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Sonic Hedgehog signaling regulates vascular differentiation and function in human CD34 positive cells

Vasculogenic CD34$^+$ cells with Sonic Hedgehog

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Abstract Identification of pivotal factors potentially present in the in situ environment and capable of influencing the function of CD34$^+$ cells, which can be used for autologous cell therapy, is of paramount interest. SHh is one of the morphogens essential for embryonic vascular development as well as postnatal neovascularization, and the activation of SHh signaling with angiogenic and vascular differentiation responses in CD34$^+$ cells by SHh treatment differed depending on the G-CSF treatment or the background disease. SHh enhanced the migration, proliferation, adhesion, and EPC colony forming capacities of G-CSF mobilized CD34$^+$ cells, increasing the vasculogenic/angiogenic potential for neovascularization. An increase in the differentiation potential of CD34$^+$ cells toward vascular lineages was demonstrated with SHh treatment involving TGFβ signaling pathway. The SHh-activated G-CSF mobilized CD34$^+$ cells directly contributed to vascular regeneration while non-activated CD34$^+$ cells showed a lower regenerative capacity in a mouse ischemic hindlimb model. SHh signaling regulates human CD34$^+$ cell fate and function, and may potentiate the therapeutic effect of G-CSF mobilized CD34$^+$ cells on ischemic diseases.

Abbreviations: SHh, Sonic Hedgehog; EPC, endothelial progenitor cell; EPC-CFA, endothelial progenitor cell-colony forming assay; EC, endothelial cell; VSMC, vascular smooth muscle cell; PTCH, patched receptor; SMO, smoothened; HUVEC, human umbilical vein endothelial cells; G-CSF, granulocyte colony-stimulating factor; HSC, hematopoietic stem cell.

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Introduction

Hedgehog (Hh) proteins are crucial regulators of organ development during embryogenesis. Sonic Hedgehog (SHh) is a prototypical morphogen known to regulate epithelial/mesenchymal interactions during embryonic development of limbs, lung, gut, hair follicles, and bone (Johnson and Tabin, 1997; St-Jacques et al., 1998, 1999). Patched (PTCH) and Smoothend (SMO) are known receptors of SHh, and PTCH inhibits SMO activation of the downstream Gli family transcription factors, which results in the activation of target genes (Bhardwaj et al., 2001; Cohen, 2003). Several recent observations have suggested an involvement of SHh in postnatal neovascularization (Kanda et al., 2003; Kusano et al., 2004, 2005; Mackie et al., 2012; Renault et al., 2010). In addition, exogenous administration of SHh has been shown to induce angiogenesis and accelerate repair of the ischemic myocardium and skeletal muscle (Kusano et al., 2004; Pola et al., 2003). Other reports also demonstrated that SHh gene therapy could significantly accelerate wound healing by inducing arteriogenesis (Asai et al., 2006) or restore nerve function in diabetic neuropathy by promoting angiogenesis (Kusano et al., 2004).

Yamazaki et al. showed that PTCH receptor expression was observed in cultured human peripheral blood-derived endothelial progenitor cells (EPCs) but not in mature endothelial cells (Yamazaki et al., 2008), suggesting that SHh might have a certain effect on human EPCs and $CD34^+$ cells. Indeed, recent studies demonstrated that a combined therapy of SHh gene transfer and mouse cultured EPC transplantation enhanced angiogenesis in ischemic skeletal muscle (Palladino et al., 2012) and that genetically modified human $CD34^+$ cells with SHh preserved cardiac function increasing angiogenesis in the ischemic myocardium following myocardial infarction (Mackie et al., 2012). Based on these evidences, we hypothesized that activation of the SHh pathway during development might regulate the fate of human EPCs, $CD34^+$ cells, and promote vasculogenic activities in ischemic tissues in situ.

