Regenerative cell therapy and pharmacotherapeutic intervention in heart failure

Part 1: Cardiovascular progenitor cells, their functions and sources

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It has been postulated that bone marrow derived endothelial progenitor cells (BM-EPCs) are essential for neovascularisation and endothelial repair and are involved in pharmacological treatment, and even its potential targets. There is no doubt that the ultimate success of angiogenic cell therapy will be determined by an appropriate stimulation of certain angiogenic progenitor cell subpopulations. Unfortunately, the biology of EPCs is still poorly understood. In particular, the understanding of endogenous microenvironments within the progenitor cell niches is critical, and will provide us with information about the signalling systems that supply a basis to develop rational pharmacotherapy to enhance the functional activity of endogenous or transplanted progenitor cells. The final success of clinical improvement of progenitor cell-mediated vascular repair and angiogenic therapy depends on a better understanding of EPC biology and a smart

therapeutic design. In the first part of this review we first briefly discuss the possible involvement of progenitor cells in chronic heart failure. In part 2 we focus on factors that beneficially affect BM-EPCs, with an emphasis on pharmacological molecular pathways involved in BM-EPC-induced neovascularisation. (*Neth Heart J* 2008;16:305-9.)

Keywords: angiogenesis, bone marrow derived stem cells, endothelial progenitor cells, heart failure

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Chronic heart failure (CHF) is the leading cause of morbidity and mortality all over the world. Although conventional pharmacological intervention has significantly improved the prognosis of patients with CHF, the mortality rate has nevertheless remained high over the last five years.^{1,2} Hence the fields of development of novel therapeutic strategies against CHF remain of utter importance. In the past decades, a novel and potentially beneficial approach has emerged: stem cell therapy. Although stem cell-based therapy provides a promising approach to salvage the damaged heart in most animal experiments, transplantation of autologous bone marrow stem cells (BMSCs) for cardiogenesis or neovasculation has yielded inconsistent outcomes in clinical trials.³⁻⁶ However, compelling new findings on stem cell niches in the heart, homing signals and telomere dysfunction of endogenous stem/progenitor cells⁷⁻⁹ have convinced us to sustain research into strategies for regenerative therapy in CHF.

Current pharmacological therapy in patients with myocardial infarction (MI) is focused on the prevention of ventricular remodelling rather than regeneration. In contrast, stem cell therapy after MI actually aims at repair, and one of its repair strategies, namely that of angiogenesis and/or neovascularisation, is based on

the model of relative ischaemia. In this model, the increased load on the surviving myocardium results in cardiomyocyte hypertrophy. However, the insufficient formation of new capillaries leads to a reduction in capillary density, in turn leading to 'relative' myocardial ischaemia.¹⁰ The relationship between cardiac angiogenesis, cardiac hypertrophy and cardiac function highlights the importance of neovascularisation.^{11,12} Thus, treatment focusing on neovascularisation through stem cell grafting could be a promising strategy in CHF.

The previous identification of endothelial progenitor cells (EPCs), extracted from human peripheral blood, by Asahara et al. in 1997¹³ and subsequent studies, has led to a novel paradigm in the field of vascular biology, namely postnatal vasculogenesis.^{14,15} Numerous studies on EPCs have confirmed the angiogenic potential of this versatile cell. Therefore, stimulation of neovascularisation by EPC, either through direct injection of these cells or through pharmacological intervention on these cells, is an attractive therapeutic target. However, one of the problems of research in this field is that the identity of actual EPCs is still controversial. Different studies use different definitions and consequently different subsets of cells. This may have contributed to the ambiguous outcomes of BM-EPCs therapy in the ongoing clinical trials.^{4,5} Moreover, this complicates the search for suitable pharmacological targets. Thus, it is essential to discuss the definition of EPCs.

Redefining endothelial progenitor cells

Bone marrow (BM) has been considered to be the major reservoir of EPC. An EPC is not a cell with an invariant phenotype but one with preserved full plasticity, which may transdifferentiate into other cell types under diverse microenvironments,^{16,17} in which cell-cell interaction has been postulated to play a vital role.¹⁸ Indeed, the difficulty in defining what an EPC is, is due to the multiple origins and whereabouts of EPC, implicating the existence of multiple identifiers for this cell type.

In earlier studies relatively simple markers to identify EPC were used, amongst which Dil-labelled acetyl low-density lipoprotein (Dil-Ac-LDL) and lectin (either Ulex or BSI) double-positive, cobblestone shaped cells. Being less costly, this method provides a means to evaluate EPC levels in animal species for which there are no suitable antibodies, or in large-scale studies. The method is not a very precise one to define EPC, as it also includes mature endothelial cells, monocytes and macrophages.

More specific EPC markers are summarised in table 1. Importantly, BM-EPC has been shown to share origin and markers with haematopoietic stem cells.¹⁹ Kiel et al. recently reported a method to distinguish HSC from progenitors,²⁰ namely by staining the 'SLAM' membrane receptors (CD150, CD244, CD48) that are not present on EPCs.^{21,22} Furthermore, it has become

Table 1. Markers used to distinguish endothelial progenitor cells (EPCs).

