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Genetically Modified Human Embryonic Stem Cells Expressing Nurr1 and Their Differentiation into Tyrosine Hydroxylase Positive Cells *In Vitro*

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

Maria Infertility Hospital Medical Institute/Maria Biotech, [1]Maria Infertility Hospital

The objective of this study is to test whether human embryonic stem cells expressing Nurr1 (Nurr1-transfected hES cells) could be expressed TH according to neuronal differentiation. As an effort to direct differentiation of hES (MB03 registered in NIH) cells to dopamine-producing neuronal cells, Nurr1 was transfected using conventional transfection protocol into MB03 cell and examined the expression of tyrosine hydroxylase (TH) after differentiation induced by retinoic acid (RA) and ascorbic acid (AA). Experimentally, cells were transfected with linearized Nurr1 cDNA in pcDNA3.1(+)-hyg overnight followed by selection in medium containing hygromycin-B (150 μg/ml). Expression of Nurr1 mRNA was confirmed by RT-PCR and protein by immuocytochemistry in the drug resistant clones. In transfected cells, 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) (2-/4+ protocol) followed by selection in N2 medium for 10 or 20 days. After 10 days in N2 medium, cells immunoreactive to anti-GFAP, anti-TH, or anti-NF200 antibodies were 38.7±2.8%, 11.0±1.2%, and 20.5±1.4%, respectively. After 20 days in N2 medium, cells expressing GFAP, TH or NF200 were 28.7±2.1%, 15.0±3.1% and 44.8±2.9%, respectively but approximately 8.0% of MB03 expressed TH protein when the cells were induced to differentiate using a similar protocol ($P<$ 0.05). These results suggest that ectopic expression of Nurr1 enhances generation of TH+ cells as well as neuronal cells when genetically modified hES cells were differentiated by 2-/4+ protocol.

Key words: *Parkinson's disease, Embryonic stem cell, Nurr1, TH*