The role of stem cells in vein graft remodelling

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Abstract

The vessel wall is a dynamic tissue that undergoes positive remodelling in response to altered mechanical stress. A typical example is vein graft remodelling, because veins do not develop arteriosclerosis until a vein segment is grafted on to arteries. In this process, it was observed that vascular endothelial and smooth muscle cells of vein grafts die due to suddenly elevated blood pressure. This cell death is followed by endothelial regeneration. Central to this theme is the essential role played by EPCs (endothelial progenitor cells) in regenerating the lost endothelium. The mechanisms by which EPCs attach to the vessel wall and differentiate into mature endothelial cells involve increased chemokine production and laminar shear flow stimulation on the vessel wall. It seems that neo-endothelial cells derived from EPCs lack mature cell functions and express high levels of adhesion molecules resulting in LDL (low-density lipoprotein) penetration and mononuclear cell infiltration into the sub-endothelial space. Among infiltrated mononuclear cells, there are smooth muscle progenitors that proliferate and differentiate into smooth muscle cells. Meanwhile, stem cells present in the media and adventitia may also migrate into arteriosclerotic lesions via the vasa vasorum that are abundant in the diseased vessels. However, the molecular events leading to the homing, differentiation and maturation of stem/progenitor cells still needs elucidation. The present review attempts to update the progress in stem cell research related to the pathogenesis of vein graft arteriosclerosis or remodelling, focusing on the mechanisms by which stem/progenitor cells participate in the development of lesions, and to discuss the controversial issues and the future perspectives surrounding this research area.

Introduction

Stem cells have the capability to transform and replenish the different tissue types that make up the body, and also represent the fundamental building blocks of organ development. In general, stem cells can be divided into two broad categories: adult (somatic) stem cells and embryonic stem cells [1]. One of the most fascinating and important aspects of stem cells is their ability to differentiate in vitro, via ‘progenitor cells’, into terminally differentiated somatic cells of all tissue types, including cardiomyocytes, SMCs (smooth muscle cells) [2,3] and endothelial cells [4]. Because of the discovery that stem cells have a role in vascular regeneration, through differentiation into endothelial cells and SMCs, stem cell research could be important not only for understanding the pathogenesis of arteriosclerosis, but also for the advancement of cell-based therapies and tissue engineering.

Autologous vein grafts remain the only surgical alternative for many types of vascular reconstruction, although the patency rate is limited due to obliterative stenosis of the grafted vessels. We demonstrated that the earliest cellular event in mouse vein grafts is cell death, i.e. apoptosis and necrosis [5]. Others have shown extensive loss of endothelial cells in the intima at the early stage of human vein grafts [6]. Following endothelial death is cell regeneration, mononuclear cell infiltration [7] and SMC accumulation which forms arteriosclerotic lesions [8]. Although the vessel wall cells contribute to lesion formation in graft arteriosclerosis, which is also called vascular positive remodelling, recent findings demonstrated the major role of stem/progenitor cells in the process [34]. Together, these findings strongly indicate the contribution of stem or progenitor cells to vein graft remodelling. The aim of this review is to provide an update on the progress in this research field, highlight the role of stem and progenitor cells in the pathogenesis of vascular remodelling, and discuss the mechanisms of stem cell mobilization and homing to the vessel wall.

Endothelial progenitor cells

Accumulating evidence indicates the presence of EPCs (endothelial progenitor cells) in the blood, which have the capacity to proliferate and migrate as well as differentiate into mature smooth muscle and endothelial cells [9–11]. EPCs are characterized by the expression of CD34 and Flk1, two antigens shared by embryonic endothelial progenitors and haemopoietic stem cells [9]. The evidence from two research groups indicates the existence of two types of circulating endothelial cells. Approx. 5% of these circulating cells are bone-marrow-derived EPCs originating from haemopoietic stem cells, which are positive for CD34 and Flk1 [9,12]. A
Vessel segments were harvested from wild-type and Tie2-lacZ mice expressing the lacZ gene only in endothelial cells, and prepared for en face staining with X-gal. Cross-grafting of vein segments between wild-type and Tie2-lacZ transgenic mice indicates that the endothelium in the vessel wall was lost and regenerated by stem/progenitor cells.

