P-36 Distinct Properties in Neural Differentiation Among Human Embryonic Stem Cell Lines

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Background & Objectives: Human embryonic stem (hES) cells derived from human blastocysts can be differentiated into every cell types composing three germ layers. Enriched populations of the particular cell types can be induced by variable culture conditions and differentiation factors, but although same differentiation methods are used, differentiation pattern into particular cell types from human embryonic stem cells and their enrichment is appeared differently in each laboratory. We studied the potential of neural differentiation among different hES cell lines for selection of proper cell line for neural induction.

Method: Human ES cell lines, SNUhES-1, SNUhES-2 and SNUhES-3, were established and characterized by immunohistochemistry (SSEA-1, 3, 4, TRA-1), RT-PCR (Oct-4), telomerase activity assay, fingerprinting karyotyping and teratoma formation. 5 days hES colonies were used for EB formation. After formation of embryoid bodies (EBs) for 4 days, hES cells were differentiated into neural progenitors for 8 days in EB medium containing N2 supplement and bFGF. Under this condition, expression levels of nestin mRNA were measured by RT-PCR and degree of nesin+ cell enrichment was beta-NGF, RA, PDGF were confirmed by Immunohistochemistry and FACS analysis. beta-NGF, RA, PDGF were used for further differentiation, and mature specific cell types were displayed by immunohistochemistry with specific antibodies.

Results: Expression levels of nestin mRNA gradually increased for 7 days and then decreased in all three cell lines. ES cell lines varied in their efficiencies to differentiate into neural progenitors. SNUhES1 cells were most efficiently differentiated into the nestin-positive cells and formed neural network well whereas SNUhES2 cells were poorly differentiated into neural precursors. When they were further differentiated into neuronal phenotypes from the neural progenitors in the presence of beta-NGF, many Tuj1+ and NF-H+ cells were detected in cells differentiated from SNUhES1 and 3 cell lines. In contrast, very few cells from SNUhES2 were differentiated into neuronal cells. Interestingly, SNUhES2 cells were efficiently differentiated into pancytokeratin+ cells under treatment of RA, indicating efficient differentiation into epithelial cells. These results suggest that hES cell lines have distinct properties in their differentiation.

Conclusions: Our hES cell lines, SNUhES 1, SNUhES 2 and SNUhES 3 have distinct properties during neuronal differentiation. It is important that selection of proper cell line for specific differentiation, because every cell line has different differentiation efficiency and preference. This research was supported by a grant (SC11011) from Stem Cell Research Center of the 21st. Century Frontier Research Program funded by the Ministry of Science & Technology, Republic of Korea