P-21

Production of Nuclear Transfer Embryos using the Somatic Donor Cells Modulated to have Male Germ Cell Functions

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The success of somatic cell cloning in the porcine gives promise to applications such as species preservation, livestock, propagation, and cell therapy for medical treatment by nuclear transfer embryonic stem cells. However, porcine cloning by somatic cell nuclear transfer has been inefficient. Even after birth, cloned porcine are found to carry a variety of abnormalities, obesity, and a shorter life span. Recent molecular analyses of cloned embryos reveal abnormal epigenetic modifications. Therefore, the prevention of epigenetic errors gas been expected to lead to the improvement of the success rate in animal cloning. Epigenetic reprogramming of a somatic nucleus was supported by the birth of cloned animals and the derivation of embryonic stem cells after nuclear transplantation into oocytes. Moreover, recent studies reported that the direct transformation of one differentiated somatic cell type into another is possible and would be advantageous for producing isogenic replacement cells. In this study, at first, we could modulate the cell fate of fibroblasts by introducing cell extracts derived from mast cell line, RBL-2H3 cells. NIH-3T3 cells were treated with streptolysin O (SLO; 230 ng/mL), which reversibly permeablizes plasma membrane and incubatedfor 30 min with the mast cell- derived cell extracts (4 mg /mL). After resealing the membrane of the cells, we incubated the cells for 3 weeks and analyzed the expression of mast cell specific genes such

as MAFA (mast cell function associated antigen) and FceRI (high-affinity IgE receptor). Our results showed that the cell extracts can activate the expression of mast cell-specific genes, implying that cell extracts can provide regulatory components required for reprogramming the cell fate to initiate a transcriptional program specific for the cell type. Moreover, mast cell-specific degranulation and cell morphology change was observed in cultured mouse fibroblasts. We could detect mast cell-specific functions even after 15 days of incubation. Next, to induce porcine fibroblasts to take on testis sertoli cell-specific properties, we modulated the cell fate of somatic donor cells by introducing cell extracts derived from porcine testis. Several porcine somaticcells including primary and stabilized porcine fibroblasts or epithelial kidney cells were treated with streptolysin O (SLO; 230 ng/mL), which reversibly permeablizes plasma membrane and incubated for 30min with the testis cell-derived cell extracts (4 mg/mL). To reseal plasma membranes, cells were allowed for 30 min in DMEM containing 30% FBS and 2 mM $CaCl_2$. After resealing the cell membranes, we incubated the cells for 3 weeks and analyzed the expression of the testis-specific genes such as protamine 1, protamine 2, SOX 9, mullerian inhibitory substance (MIS), preproacrosine (ACR), phosphoglycerate kinase 2 (PGK-2), protein C, and c-kit ligand. In the reprogrammed primary porcine fibroblasts or epithelial kidney cells, the porcine testis extracts could activate the expression of the porcine testis sertoli cell-specific genes. The male germ cell functions sustained for more than 10 days after the reprogramming process. Then, the *in vitro* matured oocytes were enucleated and a single cell (either reprogrammed or intact) was injected directly into cytoplasm of the oocytes. There constructed embryos were activated electrically and cultured *in vitro* for 7 days. We could find that the rate of blastocyst formation were significantly higher ($p<0.05$ (Chi-square test)) in reprogrammed nuclear donor cell group (27/119 (22.7 $\pm$5.0%)) than in control (intact cell) one (11/83 (13.3$\pm$3.2%)). Taken together, our results suggest that testis-derived cell extracts can be successfully used to reprogram fibroblasts to express male germ cell function improving the developmental efficiency of the nuclear transfer embryos.

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