their population doubling time and differentiation potential. Here we defined passage 30 to 40 as early passage, passage 80 to 90 as middle passage, and passage 120 to 130 as late passage. Nine colonies of hESC for each passage were observed daily to measure their area. We calculated population doubling time by regression analysis. Number of cells expressing undifferentiated cell markers (SSEA-4 and Oct-4) was determined by flow cytometry analysis. Levels of undifferentiated cell markers such as Oct-4 and Nanog were compared by RT-PCR at each stage of differentiation: undifferentiated hESC, embryoid body (EB) and reattached EB. Immunocytochemical staining was used to evaluate the expression of Oct-4 as an undifferentiated cell marker and nestin as a differentiated cell marker in reattached EBs.

Results: Population doubling time of early passage was longer than of middle and late passage. Cellular morphology was very different among passages of hESC after attachment culture of EB. The majority of reattached EBs of early passage hESC consisted of large and polygonal cells. Reattached EBs of late passage hESC remained in undifferentiated form as small and round cells. hESC of prolonged passage had higher levels of undifferentiation markers than of earlier passage even if differentiation was induced.

Conclusions: We confirmed that hESC accelerates proliferation rate and loses differentiation potential as passage number increases. Therefore, hESC of early passage might be more adequate than middle and late passage for studying of hESC differentiation.

P-27 Directed Differentiation of Human Embryonic Stem Cells into GABAergic Neurons

Cho MS[1,2], Oh SK[2,3], Kim HS[2,3], Seol HW[2], Kim YY[2], Lee L[2], Kim MH[1], Kim DW[4], Moon SY[2,3]

[1]R&D Center, Jeil Pharmaceutical CO., LTD, [2]IRMP, Medical Research Center, Seoul National University, [3]Department of Obstetrics and Gynecology, College of Medicine, Seoul National University, [4]Department of Physiology, College of Medicine, Yonsei University

Background & Objectives: Human embryonic stem (hES) cells may be a good cell source for cell replacement therapy of neurological disorders such as Parkinson's disease and spinal cord injury. In this study, we attempted to manipulate the culture conditions of hES cells at each differentiation stage to efficiently produce neural progenitors and GABAergic neurons.

Method: hES cell colonies were used to form embryoid bodies (EB). Five days after EB formation, EBs were differentiated into neural progenitors through 7-day selection culture and 7-day enrichment culture. Neural progenitors were continuously cultured for an additional 7 days in EB medium containing N2 and B27. The neuronal progenitors were mechanically separated and trypsinized. After replating the separated

progenitors, these cells were cultured for 2 weeks in differentiation medium. Various neural and neuronal markers were confirmed by immunocytochemistry and RT-PCR in each step.

Results: Neural networking was observed in attached EB during neural selection culture. After neural enrichment culture, more than 70% of the cells in network-forming EBs were nestin-positive cells. These progenitors, which express nestin, were cultured in N2 and B27 medium for 7 days to produce neuronal progenitors. Most of these cells had successfully differentiated into mature neurons in differentiation medium. Differentiated cells had expressed beta III-tubulin and neurofilament heavy chain, indicating that these cells were neurons. More than 70% of these mature neurons had expressed GABA.

Conclusions: We developed the controlled differentiation method for efficiently producing neural progenitors and neurons at each step. Using this method, we could get a high percentage of GABAergic neurons from hES cells.

P-28   Differentiation of Cardiomyocyte from Human Embryonic Stem Cell

Kim YY[1,2], Kim HS[1,2], Oh SK[1,2], Park YB[2,3], Cho MS[2,4], Ku SY[1], Kim SH[1], Choi YM[1], Kim JG[1], Moon SY[1,2]

[1]*Department of Ob. and Gyn., College of Medicine,* [2]*IRMP, MRC, Seoul National University, Central Research Institute,* [3]*Sam Jin Pharm. Co. LTD., R&D Center,* [4]*Jeil Pharmaceutical Co. LTD.*

Background & Objectives: Several studies have shown that neuronal cells, cardiomyocytes and pancreatic b-cells can be induced from hESC. In this study, we investigate whether cardiomyocytes can be generated from hESC in vitro and that differentiated cells express cardiac specific markers.

Method: We used hESC, SNUhES3 (46,XY) P87 and P126. To induce spontaneous differentiation, undifferentiated hESC were detached by collagenase IV (2 mg/ml) and cultured in suspension to form embryoid body (EB). After 30 days of suspension culture, EBs were transferred to a 1% gelatin coated Petri dish to allow cells to attach. Differentiated cells were examined for the presence of beating cells and for expression of cardiomyocyte specific genes such as GATA4, Nkx 2.5, cardiac actin, and ANF either by immunocytochemisty, RT-PCR or FACS analysis. Also, action potentials of beating cells were measured. Treatment of attached cells with 1.0 ng/ml of FGF2 and 0.6 ng/ml of BMP2 had increased expression levels of cardiomyocyte specific genes.

Results: SNUhES3-derived cardiomyocyte had expressed cardiac specific genes and had shown presence of beating cells. Beating cells had appeared 20 days after plating and beating had lasted more than 30 days