P-2 In vitro Differentiation of Human Umbilical Cord-derived Stem Cells and Human Amnion-derived Stem Cells into Hepatocyte-like Cells

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Objectives: Many types of liver diseases can damage regenerative potential of mature hepatocytes, hepatic progenitor cells or oval cells. In such cases, a stem cell-based therapy can be an alternative therapeutic option, a stem cell-based therapy can be an alternative theraperutic option. Hematopoietic stem cells or bone-marrow-derived mesenchymal stem cells have been examined for the potential. However, these cells are not easily obtained or applied. In this study, we examined that human amnion-derived mesenchymal stem cells (HAM) and human umbilical cord-derived stem cells (HUC) could be differentiate into hepatocyte-like cells as new sources of human adult stem cells.

Methods: HAM and HUC were isolated from the amnion and umbilical cord of the volunteers with informed consent after a Caesarean section. In order to differentiate the cells into hepatocyte-like cells, the cells were cultivated in hepatogenic medium using culture plates coated with fibronectin. Effects of hepatocyte growth factor, L-ascorbic acid 2-phosphate, insulin premix (ITS), fibroblast growth factor-4, dimehtylsulfoxide, oncostatin M and/or dexamethasone were examined on the hepatic differentiation. After culture for 3 weeks, the cells were analyzed by RT-PCR, immunocytochemistry, western blotting, human albumin ELISA, urea assay and periodic acid Schiff (PAS) staining.

Results: Initial fibroblast-like appearance of HAM and HUC was changed to round shape during culture in the hepatogenic medium. However, in all hepatogeic conditions, HUC secreted more amounts of albumin into medium than HAM. Urea assay also showed that HUC synthesized more amounts of urea than HAM. Expression of hepatocyte-specific genes was increased or newly synthesized by HUC cultivated in hepatogenic medium. Results of immunocytochemistry and western blotting confirmed that HUC produced albumin. PAS staining also showed that HUC could store glycogen inside of cells.

Conclusion: In conclusion, human adult stem cells were successfully isolated from amnion and umbilical cord. HUC could be better differentiated into hepatocyte-like cells than HAM in our hepatogenic conditions. So, HUC and HAM were could be used as new sources of stem cells for the cell-based therapeutics such as in liver diseases.

P-3 Differentiation of Human Amniotic Membrane-derived Stem Cells after Cryopreservation into Insulin-producing Cells

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Objectives: Beta-cell replacement is a promising approach for treatment of type 1 diabetes. However, the limited supply of suitable donors for pancreatic islets and autoimmune attack of transplanted islets are limitations of this approach. Human amniotic membrane-derived stem cells (HAM) could be a potential source of insulin-producing cells. But, the long-term cultivation of HAM may fail due to many factors. Therefore, it is necessary to cryopreservation of the cells for clinical use.

This study is aimed at investigating the characters and developmental potential of HAM from post-cryopreservation.

Methods: HAM were isolated via enzymatic procedures and cultured, and an aliquot was cryopreserved. After thawing, cells were cultured in adipogenic, chondrogenic and osteogenic inductive media, and also induced to insulin-producing cells under suitable culture conditions. Cells were analyzed by immunocytochemistry, reverse transcriptase-polymerase chain reaction (RT-PCR) and emzyme-linked immunosorbent assay (ELISA).

Results: HAM from pre- and post-cryopreservation had similar cellular morphology and similar expression of protein such as collagen, fibronectin, CD44, CD54, CD106, SSEA3, SSEA4, HLA ABC, vWF and vimentin. RT-PCR analysis of the HAM from post-cryopreservation also showed expression of specific maker, Oct4, SCF, BMP-4, FGF-5, nestin, CK18, similar to HAM from pre-cryopreservation. Under appropriate culture conditions, the cells differentiated into adipocytes, chodrocytes and osteocytes. And in medium supplemented with glucagon-like peptide (GLP-1), activin A and nicotinamide, HAM efficiently differentiated into insulin producing cells and secreted insulin and C-peptide.

Conclusion: Post-cryopreserved HAM still have developmental potential and the cells can be induced to differentiate into insulin-producing cells. So it could be used for further experiment and treatment of type 1 diabetes.

P-4 Directed Differentiation of Mouse Embryonic Stem Cells into Early Progenitors of Three Germ Layers within 3D Matrix

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Objectives: Embryonic stem cells (ESCs) can renew indefinitely and differentiate in vitro into various cell lineages depending on inducing agents used. Current protocols for cell differentiation from pluripotent ESCs involve formation of embryoid body to reinforce cell-cell interactions to generate mixed cell lineages. We hypothesized that it would be possible to achieve homogeneous cell differentiation if ESCs are cultured under a condition in which all the cells receive similar inducing signals in the presence of minimal cell interactions. To achieve this, we developed a direct differentiation culture system using micro-sized 3D matrix from the proliferated ESCs without forming EB.

Methods: Mouse D3 ESCs on STO feeder layer in sDMEM containing LIF and 15% FBS were dissociated completely into single cell suspension to prepare 3D matrix culture. Five to 10 cells were allocated in a 3D matrix gel using a glass micropipette. To prepare RNA, $0.5\sim1\times10^4$ cells were cultured in a milli-sized 3D matrix in a similar manner to that of micro-sized 3D matrix. Directed differentiation of the cells in matrix was achieved by adding 0 (control, vehicle alone), 1, 10 and 500 nM retinoic acid into sDMEM containing 2% FBS without LIF for 4 (4+0) days, respectively. Subsequently, the cells were cultured in sDMEM containing 15% FBS without LIF for 4 (4+4) and 8 (4+8) days to allow further differentiation and analyzed for early germ layer progenitors using various cell lineage markers.

Results: Different concentrations of RA showed less proliferation than that of the culture with LIF, resulting in different cell morphologies visibly. The cells in low RA concentrations at 0 and 1 nM expressed Brachyury at 4+0, whereas the cells in higher RA at 10 and 500 nM expressed nestin in immunocytochemical and RT-PCR analyses. Furthermore, GATA-4 and Foxa2 were intensively expressed in culture at 10 nM RA, suggesting endo-mesodermal cell lineages.

Conclusion: From these results, it was proposed that pure population of early progenitors can be obtained using 3D