S 11

Sang Ho Lee

School of Life Sciences & Biotechnology, Korea University

1989-Present	Assistant, Associate Professor and Professor, School of Life Sciences & Biotechnology, Korea University, Seoul, 136-701, Republic of Korea
1996-1997	Visiting scholar, Department of Anatomy, University of Cambridge, Cambridge, U.K.
1992-1992	Visiting scientist, Department of Animal Production, University of Dublin, Dublin, Ireland
1989-1990	Non-clinical research scientist, MRC Experimental Embryology & Teratology Unit, St. George's Hospital Medical School, University of London, London U.K.
1983-1989	Visiting scientist, MRC Experimental Embryology & Teratology St. George's Hospital Medical School, University of London, London U.K.
1983-1989	Department of Anatomy & Human Biology, Division of Biomedical Sciences, King's College, University of London, U.K. (PhD, Developmental Biology)
1978-1980	Korea University (MSc, Reproductive Biology)

Isolation and Therapeutic Use of Neural Stem Cells from Mixed Culture of Differentiating Embryonic Stem Cells*

Sang Ho Lee

School of Lifer Sciences and Biotechnology, Korea University, Seoul, Korea

One of many obstacles to develop stem cell therapy using embryonic stem (ES) cells is to obtaining a specific lineage of cells to match the damaged tissue. There has been no way of non-invasive isolation of a specific lineage of cells due to the lacks of technology into a single lineage of cell differentiation and cell surface markers for the target cell. In particular, because the properties of neural cell lineages are not all understood and differentiation *in vitro* is not perfect, neural stem cells (NSCs) may be a good target for clinical use in regenerating the damaged tissue as a neural progenitor. Bearing this in mind, we have been investigating the possible use of neuro-specific promoters, including tubulin α1 (Tα1) and neurotrimin (Ntm) genes for the isolation of NSCs from mixed cell population of differentiating mouse embryonic stem cells and grafting them into animal model. To initiate the study, pluripotent P19 embryonic carcinoma (EC) cells were first used by inserting green fluorescent protein (GFP) driven by Tα1 promoter. After four days of retinoic acid treatment, GFP was specifically detected in cells undergoing neuronal differentiation. Sorting of fluorescent differentiating P19 EC transfectants yielded populations highly enriched in neuronal precursors and neurons showing nestin and neurofilament in 80 and 25% of the sorted cell population, respectively (Fig. 1).

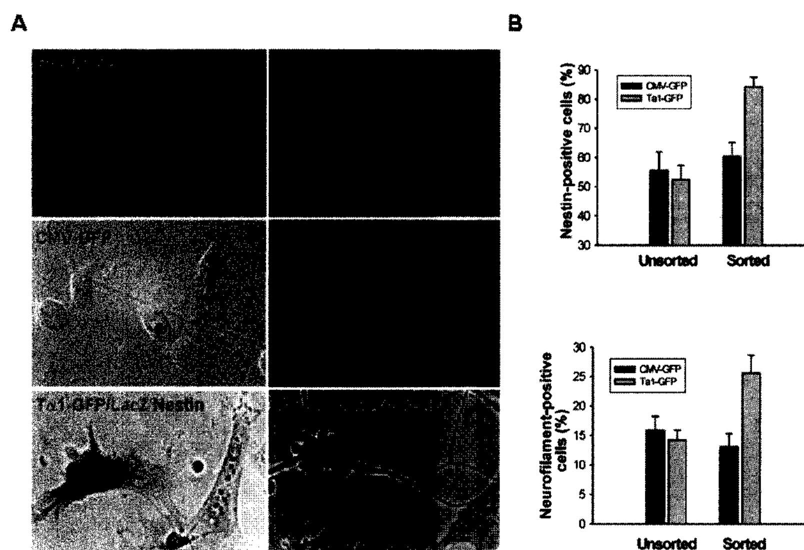

Fig 1. Reporter gene expression driven by the Tα1 tubulin promoter in neuronal progenitors and neurons of differentiating P19 EC cells in vitro (A). Tα1 -GFP-based FACS enrichment of neuronal precursors and neurons (B).

After verifying proper working of Tα1-GFP-LacZ and the enrichment or isolation of neural cell lineages, we used the dual reporter gene in mouse ES cells. Two cell lines harbouring CMV-GFP-LacZ (CMV-GL) or Tα1-GL were established. Upon neural differentiation, all the GFP-positive cells showed nestin or Tuj1 expressions. This was verified by a fluorescent activated cell sorter (FACS), showing that almost all of the cells sorted were positive with the markers (Fig. 2).

