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

neomycin resistant colonies were formed, 8 of them were subcloned, and were named TH#1/03 trough TH#8/03. By western blot analysis, 4 out of the 8 colonies selected were confirmed to express TH protein. Protein expression level of TH#2/03, TH#3/03, and TH#5/03 were approximately same as PC12 but that of TH#8/03 was about 20%. Upon establishment of subclones, expressions of GTPCH I mRNA of TH#2/03 and TH#8/03 were reconfirmed by RT-PCR. In addition, TH#2/03 and TH#8/03 clones formed EBs successfully by hanging drop method suggesting that the transfected cells retained aggregating nature of stem cells through the transfection procedure.

Conclusion: Ever since the establishment of stem cell lines, trials for generation of specific cell types did not yield satisfactory results to use in cell replacement therapy. As an alternative approach, it may be worthwhile to explore genes that are constitutively expressed and can be used for therapeutic purpose in stem cells. By this way, a simple transfection, the stem cell may provide an important clue in conquering the formidable task of human being, that is incurable disease.

P-35 Genetically Modified Human ES Cells Relieve Asymmetric Motor Behavior of PD Animal: An Implication for Carrier of Therapeutic Genes

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Objective: Since the establishment of embryonic stem cells, most of studies were concentrated on differentiation protocol, for inducing expression of genes of interest. However, in this study, we examined a possibility of using the cells as a carrier of therapeutic gene.

Material and Methods: In order to introduce therapeutic genes, human embryonic stem cells grown in monolayer were simultaneously transfected with TH and GTPCH I cDNA subcloned in pcDNA3.1(+) and pcDNA3.1(+)-hygro, respectively. After 24 hrs of transfection, transfected cells were selected using G418 (400 μg/ml) and hygromycin B (150 μg/ml) for over 15 days. Once all of the non-transfected cells died, transfected cells were expanded. Successful transfection was confirmed by western blot analysis for tyrosine hydroxylase (TH) and RT-PCR for GTP cyclohydrolase I (GTPCH I). For transplantation, PD animal models were generated by injecting 6-OHDA into two sites within substantia nigra pars compacta (AP: 4.4, ML: 1.2, DV: 7.8 tooth bar set at -2.4, and AP: 4.0, ML: 0.8, DV: 8.0, tooth bar set at 3.4) of a rat brain. Two weeks after the induction of lesion, apomorphine- and amphetamine-induced rotational behaviors were examined. Rats rotating more than 180 revolutions per hour by apomorphine and more than 300 revolutions per hour by amphetamine were selected for experiment. For transplantation, transfected cells were labelled with BrdU (50 μM) for 2 days before transplantation. Cells were implanted into two spots within striatum (AP: 1.0, ML: 3.0, DV: 5.0 and AP: 0.6 ML: 2.0, DV: 4.5) and behaviors of the animals were examined two weeks thereafter followed by immunohistochemical studies.