MULTI-LINEAGE DIFFERENTIATION POTENTIAL OF HUMAN PERIPHERAL BLOOD CD34 POSITIVE CELLS FOR OSTEOGENESIS, CHONDROGENESIS AND ADIPOGENESIS.

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INTRODUCTION

Adult human peripheral blood CD34+ cells are well known to contain intensive endothelial progenitor cells (EPCs) as well as hematopoietic stem cells (HSCs) (1). Although there were few reports that human bone marrow (BM) CD34+ cells could differentiate into osteoblasts in vitro (2, 3) and human peripheral blood CD34+ cells could differentiate into cardiomyocytes (4) and osteoblasts (5) in vivo, differentiation potential of the CD34+ cells into mesenchymal multiple lineages is still controversial. Based on these scientific backgrounds, we performed experiments to prove a hypothesis that granulocyte colony stimulating factor (GCSF)-mobilized peripheral blood CD34+ cells may have potential of osteogenesis, chondrogenesis and adipogenesis in vitro.

MATERIALS AND METHODS

Cell preparation: We prepared GCSF-mobilized human peripheral blood CD34+ cells (CAMBREX).

Pretreatment of CD34+ cells: We prepared for the supernatants of BM mesenchymal stem cell-cultured medium as conditioned medium (CM). We seeded GCSF-mobilized CD34+ cells in standard medium with 10% CM during the first one week of culture. These cells were cultured in the standard medium alone for the next 2 week and then cultured in specific medium for the last 3 weeks.

Osteogenic induction: For osteogenesis studies, $1x10^5$ of pretreated CD34+ cells were placed in six-well plate and cultured in osteogenic medium (alpha-MEM medium supplemented with 10% FBS, 2 mM L-glutamine, 60 µM ascorbic acid, 10 mM beta-glycelophosphate and 0.1µM dexamethasone) for 2-3 weeks.

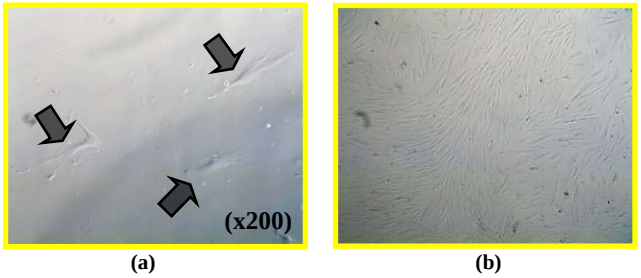
Adipogenic induction: For adipogenesis studies, $1x10^5$ of pretreated CD34+ cells were placed in six-well plate and cultured in adipogenic medium (alpha-MEM medium supplemented with 1 µM dexamethasone, 60 µM indomethacin,5 µg/ml insulin) for 3 weeks

Chondrogenic induction: For chondrogenesis studies, $1x10^6$ of pretreated CD34+ cells were placed in a 15-ml polypropylene tube and centrifuged for 10 minutes. The pellet was cultured in chondrogennic medium: high-glucose DMEM supplemented with 10 µM dexamethasone, 10 ng/ml transforming growth factor-beta 3, 50 µg/ml ascorbate-2-phosphate, 40µg/ml praline, 100 µg/ml pyruvate, and 50 mg/ml ITS+Premix (6.25 µg/ml insulin, 6.25 µg/ml transferring, 6.25 ng/ml selenious acid, 1.25 mg/ml BSA and 5.35 mg/ml linoleic acid) for 3 weeks.

Negative control: Pretreated CD34+ cells were cultured in the standard medium alone for the last 3 weeks.

RESULTS AND DISCUSSION

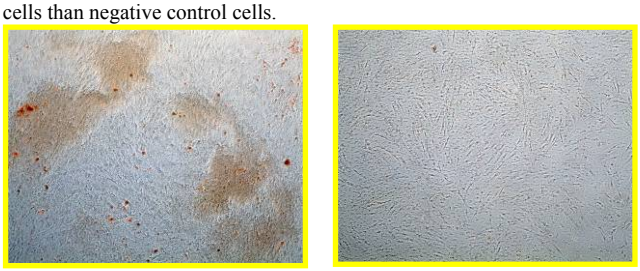
Pretreatment of CD34+ cells: After 2 weeks in primary culture, a part of cells exhibited a fibroblast-like spindle shape (Fig. 1a). These fibroblast-like cells proliferated quickly to form colonies (Fig. 1b).



(a) (b)

Fig. 1: Cell morphology after 14 days in primary culture (x40)

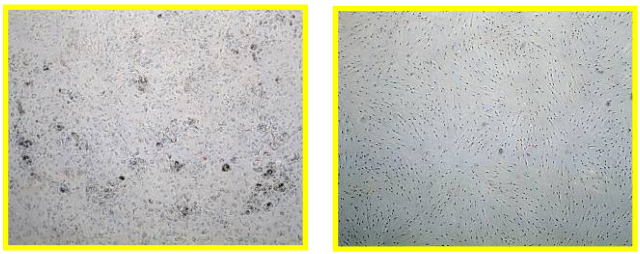
Osteogenic differentiation: After the osteogenic induction for 3 weeks, CD34+ cells demonstrated morphological transformation of the cells from long spindle-like shape to cuboidal one. In such osteogenic cells, matrix mineralization was demonstrated clearly by alizarin red staining for calcium detection (Fig. 2a). In contrast, no mineralization was observed in negative control wells (Fig. 2b). The mRNAs of osteocalcin and collagen A1 was more highly expressed in osteogenesis-induced cells than negative control cells.



(a) (b)

Fig. 2: Osteogenic induction (x40)

Adipogenic differentiation: Fresh oil red O staining revealed existence of the lipid droplets in adipogenesis-induced wells (Fig. 2a), but not in the negative controls (Fig. 2b). The mRNAs of lipoprotein lipase (LPL) and peroxisome proliferator-activated receptor gamma (PPAR γ) were highly expressed in adipogenesis-induced cells compared with negative control cells.



(a) (b)

Fig. 3: Adipogenic induction (x40)

Chondrogenic differentiation: RT-PCR for human-specific chondral markers was performed using pellets cultured in chondrogenic medium or standard one. The mRNAs of SOX9 and collagen type Ⅱ were highly expressed in chondrogenesis-induced cells compared with negative control cells (Fig. 4).

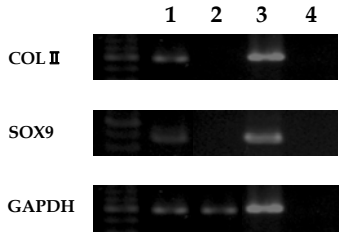


Fig. 4: RT-PCR for chondrogenic markers

CONCLUSIONS

GCSF-mobilized human peripheral blood CD34+ cells known as an enriched population for EPCs and HSCs may also have a potential of differentiating into osteoblasts, adipocytes and chondroblasts. These results suggest usefulness of the CD34+ cell transplantation for tissue regeneration in various orthopedic diseases.

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