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.

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P-28 Differentiation of Cardiomyocyte from Human Embryonic Stem Cell

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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

in vitro. Treatments of FGF2 and BMP2 had increased the expression level of cardiac specific markers in cells compared to that of untreated cells.

Conclusions: In this study, we demonstrate that hESC can differentiate into cardiomyocytes in vitro. They differentiate into beating cells and express cardiomyocyte specific genes. Also, treatment with growth factors appear to increase expression level of cardiac specific genes.

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P-29 다양한 인간 배아줄기세포주에서 미분화 및 분화 관련 유전자 발현의 특이성

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Background & Objectives: 인간 배아줄기세포는 유전적 요소, 미분화 유지 조건에 따라 미분화 상태를 유지하는 능력 (stemness)과 분화능력 (differentiation potential)에 있어 각각의 세포주마다 차이가 있는 것으로 알려져 있다. 본 연구에서는 서로 다른 인간 배아줄기세포주의 특이성을 확인하기 위해 배아줄기세포의 미분화 및 분화 표지유전자의 발현양상을 살펴보았다.

Method: 다섯가지 종류의 인간 배아줄기세포주 (SCH-1, SCH-2, Miz-1, Miz-4, Miz-6)를 MEF 또는 STO feeder layer와 공배양하여 미분화 colony, 분화된 colony, 8일간 부유 배양한 배아체 (embryoid body)로부터 RNA를 추출하여 역전사중합효소반응법 (RT-PCR)으로 cDNA를 합성한 후 실시간 중합반응법 (real-time PCR)을 수행하였다. 표지유전자로는 Oct4, Nanog, Sox2, EBAF (LeftyA), Thy1, FGF4, Rex1 등과 같은 미분화 유지 유전자와 Enolase (mesoderm), Alpha-fetoprotein (endoderm), Nestin (ectoderm) 등 삼배엽 분화 유전자를 선정하여 GAPDH에 대한 상대정량법으로 발현 양상을 분석하였다.

Results: 미분화 또는 분화과정에서 대표적인 미분화 표지 유전자인 Oct4와 Nanog 발현양상은 모든 세포주에서 유사하였지만, Sox2, EBAF, FGF4는 각각의 세포주에서 상이하게 발현되었다. 두 종류의 줄기세포주에서는 미분화 유전자로 알려진 Thy1과 Rex1 유전자의 발현이 분화과정에서 증가하는 양상을 나타내었다. 삼배엽성 유전자 중 Nestin은 미분화 colony, 분화 colony, 배아체 모두에서 유사하게 발현되었으며, Alpha-fetoprotein은 배아체 형성 후에 그 양이 유의하게 증가하였다 ($p<0.01$). 중배엽성 유전자인 Enolase는 세 종류 세포주의 분화 colony와 배아체 형성과정에서 발현양이 유의하게 증가됨을 관찰하였다 ($p<0.05$).

Conclusions: 이상의 연구결과로 보아 미분화상태를 유지하고 분화능에 관련된 유전자의 발현은 각각의 인간 배아줄기세포주마다 다소 차이가 있음을 알 수 있었다. 이러한 차이는 유전적 요소와 줄기세포의 확립 과정 및 배양 조건이 각 세포주마다 차이가 있기 때문으로 사료된다. 따라서, 보다 최적화되고 표준화된 인간 배아줄기세포의 체외배양법이 지속적으로 개발되어야 하며, 각각의 배아줄