growth factor treatment.
Fig. 2. The adult heart and its vasculature. In contrast to the embryonic heart vasculature, the adult heart vessels are quiescent. Only when provoked by stress or pathological conditions, like a myocardial infarction, does the coronary vascular bed expand. Vascular expansion in the adult heart may encompass three different mechanisms, possibly driven by distinct signals: (i) angiogenesis (sprouting of new vessels from pre-existing ones), (ii) collateral enlargement (‘adaptive arteriogenesis’) by ‘muscularization’ and enlargement of pre-existing collaterals, or (iii) vasculogenesis (assembly of new vessels by fusion and differentiation of endothelial precursor cells originating from the bone marrow). These mechanisms can be therapeutically stimulated by mainly two strategies: (i) ‘therapeutic angiogenesis’ or the administration of angiogenic growth factors (like vascular endothelial growth factor or VEGF) either as a gene or a protein, and (ii) ‘vascular regeneration’ or the supply of new vascular (precursor) cells (endothelial and/or smooth muscle).

5. Cellular sources for vascular regeneration

5.1. Endothelial cells

EPCs were initially isolated from peripheral blood and identified on the basis of their expression of VEGFR-2 and CD34, antigens shared by the angioblast and the hematopoietic progenitor [58]. These EPCs were subsequently shown to express VE-cadherin and AC133, an orphan receptor which is specifically expressed on EPCs but whose expression is lost once they differentiate into mature endothelial cells [59]. Their high proliferation rate distinguishes circulating marrow-derived EPCs in the adult from mature endothelial cells shed from the vessel wall [60].

Cells with the capacity to form endothelial cells in vitro or in vivo have been identified in different cell fractions isolated from the BM or the peripheral blood of adult animals and humans (Table 1). Some of these cell populations were shown to be effective in the formation of new vessels in ischemic tissues, including the myocardium. Kamihata et al. isolated the mononuclear fraction from porcine BM and transplantation of this cell fraction increased capillary densities and the number of visible collateral vessels [61]. Orlic et al. used the Lin⁻ c-Kit⁺ fraction from murine bone marrow for transplantation into the peri-infarct region. Transplanted cells gave rise to endothelial cells in the regenerating myocardium [62]. Transplantation of freshly obtained granulocyte-colony stimulating factor (G-CSF)-mobilized human CD34⁺ blood cells into nude rats generated an angiogenic microenvironment by incorporation into the infarct bed (vasculogenesis) and by stimulating proliferation of pre-existing endothelium in the infarct border (angiogenesis) [63]. Also a subpopulation of the CD34⁻ fraction (CD34⁻/low c-Kit⁺ Sca-1⁻; the so-called ‘side population’) of murine bone marrow was shown to contain cells with the potential to incorporate into new vessels in the peri-infarct region [64]. In some cases unfractionated bone marrow was used for transplantation. Delivery of freshly aspirated whole bone marrow increased vascularity and perfusion in the ischemic myocardium in pigs [65] and rats.
Table 1
Distinct cell sources for vascular regeneration*

| Source                     | Cell population                                | Species          | Ref. |
|----------------------------|------------------------------------------------|------------------|------|
| A. Endothelial cells       |                                                |                  |      |
| Peripheral blood           | AC133+ VEGFR-2 CD34+                           | Human            | [104]|
| Bone marrow                | Mononuclear fraction                           | Porcine          | [61] |
| Bone marrow                | Lin c-Kit†                                     | Murine           | [62] |
| Peripheral blood           | GCSF-mobilized CD34+                           | Rat              | [63] |
| Bone marrow                | CD34+/low c-Kit Sca-1+ ('side population')    | Murine           | [64] |
| Bone marrow                | Unfractionated                                 | Porcine          | [65] |
| Bone marrow                | Unfractionated                                 | Rat              | [66] |
| Peripheral blood           | Mononuclear, ex vivo expanded                   | Human            | [105]|
| Bone marrow                | CD34+CD45-cKit GlyA (‘MAPCs’)                  | Human, rat, murine, | [74]|
| Cord blood                 | CD34+                                          | Rat              | [68] |
| Embryo                     | Embryonic stem cells                            | Murine           | [70] |
| Embryo                     | Embryonic stem cells                            | Human            | [72] |
| Bone marrow                | Sca-1 c-Kit Lin+ ('hemangioblast')             | Murine           | [93] |
| Bone marrow or cord blood  | CD34+KDR+                                      | Human            | [94] |
| B. Smooth muscle cells     |                                                |                  |      |
| Peripheral blood           | Mononuclear fraction                           | Human            | [75] |
| Bone marrow                | Lin c-Kit†                                     | Murine           | [62] |
| Bone marrow                | CD34+CD45-cKit GlyA (‘MAPCs’)                  | Human, rat, murine, | [106]|
| Embryo                     | Embryonic stem cells, Flk-1+ fraction          | Murine           | [88] |