Large proportion of EPCs in blood originate from tissues other than bone marrow [13,14]. Recently, it was demonstrated that Flk1+ cells derived from embryonic stem cells can differentiate into both endothelial and SMCs [15], and that progenitor cells in bone marrow of apoE-deficient mice are significantly decreased during aging [16]. In humans, the number of EPCs in blood is inversely correlated with risk factors for coronary heart disease [10,17], e.g. smoking, hyperlipidaemia and diabetes, highlighting the involvement of these cells in cardiovascular diseases.

Stem/progenitor cells as source of lesional cell accumulation in vein grafts

As described above, vascular endothelial cells in vein grafts where biomechanical stress alters may have a higher rate of death. It is a key issue to know how dead endothelial cells are replaced and which cells are responsible for regenerating the endothelium. Owing to a lack of appropriate animal models, it has been difficult to answer these questions. To take advantage of transgenic animals, we developed and characterized a new animal model of vein graft atherosclerosis in wild-type [18] and apoE-deficient [19] mice. The lesion displayed classical complex morphological features and a heterogeneous cellular composition. Furthermore, transgenic mice expressing lacZ genes controlled by specific endothelial or housekeeping gene promoters are now available. These mice express β-gal (β-galactosidase) only in endothelial cells (TIE2-lacZ) [20], SMCs (SM-lacZ) or all types of cells (ROSA26) [21]. When these mice are crossed with apoE-knockout mice, which develop spontaneous atherosclerosis [22], then staining the tissue with X-gal (5-bromo-4-chloroindol-3-yl β-D-galactopyranoside) enables the detection of endothelial cell origins in vein grafts. Using our animal models for vein graft atherosclerosis [18,19], we performed vein isografts in two types of transgenic mice expressing β-gal in endothelial cells, including TIE2-lacZ, TIE2-lacZ/apoE−/− and wild-type mice. We demonstrated that the endothelium on vein grafts completely disappeared due to apoptosis [5], and was replaced by progenitor cells, of which approx. one-third of cells were derived from bone marrow cells [23]. Furthermore, the number of CD34+ and Flk1+ progenitor cells in the blood of apoE-deficient mice were significantly lower than those of wild-type controls, which coincided with diminished β-gal+ endothelial cells on the surface of vein grafts in TIE2-lacZ/apoE−/− mice [23]. These findings indicated the contribution of progenitor cells to regenerate damaged endothelium of the vessel wall (Figure 1).

Homing of EPCs to damaged vessels involving chemokines was documented in animal models. One of them, the
CXC chemokine SDF-1 (stromal cell-derived factor-1), which is essential for stem cell mobilization/homing and organ system vascularization [24–26], is highly expressed in human atherosclerotic plaques and effectively activates platelets in vitro [27]. Therefore a participation of SDF-1 in human atherothrombotic disease has been assumed. On the other hand, NO is thought to be a key regulator in the regulation of endothelial function and development of atherosclerosis. Notably, NO derived from eNOS (endothelial nitric oxide synthase) and iNOS (inducible NOS) is essential for the survival, migration and angiogenic response of mature endothelial cells, and has been implicated in EPC mobilization [28]. Recently, Mayr et al. [29] observed that locally produced VEGF (vascular endothelial growth factor) in vessels is significantly reduced in iNOS−/− mice, which is related to decreased EPC attachment. It has been shown that VEGF production in SMCs could be attenuated by inhibition of NO activity [30], indicating a relationship between these two factors. NO was also shown to be involved early on in angiogenesis, where inhibition of NO activity abolished the increase in capillary proliferation [31]. These results demonstrated that NO is a key factor for VEGF production in vascular SMCs stimulated by cytokines. Thus NO, together with NO-induced VEGF, may synergistically serve as chemokines for EPC homing and differentiation in damaged vessels.

