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Construction of porcine FoxN1 knockout system by RNA-guided endonucleases

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Introduction
Forkhead box protein N1 (FoxN1) regulates development, differentiation, and function of thymus epithelial cells (TECs), both in the prenatal and postnatal thymus. In mice, knockout of the FoxN1 results in two well-known defects as hairlessness and athymia. However, knockout of FoxN1 in other species has not been discovered yet. RNA-guided endonucleases (RGENs), derived from the prokaryotic type II CRISPR-Cas system, enable targeted specific genes. We successfully targeted FoxN1 in pig by using CRISPR-Cas system.
Materials and methods
RGEN design: We designed specific single-guide RNA (sgRNAs) which targets exon 2 of porcine FoxN1, and constructed Cas9 vector system.
Transfection and Microinjection: Cas9-FoxN1 vectors were transfected intoporcine fibroblastusing lipofectamin 2000 reagent according to manufacturer's protocol. Also to induce parthenogenesis, Cas9-FoxN1 vectors (4, 8, and 16 ng/μl) were microinjected into the porcineoocytes and these were activated by BTX.
T7 endonuclease I assay: The region of DNA containing the target site was amplified by PCR using the specific primer set. The PCR products were hybridized to form heteroduplex DNA, and digested by T7 endonuclease I for 1 h at 37 °C. The DNA was analyzed by gel electrophoresis using 2% agarose gel.
Results and discussion
To confirm the knockout of FoxN1, we analyzed the DNA cleavage activities by T7 endonuclease I assay both fibroblasts and oocytes. As a result, there was no significantly difference in knockout efficiency among injected DNA concentrations, 4 ng (20.7%), 8 ng (15.4%) and 16 ng (20%), respectively. However, we checked that Cas9-FoxN1 could successfully knockout the porcine FoxN1. This study suggests that knockout pig is also producible using Cas9-system by DNA microinjection into zygotes, directly.

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Expression pattern and function of claudins during tight junction maturation in the mouse pre-implantation embryo

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Introduction
The pre-implantation embryo has two distinct cell lineages at the blastocyst stage. An outer epithelial monolayer called trophectoderm and the non-polar inner cell mass. Tight junctions (TJ) form continuous intercellular contacts between neighbouring trophectoderm cells and are crucial in establishing a blastocoele cavity. TJs are composed of several transmembrane proteins including Claudins, a large family of proteins that play a central role in the formation of the epithelial barrier. Claudins (Cl) show distinct distribution patterns among epithelial tissues and several isotypes can exist in the same TJ.
Methods
mRNA extracted from different stages of mouse embryos was reverse-transcribed before amplification of cDNA for particular Claudin isotypes. Embryos at 2-cell, 8-cell, morula and blastocyst stage were fixed for immunofluorescence and confocal microscopy. Zygotes were microinjected with siRNA and development to blastocyst observed.
Results
RT-PCR showed that Cl-4 and 7 were transcribed from compaction to blastocyst stage, while mRNA for Cl-3 and 6 were present from late morula stage. mRNA for Cl-12 was only present in blastocysts and mRNA for Cl-2 and 15 was absent throughout. Immunofluorescence showed Cl-4 and 7 proteins assembling at cell–cell contacts after compaction in a dis-continuous pattern. By late blastocyst stage, Cl-3, 4, and 7 all displayed continuous staining at trophectoderm TJs, whilst Cl-12 and 15 were absent at TJs. siRNA inhibition of Cl-4 and 6 reduced percentage of blastocysts formed, whilst cavitation was not affected by Cl-7 siRNA. Our study shows that five different claudins are expressed in the early embryo and two play a crucial role in blastocyst formation. Funding by BBSRC to TPF.

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Human granulocyte–colony stimulating factor enhances viability of porcine embryos in defined oocyte maturation medium

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Granulocyte colony-stimulating factor (G-CSF) enhances the proliferation, differentiation and survival of cells. In addition, G-CSF is a non-invasive biomarker of human oocyte developmental competence for embryo implantation. In human follicle G-CSF concentration increased during the ovulatory. At present experiment, the cumulus oocyte complexes (COCs) were aspirated from superficial follicles (1–3 and 3–6 mm). COCs from small and medium follicle were matured in protein-free maturation medium supplemented with concentrations of hG-CSF (0, 10 and 100 ng/ml). After 44 h of IVM, the ratios of nuclear maturation have no difference. The intracellular ROS levels of oocytes from both follicle groups matured with 10 ng/ml were significantly ($P<0.05$) decreased. However, all groups showed no significantly difference in GSH levels. After PA, the cleavage and blastocyst stage was significantly ($P<0.05$) high in 100 ng/ml with small and 10 ng/ml with medium follicle. Cell numbers of blastocyst from both follicle groups were significantly high in 10 ng/ml. After IVF, the blastocyst stage was significantly ($P<0.05$) increased in 10 ng/ml with medium follicle. Monospermy and fertilization efficiency were significantly high in the 100 ng/ml of small and 10 ng/ml of medium follicle. We examined the *Has2* and *Bax* transcript levels were significantly decreased in cumulus cells from small follicle treatment and the *Bcl2* and *ERK2* were significantly increased from medium follicle treatment. In conclusion, these results indicate that hG-CSF improve the viability of porcine embryos. Research supported, in part, by a grant from the National Research Foundation of Korea Grant Government (NRF-2012R1A1A4A01004885, NRF-2013R1A2A2A04008751), Republic of Korea.

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Chromatin remodelling and histones mRNA accumulation in bovine germinal vesicle oocyte

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In several mammalian species, a major remodelling of the germinal vesicle chromatin is known to occur towards the end of the oocyte growth. Various chromatin configurations have been identified. However, the mechanisms involved in this remodelling process are yet not completely understood. In the bovine species, four distinct and progressive states of chromatin compaction have been characterized and are linked to a gradual acquisition of the developmental potential. Germinal vesicle oocytes were collected and separated in four groups according to their degree of chromatin condensation, ranging from a diffused state to a fully compacted configuration. To better understand the molecular changes undergoing in the oocyte during that critical period, transcriptomic analysis was performed with the EmbryoGENE microarray platform (custom Agilent 44K) in order to identify mRNA modulations occurring during the remodelling process. An important proportion of genes showed a reduced mRNA level as the chromatin becomes more compacted, which strongly correlates with the decreased transcriptional activity at the end of oocyte growth. However, among the transcripts presenting an increased mRNA level, many were associated with the histone genes. Depending on the specific histone (H2A, H2B, H3, H4 or linker H1), an important mRNA accumulation occurs in the oocyte before ovulation. This dataset then offers a unique opportunity to picture the stock of accumulated histone mRNAs either to complete the build-up of a compacted chromatin, but also to ensure the protamine-histone replacement following fertilization and the completion of the first three cell cycles.

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