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In Vitro Neural Cell Differentiation of Genetically Modified Human Embryonic Stem Cells Expressing Tyrosine Hydroxylase

Hyun-Ah Shin, Keum-Sil Lee, Hwang-Yun Cho, 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

This study was to examine *in vitro* neural cell differentiation pattern of the genetically modified human embryonic stem cells expressing tyrosine hydroxylase (TH). Human embryonic stem (hES, MB03) cell was transfected with cDNAs cording for TH. Successful transfection was confirmed by western immunoblotting. Newly transfected cell line (TH#2/MB03) was induced to differentiate by two neurogenic factors retinoic acid (RA) and b-FGF. Exp. I) Upon differentiation using RA, embryoid bodies (EB, for 4 days) derived from TH#2/MB03 cells were exposed to RA (10^{-6} M)/AA (5×10^{-2} mM) for 4 days, and were allowed to differentiate in N2 medium for 7, 14 or 21 days. Exp. II) When b-FGF was used, neuronal precursor cells were expanded at the presence of b-FGF (10 ng/ml) for 6 days followed by a final differentiation in N2 medium for 7, 14 or 21 days. Neuron differentiation was examined by indirect immunocytochemistry using neuron markers (NF160 & NF200). After 7 days in N2 medium, approximately 80% and 20% of the RA or b-FGF induced Th#2/MB03 cells were immunoreactive to anti-NF160 and anti-NF200 antibodies, respectively. As differentiation continued, NF200 in RA treated cells significantly increased to 73.0% on 14 days compared to that in b-FGF treated cells (53.0%, $P<0.05$), while the proportion of cells expressing NF160 was similarly decreased between two groups. However, throughout the differentiation, expression of TH was maintained ($\geq$90%). HPLC analyses indicated the increased levels of L-DOPA in RA treated genetically modified hES cells with longer differentiation time. These results suggested that a genetically modified hES cells (TH#2/MB03) could be efficiently differentiated *in vitro* into mature neurons by RA induction method.

Key words: ***Human embryonic stem cell, TH, Differentiation, RA, b-FGF***