THERAPEUTIC POTENTIAL OF VASCULOGENESIS AND OSTEOGENESIS PROMOTED BY PERIPHERAL BLOOD CD34-POSITIVE CELLS FOR FUNCTIONAL BONE HEALING

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Introduction

Significant proportion (5-10%) of fractures fail to heal and result in delayed unions or nonunions, mainly caused by a lack of vascularization. Human circulating CD34+ cells, which are recently proved to contribute to vasculogenesis under appropriate environment (1), have been also reported to differentiate into osteoblasts (OBs) (2). Therefore, we performed a series of experiments to prove a reasonable hypothesis that functional fracture healing may be supported by vasculogenesis and osteogenesis via developmental plasticity of CD34+ cells.

Material and methods

The institutional animal care and use committees of RIKEN Center for Developmental Biology approved all animal procedures including human cell transplantation.

Isolation of mononuclear cells and CD34+ cells: Mononuclear cells (MNCs) from human peripheral blood (hPB)(human healthy volunteer) are obtained by density gradient centrifugation (1.077g/cm³). CD34+ cells from MNCs are enriched by magnetic cell sorting (auto MACS).

Animal Model: A reproducible model of femoral fracture was created in nude rats (F344/nude rat, 8~12W) with the periosteum cauterized which are obtained by density gradient centrifugation (1.077g/cm³) (MNCs) from human peripheral blood (hPB)(human healthy volunteer) performed a series of experiments to prove a reasonable hypothesis that CD34+ cell fraction had a purity of > 97%, as determined by FACS analysis. RT-PCR analysis of the CD34+ cells revealed weak expression of the human-specific gene of CD31 (hCD31) and osteocalcin (hOC), but not of another endothelial cell (EC) marker, VE-cadherin (hVE-cad), and another bone-related marker, collagen1A1 (hCol1A1) (Fig. 1).

Results

The MNCs contained a CD34+ cell fraction at the rate of < 0.5% and the CD34+ cell fraction had a purity of > 97%, as determined by FACS analysis. RT-PCR analysis of the CD34+ cells revealed weak expression of the human-specific gene of CD31 (hCD31) and osteocalcin (hOC), but not of another endothelial cell (EC) marker, VE-cadherin (hVE-cad), and another bone-related marker, collagen1A1 (hCol1A1) (Fig. 1).

Discussion

Our findings indicate that CD34+ cell fraction contains not only endothelial progenitor cells but also a few number of multipotent stem and/or committed osteogenic progenitor cells, subsequently representing the osteogenic activity under the microenvironment of fracture. This mechanism may, at least in part, contribute to enhanced functional recovery of bone healing following CD34+ cell transplantation. In conclusion, our data suggest therapeutic potential of circulating human CD34+ cells in promoting an environment conductive to neovascularization and osteogenesis in damaged skeletal tissue so that fractures can completely heal.

References


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