Tubule Formation on Matrigel The Matrigel was added to each well of a 24 well tissue culture plate and allowed to solidify at 37℃ for at least 30 minutes. Following gelatinizing, 0.2 ml of a cell suspension containing $5\times10^4\sim1\times10^5$ D3 ES cells were placed on top of the Matrigel. Immunostaining Cells were fixed, permeabilized, then incubated at room temperature for one hour with primary antibodies, washed, and incubated for 30 minutes in the secondary antibody. After a final wash, cells were observed by fluorescence microscopy. Reverse Transcriptase-Polymerase Chain Reaction Total RNA was extracted from differentiated EBs with TRIZOL. Reverse transcription was performed using an Superscript II. RT product was used to perform PCR with a Taq DNA polymerase. An initial 3 minute 94℃ hotstart was used followed by cycles consisting of 45 seconds denaturation at 94℃, 45 seconds annealing at 55℃ and 45 seconds extension at 72℃. A 10 minute extension was done at 72℃ after the final cycle. 35 cycles were done for HPRT, Oct-4, Tie-2, VE-cadherin, PECAM and AC133.

Results: 1. EGM-2 culture conditions were effectively induced endothelial differentiation in mES cells. And differentiated mES cells were expressed endothelial specific genes, such as PECAM, VE-cadherin, Tie-2 and AC133. 2. Immunofluorescence data demonstrate several endothelial markers during EB differentiation, reaching a maximum at days 5+2~5. Oct-4, which specific transcription factor and key control molecule for undifferentiation was also expressed during EB formation. However, level of expression were sequentially decreased during cultivation. Some endothelial specific markers were expressed in high levels (Flk-1, PECAM, VE-Cadherin) orlower levels (Tie-2, CD34) became notable after EB formation and differentiation.

Conclusions: We established more simple, efficient differentiation methods of endothelial progenitor cells from mouse ES cells using EGM2 media. Thistool can provide of mass differentiation source of endothelial progenitor cells.

P-24 In vitro Differentiation of Human Embryonic Stem Cells into Osteoblast-like Cells

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Background & Objectives: Bone is maintained by a balance between the synthesis of bone matrix by the osteoblasts and degradation by osteoclasts. Recently, osteogenesis-related transcription factors and osteoblast-specific markers has led to a rapid advancement in understanding the process of osteoblast differentiation. However, the early steps of osteoblast differentiation remain to be identified. In this study, we examine the osteoblast differentiation process in human embryonic stem cells. This experiments have been optimized the culture conditions to achieve osteoblast differentiation from human embryonic stem cell, as measured by both the deposition of a mineralized bone matrix, and the expression of osteoblastic marker genes.

Method: 1) Cell culture and differentiation of embryonic stem cells. Human ES cells were grown on

STO cells in DMEM/F12 media (Gibco) supplemented 20% Serum Replacement (Gibco), 1 mM L-glutamine (Gibco), 1% nonessential amino acids (Gibco), 100 mM b-mercatoetanol (Gibco), and 4 ng/ mL bFGF (Sigma). To induce formation of EBs, ES cells were transferred to plastic Petri dishes to allow their aggregation and prevent adherence to the plate. Human EBs were grown in the same culture medium, except that it lacked bFGF. The EBs were cultured for 3 days and then plated on tissue culture plates coated with Gelatin (Sigma). After 2 more days, the cells were grown in the presence of 0.05 mM AA (Sigma), 10 mM bGP (Sigma), 10^{-7} M Dex, and 5×10^{-8} M VD3 (Sigma). 2) Histochemical analysis. Mineralized hES cells were detected by the Von Kossa method (2% silver nitrate solution, Sigma) and by Alizarin Red S staining (1% Alizarin Red S solution, Sigma) for mineralized 3) RNA extraction and RT-PCR. The extraction of RNA was performed using Trizol (Invitrogen) following the instructions of the manufacture. After DNAse (Invitrogen) treatment of the samples, reverse transcription was carried out using Superscript Reverse Transcriptase (Invitrogen). PCR reactions (20 μl) were set up using Tap DNA polymerase (Takara). Standard PCR conditions were as follows; 3 min at 95℃; followed by cycles of 30 sec denaturation at 95℃, 45 sec annealing at 60℃, and 1 min extension at 72℃. The primer sequences were as follows: beta-actin (F: GCTCGTCGTCGACAACGGCT, R: CAAACTTGATCTGGGTCATCTTTCTC), OCT4 (F: GAGA-ACAATGAGAACCTTCAGGAGA, R: TTCTG GCGCCGGTTACAGAACCA), OPN (F: CATCTCAG-AAGCAGAATCTCC, R: CCATAAACCACACTATCACCTC), Cbfa1 (F: CCGCACGACAACCGCA-CCAT, R: CGCTCCGGCCCACAAATCTC), BSP (F: CAGTAGTGACTCATCCGAAG, R: CTCCTCT-TCTTCTTCATCAC), bALP (F: GGGGGTGGCCGGAAATACAT, R: GGGGGCCAGACCAAAGA-TAG), OC (F: ATGAGAGCCCTCACACTCCTC, R: GCCGTAGAAGCGCCGATAGGC)

Results: 1. The comparison of cultured cells between base media with Dex/ VD3 and control media showed different morphologies. hES cells treated with Dex/ VD3 had more spindle-shaped cells which is derived from mesenchymal origin. 2. The deposition of calcium and phosphate minerals detected by Von Kossa staining and Alizarin Red S staining. No calcification was seen in control cultures without additives. The number of calcified cells appeared to be greater after addition of bGP, AA, and additional Dex/ VD3 to the culture system. 3. In order to further confirm lineage commitment, we have analyzed the expression of osteoblastic specific markers. hES cultures exposed to base media with Dex/ VD3 showed as increase in the expression level of a range of osteogenic markers: transcription factor involved in osteogenic commitment (Cbfa1), elements of the matrix mineralization process (OC, OPN, BSP).

Conclusions: We present the conditions to enable the differentiation of hES cells into osteoblast like cells. We showed that Dex/ VD3 added with base media enhances the deposition of a bone matrix. In vitro differentiation of ES cells into osteoblast-like cells should provide a powerful model system to study the early events regulating the decision of pluripotent stem cell to differentiate into osteocytic lineage.