CD34 has been recognized as one of the best-characterized human hematopoietic stem cell-related cell surface antigens defined to date (Sutherland et al., 1993). Its expression is down-regulated during hematopoietic development and it is not found on mature peripheral blood cells. In fact, the function of CD34 was reported to be an adhesion molecule that allows stem cells to remain in the proper stromal environment for hematopoiesis in the bone marrow (Gordon et al., 1987), and transplantation of bone marrow- or cord blood-derived $CD34^+$ cells was shown to reconstitute ablated bone marrow following lethal irradiation as part of therapeutic approaches aiming for the cure of hematological disorders involving leukemia (Link et al., 1996), malignant lymphoma (Tomblyn et al., 2007), and refractory anemia (Korbling et al., 1995). On the other hand, putative endothelial cell (EC) progenitors or angioblasts were isolated from adult human peripheral blood for the first time by selection on the basis of expression of the cell surface antigen $CD34^+$ and recognized as EPCs in 1997 (Asahara et al., 1997). Since then, a number of preclinical animal studies (Iwasaki et al., 2006; Jujo et al., 2010; Wang et al., 2010) and clinical studies (Kawamoto et al., 2009; Manginas et al., 2007) have indicated the therapeutic potential of EPCs including peripheral or bone marrow (BM)-derived $CD34^+$ cells for vascular regeneration in the ischemic limb or ischemic heart disease. The several lines of evidences accumulated in these studies clearly supported the therapeutic efficacy of $CD34^+$ cell transplantation in ischemic tissues, however, a clear and distinct mechanism of action for the direct or indirect $CD34^+$ cell contribution to neovascularization remains to be established.

Previous studies identified concurrent dose-dependent effects of $CD34^+$cells on the incorporation into vascular endothelial cells and the vascular recovery of ischemic tissues following transplantations (Iwasaki et al., 2006). However, other studies have demonstrated that the major positive effect of $CD34^+$ cell transplantation on vascular regeneration in ischemic tissues was due to the production of various stem/progenitor chemokines, angiogenesis-related cytokines, angiogenic growth factors, anti-inflammatory cytokines, and survival factor, promoting resident EC proliferation and migration (Ii et al., 2005). We and others have experienced difficulties in regard to the commitment of $CD34^+$ cells into totally differentiated endothelial cells in vitro. Several publications also questioned the involvement of EPCs in new blood vessel formation during tumor angiogenesis or in ischemic diseases, by showing perivascular lodging cell void of endothelial lineage profiles but expressing smooth muscle cell lineage markers (Rajantie et al., 2004).

A possible unifying explanation for these diverse and to some extent controversial aspects of EPC biology may be the current lack of knowledge about the pivotal factors present in the in situ environments of EPCs, depending on as well as influencing the molecular make-up and interactions of the transplanted cells with the recipient tissues, and being thus possibly involved in the induction and specification of the cell fate and regenerative function of EPCs and $CD34^+$ cells in ischemic tissues. We hypothesize that SHh may be one of these pivotal players present in situ and possibly involved in the regulation of the fate of EPCs, promoting the differentiation of $CD34^+$ cells and EPCs into regenerative vascular cells under ischemic conditions. To evaluate the possible effects of SHh on $CD34^+$ derived EPCs, we designed and performed a series of in vitro and in vivo experiments.

Methods

Human recombinant Sonic Hedgehog (SHh) protein

Human recombinant SHh protein, N-terminus (Cat #: 1314-SH-025) was purchased from R&D Systems, Inc. (MN). Human SHh cDNA encodes a 45 kDa precursor protein. An autocatalytic reaction yields a 19 kDa amino-terminal domain SHh-N protein containing cholesterol and palmitate, and a 25 kDa carboxy-terminal domain SHh-C protein. The N-terminal domain retains all known signaling capabilities, while the C-terminal domain is responsible for the intramolecular processing, acting as a cholesterol transferase. SHh can act as both a short-range contact dependent factor and as a long-range, diffusible morphogen.

Cells

G-CSF mobilized human peripheral blood $CD34^+$ cells were purchased from ALL CELLS (Emeryville, CA) and used for