PA-19

Ex Vivo Expansion of Hematopoietic Stem/ Progenitor Cells by Coculture using Insert

Kyung Suk Kim[1], Haekwon Kim[2], Byung Rok Do[3], Seah Park[2], Hyuck Chan Kwon[1], Hyun Ok Kim[4], Jung Ae Im[1]

Medical Research Center, Major Woman's Care Center[1], Department of Biotechnology, Seoul Women's University[2], Biotechnology Research Institute, KTBiosys[3], Department of Laboratory Medicine, Yonsei University[4]

Coculture of HSC with bone marrow-derived mesenchymal stem cells (BM-MSCs) is one of used methods to increase cell numbers before transplant to the patients. However, because of difficulties to purify HSCs after coculture with BM-MSCs, it needs to develop a method to overcome the problem. In the present study, we have examined whether a culture insert placed over a feeder layer might support the expansion of HSCs within the insert. $CD34^+$ cells isolated from the umbilical cord blood by using midiMACS were divided into three groups. A group of 1×10^5 cells were grown on a culture insert without feeder layer (Direct). The same number of HSCs was directly cocultured with BM-MSCs (Contact). The third group was placed onto an insert below which BM-MSCs were grown (Insert). To distinguish feeder cells from HSCs, BM-MSCs was pre-labeled fluorescently with PKH26 and 1×10^5 cells were seeded in the culture dishes. After culture for 13 days, the expansion factor (x) of HSCs that were grown without feeder layer (Direct) was 26.6 ± 8.4. In contrast, the number of HSCs directly cocultured with feeder layer was 59.6 ± 0.5 and that of HSCs cultured onto an insert was 46.9 ± 8.4. The percentage of BM-MSCs cells remained being fluorescent was 97.9 ± 0.3 % after culture. Immunophenotypically large proportion of cultured cells were founded to be differentiated into myeloid/monocyte progenitor cells. The ability of BM-MSCs, fetal lung, cartilage and brain tissue cells to support ex vivo expansion of HSCs was also examined using the insert. After 11 days of coculture with each of these cells, the expansion factor of HSCs was 15.0, 39.0, 32.0 and 24.0, respectively. Based upon these observations, it is concluded that the coculture method using insert is very effective to support ex vivo expansion of HSCs and to eliminate the contamination of other cells used to coculture wth HSCs.

Key words) *Hematopoietic stem cell, Ex vivo expansion*