Method: 영양세포는 STO (CRL-1503, ATCC)를 사용하였으며, 인간 배아줄기세포는 SNUhES3을 이용하였다. STO 세포의 성장억제를 위해 40 Gy (Gray)의 gamma-irradiation을 하거나 mitomycin C (0, 5 mg/ml)를 2시간반 동안 처리해 주었다. 인간 배아줄기세포의 성장을 측정하기 위해서는 세포군집의 면적을 비교하였으며, 그들의 미분화상태를 확인하기 위해 Oct-4, TRA 1-60, SSEA-3 그리고 SSEA-4에 각각 해당하는 항체로 면역염색을 하였다. 면역형광의 강도와 비율분석에는 confocal laser scanning microscope과 flow cytometer가 사용되었다.

Results: 인간 배아줄기세포의 형상은 두 군간에 차이를 보이지 않았다. 군집의 면적변화를 이용해 성장비율을 분석한 결과 인간 배아줄기세포들을 새로운 배양접시로 옮기는 7일째 되는 날에는 두 군 모두에서 3일째된 날과 비교해 같은 면적 증가비율을 나타냈다. 또한 Oct-4, TRA 1-60, SSEA-3, SSEA-4 등 배아줄기세포에서 발현되는 여러가지 미분화 인자들의 발현을 분석한 결과 두 군 모두에서 전사인자인 Oct-4가 핵과 일치하는 위치에 다량 발현되는 것을 관찰하였다. 또 다른 배아줄기세포 특유의 인자인 SSEA-3, SSEA-4와 TRA 1-60도 역시 강하게 발현되는 것을 두 군 모두에서 확인할 수 있었다. 또한 flow cytometer를 이용해 위와 같은 인자들의 발현 정도를 분석한 결과 Oct-4, TRA 1-60, SSEA-3 그리고 SSEA-4가 confocal laser scanning microscope에서 관찰된 결과와 유사한 정도를 나타내고 있음을 확인하였다.

Conclusions: SNUhES3는 Mitomycin C를 처리해준 STO 세포와 gamma-irradiation된 STO 세포위에서 모두 같은 성장 정도를 나타냈으며 배아줄기세포 특유의 인자들이 모두 같은 발현양상을 보여 인간 배아줄기세포가 배양기간 동안 미분화된 상태로 유지되는 것을 보여줄 수 있었다. 따라서 번거로운 irradiation 과정을 거치지 않고 수행될 수 있는 Mitomycin C처리 방법이 영양세포의 성장을 적절히 억제시키고 인간 배아줄기세포의 지지역할을 할 수 있는 적합한 방법임을 확인할 수 있었다.

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P-26 Proliferation Rate and Differentiation Potential During Extended Culture of Human Embryonic Stem Cells

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Background & Objectives: Many studies demonstrated human embryonic stem cells as a promising target for novel strategies in the cell therapy because of its pluripotentiality and infinite proliferative ability. But we encountered changes in proliferation rate and differentiation potential during extended culture of hESC. Thus, we investigated proliferation rate and differentiation potential of successively subcultured hESC.

Method: The hES cell line (SNUhES3) established at Seoul National University was used to determine

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.

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

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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