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Transgenesis and Nuclear Transfer Using Porcine Embryonic Germ Cells

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Embryonic germ (EG) cells are undifferentiated stem cells isolated from cultured primodial germ cells (PGC). These cells share many characteristics with embryonic stem cells including their morphology and pluripotency. Undifferentiated porcine EG cell lines demonstrating capacities of both *in vitro* and *in vivo* differentiation have been established. Since EG cells can be cultured indefinitely in an undifferentiated state, whereas somatic cells in primary culture have limited lifespan, EG cells may provide continuous source of karyoplast in nuclear transfer (NT). In addition, genome-wide demethylation of DNA occurs in preimplatation embryos as well as PGC. Nuclear transfer embryos using EG cells rather than somatic cells may be close to embryos from normal fertilization in their DNA methylation status. If combined with NT technique, EG cells may potentially be useful for genetic manipulation in pigs. In this study the efficiency of transgenesis and NT using porcine fetal fibroblast and EG cells was compared. Two different techniques were used to perform NT. When conventional NT procedure (Roslin method) was used, the rate of development to the blastocyst stage was higher in EG cell NT than somatic cell NT (38/254, 15.0% vs. 29/233, 12.4%). In piezo-driven micromanipulation (Honolulu method), EG cell NT embryos also develop better than somatic cell counterpart (15/126, 11.9% vs. 12/160, 7.5%). Although statistical difference was not observed, EG cell NT tends to result in greater rate of blastocyst development regardless of NT mothods used in this study. To investigate if EG cells can be used for transgenesis in pigs, GFP gene was introduced into porcine EG cells. Nuclear transfer embryos using transfected EG cells gave rise to blastocysts (8/40, 20%), and all embryos expressed GFP based on observation under fluorescence microscope. In this study the possibility of using EG cells as karyoplast donor in NT procedure was tested. The results suggests that EG cell NT may have advantages over somatic cell nuclear transfer, and transgenic pigs may be produced using EG cells.

Key words : ***Porcine embryonic germ cell, Transgenesis, Nuclear transfer***