# DEVELOPMENT OF PORCINE EMBRYONIC STEM CELL TECHNOLOGY IN TAIWAN

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## INTRODUCTION

Embryonic stem (ES) cells can self-renew and maintain undifferentiated characteristics under suitable in vitro conditions. They are able to differentiate in vitro, spontaneously or responding to suitable signals, into cells of all somatic cell types of the body, including the germ-line. Consequently, ES cells will be a valuable source for cell replacement therapy in numerous pathologies, and make a promise of cell transplantation and biomedical engineering in the future. Though the human ES (hES) cells hold promise in degenerative disorders such as Parkinson and Alzheimer, or in the treatment of spinal cord injury, the pre-clinical researches must be proved in suitable animal models to present bio-safety and long-term efficiency of transplanted ES cells and/or their derived cells before clinical therapies applied.The swine, a common livestock species, has the potential to serve as a great research model for human biomedicine. They share a high similarity in anatomic, immunologic and physiologic characteristics with human, and the sizes of their organs are fairly comparable to that of human. The pigs have been considered an optimal model for pre-clinical development of therapeutic approaches because their morphological and physiological functions are similar to human. Although the establishment of pluripotent ES cell lines from domestic species is much more difficult than that in murine species, porcine ES (pES) cell lines have been successfully derived from pre-implantation blastocysts. Moreover, pES cells were very similar to hES cells in many characteristics, including colony morphology, feeder-dependent and refractory to leukemia inhibitory factor (LIF) in culture, and expression of stage-specific embryonic antigen 3/4 (SSEA3/4) but not the SSEA1 which is characterized to mouse ES (mES) cells. Therefore, the pES cells are adequate to serve as an excellent model in the study and development of regeneration medicine in human, especially the traceable pES cells and their deriving cells in the therapeutic approaches involving cell transplantation.

**Key words: Porcine embryonic stem cells, Parkinson's disease, Spinal cord injury**

## ESTABLISHMENT AND CHARACTERIZATION OF NOVEL PORCINE EMBRYONIC STEM CELL LINES EXPRESSING GFP

The purpose of this study was to establish transgenic porcine embryonic stem cell lines which can stably express report gene. The pES cells of line M215-3 used in this study were derived from the inner cell mass (ICM) in preimplantation blastocysts of the Taiwan Livestock Research Institute Black Pig No. 1 (a topcrossing breed established from Taoyuan and Duroc pigs). The cells isolated and derived from ICM were all cultured and maintained on a feeder layer of mitomycin C inactivated STO cells (mouse embryonic fibroblasts, CRL-1503, ATCC, Rockville, MD) in gelatin-coated Multidish 4 Wells (Nunc 176740, Roskilde, Denmark) in ES-cell culture medium (ESM) at 39℃ with an atmosphere of 5 % $CO_2$ in air. Established pES cell line at passage 44 was transfected with pAAV-hrGFP Control Plasmid by electroporation-mediated, viral vector-mediated and liposome-mediated strategies. A total of 28 GFP-expressing pES cell colonies were obtained following electroporation with 2 DC pulses of 150 V/cm for 10 msec and three GFP-expressing pES (pES/$GFP^+$) cell lines were established. These pES/$GFP^+$ cell lines stably expressed exogenous GFP and continuously proliferated in vitro for more than 90 passages in 20 months. They maintained normal karyotype of 36 +XX and typical characteristics of pluripotent stem cells, including expression of pluripotent markers Oct-4, AP, SSEA-4, TRA-1-60, and TRA-1-81, formation of embryoid bodies under suspension culture. They were able to differentiate in vitro into neural and cardiomyocytic lineage, respectively, under suitable induction. To our knowledge, there has been no report of establishing GFP-expressing pES cell lines. These novel pES/$GFP^+$ cell lines established in this study might serve as a non-rodent model and would benefit to the studies involving ES cell transplantation, cell replacement therapy and tissue regeneration due to their traceable capacity.

## DIRECTED DIFFERENTIATION INTO NEURAL LINEAGES AND THERAPEUTIC POTENTIAL OF PORCINE EMBRYONIC STEM CELLS IN RAT PARKINSON'S DISEASE MODEL

Parkinson's disease is a degenerative disorder characterized by progressive and selective loss of dopamine (DA) neurons in the midbrain substantia nigra. This disorder has being a prime target for cell replacement therapy, given over a decade of successful clinical experiences with fetal ventral mesencephalic cell transplantation in PD patients. However, fetal cells transplantation has significant technical, ethical, and practical limitations, partly due to limited availability and variable outcomes. Due to the self-renewal capacity and

multi-lineage developmental potential, stem cells could be ideal sources for cell replacement therapy. The challenge in using ES cells for regenerative medicine has been to direct the wide differentiation potential toward the derivation of a specific cell fate. The successful induction protocols have provided a powerful tool to control the development and function of ES-derived midbrian DA neurons and made a promise of ES cell therapy on PD in the future.

