Circulating CD34-Positive Cells Promote Early Osteogenic differentiation of Human Fracture Hematoma-Derived Progenitor Cells in Vitro

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INTRODUCTION

Human circulating CD34+ cells, an endothelial progenitor cell (EPC)-enriched population [1], have been also reported to differentiate into osteoblasts [2]. Previously, we demonstrated that human circulating CD34+ cells, systemically transplanted into immunodeficient rats with nonhealing fracture, were recruited into fracture sites, contributed to a favorable environment for fracture healing by enhancing vasculogenesis and osteogenesis, and finally led to functional recovery from fracture [3].

Hematoma occurring at a fracture site is known to play an important role in fracture healing. Recently, we demonstrated that mesenchymal progenitor cells (MPCs) with multilineage differentiation potential exist in human fracture hematoma, indicating its critical role in the process of fracture healing [4]. However, the influence of circulating CD34+ cells on the differentiation of fracture hematoma-derived MPCs (HMPCs) into osteogetic cells in the process of fracture healing is not yet well understood. We therefore studied the effect of peripheral blood-derived CD34+ cells on human HMPCs differentiation towards an osteoblastic phenotype by indirect contact co-cultures.

METHODS

Circulating CD34+ cell preparation: G-CSF-mobilized peripheral blood-derived CD34+ cells from healthy male were purchased from Cambrex Bioscience.

Patient characteristics: This study was approved by the institutional ethical committee and informed consent was obtained from all study subjects. Fracture haematomas were obtained from 3 patients with a mean age of 35.6 years (16 to 49) during osteosynthesis, a mean of 6 days (2 to 10) after fracture. The fracture sites involved were clavicle (1 patient) and fibula (2 patients).

Isolation and culture of human fracture HMPCs: Hematoma which had formed fibrin-clots was removed from the fracture site. Specimens were minced and digested with Collagenase II. Isolated cells were cultured in the growth medium, α-MEM containing 10% fetal bovine serum and antibiotics. At confluence, the adherent cells were harvested with trypsin-EDTA and passaged for further expansion.

Co-cultivation of HCs with CD34+ cells: For non-contact co-culture, HMPCs at passage 2 were seeded into 24-well plates at a density of 3 x 10^4 cells/well. After 24 hour, the upper chambers of 24-well transwell plates (pore size 0.4 μm) were inserted above the HC monolayer and CD34+ cells at a density of 1 x 10^6 cells/chamber were added to the top chambers (Fig. 1). The co-cultures were incubated for 10 days in the growth medium. HCs without co-culture of CD34+ cells served as control.

Culture forming unit-fibroblasts (CFU-F) assay: After co-culturing for 10 days, HMPCs were harvested and replated at 100 cells per 60-cm^2 culture dish. Colony forming unit-fibroblasts (CFU-F) were counted.

Osteogenic stimulation: After 10 days, transwell filter chambers with CD34+ cells were removed, and medium was then replaced by osteogenic differentiation medium consisting of the growth medium, 10 mM β-glycerophosphate, and 50 μg/ml of ascorbic acid.

Alkaline phosphatase (ALP) activity assay: ALP activities of harvested samples were assayed by measuring the release of p-nitrophenol from p-nitrophenyl phosphate as substrate.

Real-time Polymerase Chain Reaction (PCR) Analysis: Expression of osteoblast-related genes, runt-related gene 2 (Runx2), ALP, and GAPDH was measured by real-time PCR. The level of each target gene was normalized to GAPDH levels and expressed relative to the day-0 control culture levels (ΔΔCt methods).

RESULTS

Gene expression of osteoblast-related genes: Real-time PCR analysis revealed that gene expression of ALP and Runx2 in co-cultured HMPCs was significantly higher than in control HMPCs at day 7. The fold change was 4.0 for Runx2, and 2.9 for ALP in co-cultured HMPCs.

DISCUSSION

Our results showed that circulating CD34+ cells enrich MPC population in HMPCs and promote early osteogenic differentiation potential of HMPCs by co-culture, indicating that circulating CD34+ cells may select or prime osteogenic progenitors in the heterogeneous fracture HMPCs. This is the first report that demonstrated the biological response of cells derived from the actual human fracture site to circulating CD34+ cells. This study may provide significant evidence for the critical role of circulating CD34+ cells and fracture hematoma during fracture healing.

REFERENCES: