P-6 Shh Gradients Guide Differentiation of Motoneuron with Different Positional Identities from P19 EC Cell Derived-neurospehres

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Objectives: Motoneurons are the key effector cell type to control motor functions occurring in majority parts of the body. Thus, loss in the motoneurons results in varying degrees of neurological disorders including amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy. Recent studies have demonstrated that motoneurons can be generated from both mouse and human ES cells, functionally active in vitro, and eventually incorporated into developing embryo upon transplantation. With the advent of refined conditions for directed cell differentiation for pluripotent cells in vitro, we examined a possible procedure to direct motoneuron differentiation from P19 embryonic caricinoma stem (EC) cells with positional information as a well-established developmental model in vitro.

Methods: P19 EC cells were cultured in α-MEM containing 10% fetal bovine serum (FBS) for proliferation, and neurosphere (NS)-forming cells were generated using NBM medium with 2% B27 for 2 to 4 days and stored for subsequent cell differentiation. From the NSs, further neuronal differentiation was induced under varying conditions including EGF/bFGF, EGF/bFGF/LIF, EGF/PDGF and bFGF/PDGF for 12 days.

Results: The NSs generated from P19 EC cells not only expressed robustly neural cell lineage markers including nestin and Sox2, but also showed bromodeoxyuridine (BrdU) incorporation in most of cells in the NS. The differentiating cells showed expressions of Sox2, Nkx3.1, Ngn2, Nestin, Tuj1, and MAP2b. Further directed motoneuron differentiation was induced in the NBM medium mentioned above containing conditioned medium obtained from the culture of Shh-overexpressing HeLa cells. Analysis of the motoneurons generated with different Hox gene markers suggests that varying Shh concentration gradients drive the cells to motoneurons with different positional identities.

Conclusion: It was proposed that the new procedure should provide motoneurons that are more appropriate in a specific part of the body when transplanted.

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P-7 Guidance of Axon Growth on SiO2 Micropattern Surfaces During Differentiation of Neuroblastoma N2a Stem Cells

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Objectives: During embryogenesis and postnatal development, it is known that growth of neuronal axon is guided by various chemical cues including intrinsic and extrinsic molecules. Other mechanism(s) may be involved in this process although the other exact factors causing the axonal guidance remain obscure at present. To investigate whether the physical

geometry could contribute differentiating neuronal cells, we used mouse N2a neuroblastoma stem cells on different micropatterns of convex pillars or walls.

Methods: N2a cells were plated on poly-L-lysine-coated SiO2 surfaces at a concentration of 1×10^5/ml. After the cells were attached firmly for 18h, the cells were induced to differentiate into neurons in the presence of dibutyryl cyclic AMP (dbcAMP) and 17β-estradiol for 48~96 h. The cell were fixed in 2% paraformaldehyde after washing in phosphate buffered saline, pH 7.4, and stained with a lipohillic dye, 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO) to clearly show axonal growth. Cells were also analyzed by scanning electron microcopy.

Results: Different patterns of SiO2 gave a marked guidance of axonal growth according to the formed walls of SiO2. The micropatterns also influenced orientations of axonal growth and neurite development.

Conclusion: Thus, the results suggest that physical microenvironment could also serve as a mechanical cue for the guidance of axonal growth in differentiating neuronal cells.

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P-8 Maintenance and Differentiation of Embryonic Stem Cells without Feeder Layer

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Objectives: Although certain cell lines from mouse (mESCs) and human embryonic stem cells (hESCs) can be grown under feeder-free conditions, most of the cell lines can be maintained only on various feeder cells. Feeder-free ES cell culture system is one of critical components towards generating and maintaining clinical grade of hESC lines for the application of cell replacement therapy in near future. To overcome the feeder-dependent or conditioned medium culture systems for proliferation and differentiation of ESCs, we employed a mixture of polymeric natural product gel as a substrate to hold ESCs in suspension to develop a feeder-free system.

Methods: Both mESC (J1 and D3) and hESC lines (Miz-Med-4 and 6) were cultured according to conventional ES cell culture system established in sDMEM containing LIF. These cells were encapsulated in the suspension matrix gel in either single cells or multi-cells in the matrice depending on the sizes of matrix gel.

Results: Both J1 and D3 mESCs were capable of proliferation under the identical condition used in conventional culture without feeder layer. hESCs (Miz-Med-4 and 6) also showed cell proliferation as judged by diameter of the cell colony formed within the matrix even from a single cell. The mES cell colony formed within the matrix showed pluripotent cell lineage makers, including alkaline phosphatase, SSEA-1, nanog, Oct-4 and Sox2, commonly used. Upon the removal of LIF from the medium, both mESCs and hESCs showed varying degrees of cell differentiation depending on the duration of induction of cell differentiation.

Conclusion: The developed feeder-free ESC culture system allowed us to eliminate preparation and maintenance of feeder layer for cell proliferation, and subsequent steps of embryoid body formation and replating ESCs onto substrates for cell differentiation. The newly-established ESC culture system should provide opportunities to develop clinical grade of