matrix by both minimizing cell-cell interactions and providing a uniform milieu to differentiating ESCs with the inducing agent.

* Supported by the 21st C Frontier R&D projects (Korea Ministry of Commerce, Industry and Energy) and partially by RCTCP, Chungnam National University (KOSEF).

P-5 Isolation of Single Cell-derived Mouse Embryonic Stem Cell Clones from Mixed Cell Population

Sung Sik Choi[1], Byung Hee Hyun[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, KIST, Seoul 136-791, Korea

Objectives: Since mouse embryonic stem cells (ESCs) have generally been cultured on the feeder layer in vitro for long time, the pluripotency of ESCs depends entirely on the quality of the feeder layer. Therefore, minor alterations in the microenvironment of feeder layer could affect status of the cells, generating slightly different cell lineages without notice. Thus, some of the ESC lines turn into preferring to differentiate into certain directions upon induction. It would be a laborious task to separate pluripotent cells from the mixed cell population with early germ layer stem cells. In particular, establishment of a single cell-derived cell clones should be a useful tool to study further. To isolate single cell-derived ESCs, we used a 3D matrix culture system in present study.

Methods: Mouse J1 ESCs were cultured on STO feeder layer in sDMEM containing LIF and 15% FBS for proliferation. To obtain stable GFP expressing J1 ESCs (J1-GL), cells were selected with G418 for 1 week. 3D matrix culture was performed by making single cell suspension for every 4 days. At the end of culture, high or low of GFP expressing cell clones were selected for candidate colonies. They were then used for further proliferation and establishment of single cell-derived ESC lines, designated J1-GL-H and J1-GL-L, respectively.

Results: The isolated J1-GL-H and J1-GL-L ESCs showed similar proliferation rates to those of wild type and the mother cell clone (J1-GL). To verify whether these cell lines have pluripotency as in wild type J1 or original J1-GL, various stem cell markers including alkaline phosphatase, SSEA-1, nanog, Oct-4 and Sox2 were examined cytochemically and at RNA level. Upon retinoic acid (RA) treatment with different concentrations, the established cell lines underwent differentiation into 3 germ layer lineages.

Conclusion: Taken these results including cell proliferation and differentiation together, the developed technology should be useful in establishing homogeneous cell population and isolation of single cell-derived clone safely without using feeder layer cells. Furthermore, the ESC culture system should provide opportunities toward obtaining clinical grade of human ESCs as well as studying signaling molecules in a controlled way by eliminating cell-cell interactions and growth factors secreted by feeder layer.

* Supported by the 21st C Frontier R&D projects (Korea Ministry of Commerce, Industry and Energy) and partially by RCTCP, Chungnam National University (KOSEF).