* Selected list; see text for details.

In other cases, to obtain larger numbers of cells, isolated cells were expanded ex vivo before transplantation by culture for 7 days in the presence of angiogenic growth factors [67]. Cord blood constitutes an alternative source of EPCs. Human cord blood was shown to contain a higher concentration of EPCs as compared to peripheral blood and cord blood derived EPCs were reported to show faster differentiation rates than peripheral blood derived EPCs [68]. The CD34+ fraction of umbilical cord mononuclear cells contributed to neovascularization in the ischemic hindlimbs of nude rats and increased blood flow [68] (Table 1).

Totipotent murine embryonic stem cells were shown to differentiate into endothelial cells in vitro [69,70]. After removal of leukemia inhibitory factor, these cells differentiate into cells of the different germ layers and form embryoid bodies in which vascular-like structures can be identified consisting of endothelial cells. Endothelial differentiation could be enhanced by adding angiogenic growth factors (VEGF and FGF-2) to the culture [71]. Recently, endothelial cells were derived from cultured human embryonic stem cells by the use of antibodies against platelet endothelial cell-adhesion molecule-1. These endothelial cells participated in new vessel formation after implantation into severe combined immunodeficiency mice [72].

5.2. Smooth muscle cells

Only recently, one report in humans has shown SMC outgrowth after culturing blood mononuclear cells in the presence of PDGF-BB [75] (Table 1). However, several animal studies have given indirect evidence for the existence of circulating SMC progenitors from the BM. After heterotopic cardiac [76,77] or aortic [76] transplantation in mice, most of the neointimal α-actin positive SMCs in the donor coronary arteries or aortas were from host origin, suggesting that these SMCs might at least in part be derived from BM-derived smooth muscle progenitor cells. In support of this concept, transplantation of β-galactosidase-expressing BM into irradiated aortic allograft recipient mice revealed that part of the neointimal SMC-like population consisted of marrow-derived cells [78]. Two recent studies revealed that bone marrow-derived SMCs (and endothelial cells) importantly (10–50%) contribute to neointima formation and re-endothelialization in the context of transplant atherosclerosis, balloon injury and primary atherosclerosis [79,80]. However, other reports demonstrated a more modest (1–10%) contribution of BM-derived cells during these events [81–83]. Orlic et al. revealed that after implantation of Lin- c-Kit+ bone mar-
row cells into the ischemic myocardium, 44% of the newly formed SMCs were BM-derived [62]. Analysis of human hearts from sex-mismatched transplantations, have, however, revealed conflicting results, reporting substantial (up to 60%) contribution of bone marrow derived cells (including SMC and cardiomyocyte precursors) in some [84], but rather low (0.04–2.6%) in other studies [85–87]. The persistence of bipotential vascular progenitors giving rise to both endothelial cells and SMCs and their contribution to adult neovascularization, as recently described in the embryo [88], requires further study.

