

**Optimization of Human Embryonic Stem Cells into Differentiation of
Dopaminergic Neurons *In Vitro*: II. Genetically Modified
Human Embryonic Stem Cells Treated with RA/AA or b-FGF**

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Since the establishment of embryonic stem cell, pluripotency of the cells was known to allow differentiation of the cells into various cell types consisting whole body. Several protocols have been developed to induce expression of specific genes. However, no precise protocol that will generate a single type of the cells from stem cells has been reported. In order to produce cells suitable for transplantation into brain of PD animal model, which arouse due to a progressive degeneration of dopaminergic neurons in midbrain, human embryonic stem cell (hESC, MB03) was transfected with cDNAs coding for tyrosine hydroxylase (TH). Successful transfection was confirmed by western immunoblotting. Newly transfected cell line (TH#2/MB03) was induced to differentiate by the two neurogenic factors retinoic acid (RA) and b-FGF. Exp. I) Upon differentiation using RA/ascorbic acid (AA), embryoid bodies (EB, for 4days) derived from hES cells were exposed to RA (10^{-6} M)/AA (50 mM) for 4 days, and were allowed to differentiate in N2 medium for 7, 14, 21, or 28 days. Exp. II) When bFGF was used, neuronal precursor cells were selected for 8 days in N2 medium after EB formation. After selection, cells were expanded at the presence of bFGF (20 ng/ml) for another 6 days followed by a final differentiation in N2 medium for 7, 14, 21 or 28 days. By indirect immunocytochemical studies, proportion of cells expressing NF200 increased rapidly from 20% at 7 days to 70 % at 28 days in RA/AA-treated group, while those cells expressing NF160 decreased from 80% at 7 days to 10% at 28 days upon differentiation in N2 medium. However, in differentiation by RA/AA treatment system, there was a significant increase in proportion of neuron maturity (73%) at day 14 after N2 medium. TH#2/MB03 cells expressing TH are >90% when matured at the absence of either bDNF or TGF- α . These results suggested that TH#2/MB03 cells could be differentiated *in vitro* into mature neurons by RA/AA.

Key words) *Human embryonic stem cell, Genetic modification, Dopaminergic neuron, Retinoic acid, Immunocytochemical study*