

Promoter/enhancer-based reporter system for the identification and isolation of a specific phenotype from human ES cells.

Takahiro Nakano, PhD

Human embryonic stem (hES) cells may generate all major somatic cell types. Consequently, establishment of defined and reproducible phenotype-specific differentiation and isolation from hES cells will most likely provide a dynamic tool for stem cell research and potential application in diseases. The heterogeneity of phenotypes induced in embryonic bodies prompted me to develop a method for identifying and purifying a specific phenotype. To this end, the promoter/enhancer is useful to selectively direct EGFP expression in live cells in a phenotype-specific manner. Therefore, I will describe a fast method to identify phenotype-specific promoter/enhancer sequences using motor neuron (MN)-specific Hb9 enhancer as an example.

Next, high efficiency induction and isolation of functional MNs from hES cells was achieved using an Hb9 enhancer-based EGFP expression plasmid followed by FACS. These results demonstrate the utility of promoter/enhancer-based FACS for the isolation of a specific phenotype from hES cell populations as a means of purifying clinically appropriate cells for therapy. This technique is also a rational and realistic way of producing enriched populations of phenotype-specific cells for drug screening purposes.

In the future, I would like to 1) identify novel phenotype-specific promoters/enhancers that could be applicable for clinical studies, 2) establish stable hES lines carrying an enhancer/promoter-based reporter system, 3) screen for optimal differentiation conditions to induce a specific phenotype from the engineered hES cells based on reporter expression, and 4) conduct transplantation studies after enriching specific-phenotypes by FACS.

Speaker: Dr. Nakano was born in the US and raised in Japan and obtained his DVM from School of Veterinary Medicine, Osaka Prefecture University, Japan in 1995, and then received a PhD (in Developmental biology) from Kumamoto University Graduate School of Medicine, Japan in 2000. Dr. Takahiro Nakano is currently a Senior Scientist at Q Therapeutics, Inc. (Salt Lake City, USA) where he develops human oligodendrocyte progenitor cell (hOPC)-based therapies for demyelinating diseases. His work includes isolation and characterization of hOPCs. He then uses these cells to conduct transplantation studies using mouse disease models. Prior to that, Dr. Nakano was a post doctoral research fellow at Cornell University Medical Center (New York, USA). He identified novel Hb9 enhancers that drive gene expression in spinal motor neurons, and used it for the induction and Hb9 enhancer-directed FACS isolation of spinal motor neurons from hES cells. Similarly, he was involved in the induction and Ngn2 enhancer-directed FACS isolation of mesencephalic dopaminergic neurons from hES cells. As a result, he filed three patent applications.