5.3. Hematopoietic stem cells

Depending on the location, endothelial cells in the embryo derive either from angioblasts (committed to the endothelial lineage) or from hemangioblasts, (common precursors for endothelial and hematopoietic cells). Endothelial and hematopoietic stem cells (HSCs) not only share a common origin, the latter can also stimulate the assembly of endothelial cells into nascent blood vessels in the embryo. Indeed, HSCs are present at sites of active vascular expansion. By producing angiopoietin (Ang)-1, they stimulated endothelial growth in the embryo [89].

HSCs are also present in adult BM and can be mobilized in response to angiogenic factors like VEGF, PIGF and Ang-1 [90,91]. Conversely, blocking of VEGFR-1—but not VEGFR-2—attenuated their recruitment [37,90]. Recently, co-recruitment of VEGFR-1–BM-derived hematopoietic (precursor) cells and VEGFR-2–EPCs, was shown to contribute to tumor growth and vascularization, suggesting a mutually vascular supporting role for these cell types [92]. Possibly, co-mobilization of VEGFR-1–hematopoietic (stem) cells is essential for incorporation of EPCs into newly formed vessels. In agreement with the involvement of VEGFR-1–cells in angiogenesis, we demonstrated that VEGFR-1 blocking resulted in reduced angiogenesis in ischemic retinopathy, tumor growth and rheumatoid arthritis [37]. The existence and identity of hemangioblasts in adult tissues and their contribution to postnatal vessel growth has remained uncertain. Proof of concept requires experiments in which is demonstrated that a single cell can give rise to both endothelial and hematopoietic cells. Recently, Grant et al. demonstrated that transplantation of individual Sca-1–c-Kit–Lin–BM cells contributed to both hematopoietic and endothelial cells [93], suggesting that these transplanted cells had hemangioblastic potential. In addition, Pelosi et al. reported that a small subset of CD34–KDR–cells from bone marrow or cord blood have long-term proliferative potential and can differentiate in endothelial and blood lineage [94]. Although a single MAPC generated cells of all germ layers including endothelium, in vitro differentiation into hematopoietic cells was never observed [73,74]. However, in vivo, MAPCs gave rise to cells of all blood lineages [74].

6. Vascular and myocardial regeneration

Although vascular regeneration can relieve ischemia, rescue viable myocardium and accelerate the post-infarction healing process, the necrotic area will be replaced by a collagenous scar instead of contractile cardiomyocytes. Functional cardiomyocyte loss causes progressive ventricular dysfunction leading to end-stage cardiac failure. Although recent findings have indicated that cardiomyocytes can divide [95], the level of proliferation reported in these studies is likely insufficient to rescue cardiomyocyte loss resulting from a large infarct. Therefore, myocardial regeneration or repopulation of post-infarction scar with contractile cells has been proposed as a potential therapeutic strategy to prevent progression to fatal cardiac failure.

Like for vascular regeneration, different cell types have been tested for their ability to engraft the injured area and improve cardiac function: fetal and neonatal cardiomyocytes, skeletal myoblasts, BM-derived cells and embryonic stem cell-derived cells (reviewed in [96]). An important issue is that the success of cardiac cell transplantation depends on the long-term survival and functional integration of sufficient numbers of grafted cells. Recently, Zhang et al. estimated that cell survival falls to about 10% a week after transplantation of rat neonatal cardiomyocytes into cryo-injured hearts [97]. Likely, an adequate microenvironment that supports nutrient delivery and waste removal is necessary to sustain survival, growth and differentiation of the transplanted cells. Therefore, a concurrent revascularization must keep pace with repopulation of the infarct with new myocardial cells. Two recent studies have combined myocardial regeneration with stimulation of blood vessel growth by an angiogenic factor. Yang et al. implanted VEGF-transfected embryonic stem cell-derived cardiomyocytes into injured mouse hearts and the density of newly formed capillaries was significantly higher than in animals implanted with non-transfected cells [98]. In a second study, neonatal cardiomyocytes transfected with hepatocyte growth factor were implanted in the infarcted area of rat hearts and a significant improvement in myocardial perfusion was obtained but not when non-transfected cells were used [99]. Alternatively, the transplanted cells may give rise to both cardiomyocytes and vessels. Orlic et al. showed that the Lin–c-Kit–fraction from murine BM gave rise to cardiomyocytes, endothelial cells and SMCs [62]. Transplantation of the ‘side population’ resulted in engraftment of both endothelial cells and cardiomyocytes [64].

