P-18 Human Amnion-derived Mesenchymal Stem Cells: a Potential New Tool for in vivo Applications

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Background & Objectives: Human Mesenchymal Stem Cells (hMSC) have shown to be capable of differentiating into multiple lineages of cells and thus are potential source of therapeutic uses. Human amnion is an easy-to-obtain novel source of hMSCs and does not pose any ethical issue. In the present study, we isolated human amnion-derived mesenchymal stem cells (hAMC) from amniotic membrane and analyzed their potential for in vivo application. For this purpose, we utilized a lentiviral delivery system.

Method: A human amniotic mesenchymal stem cell line was established from an amnion by enzymatic digestion with trypsin and collagenase A. FACS analyses were initially performed to characterize and quantitate expression of various cell surface markers. For in vivo studies, we established a hAMC cell line expressing EGFP by using a lentivirus system.

Results: Isolated hAMCs were cultured in the presence of 10% FBS and antibiotics in DMEM up to 10 passages. These cells (KU1-hAMC) were similar to MSCs obtained from human bone marrow and other sources, expressing representative mesenchymal cell surface markers CD105 (SH2) and CD90 (Thy-1). KU1-hAMC cells express also CD73 (SH3, SH4), CD49d (integrin subunit), and CD44, but not CD45 (hematopoietic marker), CD34 (hematopoietic/endothelial marker), CD31, and CD106 (Ig superfamily makers). Among three MHC markers, HLA-ABC (class I), HLA-DR (class II), and HLA-G (soluble), HLA-ABC is expressed but not others. Karyotyping of KU1-hAMC at 10th passage showed chromosomal normality. To investigate in vivo application potential for these cells, we infected lentivirus expressing EGFP under the human EF-1 promoter to KU1-hAMC cells. Up to 99% of cells were successfully infected and expressed EGFP. We then transplanted EGFP-expressing KU1-hAMC cells into immunocompetent mice. Intraluminal injection into the uterus and systemic intravenous injection were performed and presence of EGFP-expressing cells within tissues and organs were examined 7~10 days later. Large EGFP-positive cells were observed in the uterus (intraluminal injection) and in the liver (intravenous injection). However, whether these cells have truly incorporated into cell populations within tissues and organs remains to be determined.

Conclusions: Our results demonstrate that human mesenchymal stem cells derived from amniotic membrane are pluripotent stem cells and that these cells can be successfully engineered by application of lentiviral system for in vivo studies. Thus, further investigation on cell type-specific differentiation pathways of hAMC cells will provide new information and tools for therapeutic applications.