P-23 Identification of Supporting Factors for Human Embryonic Stem Cells Maintenance in Feeder Cells

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Background & Objectives: Maintenance of human embryonic stem cells (hESCs) is necessary for their support of feeder cells like mouse embryonic fibroblasts (MEFs). Thus, studies on feeder cell in hESCs research have been actively investigating to set a goal of establishment of human feeder cell line and feeder-free or xeno-free culture system. However, accurate supporting mechanism or factors of feeder cell in hESCs are poorly understood up to date. We investigated for the purpose of searching unidentified supporting factors of feeder cells.

Method: For this experiment, as we selected two feeder cell lines (MEF and STO), it made a comparative study of the difference between induced mitotic arrest and activity groups. For search differentially expressed genes between both groups, we examined using modified Differential Display RT-PCR method (GeneFishing technique), and repeated this experiments three times to each sample.

Results: We performed using 20 of different primers, observed the expression pattern of diverse bands between two groups. We selected 34 genes differentially expressed between two groups including cell-line specific band. For further identification, we eluted them from the gels and sequenced.

Conclusions: In conclusion, the expression patterns of two lines in general were similarly observed, but some differentially expressed bands of each line were identified.

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P-24 Effect of Glucose Concentrations on Human Embryonic Stem Cell Culture

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Background & Objectives: Regeneration therapy is among the most promising approaches currently

being vigorously researched. This approach could guide us towards a fundamental cure for many diseases requiring tissue or organ replacements. Among the potential cell sources, human embryonic stem cells (hESC) could play a necessary part for the therapy as a cell source, especially due to efficient control of development in vitro. The differentiation of hESC into insulin-producing cell (IPC) clusters has been extensively studied for possible treatment of diabetes. But unfortunately, the clusters have yet to show proper function. We focused on long-term exposure of cells to high glucose concentrations affecting the IPC's glucose responsiveness. We believe that this exposure to high glucose levels, common in hESC media, may hinder IPC clusters to secrete insulin upon glucose stimulation. Prior to hESC culture in low glucose conditions, we tested whether that milieu properly maintained hESC characteristics such as pluri-potency and self-renewal over prolonged passages.

Method: SNUhES3, a hESC line established in our laboratory, was gradually adapted into low glucose conditions (5.0 mM) from high glucose conditions (17.5 mM) over 4~5 weeks. The cells cultured in either low or high glucose levels were compared by various methods. Growth pattern and growth rate were checked under microscopy and immunocytochemistry for the protein markers verifying undifferentiated state of the cells. For the differentiated cells, their genetic expression indicating embryonic 3-germ layers were also performed.

Results: Here, we report that SNUhES3 in low glucose concentrations around 8 mM showed normal hESC characteristics as well as karyotype. Furthermore, the cells in lower glucose medium showed higher growth rate by 2-fold and increased production of cystic embryoid bodies amounting to 70% of the population. We believe that these qualities may be merits for the further cell differentiation.

Conclusions: The results suggest that hESC culture in low glucose concentrations would be a practical and effective step in inducing functional IPC clusters.

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P-25 Gamma-Irradiation과 Mitomycin C처리된 영양세포가 인간 배아줄기세포 배양에 미치는 영향

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Background & Objectives: Irradiation은 세포의 성장을 억제하는데 지금까지 널리 사용되어온 방법 중의 하나이다. 그러나 기기사용자가 방사선에 피폭될 가능성이 있기 때문에 안전관리와 사용을 위해 특정한 교육을 받아야 한다는 단점이 있다. Mitomycin C는 항암제의 하나로써 DNA 합성을 차단하여 세포성장을 억제시킨다. 배양기간 동안 배아줄기세포가 미분화된 상태를 유지하기 위해 필요한 영양세포의 성장을 억제시키는 이들 두가지 방법이 인간 배아줄기세포 배양에 미치는 영향을 비교 분석하였다.