7. Therapeutic perspectives

Hopes are raised that vascular regeneration in the heart will be of therapeutic value in the future. However, some issues need to be resolved before its therapeutic potential can be rigorously tested in patients. First, reports on the
contribution of vascular progenitors to neovascularization of ischemic tissues have been quite variable in animal models. After transplantation of human CD34+ cells into nude rats, human cells were detected in 25% of newly formed vessels in the infarcted myocardium [63]. After transplantation of the Lin-c-Kit+ fraction from murine BM, the percentages of new endothelial cells and SMCs of donor origin were 44 and 49%, respectively [62]. Similarly, distinct degrees of engraftment have been reported in hindlimb ischemia. Transplantation of human CD34+ cells into nude mice resulted in incorporation of human cells in ~13% of the capillaries in the ischemic limbs [58]. After ex vivo expansion of human EPCs (by culture for 7–10 days in the presence of angiogenic growth factors), human cells were found in ~56% of the vessels of the ischemic limb [51]. The percentages mentioned above might be an understimation, as EPCs and/or other bone marrow cells may provide a local source of angiogenic and inflammatory factors that further enhance new vessel formation [63,66].

Secondly, the transplanted cells should contribute to the formation of functional vessels that restore blood flow to the ischemic region and improve cardiac function. Transplantation of either ex vivo expanded hEPCs [67], freshly isolated CD34+ cells [63] or total bone marrow [65] resulted in increased vessel formation and improved cardiac function, indicating that the newly formed vessels were functional.

Finally, it currently remains unknown which cellular source might be preferable for vascular regeneration of the ischemic myocardium, since studies directly comparing the different cell sources are lacking. In addition, the optimal cell number required for successful vascular regeneration in the heart is unknown. Based on their studies of human EPC transplantation into nude mice, Iwaguro et al. calculated that satisfactory reperfusion of the hindlimb would require 0.5 to 2×10⁷ EPCs/g and that 12 l of human blood would be needed to collect such a number of EPCs. Transfection of EPCs during ex vivo expansion with a VEGF-encoding adenovirus resulted in a 30-fold reduction in EPC requirement for limb revascularization [100]. This was possibly due to the proliferative and survival effect of VEGF on EPCs and/or by modulation of adhesion receptor expression [100]. The yield of EPCs from blood might also increase after mobilization with cytokines (VEGF, angiopoietin, G-CSF) [51,63,91].

Vascular regeneration might complement therapeutic angiogenesis, based on the administration of recombinant growth factors or on gene transfer, for patients in whom resident endothelial cells fail to sufficiently respond to growth factor treatment. In support of this strategy, it was recently shown that transplantation of young adult BM-derived EPCs reversed the aging-related angiogenic dysfunction by restoring the cardiomyocyte-induced expression of PDGF-B, the latter being important for maintaining vascular integrity [101]. In addition, EPCs might be used as vectors to deliver angiogenic factors to the ischemic tissue thereby providing an additional stimulus for neovascularization [100].

Despite many issues to be clarified, results of the first clinical trials have recently been reported. Strauer et al. saw a decrease in infarct area, better heart function and improved myocardial perfusion after intracoronary transplantation of autologous mononuclear bone marrow cells (consisting of 0.65% AC133+ and 2.1% CD34+ cells) in patients 7 days after myocardial infarction [102]. In a similar set-up, Assmus et al. reported that transplantation of either bone marrow mononuclear cells or blood-derived ex vivo expanded progenitors (of which 90% carried endothelial markers), increased myocardial viability, perfusion and function with comparable efficiency [103]. Although these results need to be confirmed in large-scale randomized placebo-controlled trials, they are encouraging to continue in this rapidly progressing scientific field.

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