This study was conducted to direct porcine embryonic stem (pES) cells differentiating into neural lineages and to investigate therapeutic potential of GFP-expressing pES (pES/GFP$^+$) in the rat model of Parkinson's disease (PD). Directed differentiation of pES into neural lineages was induced by suspension culture in medium containing RA, SHH, and FGF combinations without going through embryoid body formation. A high yield of nestin-expressing neural precursors was found in all treatments on day 2 after the 12-day induction. On day 6 after re-plating, more than 86.2 % and 83.4 % of the differentiated cells stained positively for NFL and MAP2, respectively. The expression of TH, ChAT, and GABA specific markers were also observed in these NFL-positive neural cells. The undifferentiated pES/GFP$^+$ cells and their neuronal differentiation derivatives were transplanted into the Sprague-Dawley (SD) rat's brain, and their survival and development was determined by using live animal fluorescence optical imaging system every 15 days. The results showed that fluorescent signals from the injection site of SD rats' brain could be detected through the experimental period of 3 months. The level of fluorescent signal detected in the treatment group was two folds of that of the control group. The results of behavior analysis showed that PD rats exhibited stably decreased asymmetric rotations after transplantation with pES/GFP$^+$-derived D18 neuronal progenitors. The dopaminergic differentiation of grafted cells in the brain was further confirmed by immunohistochemical staining with anti-TH, anti-DA, and anti-DAT antibodies. These results suggested that the differentiation approach we developed would direct pES cells to differentiate into neural lineages and benefit the development of novel therapeutics involving stem cell transplantation.

## TRANSPLANTATION OF PORCINE EMBRYONIC STEM CELLS AND THEIR DERIVED NEURONAL PROGENITORS IN SPINAL CORD INJURY RATS MODEL

Spinal cord injury (SCI) in which fragments of broken vertebrae and ligaments compressed the cord is one of the leading causes of disability. Axonal regeneration, delayed death of neurons, oligodendrocytes and glia cells, and axonal demyelination of intact fiber ed traced around the injured site then cause remarkable morbidity and mortality and restrict therapeutic options. Embryonic stem (ES) cells transplantation had been considered a cell

therapy approach for promoting neural repair and functional recovery from SCI. Optimal SCI approaches minimized the progression of secondary injury, manipulated the neuroinhibitory spinal cord environment, replaced lost tissue with transplanted cells, remyelinated denuded axons, and maximized the intrinsic regenerative potential of endogenous progenitor cells. ES cell-based transplantation has been studied several times in SCI animal models. Results indicated that the grafting of ES cell-derived oligodendrocytes might have remyelinated fibers along the neuronal axons of long projection neurons and did not cause harm, that ES cell-derived neurons could survive and integrate after injection into the injured rat spinal cord, and that long-term therapy could be achieved by ES cells transplantation. Also, the ES cell-derived gliogenic neural stem/progenitor cell transplantation improved functional recovery after SCI.

The purpose of this study was to investigate therapeutic potential of the green fluorescent protein expressing porcine embryonic stem cells (pES/GFP$^+$) in the rat model of spinal cord injury (SCI). The undifferentiated pES/GFP$^+$ cells and their neuronal differentiation derivatives were transplanted into the Long Evans (LE) rat's contused spinal cord, and their in situ development was determined by using live animal fluorescence optical imaging system every 15 days. After pES/GFP$^+$ cells transplantation, the behavior functional recovery of SCI rats was assessed with the Basso, Beattie, and Bresnahan Locomotor Rating Scale (B.B.B. scale), and the growth and differentiation of the grafted pES/GFP$^+$ cells in SCI rats were analyzed by immunohistochemical staining. The results showed that the relative GFP expression level was decreased along 3 months duration after transplantation. The pES/GFP$^+$-derived cells positively stained with neural specific antibodies of anti-NFL, anti-MBP, anti-SYP, and anti-Tuj 1 were detected at the transplanted position. The SCI rats grafted with the D18 neuronal progenitors showed a significant functional recovery of hind limbs and exhibited the highest B.B.B. scale of 15.20 ± 1.43 at week 24. The SCI rats treated with pES/GFP$^+$-derived neural progenitors demonstrated a better functional recovery. Transplantation of pES-derived D18 neuronal progenitors has SCI treatment potential and functional behavior improvement of grafted pES-derived cells in SCI model rats suggested the potential for further application of pES cells in the study of replacement medicine and functionally degenerative pathologies.

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