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Review

Circulating endothelial/skeletal progenitor cells for bone regeneration and healing

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ABSTRACT

An emerging strategy in the regeneration and repair of bone is to use stem cells, including bone marrow mesenchymal stem cells, which are the most investigated and reliable source for tissue engineering, as well as circulating skeletal stem/progenitor cells, which are receiving abundant attention in regenerative medicine due to their ease of isolation and high osteogenic potential. Because failures in fracture healing are largely due to poor vascularization among many environmental factors, we highlight the first proof-of-principle experiments that elucidated the collaborative multi-lineage differentiation of circulating CD34 positive cells – a cell-enriched population of endothelial/hematopoietic progenitor cells – into not only endothelial cells but also osteoblasts. These cells develop a favorable environment for fracture healing via vasculogenesis/angiogenesis and osteogenesis, ultimately leading to functional recovery from fracture. This review will also highlight current concepts of circulating stem/progenitor cell-based therapy and their potential application for bone repair.

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Introduction

Whereas embryonic stem cells in the blastocyst stage can generate into any differentiated cell type, most adult stem cells have a limited potential for postnatal tissue/organ regeneration. Among the phenotypically characterized adult stem/progenitor cells [5,29,54], the hematopoietic system has traditionally been considered as an organized, hierarchical system that is spearheaded by multipotent, self-renewing stem cells at the top, followed by lineage-committed progenitor cells in the middle, and ends with lineage-restricted precursor cells – which give rise to terminally differentiated cells – at the bottom [62]. Recently, however, a new population of stem cells has been added to this schematic, notably, the adult human peripheral blood (PB) CD34+ cells. These cells reportedly contain intensive endothelial progenitor cells (EPCs) as well as hematopoietic stem cells (HSCs) [2], and – as only recently discovered following the discovery of bone marrow (BM)-derived and circulating EPCs in adults – promote embryonic vasculogenesis [1,2,47,50].

A fundamental aspect of tissue engineering research has been to identify various stimuli through which to direct stem cell activity toward tissue regeneration. To this end, recent research has demonstrated that EPCs respond to tissue ischemia as well as cytokines by

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mobilizing from the bone marrow (BM) into PB, ultimately migrating to regions of neovascularization to differentiate into mature endothelial cells and further promote vasculogenesis [57]. As a result of this finding, many researchers have applied EPCs to promote the therapeutic neovascularization in animal models of limb ischemia, myocardial infarction, and liver disorders [3,20,22,24,25,27,28,34,43]. Promising results have particularly been noticed in the immunodeficient rat model of acute myocardial infarction following the transplantation of either human CD34+ cells or EPCs expanded ex-vivo into the site of myocardial neovascularization. Following this implantation, these cells differentiate into mature endothelial cells, augment capillary density, inhibit myocardial fibrosis and apoptosis, and ultimately preserve the left ventricular function [20,24,25,27]. Based on these findings, clinical trial using PB CD34+ cells has been initiated with promising results [30,37,59].

More recent investigations on, promoting tissue neovascularization with EPC has led to broader applications for these cells among various areas of regenerative medicine. Some of these areas include the regeneration of brain tissue, as has been successfully performed by the systemic administration of human cord blood-derived CD34+ cells to immunocompromised mice within 48 h of sustaining a stroke; in these mice, neovascularization was induced along the ischemic zone, providing a favorable environment for neuronal regeneration [56]. Other areas in which PB CD34+ cell transplantation has promoted tissue healing via revascularization include full-thickness skin wounds of diabetic mice [53]. In the latter, our group utilized human cells that were mobilized by the granurocyte colony stinulating factor (G-CSF) contributing to ligament healing via vasculogenesis in the rat medial collateral ligament injury model [58]. Finally, our group has also reported several successful outcomes when utilizing PB CD34+ cells/EPCs for fracture healing [39–41].

In this review, based on our findings and literatures in this fields, we will highlight the concepts of circulating stem/progenitor cell-based therapy and their potential application for bone repair.

Bone and vascularity

Failures in fracture healing are caused due to many systemic and local factors, including immune depression, hormonal milieu, nutrition, mobility, high-energy fracture, extensive soft tissue damage, infection, irradiation, lack of contract between the bone ends, and the actual loss of bone substance. Among them, severe skeletal injuries consisting of fractures with a compromised blood supply result in either delayed unions or established non-unions. An essential requirement for these fractures to heal, then, is to restore the local blood flow, which has traditionally been performed through complex vascular procedures or soft tissue transfers with adequate blood supply in certain types of injuries [16,23]. From the standpoint of regenerative medicine and tissue engineering, neovascularization is accordingly emerging as a bone development and regeneration strategy that appears to benefit quite well from an osteogenic reciprocity that exists between endothelial cells and osteoblasts [23].

