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Improvement of Motor Behavior of Parkinson's Disease Animal Model by Nurr1-Transfected Human Embryonic Stem Cells

Chang Hyun Lee, Hwang Yun Cho, Yong Sik Kim[1], Eun Young Kim, Won Don Lee[2], Sepill Park and Jin Ho Lim[2]

Maria Infertility Hospital Medical Institute/Maria Biotech,

[1]Seoul National University, College of Medicine, [2]Maria Infertility Hospital

The purpose of this study is to evaluate an efficacy of *in vitro* differentiated human embryonic stem (hES, MB03) cells expressing Nurr1 in relief of symptomatic motor behavior of Parkinson's disease (PD) animal models. To enhance the direct differentiation of hES (MB03 registered in NIH) cells to dopamine-producing neuronal cells, Nurr1 cDNA (pcDNA3.1 (+)-hyg) was transfected using conventional transfection protocol into MB03 cell. Expression of Nurr1 mRNA was confirmed by RT-PCR and protein by immunocytochemistry in the drug resistant clones. For *in vitro* differentiation, one of the positive clones (Nr#24/MB03) was allowed to form embryoid body (EB) for 2 days and were induced to differentiate for another 4 days using RA (1 μM) and AA (50 mM). Additionally treated in N2 medium for 20 days, cells immunoreactive to anti-GFAP, anti-TH, or anti-NF200 antibodies were 28.7±2.1%, 15.0±3.1% and 44.8±2.9%, respectively, but 8.0% of MB03 cells expressed TH protein in the same differentiation protocol (P<0.05). In order to examine therapeutic effects of the differentiated cells in PD animal model, rats were unilaterally lesioned by administration of 6-hydroxydopamine HCl into medial forebrain region. Confirmation of successful lesion by apomorphine-induced rotational behavior, differentiated cells were transplanted into the striatum (AP 1.0 mm, ML 3.5 mm, DV -5.0 mm; AP 0.6 mm, ML 2.5 mm, DV -4.5 mm). Improvements of asymmetric motor behavior by the transplantation were examined every two weeks after the surgery. In two weeks, numbers of rotation by the experimental rats were -14.8±33.9% (p<0.05) of the

number before transplantation, however, the ratio increased slightly to 13.6±56.3% in 8 weeks. In contrast, the ratio of sham-grafted animals ranged from 112.3±8.5% to 139.2±28.9% during the examination. This result suggests that Nr#24/MB03 cells differentiated *in vitro* were survived at least for 8 weeks when grafted into brains of PD animal model, and that symptomatic motor behavior was improved.

Key words) *Human embryonic stem cell, Parkinson's disease, Nurr1, Motor behavior*