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

Sun Min Jung[1], Byung Hee Hyun[1], Sung Sik Choi[1], Dong Ho Lee[1], Ji Yoon Kang[2], Sang Ho Lee[1]

[1]School of Life Sciences and Biotechnology, Korea University, Seoul 136-701,
[2]Intelligent Microsystem Center, KIST, Seoul 136-791, Korea

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

Dong Ho Lee[1], Jin Woo Lee[2], Sung Sik Choi[1], Sang Ho Lee[1]

[1]School of Life Sciences and Biotechnology, [2]Department of Electrical Engineering,
College of Engineering, Korea University, Seoul 136-701

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