The progenitor cell lineages giving rise to endothelial and osteoblastic cells have recently been thought to overlap, contrary to prior beliefs. The data supporting this shifting paradigm has ranged from an analysis of markers for cellular differentiation to data on cell engraftment and isolation from various tissues. *In vitro*, CD34+ and CD133+ cells were not only hematopoietic and vasculogenic, but they were also capable of differentiating into osteoblasts [4,8,60], while *in vivo*, a non-adherent side population of BM cells containing primitive cells was capable of generating into both hematopoietic and osteogenic lineages.[44] Interestingly, purified human hematopoietic/endothelial cells expressing the CD34+ marker have been previously used to generate the antibody Stro-1, which is now widely used to identify mesenchymal stem cells [52]. The combined results from these studies suggest that these hematopoietic/endothelial progenitors may share some phenotypic and perhaps functional (i.e., plutipotency) traits with mesenchymal stem cells, permitting them to give rise to osteoblasts. In a separate series of experiments, a murine BM side population (SP) of cells isolated from murine BM, which contain hematopoietic repopulating cells [17], can engraft in bone after intravenous transplantation [11]; the absence of rejection indicates that these hematopoietic cells may share phenotypic traits that are crucial to prevent immune rejection. Based on these reports, our group sought to confirm the overlapping origin of endothelial and osteogenic markers by running single cell reverse-transcriptase polymerase chain reaction (RT-PCR), and showed that 20% of human peripheral blood CD34+ cells expressed the mRNA for osteocalcin [39].

The presence of the CD34 cell marker among cells with potential for osteogenic differentiation has been further elucidated by other groups. Eghbali-Fatourechi et al. have shown that this marker exists among human circulating osteocalcin or alkaline phosphatase positive cells, and that approximately 40% of osteocalcin positive and 50% of alkaline phosphatase positive cells can be obtained from random blood donors co-expressed CD34., while, conversely, 30% of circulating CD34+ cells co-stain with the osteocalcin antibody [14]. Although several reports suggest that there HSC- or EPC-rich cell populations are committed toward osteogenic differentiation, the morphological and physiological incorporation of these cells for medical application has never been proved until our series [39–41].

As it appears from the above-reviewed studies CD34+ cells are committed to not only endothelial cells but also mural perivascular cells (i.e., pericytes and smooth muscle cells) [20,64]. Similarly, it has been quite recently reported that vascular pericytes may arise from CD34+ cells [19]. In addition to these reports, quite recently, Zengin et al. reported the existence of EPC and stem cells in a distinct zone between smooth muscle and adventitial layer of human adult vascular wall that are capable to differentiate into mature endothelial cells, hematopoietic and local immune cells, such as macrophages [65]. Furthermore, there exists increasing evidence that vascular pericytes are also capable of forming osteoblasts [10], the latter being capable of differentiation into osteoblasts. These findings suggest CD34+ cells may be involved and pooled as vascular progenitor cells in the vascular wall, which transform toward osteogenic lineage in response to various environmental cues.

Circulating BM-derived endothelial/osteoprogenitor cells

Human PB CD34+ cells/EPCs are being investigated to specifically address the problem of delayed and atrophic non-unions in fracture healing, which has a significantly high (5–10%) annual incidence among all long bone fractures and result from an inadequate local blood supply around the zone of injury [38,51]. Because securing an adequate blood supply to this area is crucial for bone healing to occur [9,16], as would be evidenced radiographically by the formation of bridging callus along a former fracture gap, an emerging focus in regenerative medicine is to develop EPCs to promote neoangiogenesis. EPCs are appealing for this task in large part because the link between angiogenesis and the development of native bone on a larger scale has led to the discovery on a cellular level that there exists a developmental reciprocity between endothelial cells and osteoblasts [23]. EPCs are also appealing for this task because a more traditional approach for enhancing the local vascularity along a non-union or delayed union has been to perform vascular bone grafting, which requires painstaking microvascular surgical skills [51].

We first reported that human PB CD34+ cells that were recruited to the fracture site following systemic delivery, developed a favorable environment for fracture healing by enhancing vasculogenesis/angiogenesis and osteogenesis, and finally led to functional recovery from fracture [39] (Fig. 1). Briefly, we systemically transplanted PB CD34+ cells, mononuclear cells (MNCs) or PBS into non-healing femoral fracture model of immunodeficient rats. Bone healing assessed by