



## Production of hCD46-Transgenic Clone Embryos Using Embryonic Germ Cell Nuclear Transfer in Pigs

Ji Young Won, Kwang Sung Ahn, Jee Hyun Kang and Hosup Shim

*Department of Physiology, Dankook University School of Medicine, Cheonan, Korea*

Production of transgenic pigs carrying human complement-regulatory protein genes may assist to overcome hyperacute rejection (HAR), the first and currently the most critical immunological hurdle in the development of xenogenic organs for human transplantation. However, not only the production of transgenic pigs is time-consuming and costly but usefulness of such pigs in transplantation to humans is unpredictable. In this study, we developed an in vitro procedure using porcine embryonic germ (EG) cells, undifferentiated stem cells derived from culture of primordial germ cells, to pre-evaluate effectiveness of transgenic approach. Porcine EG cells were maintained in feeder-free state in DMEM containing 15% (v/v) fetal bovine serum and 1,000 units/ml leukemia inhibitory factor. Human complement downregulator hCD46 gene under the regulation of cytomegalovirus promoter was introduced into porcine EG cells. Transfected cells were selected by antibiotic treatment and confirmed by PCR. To test the resistance of hCD46-transgenic EG cells to human xenoreactive natural antibody and complement, EG cells were cultured for 24 hours in DMEM containing 15% (v/v) normal human serum. The treatment of human serum did not affect the survival of hCD46-transgenic EG cells, whereas with the same treatment approximately one half of non-transfected EG cells failed to survive ( $p < 0.01$ ). Transgenic EG cells presumably capable of alleviating HAR were used as nuclear donor for subsequent transfer of nucleus into enucleated oocyte. Among 235 reconstituted oocytes, 35 (14.9%) developed to the blastocyst stage. Analysis of individual nuclear

transfer embryos by PCR indicated that 80.0% (28/35) of embryos contained transgene hCD46. The PCR-negative embryos might be due to an incomplete antibiotic selection of cells after transfection. Overall, the results of present study demonstrate that the cell culture-based pre-evaluation of xenotransplantation may secure the usefulness of transgenic pigs prior to actual production. Further experiments on differentiation of transgenic EG cells into various cell types, cytolytic analysis of such cells to assess efficiency of xenotransplantation and subsequent production and transfer of transgenic clone embryos to recipients may provide a useful new procedure to accelerate xenotransplantation research.

Keywords: *embryonic germ cell, transgenesis, cytolysis, nuclear transfer, xenotransplantation*