Markers	BM-EPCs	Circulating EPCs (early)	Circulating EPCs (late)
CD133	++	-	-
CD117	++	-	-
Sca-1	++	+	+
CD34	++	+	+
CXCR-4	+	+	+
VEGFR-2	+	+	+
CD31	+	+	+
VE-cadherin	-	+	+
VWF	-	+	+
E-selectin	-	+	+
ENOS	-	+	+
Dil-Ac-LDL	+	+	+
Lectin	+	+	+
CD105	-	+	+
CD45	-	+	-
CD14	-	+	-

clear that EPCs undergo at least three stages during their specific journey of maturation into the endothelial lineage, namely BM-EPC, early and late circulating EPC (figure 1). Late EPCs, characterised by CD34+/CD45-, are different from early EPCs in secreted growth factors and possess outgrowth capabilities.^{23,24} In contrast, the early circulating EPC is a myeloid-derived endothelial-like cell with a limited vascular tube formation capacity on Matrigel.²⁵

More recently, studies from Ingram's group redefined the concept of EPCs,²⁶ using angiogenic potency as a determinant. They distinguished endothelial cell colony-forming units (CFU-ECs), descendents of myeloid cells, from endothelial colony-forming cells (ECFCs), which derive from mononuclear cells, and concluded that ECFCs are the 'real EPCs' able to form perfused vessels *in vivo*.²⁶ This finding underscores that the cell subtype should be cautiously considered in future cell therapy studies and studies that aim at identification of pharmacological target molecules.

Besides the identity of the transplanted EPC itself, the fate of progenitor cells in its resident microenvironments, known as 'niches' that are also involved in the different phases of progenitor cell recruitment, plays a crucial role in the success of cell therapy. From studies on these niches, signal molecules and transduction pathways involved in EPC function can be identified that may provide targets for pharmacotherapeutic strategies. Therefore, we discuss here the sequence of events in progenitor cell recruitment and subsequently we will summarise the niches involved.

Figure 1. EPC mobilisation and interactions between bone marrow niches and ischaemic niches in the heart. In peripheral organs, ischaemic injury provokes increases of angiogenic factors, such as HIF-1 α , SDF-1 and VEGF, which in turn causes activation of matrix metalloproteinase-9 (MMP-9) and increases the cleavage of soluble kit ligand (sKitL, such as stem cell factor), and thus initiates recruitment and mobilisation of bone marrow endothelial progenitor cells (BM-EPCs) from bone marrow niches into the peripheral circulation. The initiation is followed by shedding of c-Kit+/VEGFR2+/CXCR-4+/Sca-1+ progenitors from endosteal or bone marrow stromal niches to the vascular sinusoidal of the bone marrow and their subsequent transmembrane movement compels BM-EPCs to differentiate into circulating EPCs. In bone marrow, after receiving peripheral signals, stem cells divide into a mother cell (self-renewal: semi-circular arrow) and a daughter cell (in this figure a haemangioblast) by asymmetric division. BM-EPCs are of haematopoietic origin and possibly derived from these haemangioblasts, which are characterised by c-Kit+/Sca-1+/Lin-/CD133+/CD34+/VEGFR-2+/CXCR-4+, and capable of giving rise to late endothelial outgrowth. The early circulating EPC is characterised by CD34+/CD45+/CD14+ whereas the late circulating EPC is negative for CD45 and CD14.

Phases in EPC-induced neovascularogenesis and pharmacological intervention

For optimal development and application of pharmacological and/or molecular therapy on progenitor cells, a comprehensive understanding of the basic processes of blood vessel development and repair is essential. The vascular system mainly originates from two fundamental processes: vasculogenesis and angiogenesis.²⁷ Vasculogenesis is defined as differentiation of EPCs and formation of a new, primitive vascular network and is believed to occur in both the embryonic and the postnatal stage.²⁸ During angiogenesis in adult tissues, EPCs are mobilised to a local niche (e.g. the infarcted heart) where they settle to form the primary vascular plexus and to release paracrine, proangiogenic factors.²⁹ This stimulates endothelial proliferation, sprouting or splitting (non-sprouting) of preexisting

blood vessels, a process termed angiogenesis.^{30,31} In summary, cardiac neovascularisation can be divided into five phases, involving various niches:

1. Endothelial permeability and mobilisation of EPCs from bone marrow niches to circulating blood;
2. Homing to the heart;
3. Basement membrane degradation in coronary vessels and proteolysis to allow migration into the cardiac niches or ischaemic niches;
4. EPC/EC proliferation and primary vascular plexus formation;
5. Vessel formation and stabilisation.

Many signalling factors play a crucial role in the different stages of EPC-induced neovascularisation, and most of them are involved in more than one of these phases (see Part 2 of this review, which will be published in the October issue of this journal). Both stimulatory

and inhibitory signals are involved in guiding the processes, and can be found within the various niches. We will now summarise the niches where progenitor cells are found.