Once EPCs attach to the area of damaged endothelium, they have to differentiate into mature endothelial cells in order to acquire complete endothelial function. I hypothesize that the direction of EPC differentiation can be determined by local microenvironments, e.g. growth factors/cytokines and haemodynamic forces. Yamamoto et al. [32] reported that shear stress generated by blood flow or tissue fluid flow can accelerate the proliferation, differentiation, and capillary-like tube formation of EPCs derived from human peripheral blood. Shear stress markedly increased the EPC expression of VEGFR (VEGF receptor)/KDR (kinase insert domain-containing receptor), ICAM-1 (intercellular adhesion molecule 1) and VE-cadherin (vascular-endothelial cadherin). The mechanisms of shear-induced progenitor differentiation seem to involve several signal initiators and transducers, as identified recently by Zeng et al. [33]. They found shear stress can rapidly activate VEGFR–Akt–eNOS pathways, in which Akt can also induce HDAC3 (histone deacetylase 3) phosphorylation. One of the downstream targets for HDAC3 is p53, which is up-regulated by shear stress, and in turn activates p21. These mechanistic findings for shear-stress-induced stem cell differentiation towards endothelial cells could significantly enhance our knowledge not only for understanding EPC differentiation but also for searching new drugs to promote this process.

Smooth muscle progenitors in blood

It has been believed for a considerable time that the phenotype of SMCs within neointimal lesions differs from medial cells, i.e. contractile and secretory SMCs, which is known to be essential for the migration and proliferation of SMCs in the pathogenesis of arteriosclerosis. However, growing evidence indicates that SMCs in the lesions may be derived from stem cells [34]. Simper et al. [35] demonstrated for the first time that smooth muscle progenitors were present in circulating blood. They looked for these smooth muscle progenitors in the human circulation by culturing mononuclear cells from the peripheral circulation in a PDGF (platelet-derived growth factor)-BB enriched growth medium. Concomitantly, a recent report confirmed the presence of blood smooth muscle progenitors that are derived from CD14/CD105-double-positive cells [36]. Furthermore, they also investigated their integrin profile to provide clues into the homing process of these progenitor cells. Two studies by Simper et al. [35] and Deb et al. [37] showed a distinct profile of these cells from that of endothelial outgrowth cells. Both are in agreement that the β1 integrin is present in greater quantities on smooth muscle outgrowth cells in comparison with endothelial outgrowth cells. The smooth muscle outgrowth cells also showed a greater adherence to fibronectin, which is known to be adhesive with β1 integrin. Further studies might allow the targeting of β1 integrins in vivo and thus prevent differentiating progenitors from entering sites of arterial injury.

Smooth muscle progenitors in the vessel wall

Tintut et al. [38] first identified a subpopulation of vascular cells derived by dilutional cloning of bovine aortic medial cells, and showed that they undergo osteoblastic differentiation and mineralization, which have the potential to produce multiple lineages similar to mesenchymal stem cells but with a unique differentiation repertoire. Moreover, Hu et al. [39] found that the adventitia in aortic roots harbours large numbers of cells containing stem cell markers such as sca-1+, c-kit+, CD34+ and Flk1+ cells. Explanted cultures of adventitial tissues displayed a heterogeneous outgrowth, e.g. the formation of round-shaped cell islands surrounded by fibroblast-like cell monolayers. Isolated progenitor cells were able to differentiate into SMCs in response to PDGF-BB stimulation in vitro. When progenitor cells carrying the lacZ gene were transferred to the adventitial side of vessel grafts in apoE-deficient mice, β-gal+ cells were found in atherosclerotic lesions of the intima, and enhanced the development of atherosclerotic lesions [39]. In addition, a recent study demonstrated that healthy arteries host progenitor cells in adult mouse, termed ‘arterial side-population cells’, which represent approx. 6% of medial cells [40]. In humans, a small number of progenitor cells were identified within neointimal lesions and the adventitia with variable expression of CD34, sca-1, c-kit and VEGFR2 markers, but no CD133 expression [41]. Thus vascular progenitor cells exist within the vessel wall, and an increased number of progenitor cells can be identified in the adventitia of human vessels. These cells might be a source for the SMCs, macrophages and endothelial cells that form atherosclerotic lesions.
Progenitor cell origins of lesional SMCs

