retinoic acid (RA), a known stimulant, on differentiation into cardiomyocytic lineage were assessed by adding at a concentration of 0.75% and 10^{-6} M, respectively. Contracting areas were mechanically dissected and then enzymatically dispersed using trypsin-EDTA. Cells were plated on glass coverslips, incubated for 48 h fixed using 4% paraformaldehyde and incubated with primary antibodies for 1 h at 37℃. Staining of sarcomeric α-actinin was performed using anti-sarcomeric α-actinin mAb's at a dilution of 1:800. After three washes with PBS, cells were incubated with FITC-conjugated anti-rabbit IgG antibodies for α-actinin. Preparations were examined using fluorescence microscopy.

Results: Rhythmically contracting areas appeared at 15-17 days after plating. Spontaneously contracting areas appeared in 9.3% (DMSO) and 2% (RA) of the EBs, respectively. Cells from the spontaneously contracting areas within EBs were stained positively with anti-α-actinin.

Conclusion: This study showed that the parthenogenetic mouse ES cell-derived cardiomyocytes displayed structural properties of cardiomyocytes and that the DMSO enhanced development of cardiomyocytes.

P-14 Induction of Tyrosine Hydroxylase by Nurr-1 in hES Cells

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Objective: 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.

Materials and Methods: 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 μg/ml). They were plated on p60 to have approximately 1.0×10^5 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 μg/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 α-Nurr-1 antibody (Santa Cruz. USA) and α-TH antibody (sigma. USA).

Results: Based on immunocytochemical staining, transfected hES cells were indistinguishable from non-transfected hES cells in terms of its morphology and expression of nestin, βIII-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 were also TH-positive, suggesting that an ectopic expression of Nurr-1 induced expression of tyrosine hydroxylase whose expression is rather confined within dopaminergic cells.

Conclusion: Therefore, this result suggests that Nurr-1 may be implicated in the transcriptional control of TH gene expression.