화유도하였다.

Results: RT-PCR과 Immunostaing 결과 여러 가지 growth factor가 첨가된 분화유도 조건에서 endothelial marker의 발현임 증가됨을 확인하였으며, FACS analysis 결과 endtothlial marker의 발현을 확인하였다.

Conclusions: 실험결과 VEGF, EGF, bFGF, EPO를 이용하여 혈관형성세포로의 분화를 유도한 결과 혈관세포의 표식인자인 PE-CAM, VE-CADherin, vWF의 발현을 확인하였으며 RT-PCR 결과 혈관세포 특이 유전자인 PE-CAM, Flk-1, Tie-1, 2의 발현이 시간이 지남에 따라서 증가함을 확인하였다. 따라서 인간배아줄기세포에서 혈관형성세포로의 분화유도가 더 효과적으로 이루어 짐을 확인하였다.

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P-20 In Vitro Differentiation of Insulin Producing Cells from Human Embryonic Stem Cells

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Background & Objectives: Human embryonic stem (ES) cells are derived from the inner cell mass of blastocyst and are capable of differentiating into a variety of cell lineages. Human ES cells can be new potential sources for cell therapy to cure degenerative diseases, such as diabetes mellitus. The objective of this study is to improve the culture conditions for differentiation of insulin-producing pancreatic beta cells using human ES cells.

Method: Insulin -producing islet like clusters were induced from human ES cells in vitro by 5-step protocol, as in mouse system. Undifferentiated human ES cells were cultivated on feeder layer of STO cells for 6~7 days (stage 1). Embryoid bodies (EB) were generated in suspension culture for 7 days (stage 2), and transferred to plates with coated poly-L-ornithine and fibronectin in ITSFn medium for 10 days (stage 3). The selected nestin positive cells were expanded in N_2 medium contained B27 supplement and 10 ng/ml bFGF for 6~7 days (stage 4). The pancreatic differentiation and maturation of these cells was carried out by addition of two factors, 10 mM nicotinamide and 10 μM LY294002 (inhibitor of phosphoinoside 3-kinase) into and withdrawal bFGF out of medium for 20 days (stage 5).

Results: Distinct morphology cell clusters were observed after differentiation of final maturation process. Immunocytochemical analysis demonstrated that insulin and glucagon expressed in the all clusters. Cytokeratin19, pancreatic ductal cell marker, is expressed in the cluster. Most cells in the clusters expressed insulin and C-peptide. Expression of glucagon was localized in the some parts of cell clusters. Pancreatic polypeptide (PP) is expressed partially in the cluster. RT-PCR analysis showed the insulin was expressed in

the cell after stage 5, but not on EB at stage 2. Similar results were obtained for expression of glucagon, except for weak expression in EB at stage 2. Expression of amylase, the exocrine enzyme, was observed not only in the EB but also in the differentiated ES cells.

Conclusions: Taken together, human ES cells can differentiate into pancreatic endocrine cells in vitro culture, supporting that human ES cells can be used as a source for replacement therapy of diabetes mellitus.

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P-21 SPA-1 Functions as a Regulator of Human Embryonic Stem Cells Maintenance and Differentiation

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Background & Objectives: In the present study, we showed a regulator which was interested in our unpublished data and suggested that it could be a key molecule for human embryonic stem cells (hESCs) dynamics. We focused on human spa-1 (signal-induced proliferation-associated gene 1) which encodes a 1042-amino acids polypeptide which contains leucine zipper motif, PDZ and Rap1GAP domains. spa-1 is expressed restrictively in human adult tissues (bone marrow, thymus, and spleen) and involved in intracellular signaling cascade. We hypothesized that spa-1 is a candidate for regulator which is involved in hESCs maintenance/differentiation.

Method: spa-1 and germ layer marker genes (pluripotency, endoderm, mesoderm, and ectoderm) expression profiles were analyzed in differentiated hESCs (7, 14, and 21 days embryoid bodies) using semi-quantitative RT-PCR. Also RNA interference (RNAi) was performed to suppress endogenous spa-1 expression in hESCs.

Results: During hESCs differentiation, spa-1 transcripts were decreased and interestingly, its expression pattern was similar to that of pluripotent marker genes (human oct-3/4 and nanog). siRNA expression vector (pSUPER.retro.puro) against spa-1 was transfected into hESCs, and physiological roles of spa-1 in hESCs are under analyses.

Conclusions: According to findings we observed so far, spa-1 might be a new molecule which is involved in the hESC dynamics, and its roles in related to other pluripotent marker genes such as oct3/4 and nanog remain to be elucidated.