Because of the availability of transgenic animals expressing specific markers in the cells, the search for the evidence of smooth muscle progenitors contributing to SMC accumulation in neointima was initiated almost simultaneously by several groups [42–44]. One method for tracing and distinguishing cells makes use of ROSA26 or GFP (green fluorescent protein) mice expressing the lacZ or GFP gene in all cell types. SM-LacZ mice, which only express the lacZ gene in SMCs, also exist. Using such methods, researchers have devised experiments to investigate the contribution of smooth muscle progenitors in vein arteriosclerosis. Hu et al. [42] provided the evidence that SMCs in the neointima were derived from progenitor cells of both the vessel wall and circulating blood.

The mechanisms of smooth muscle progenitor recruitment

Similarly to the recruitment of other cells, chemokines would be needed for progenitor cells to migrate into the intima. PDGF-BB is recognized to be a chemokine for SMCs in vitro and in vivo [45]. Using the in vitro Boyden chamber assay system, we found that PDGF-BB has a role in attracting sca-1⁺ progenitor cells derived from the adventitia, suggesting that PDGF might serve as a chemokine for vascular progenitors [39]. The second candidate protein is SDF-1, which plays a key role in stem cell mobilization and possibly in stem cell homing as well [46]. It has been shown that SDF-1 binds to platelets at the site of injury, triggers CXCR4 (CXC chemokine receptor)- and P-selectin-dependent arrest of progenitor cells on injured arteries or matrix-adherent platelets, preferentially mobilizes and recruits sca-1⁺/PDGFR (PDGF receptor)-β⁺/lineage progenitors for neointimal SMCs without being required for their differentiation [47]. Hence, the SDF-1–CXCR4 axis is pivotal for vascular remodelling by recruiting a subset of SMC progenitors in response to vascular injury, epitomizing its importance for attracting smooth muscle progenitors from circulating blood into the intima of the artery and vein grafts [46].

Conclusion and perspectives

As described above, in response to endothelial damage, EPCs may provide a circulating pool of cells that could form a cellular patch at the site of lost endothelial cells, or serve as a cellular reservoir to replace dysfunctional endothelium covering vein graft neointimal lesions. Circulating EPCs contribute to ongoing endothelial repair [10,17,48–50]. Furthermore, freshly attached EPCs or neo-endothelial cells derived from blood may lack some of the mature endothelial functions [34]. It may take several days or weeks for these neo-endothelial cells to differentiate into mature endothelium, during which LDL (low-density lipoprotein) deposits in the intima, and blood mononuclear cells, including smooth muscle progenitors, adhere to neo-endothelial cells, migrate into subendothelial space, and take up modified LDL to form foam cells (Figure 2). In this process, disturbed blood flow in vascular grafts is a key factor influencing EPC differentiation [34]. During endothelial injury and replacement by EPCs, the vessel wall may also release chemokines, e.g. PDGF-BB and SDF-1, which attract smooth muscle progenitors from the blood as well as the vessel wall via the vasa vasorum. These progenitor-derived neo-SMCs within lesions are immature and produce inflammatory cytokines, i.e. have a secretory phenotype and form foam cells. Thus three major cell components, endothelial cells, SMCs and macrophages, in arteriosclerotic lesions of vein grafts are, at least in part, derived from stem/progenitors, possibly from a common precursor such as the CD14 monocyte.

In summary, the introduction of new techniques has provided a great amount of information at the cellular and molecular levels during the last decade, hence expanding our knowledge of the pathogenesis of arteriosclerosis or vascular positive remodelling. Recent advances in vascular biology namely with regard to the impact of progenitor cells in lesion formation, has formed the basis for the hypothesis presented in this article. By testing further the hypothesis of vessel remodelling, emerging information should permit
development of new diagnostic tools and strategies for prevention and therapy of vascular disease.

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References

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