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

마리아 생명공학연구소/마리아 기초의학연구소, [1]건국대학교, [2]마리아 병원

최경희 · 이영재 · 김은영 · 이창현 · 길광수 · 조현정
박세필 · 정길생[1] · 임진호[2]

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.

Results: After transfection and selection for 15 days, approximately forty five drug-resistant colonies were formed. To avoid individual difference, all of the colonies were combined and named bk-THGC/03. By western blot analysis, expression level of TH protein in bk-THGC/03 was approximately 10% of PC12 but that of GTPCH I was in a similar level to PC12. By microscopic examination, a dramatic change of cell shape was noticeable showing lots of vacuole-like structures within cytoplasm. In addition, bk-THGC/03 grew much slowly (doubling time of an approximately 40 hrs) than MB03. Upon transplantation, bk-THGC/03 soothed asymmetric motor behavior of PD animal model, while the asymmetric behavior of sham-grafted animals worsened. Immunohistochemical studies revealed that the transplanted cells retained TH expression at least for two weeks in the rat brain. Furthermore, anti-BrdU staining showed presence of human cells within the striatum of experimental animals.

Conclusion: This study showed that embryonic stem cells can be modified genentically in vitro and can be used as a carrier of therapeutic gene for cell replacement therapy as well as their pluripotency.