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Current Position

Professor	*Director*	*Scientific Director*
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1993 - Current	Scientific Director, CHA Fertility Center of CHA General Hospital Pochon CHA University	
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2000 - Present	CEO, CHABIOTECH, CO., Ltd.	
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1993	Ph.D.	Graduate School of Kon-Kuk University, (Major: Molecular developmental biology)
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Guided Differentiation of Human Embryonic Stem Cells

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Mammalian embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of the early embryo blastocysts and have their remarkable ability to differentiate into the specialized cells of the three embryonic germ layers. These capacities of ES cells are important characters to serve the useful cells for transplantation therapies of various incurable diseases on the clinical approach. However, it is difficult to regulate the differentiation of ES cells because the mechanism of differentiation of ES cells has not yet been discovered. Many researchers have been use genetic modifications of ES cells to increase the efficiency of differentiation into interest cells to be used.

Since Doetschman colleagues have been reported on genetic modification in mouse ES cells, various genetic modifications in ES cells were applied to study on function of targeted transgene expression during cellular differentiation. Efficient genetic manipulation of ES cells is an essential technique needed to establish ES cells as a widely used research tool and the efficiency according to genetic manipulation depends on the stable transfection of target gene via various plasmids carrying viral and cellular promoters such as those for cytomegalovirus (CMV), chicken β-actin (CA), and human polypeptide chain elongation factor 1α (EF-1). Although the CMV promoter was strongly activated in many cell lines, and applied as a standard vector system, the limitation of CMV promoter was reported to have a different efficiency in specific tissues and whole animal models. Due to these reasons, EF-1 promoter was developed to be induced prolonged gene expression in progenitor cells such as hematopoietic and mesenchymal cells. The molecular properties of the EF-1 promoter have been described, who found that this promoter has high level activity with respect to promoting gene expression and may be used to generate stably transfected cell lines. In addition, EF-1 promoter expression is relatively insulated from changes in cell physiology and is cell type independent.

The relative strengths of different promoters have not yet been well established in mammalian ES cells. However, it has been reported that the EF-1 promoter and CMV immediate early enhancer fused to CA promoter were robustly drove reporter gene expression in mouse ES cells, while the CMV promoter was inactive. In addition, it was reported that the CMV promoter is transcriptionally active in undifferentiated mouse embryonic stem cells.

Recently, Gropp et al. demonstrated that long-term transgene expression in mammalian ES cells is available by transfection of lentiviral vectors, which is a contained target gene for lineages specific differentiation. This technique has been shown to be highly effective and could therefore emerge as

a popular tool in human ES cell genetic modification. These experiments was attempted to determine the optimal promoter system for their genetic manipulation in mouse, monkey and human ES cells by using lentiviral vector system. We show that the EF-1 promoter show a strong activity in both of them, whereas the CMV promoter has only weak activities in mammalian ES cells. Additionally, we analyzed the efficiency of these promoters in different stages of *in vitro* differentiated mouse, monkey, and human ES cells and have generated stable enhanced green fluorescent protein (EGFP) positive human ES cell line.

The efficiency of EF promoter is higher than those of CMV promoter in all ES cells. Especially, the expressions of EGFP from the EF-1 promoter and CMV promoter in mouse ES cells were 82.51% and 3.55%, respectively. Otherwise, EF-1 promoter (14.74%) efficiently droved gene expression compared with CMV promoter (3.69%) in human ES cells. Continuously, we have generated stable EGFP positive human ES cell line (EGFP-CHA3 human ES cells), expressed high levels (~95%) of EGFP from EF-1 promoter and maintained for up to 32 weeks with undifferentiated proliferation. Newly established EGFP-CHA3 human ES cell lines were characterized by various undifferentiating markers and teratoma formation. From these results, genetic modification by lentiviral vector with specific promoter in ES cells will use a powerful tool for guided differentiation as well as gene therapy.

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