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Optimization of Human Embryonic Stem Cells into Differentiation of Dopaminergic Neurons *In Vitro*: I. Additive Effect of Neurotrophic Factor on Human Embryonic Stem Cells

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Embryonic stem cells are capable of differentiating into a variety of cell lineages. However, the ultimate results of differentiation in vitro greatly depend on the duration of treatment and kinds of differentiating inducers added. In order to investigate the efficiencies of various differentiation inducers and the methods of treatment, we examined differentiation patterns of human embryonic stem cell (hESC, MB03) according to several different protocols. Exp. I) Upon differentiation using retinoic acid and ascorbic acid (RA/AA), embryoid bodies (EB, for 4days) derived from hESC was exposed to RA (10^{-6}M) and 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. Exp. III) In addition, to examine the effects of neurotrophic factors in the production of mature neurons, groups of cells were exposed to either BDNF (5 ng/ml) or TGF-α (10 ng/ml) during the 28 days of final differentiation. Differentiation patterns of RA/AA or bFGF treated groups were very similar; approximately 82% and 83% of the cells, respectively, were positive for anti-NF200 antibody, while it was about 10% and 11%, respectively, for anti-NF160 antibody in 28 days in N2 medium. Alsor, cells expressing TH were as low as 5%, while the cells doubled when matured at the presence of either BDNF or TGF-α. Cells immunoreactive to anti-GAD antibody were approximately 20%. These results suggest that a maturation step rather than differentiation induction step, which is formation of EB, effects more decisively to the ultimate differentiation pattern.

Key words) ***Human embryonic stem cell, In vitro differentiation, TH, GAD, Dopaminergic neuron.***