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Ex vivo Expansion of Hematopoietic Stem/Progenitor Cell during the Co-culture with Human Bone Marrow Stromal Cell

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Hematopoietic stem cell (HSC), the representative adult stem cell residing in BM, is used for transplantation to normalize the myeloablated status caused by chemotherapy, autoimmune disease, and genetic defect. With the limitation of stem cell source for transplantation it is needed to expand HSC *in vitro*. Use of cytokines which are well known for their activity on HSCs is believed to be the most reliable *in vitro* culture method. Despite of many valuable progress in *in vitro* expansion of HSC, clinical application of *in vitro* expanded HSC has not been successful. This is attributed to the fact that the regulation of HSC fate in the bone marrow (BM) is a very complex process which many cells and factors participate in and for that reason it is very difficult to set up the *in vitro* culture method to proliferate the HSC still maintaining the stemness. If cytokines alone are not enough to support the *in vitro* expansion, a new strategy must be applied. This can be started from the '*in vivo* mimicry', which means adoption of the *in vivo* mechanism that acts on within the BM to regulate the self renewal and differentiation of HSC to *in vitro* culture method. For this purpose this study was composed to develop the *in vitro* co-culture method for the expansion and/or lineage differentiation of HSC by using bone marrow stromal cell (BMSC), which functions as a supporting stromal layer for HSC in the BM. Epigenetic regulation may have an important role in maintaining the stemness and lineage differentiation of HSCs. Though it has potent activity to maintain the stemness, it also causes severe cell death. This study evaluated the effects of 5-aza-deoxycytidine (aza-D) and trichostatin A (TSA) in the proliferation and maintenance of CD34+ cell driven by the combined cytokines of thrombopoietin (TPO), flt-3 ligand (FL), stem cell factor (SCF), and interleukin-3 (IL-3), and compared the results in concern of single difference, the presence or absence of BMSCs. Though co-culture of HSC with BMSC was always superior than culture without BMSCin concern of total and CD34+ cell expansion, BMSC alone is not sufficient to support the HSC expansion independently, so additional exogenous cytokines are still needed. The limited growth factor production and feedback inhibition in endogenous cytokine production caused by exogenous cytokines may be the one reason. On the whole in presence of exogenous

cytokines, co-culture with BMSC resulted in higher expansion of total nucleated, CD34+, and CD34+38- cells than culture with cytokines alone (without BMSC) or with BMSC alone (without cytokines). During the short term culture CD34+ and CD34+38- cell numbers were increased and maximized till D-6 but afterthen decreased rapidly. This is due to consecutive decline in CD34+ and CD34+38- frequency in spite of the exponential increase in total cell numbers. The addition of aza-D and TSA gave an advantage in retaining CD34+ and CD34+38- frequency highly, but large portion of the cells were faced to apoptotic cell death. During the co-culture BMSC elevated the survival of CD34+ cell by protecting apoptotic cell death caused by aza-D and TSA. In conclusion co-culture system of HSC with BMSC in *in vitro* manipulation of HSC may be beneficial in concern of cell proliferation and survival against intrinsic or extrinsic stress.

Outcomes of HSC expansion in culture containing direct contact between HSC and BMSC, transwell culture, and culture without BMSC were compared to evaluate the role of direct contact and soluble factor from BMSC. Total nucleated, CD34+, and CD34+38- cell expansion were superior in culture containing direct contact with BMSC than transwell culture and culture without BMSC, even though apoptotic cell death in transwell culture was diminished as like in direct contact. These results imply that the role of BMSC for supporting the *in vitro* hematopoiesis as a stromal feeder layer in co-culture is mainly mediated by direct contact and proximal signalling via extracellular matrix (ECM), and soluble factors derived from BMSC may be worked as a protectant against apoptotic cell death induced in HSC.

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