

Isolation, differentiation and in vivo application of pluripotent stem cells

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Stem cells provide the great hope for numerous areas, such as embryonic development, differentiation, nuclear cloning, cell transplantation, tissue engineering, gene discovery, drug screening, and so on. With the isolation of human pluripotent stem cell lines, the significance of stem cell research and its applications will continuously increase. We are interested in various aspects of stem cell biology, especially using embryonic stem (ES) cells and embryonic germ (EG) cells. We here report the establishment and characterization of new embryonic germ (EG) cell lines derived from primordial germ cells of developing gonads from therapeutically aborted human embryos.

Morphological and marker analyses show that our new EG cells (CHA-hEG cells) meet the criteria of the human EG cells, firstly reported by Shamblott et al. Detailed analysis on growth and differentiation properties, however, revealed that CHA-hEG cells differ from those previously reported in many aspects. They grow faster, and can be easily dissociated into single cells that can be clonally separated and proliferated as colonies. The culture condition is much simpler than those previously reported, although presence of feeder layers is apparently indispensable. These new properties makes it possible that our cells can be grown at a large scale under relatively simple culture conditions.

Dissociated EG cells can form embryoid bodies in suspension culture, and can be differentiated into various cell types spontaneously, including functional cardio-myocytes. Using the optimized conditions developed for mouse embryonic stem (ES) cells, we show that CHA-hEG cells can be differentiated directionally into specific cell types, such as dopamine-producing neurons. Teratoma analysis revealed that CHA-hEG cells can be differentiated into a wide range of cell types, confirming their pluripotency in vivo. Taken together, our new human EG cells share many similar properties with the well characterized mouse ES cells, providing a better opportunity for differentiation and transplantation studies.

We have also made much progress using mouse ES cells. Based on both DNA chip and protein analyses, we have recently discovered dozens of differentially expressed genes and proteins during ES cell differentiation, which are currently under characterization.

Given that ES cells can be differentiated into a wide variety of different cell types, they can serve as an ideal source for repairing damaged tissues or organs. We use three types of biodegradable polymers (PGA, PLGA and Collagen) as matrices on which ES cells are placed. Morphological and histological analyses strongly indicate

that ES cells can be differentiated into many cell types, following attachment onto the matrices. In some cases, heart beating is also observed, in which the formation of functional cardiomyocytes is confirmed using a cardiomyocyte-specific antibody, Troponin-T. This result strongly suggests the plausibility of this approach for making artificial organs, such as artificial hearts.

We are also interested in understanding the optimal in vivo parameters for the transplantation of ES cells into animal models. Our results strongly indicate that cell numbers to be injected is critically important, in which low numbers of ES cells resulted in various differentiated cell types in a localized manner at the injection site, whereas higher numbers led to the widespread formation of tumors, independent of the injection site. In most cases, ES cells injected into brains are shown to differentiate into various neuronal cell types, which will be useful for curing various forms of neurological disorders. Based on this observation, we are currently transplanting our ES and EG cells into the animal models of Parkinson's disease and strokes.

This work was supported by the Korean Ministry of Health and Welfare (01-PJ1-PG1-01CH11-0003).