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# Induction of Tyrosine Hydroxylase by Nurr-1 in hES Cells

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As an effort to direct differentiation of human embryonic stem cells (hES, MB03) to dopamine-producing neuronal cells, we expressed Nurr-1 in hES and examined the expression of tyrosine hydroxylase (TH) after bFGF induction. To introduce Nurr-1, hES cells were maintained in humidified chamber with 5% $CO_2$ and 95% air in DMEM/F12 supplemented with FBS (10%), penicillin (100 U/ml), and streptomycin (100 $\mu$g/ml). They were plated on p60 to have approximately $1.0 \times 10^5$ cells on the day of transfection. For transfection, Nurr-1 cDNA in pcDNA3.1-hyg (InVitrogen, USA) was mixed with transfecting reagent and added directly onto the culture medium. After 18-24 h, transfecting medium was replaced by selection medium containing 100 $\mu$g/ml of hygromycin B and left under selection until all of the non-transfected cells died. In order to see the effect of Nurr-1 on the expression of TH, successfully transfected cells were expanded and stained with $\alpha$-Nurr-1 antibody (Santa Cruz. USA) and $\alpha$-TH antibody (sigma. USA). Based on immunocytochemical staining, transfected hES cells were indistinguishable from non-transfected hES cells in terms of its morphology and expression of nestin, $\beta$ III-tubulin, and GFAP. These results suggest that the transfection procedure and/or the ectopic expression of Nurr-1 does not exert any effects on the differentiation and/or morphology of hES cells. However, double staining revealed that the Nurr-1 positive MB03 was also TH-positive, suggesting that an ectopic expression of Nurr-1 induced expression of TH whose expression is rather confined within dopaminergic cells. Therefore, this result suggests that Nurr-1 may be implicated in the transcriptional control of TH gene expression.

Key words) ***Human embryonic stem cell, Nurr-1, Tyrosine hydroxylase (TH), Trnasfection***