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In Vitro Neural Cell Differentiation Derived from Human Embryonic Stem Cells: I. Effect of Neurotrophic Factors on Neural Progenitor Cells

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This study was to investigate the effect of neurotrophic factors on neural cell differentiation *in vitro* derived from human embryonic stem (hES, MB03) cells. For neural progenitor cell formation derived from hES cells, we produced embryoid bodies (EB: for 5 days, without mitogen) from hES cells and then neurospheres (for 7 - 10 days, 20 ng/ml of bFGF added N2 medium) from EB. And then finally for the differentiation into mature neuron cells, neural progenitor cells were cultured in i) N2 medium (without bFGF), ii) N2 supplemented with brain derived neurotrophic factor (BDNF, 5ng/ml) or iii) N2 supplemented with platelet derived growth factor-bb (PDGF-bb, 20ng/ml) for 2 weeks. Identification of neural cell differentiation was carried out by immunocytochemistry using human nestin (1:100; Chemicon), β_{III}-tubulin (1:250; Sigma), MAP-2 (1:100; Sigma) and GFAP (1:500; DAKO). It was carried out using standard protocol. When *in vitro* neuron cell differentiation from neural progenitor cells was examined by double staining, neurotrophic factors (PDGF-bb and BDNF) treated cell groups were high expressed MAP-2 and GFAP than non-treated cell group. Especially, the highest expression pattern of MAP-2 and β_{III}-tubulin was indicated in BDNF treated group. These results suggest that BDNF as well as PDGF-bb related to the *in vitro* neural cell development in neural progenitor cells derived from hES cells.

Key words) ***Human embryonic stem cell, Neural progeniter cell, In vitro differentiation, Neurotrophic factor, Mature neuron cell***