P-32 Alteration of Gene Expression Pattern during the Course of Differentiation of hES Cells into Neural Cells

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Objective: Studying molecular mechanisms that regulate cellular differentiation is very important to define differentiation stages of hES cells. Using a microarray-based expression analysis, we have examined differential gene expression profiles during hES (MB03) cell differentiation into neural cells.

Materials and Methods: For microarray, RNA was isolated from each stages of differentiation in vitro; i) undifferentiated hES cells, ii) embryoid bodies (EBs) forming stage, iii) differentiating stage, iv) differentiated cell stage. Growth conditions in each stages were as follows; EBs (for 4 days) were produced from hES cells in suspension culture, differentiating stage cells were in N2 medium supplemented with 20 ng/ml of b-FGF for 7 days on gelatin coated dish, and then finally differentiated cells were grown in N2 neural cell differentiation medium without b-FGF for 2 weeks. To examine gene expression profile during the course of hES cell differentiation, mRNAs from four stages were labeled and competitively hybridized to cDNA chip containing 3,000 mesenchymal cell derived gene sequences.

Results: Analysis of cDNA microarray identified 17 up-regulated genes and 4 down-regulated genes from stage i to ii, 6 up-regulated genes and 8 down-regulated genes from stage ii to iii, while 2 up-regulated genes and 2 down-regulated genes from stage iii to iv. These changes were reproducible and represent changes in the expression of development and differentiation enhancing factor, translation initiation factor, and cell surface antigens.

Conclusion: These identifications provide clues in understanding important molecular information for further functional studies of hES cell differentiation, although it was not sufficient to evaluate the large-scale changes in gene expression during neural cell differentiation.

P-33 *In vitro* Differentiation of P-mES and mES Cells into Functional Neurons

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Objective: This study was to investigate the probabilities of parthenogenetic mouse embryonic stem (P-mES02) cells and *in vitro* fertilization mES (mES01) cells differentiating into tyrosine hydroxylase expressing neuronal cells *in vitro* by several different factors.

Materials and Methods: P-mES or mES cells were cultured in ES media without mLIF 4 days until ES cell aggregations (embryoid bodies, EBs) were formed. To induce the neural cell differentiation *in vitro*, the

EBs were exposed to retinoic acid (RA), ascorbic acid (AA), and lithium chloride (LiCl) for another 4 days (4-/4+ protocol), and then they were dissociated with trypsin followed by culture in N2 medium for 7 to 14 days. Immunocytochemistry assay was performed to identify the properties of the differentiated cells.

Results: Immunofluorescence analysis revealed that increased numbers of MAP-2 (64% and 61%) and GFAP (25% and 20%) were expressed in P-mES cells and mES cells at 14 days of differentiation. Also, at that time, we found that TH induction was increased to nearly 45% and 40%, respectively. while the GABA expression was low (13% and 15%). Especially, in both cell lines, TH expression in RA plus AA treatment group was higher than the other treatment groups (Non, RA, RA + LiCl, RA + LiCl + AA).

Conclusion: P-mES cells or mES cells can be successfully induced into neuronal cells *in vitro*, which could provide resourceful materials for neural transplantation.

P-34 Establishment of hES Cell Lines that Stably Express TH Proteins

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Objective: The most attractive feature of embryonic stem cells would be by far its pluripotency, however, no solid differentiation protocol developed has generated pure culture of any specific cell types. In this study we examined a possibility of using stem cells as a carrier of therapeutic gene in combination with its pluripotency.

Material and Methods: Expression of GTPCH I mRNA was examined by RT-PCR using MB03 in colony forming state and trypsin-dissociated monolayered cells. To introduce tyrosine hydroxylase (TH) cDNA subcloned in pcDNA3.1(+) into MB03, approximately 5×10^4 cells were plated onto p100 dish a day before transfection. For transfection, 6 μg of the transfecting DNA was mixed with FuGene 6 (Amersham) and laid on top of culture medium of MB03. After incubation for 24 hrs at 37℃, transfection medium was replaced with fresh medium containing 400 μg/ml of neomycin. Selection was continued for approximately 15 days until single cell-derived colonies were formed and 8 of them were subcloned using subcloning cylinder. Once expanded, successful transfection was confirmed by western blot analysis using anti-TH antibody. To form embryoid body (EB), 20 μl of medium containing approximately 600 cells were spotted on the lid of culture dish and incubated for 4 days.

Results: In order to examine a usefulness of MB03 for cell therapy for Parkinson's disease (PD), expression of GTPCH I was examined since the enzyme plays a critical role in synthesizing tetrahydrobiopterin (BH4), cofactor of TH activity. By RT-PCR, not only trypsin-dissociated and monolayered MB03 expressed GTPCH I mRNA but MB03 in colony state also expressed the mRNA. However, TH expression was neither detected by RT-PCR nor by western blot analysis. Expression of GTPCH I is of a great advantage for using in PD cell therapy that MB03 was transfected with TH cDNA. After selection with G418, over 50