in differentiation medium were examined by RT-PCR, some specific factors represented three embryonic germ layers were determined in vitro (NF-M, keratin, enolase, cAct and amylase).

Conclusion: The established hES cells derived from frozen-thawed blastocysts can be maintained on feeder-free condition without loss of human cell characteristics.

P-12 In vitro Differentiation of Human Embryonic Stem Cells into Cardiomyocytes

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Objective: This study was to investigate differentiation of human embryonic stem (hES) cells into cardiomyocytes according to treatment factors and culture duration.

Materials and Methods: To differentiate into cardiomyocytes, embryoid bodies induced from established hES (MB03) cells were cultured in 0.75% DMSO, 0.75% DMSO + 1 μM retinoic acid (RA), 1 μM retinoic acid (RA) and 10 ng/ml bFGF added DMEM (+10% hyclon FBS) medium for 1, 2 and 3 weeks. To demonstrate differentiation into cardiomyocytes, we did RT-PCR with primers about cardiomyocytes once a week for 3 weeks.

Results: In RT-PCR, MLC-2A and MLC-2V as cardiomyocyte markers were expressed in all groups from 1 week. Especially, cardiac actin was just expressed in 0.75% DMSO treatment from 2 weeks.

Conclusion: This study showed that DMSO has an effect on differentiation of hES cells into cardiomyocytes from 1 week to 2 weeks.

P-13 Functional Cardiomyocytes Formation Derived from Parthenogenetic Mouse Embryonic Stem Cells

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Objective: This study was to establish the use of parthenogenetic mouse ES cells as a reproducible differentiation system for mouse cardiomyocytes.

Materials and Methods: To induce differentiation, parthenogenetic mouse ES cells were dispersed by dissociation and the formation of ES cell aggregates in differentiation medium. After 7 days in differentiation culture, the embryoid bodies (EBs) were plated onto gelatin-coated dish. Cultures were observed daily using an inverted light microscope to determine the day of contraction onset and total duration of continuous contractile activity for each contracting focus. The effects of dimethyl sulfoxide (DMSO) and

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.