배양한 후 2.5% glutaraldehyde에 고정하였다. 전자현미경실에 의뢰하여 SNUhES4는 SEM과 TEM을 진행하였고 SNUhES3는 TEM을 찍은 후 분석하였다.

Results: 미분화된 인간배아줄기세포는 SEM을 통해 관찰하였을 때 세포표면이 대사가 왕성한 세포에서 보이는 microvilli가 많이 존재하였고 bleb도 관찰되었다. TEM을 통해 관찰하였을 때 각각의 줄기세포들 대부분은 핵이 차지하였고 상대적으로 세포질의 양은 매우 적어 다른 소기관들은 관찰할 수 없었다. 그러나 세포군의 바깥쪽에 존재하는 세포의 경우는 핵이 길게 늘어져 있고 세포들간에 desmosome이 발달한 상피세포와 비슷한 모양을 보였다. 이 세포들에서도 소기관은 또한 보이지 않았다. 세포군의 전체에서 때로는 세포분열중인 세포가 관찰되었고 phagocytosis나 apoptosis가 일어나는 세포도 관찰할 수 있었다. 한편 분화가 진행중인 배아체의 경우 미분화된 줄기세포에서는 볼 수 없었던 mitochondria, SER과 RER 같은 세포질내 소기관이 발달하였다. 세포들 사이에는 fiber가 많이 발달하여 존재하였으나 세포들이 서로 모여 관이나 막을 형성한 모습도 관찰되었다.

Conclusions: 미분화된 인간배아줄기세포는 TEM사진하에서 세포의 대부분을 핵이 차지하고 다른 소기관이 발달하지 않은 것으로 보아 세포분화의 정도가 상당히 낮음을 알 수 있었으며 분화가 진행됨에 따라 세포질내 소기관이 발달되는 것을 알 수 있었다. 따라서 이러한 세포 각각의 변화를 체계적으로 관찰 한다면 삼배엽으로의 분화유도에 있어서 기초자료로 제공될 것으로 사료된다.

위 연구는 21세기 프론티어연구개발사업단인 세포응용연구사업단의 연구비 지원 (SC11011)으로 수행되었습니다.

P-45 Human Amniotic Fluid Cells Prolonged Expansion Culture of Human Embryonic Stem Cell

SK Oh, HW Seol[2], HJ Ahn[2], YY Kim[2], MJ Kang[1,2], HS Kim[1,2], SY Gu[1,2], SH Kim[1,2], YM Choi[1,2], SY Moon[1,2]

Deprtment of Ob & Gyn. College of Medicine[1], Lab. of Stem Cell, Institute of Reproductive Medicine and Population, Medical Research Center[2], Seoul National University, Seoul, Korea

Background & Objectives: This study was performed to evaluate the possibility of prolonged culture of SNUhES2 cells on human amniotic fluid (HAF) cells (9 passages), which is storaged after karyotyping, with treatment (2.5 hrs) and non-treatment of mitomycin C, respectively.

Method: HAF feeder layer was prepared in the presence of Chang medium (Irvine Scientific) or STO medium (90% DMEM, 10% FBS) at 37℃ in a 5% CO_2 in air atmosphere. SNUhES2 on HAF were passaged mechanically every seven days with ES culture medium (80% DMEM-F12, 20% SR, bFGF).

Results: Both HAF feeders with treatment and non-treatment of mitomycin C support the growth of undifferentiated state of SNUhES2 for at least 24 passages thus far. SNUhES2 colonies on each of HAF feeders appeared slightly angular and flatter shape as compared with circular and thicker colonies observed with STO feeder layer and showed higher level with complete undifferentiation in seven days. Like HES cells cultured on STO feeders, SNUhES2 grown on HAF cells had normal karyotype, tested positive for

alkaline phosphatase activity, detected high telomerase activity, expressed Oct-4, SSEA-3, SSEA-4, Tra-1-60 and Tra-1-81 and formed embryoid bodies (EBs).

Conclusions: HAF cell supported undifferentiated growth of HES cell and therefore these results may help to provide a clinically practicable method to expand HES cells for cell therapies. This research was supported by a grant (SC11011) from stem cell research center of the 21st. century Frontier research program funded by the ministry of Science & Technology, Republic of Korea.

P-46 Transforming Growth Factor-α Increases the Yield of Functional Dopaminergic Neurons from in vitro Differentiated Human Embryonic Stem Cells Induced by Basic Fibroblast Growth Factor

KS Lee, HA Shin[1], HY Cho[1], EY Kim[1], YJ Lee[1], KC Wang[2], YS Kim[3], HT Lee[4], KS Chung[4], SP Park[1], JH Lim[5]

Maria Infertility Hospital Medical Institute/Maria Biotech[1], Seoul National University Deprtment of Neurosurgery[2], Seoul National University Deprtment of Pharmacology[3], Konkuk University[4], Maria Infertility Hospital[5]

Background & Objectives: In this study, we examined the in vitro neural cell differentiation patterns of hES cells (MB03), following induction by basic fibroblast growth factor (bFGF) or retinoic acid (RA). The effects of neurotrophic factors, such as brain derived neurotrophic factor (BDNF) or transforming growth factor (TGF-α), on differentiating hES cells were additionally investigated.

Methods: Exp. I) Embryoid bodies (EB) were derived from hES cells for 4 days. When bFGF was used, neuronal precursor cells were selected for 8 days in ITSFn medium after EB formation. After selection, cells were expanded at the presence of bFGF for another 6 days followed by a final differentiation in N2 medium for 7, 14, 21 days. Exp. II) EBs derived from hES cells were exposed of RA for 4 days, and were allowed to differentiate in N2 medium for 7, 14, 21 days. Exp. III) In addition, to examine the effects neurotrophic factors in the production of mature neurons, groups of cells were exposed to either BDNF or TGF-α during the 21 days of final differentiation.

Results: bFGF or RA treated hES cells were resulted in similar neural cell differentiation patterns at the terminal differentiation stage, specifically, 75% neurons and 11% glial cells. Additionally, treatment of hES cells with BDNF or TGF-α during the terminal differentiation stage led to significantly increased tyrosine hydroxylase (TH) expression, compared to control ($p<0.05$). In contrast, no effect was observed on the rate of mature or glutamic acid decarboxylase-positive neurons. Immunostaining and HPLC analyses revealed the higher levels of TH (20.3%) and dopamine in bFGF and TGF-α treated hES cells than in RA or BDNF treated hES cells.

Conclusion: The results indicate that TGF-α may be successfully used in the bFGF induction protocol to yield higher numbers of functional dopaminergic neurons from hES cells.