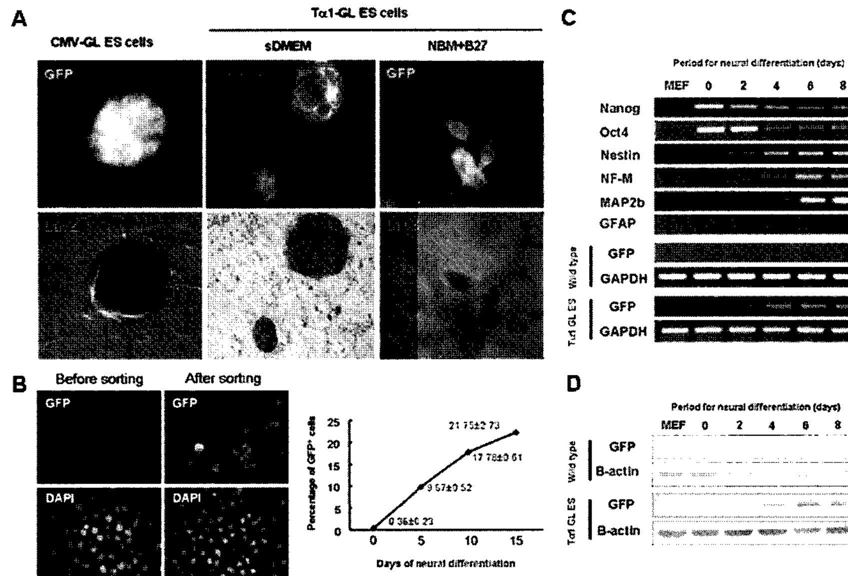

Fig 2. Establishment of two mouse cell lines, CMV-GL and Tα1-GL ES cells showing concurrent expressions of GFP and LacZ (A). Increasing population of GFP+ cells and sorting of the GFP+ cells during the neural differentiation of Tα1-GL ES cells (B). Expressions of neural cell markers (C) and GFP during neural differentiation (D).

Transplantation of the FACS-sorted GFP+ cells into mouse embryonic or adult brains further demonstrated that the grafted cells become integrated and traceable as concurrent GFP- and nestin-expressing cells for their fate and potential by either GFP expression or X-gal staining within the

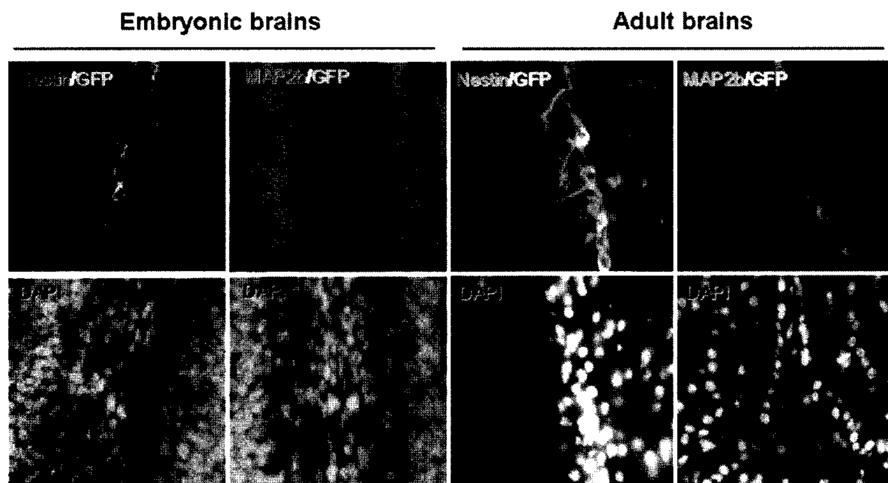

Fig 3. Integration of FACS-isolated NSCs in emebryonic and adult brains after grafting.

brain (Fig. 3). The newly established ES cells harbouring Tα1-GL should provide opportunities to isolate pure nestin-positive cells, and this is the first demonstration of the integration of the transplanted pure neural stem cells derived from ES cells. The isolation system of all nestin-positive NSCs should serve as an important tool for the study of stem cell signals and the developmental analysis of the grafted NSCs. It should be also applicable to human ES cells for the development and use for stem cell therapy with more careful studies.

* This work was supported by Research Center for Transgenic Cloned Pigs, which is funded by Ministry of Science and Technology/KOSEF. The work was also partially supported by the Intelligent Microsystem Center, KIST, which carries out one of the 21st century's Frontier R & D Projects sponsored by the Korea Ministry of Commerce, Industry and Energy.