embryo bodies (EB: for 5 days, without mitogen) from hES cells and then neurospheres (for 7~10 days, 20 ng/ml of bFGF added N2 medium) from EB. And then for the differentiation into neuronal cells, neurospheres were cultured in N2 medium (without bFGF), supplemented with brain derived neurotrophic factor (BDNF, 5 ng/ml) or platelet derived growth factor (PDGF, 20 ng/ml) for 1 or 2 weeks. Identification of neural cell differentiation was carried out by immunocytochemistry using human nestin (1:100; Chemicon), β_{III}-tubulin (1:250; Sigma), MAP-2 (1:1000; Sigma) and GFAP (1:500; DAKO). It was carried out using standard protocol.

Results: In vitro neural cell differentiation derived from hES cells, neurotrophic factor (PDGF and BDNF) treated neural progenitor cells were high expressed β_{III}-tubulin, MAP-2 and GFAP. Especially, the cells in the presence of BDNF were expressed with MAP-2 and β_{III}-tubulin.

Conclusion: These results suggest that BDNF as well as PDGF related to neural development in neurospheres derived from hES cells.

O-18 Human Embryonic Stem Cell Transplantation in Parkinson's Disease Animal Model: I. In vivo Transplantation in Intact Rat Brain

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

최경희 · 주완석[1] · 김용식[1] · 김은영 · 박세필 · 임진호[2]

Objective: This study was to examine whether the in vitro differentiated neural cells derived from human embryonic stem (hES, MB03) cells can be survived and expressed TH in grafted intact rat brain.

Materials and Methods: To differentiate in vitro into neural cells, embryoid bodies prepared from hES cells were cultured in b-FGF added N2 medium for three weeks. Being grafted neural cells were devided into early (10 days) and late neural cells (>20 days) according to culture duration and their cell types were determined by indirect immunocytochemistry. For transplantation, neural cells (1×10^7 cells/ml) in both groups were grafted to the striatum of intact rats. Based on this data, as a preliminary test, early neural cells were grafted to the striatum of 6-hydroxydopamine (6-OHDA) lesioned (parkinson's disease; PD) rats. After 2 weeks, we confirmed the survival, neural pattern and TH expression of grafted hES cells in intact rats or PD rats by immunohistochemical analysis.

Results: When the survival of grafted hES cells was examined, the early neural cell group indicated higher NeuN+, MAP2+, tubulin-βIII+ and GFAP+ than late neural cell group in grafted sites of intact rats. This result demonstrated that the differentiation of grafted hES cells be increased simultaneously in both of neuronal and glial cell type. Early neural cell grafted intact rats expressed TH. Also, we confirmed that the early neural cells grafted in PD rats expressed TH although the analysis term of grafted hES cells is too short.

Conclusion: This result suggested that in vitro differentiated early neural cells derived from hES (MB03) cells can be survived and expressed TH in intact and PD rats.