Stem/progenitor cell niches in the bone marrow and the heart

The concept of stem cell niches was first proposed by Schofield in 1978.³² In fact, a stem cell niche is not an undefined place in which stem/progenitor cells simply dwell, but a three-dimensionally structured entity where cross-talk occurs between stem cells, and where supporting cells guide or direct both stem cell self-renewal and progenitor cell differentiation after receiving extrinsic signals from the circulatory system.³³ Stem cell niches have been identified in the bone marrow as well as in peripheral tissues, amongst which the myocardium. The stem cell niches in the bone marrow have been intensively investigated. So far, two bone marrow niches have been identified.^{20,34}

Bone marrow niches

Haemangioblasts are the major source for generation of haematopoietic stem/progenitor cells (HSCs/ HPCs) and EPC in the bone marrow. The micro-environment in this bone marrow source is divided into two compartments: the vascular zone and the endosteum zone. The vascular zone is formed of niches that contain endothelial cells and pericytes together with supporting cells, such as bone marrow stromal cells and fibroblasts. The endosteum zone consists of niches with osteoblasts/osteoclasts and their supporting cells.³⁴ The two niches together regulate mobilisation of the otherwise quiescent stem cells (figure 1). In this mobilisation process the oxygen concentration gradient between the osteoblastic niche and the vascular niche is the switch that turns on the differentiation of haemangioblasts into EPCs or the recruitment and differentiation of vascular progenitor cells. This recruitment involves an important signalling mechanism, as outlined in Part 2 of this review.

Another contributor to progenitor recruitment is the osteoclast (figure 1). Bone-resorbing osteoclasts play a critical role in the recruitment of vascular progenitor cells, as further specified in Part 2 as well. Therefore, in bone marrow niches stem cell recruitment is regulated by the balance between the vascular zone and the endosteal zone. Pharmacological stimulation of the niches in these zones may enhance stem cell recruitment after myocardial infarction. Apart from these bone marrow niches, two cardiac stem cell niches have been identified, involving resident cardiac stem cells and bone marrow-derived cells, respectively.

Resident cardiac stem cell niches

The previous notion that the heart is a terminally differentiated organ without self-renewal potential after birth has been challenged by the successful isolation of

cardiac stem cells (CSCs) from adult cardiac tissue.^{35,36} From a therapeutic standpoint, the c-kit⁺ stem cells could bipotentially differentiate into both a EPC and a cardiac progenitor cell (CPC), a progeny of the CSC,^{37,38} which may have more advantages for cell therapy in CHF when compared with the more restricted EPCs. From the stem cell biology point of view, the identification of c-kit⁺ CSCs in cardiac tissue has driven researchers to seek CSC niches in the heart. Until recently, CSC niches in adult mouse heart have been predominantly addressed by Urbanek and co-workers.⁷ Urbanek et al. reported that CSCs niches consist of lineage negative, c-kit positive and stem cell antigen-1 positive (Lin⁻/c-kit⁺/Sca-1⁺) CSC and CPC and extracellular matrix (ECM) components, including fibronectin and a subtype of laminin. Myocytes and fibroblasts serve as supporting cells for CSCs, whereas endothelial and smooth muscle cells are intimately connected with CPCs.⁷ This suggests that cell-specific interactions and, hence, specific signalling mechanisms guide either CSCs or CPCs. The importance of local CSCs in cardiac maintenance and regeneration has been elegantly studied by Hsieh and colleagues.³⁹ Their fate mapping study provides the first evidence that resident CSCs are partly responsible for cardiomyocyte turnover after ischaemic injury, but not during normal ageing.

Although the CSC niche may prove to be an important regenerative tissue, the pharmacological targets to stimulate this source are far from unravelled. Far better characterised, in this respect, are BM-derived progenitor cells that home to the ischaemic myocardium: the postinfarction myocardial ischaemic niche.

BM stem cell recruitment in cardiac ischaemic niches

Endogenous stimuli such as tissue ischaemia have been demonstrated to promote mobilisation of EPCs from the bone marrow to peripheral ischaemic organs.⁴⁰ The circulating number of EPCs is elevated after MI, and further homing to ischaemic niches. This ischaemic niche is crucial for local cardiac neovascularisation, as will be more elaborately discussed below.

The pre-existent coronary collateral vessels support the vascular niche in the infarcted heart, possibly by increased expression of bFGF, and of SDF-1 from engrafted BM-EPC, leading to improved BM-EPC implantation.⁴¹ It has been verified that the efficiency of BM-EPC transplantation depends on the hosting ischaemic myocardium.⁴² Transplantation of BM-EPC gives rise to a transient angiogenic effect as it disappears with transplanted cells fading out, whereas the sustained therapeutic effects are raised and attributed to the endogenous BM cells homing to ischaemic myocardium after BM-EPC transplantation, although definitive evidence has not yet been presented.⁴² Therefore, the local ischaemic niches in the heart play a vital role in cell-based therapy for CHF. Promotion

of a favourable microenvironment in ischaemic myocardium is another potential target for pharmacotherapy.

Pharmacotherapy to improve regenerative therapy can be based on the various signalling factors that can be found in the stem and progenitor cell niches. These signalling factors and beneficial pharmacotherapy will be summarised in Part 2 of this review. ■

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