学 位 論 文 の 要 旨

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学 位 論 文 名　Effective *Ex Vivo* Expansion of Hematopoietic Stem Cells Using Osteoblast-Differentiated Mesenchymal Stem Cells Is CXCL12 Dependent

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論 文 内 容 の 要 旨

INTRODUCTION

Hematopoietic stem cells (HSCs) are cells that possess both self-renewal and differentiation abilities. The self-renewal ability of HSC, however, was usually impaired in long-term *ex vivo* culture. Recently, cell-to-cell and cell-to-matrix interactions, which allow interactions of adhesion molecules and surface-bound cytokines or chemokines with their receptors, have been focused as an essential event to proliferate and differentiate HSCs. Maintenance of these HSCs is also reported to be regulated and controlled by the bone marrow (BM) microenvironment around the bone surface of epiphysis as called "niche". Therefore, successful expansion of HSCs is essential for coculturing on the cell feeder layer, which is believed to be representative of the stem cell "niche". Mesenchymal stem cells (MSCs) which comprise one of the cell feeder layers might fulfill the requirement of the stem cell-niche by maintaining the necessary hematopoietic microenvironment to support stem cell function. Among BM cell components, osteoblastic cells may be a regulatory component

of the BM microenvironment. We hypothesized that osteogenic differentiated-MSCs can act as stromal cells and support *ex vivo* long-term growth and maintenance of HSCs.

MATERIALS AND METHODS

Human MSC cell line, HM3.B10 (B10) was generated by transformation of v-*myc* gene into primary human BM cells. B10 and osteoblast-differentiated B10 (Ost-B10) were used as a feeder layer. G-CSF-mobilized $CD34^+$ cells from peripheral blood (PB-$CD34^+$ cells) and cord blood-derived $CD34^+$ cells (CB-$CD34^+$ cells) were used as a HSC.

To expand HSCs in vitro, we cultured PB-$CD34^+$ cells and CB-$CD34^+$ cells with several cytokines and feeder layers including B10 and Ost-B10. To investigate the multipotency of HSCs after expansion were assayed by employing methylcellulose culture system with recombinant cytokines. To confirm the self-renewal ability, we performed a modified long-term culture initiating cell (LTC-IC) assay. To demonstrate the effect of CXCL12 which significantly increased in osteoblastic differentiation, we silenced CXCL12 using siRNA of CXCL12.

RESULTS AND DISCUSSIONS

Both B10 and Ost-B10 showed similar efficacy on total HSC expansion in the presence of the cytokine mix, however, Ost-B10 cells showed a higher potency in $CD34^+CD38^-$ cells ,human primitive progenitors, than B10 cells. Cytokines alone failed to expand the HSC, however, the cytokines and feeder cells showed a synergistic effect on HSC expansion. Similar effects of Ost-B10 cells were observed on CB-$CD34^+CD38^-$ cells expansion. Colony forming assay and LTC-IC assay revealed that, osteoblasts differentiation increased the efficacy of colony forming, especially CFU-GM, and clonogenic cells expansion. Importantly, we have established a stable culture system, and can stably maintain the feeder cells in a differentiated state, providing an effective means to expand HSCs and thereby contribute to

HSC-based therapies.

We analyzed the expressional changes of CC and CXC chemokines during osteoblastic differentiation of B10. Osteoblastic differentiation significantly increased several CXC chemokines mRNA expression including CXCL4 and CXCL12.

Because ost-B10 exhibited increased $CD34^+CD38^-$ cell expansion efficiency, and CXCL4 and CXCL12 expression were increased after osteoblastic differentiation, we investigated the role of CXCL4 and CXCL12 on HSC proliferation. HSCs were cultured in cytokine-supplemented medium and on a B10 feeder layer, in the presence or absence of CXCL4 or CXCL12. Although chemokines increased total cell numbers, this trend did not reach significance. Interestingly, $CD34^+CD38^-$ cell numbers were significantly increased with addition of CXCL12, whereas CXCL4 did not show this effect.

To further investigate the significance of CXCL12 on expansion of $CD34^+CD38^-$ cells in HPC culture, we silenced CXCL12 in Ost-B10 feeder cells. The CXCL12 secretion level in Ost-B10 cells was approximately 8-fold higher than in the B10 cells. Silencing CXCL12 in Ost-B10 feeder cells decreased the cytokine secretion to below that in B10 cells, and simultaneously decreased the expansion of $CD34^+CD38^-$ cells. However, although silencing CXCL12 significantly decreased CXCL12 secretion in the B10 feeder cells, the expansion of $CD34^+CD38^-$ cells was not subsequently affected. Our study has established that osteoblast-differentiated MSC-derived CXCL12 contributes to human HSC expansion.

CONCLUSIONS

In this *ex vivo* cell culture system, effective HSC expansion was achieved by using osteoblast-differentiated MSCs as feeder cells in the presence of cytokines, and we demonstrated that maintenance of HSCs by osteoblast-differentiated feeder cells was dependent on CXCL12 expression.