Bioresource Engineering Laboratory
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(0) Research field

CPR Subcommittee: Biology

Keywords:

Developmental biology, embryo, epigenetics, fertilization, mouse

(1) Long-term goal of laboratory and research background

To understand how new life is established at fertilization, how the germline genomes are established during development, and how the resultant embryonic genomes are programmed to contribute to two developmental lineages (the embryonic and placental lineages) in the mammalian life cycle. These are the key questions of developmental epigenetics. Clues should exist within the genomes of normally developing germ cells and embryos, but the information obtained from conventional analyses with these "normal" materials may be limited. Therefore, we reconstruct special embryos using reproductive engineering techniques including microinsemination (sperm or spermatid injection), nuclear transfer cloning and establishment of new stem cell lines, and undertake systematic and comparable analyses. Most studies are performed using laboratory mice (*Mus musculus*) because a variety of reproductive engineering techniques as well as a large amount of genomic information are available for this species. Our strong point is that we can make the best use of the mouse reproductive engineering techniques and combine them with cutting-edge genomic analyses. This strategy may provide us with the opportunity to solve the important questions above, which cannot be achieved by the conventional experimental methods.

(2) Current research activities (FY2019) and plan (until Mar. 2025)

(A) Analysis of the epigenetic status of somatic cell nuclear transfer (SCNT) embryos

SCNT is the sole reproductive engineering technique that produces an animal from a single somatic cell. Although it can be broadly applied for farm animal industry, pharmaceutical industry and regenerative medicine, the live birth rate is relatively low. Previously we have improved the efficiency to nearly 20% by correcting abnormal X chromosome inactivation and removing H3K9me3, a repressive histone modification, in SCNT embryos. However, SCNT-specific placental phenotype (enlargement) was not improved. In FY2019, we have successfully normalized the abnormal placental phenotype by the recovery of the normal monoallelic expression of imprinted micro RNAs located in intron 10 of the *Sfmbt2* gene (Fig. 1). However, post-implantation defects were still observed, indicating that additional epigenetic barriers impede SCNT cloning.

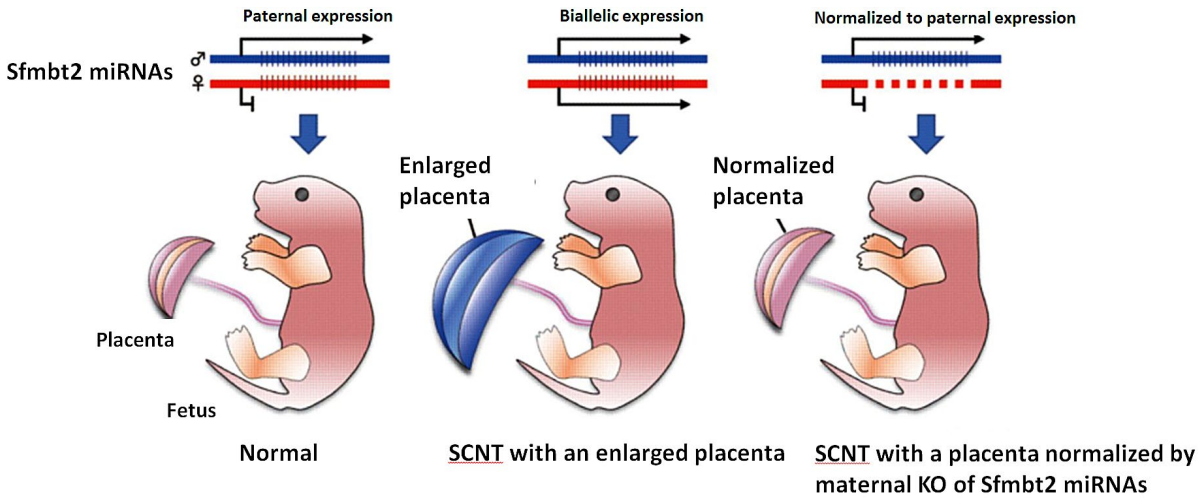

Fig. 1. Biallelic expression of imprinted *Sfmbt2* miRNAs is responsible for SCNT-specific placental enlargement. Normalization of the paternal expression of the miRNAs by maternal knockout results in formation of a near-normal size placenta following SCNT.

Plan until Mar. 2025 we will increase the live birth rate of SCNT cloned mice to the practical level (about 30-40% per transferred embryos) by modulating the epigenetic status of SCNT embryos; e.g., correction of the aberrated imprinted gene expressions and removal of multiple repressive histone modifications. We hope that our results will lead to the long-awaited technical breakthrough of the mammalian SCNT technique.

(B) Understanding the epigenetic basis of establishment of extraembryonic lineage

In mammals, the first lineage specification occurs at implantation, giving rise to the embryonic and extraembryonic lineages. We found that histone H3.1 is responsible for establishment of the epigenome structure of the extraembryonic lineage. Genome-wide ChIP analysis of trophoblast stem cells (TSCs) and embryonic stem cells (ESCs) using antibodies specific for H3 variants revealed that H3.1/H3.2, but not H3.3, discriminated these two stem cell types. In TSCs, H3.1/H3.2 was highly associated with H3K9me3 but was absent in H3K4me3-enriched regions, indicating that H3.1/H3.2 may exerts stronger repressive effects in TSCs than ESCs. In addition, the TSC genome uniquely contained large H3.1/H3.2- and H3K9me3-enriched domains (~ 10 Mb) in gene-poor regions. This feature was common to the early extraembryonic ectoderm in mice and humans. After knockdown of CAF1, a H3.1 chaperone, TSCs ectopically expressed Oct3/4 while repressing Cdx2 and Elf5. Our findings indicate that H3.1 may safeguard epigenome stability during placental formation and maintenance.

Plan until Mar. 2025 We will accumulate information on the epigenetic status specific for extraembryonic tissues and the mechanisms responsible for their differentiation. This will lead to understanding the whole picture of mammalian development specialized by formation of the placenta.

(C) Understanding the mechanisms of mammalian fertilization

During natural fertilization, mammalian spermatozoa must pass through the zona pellucida before reaching the plasma membrane of the oocyte. It is assumed that this step involves partial lysis of the zona by sperm acrosomal enzymes, but there has been no unequivocal evidence to support this view. We found that acrosin, an acrosomal serine protease, plays an essential role in sperm penetration of the zona. We generated acrosin-knockout (KO) hamsters, using an in vivo transfection CRISPR/Cas9 system. Homozygous mutant males were completely sterile. Acrosin-KO spermatozoa ascended the female genital tract and reached ovulated oocytes in the oviduct ampulla, but never fertilized them. In vitro fertilization (IVF) experiments revealed that mutant spermatozoa attached to the zona, but failed to penetrate it (Fig. 2). When the zona pellucida was removed before IVF, all oocytes were fertilized. This indicates that in hamsters, acrosin plays an indispensable role in allowing fertilizing spermatozoa to penetrate the zona. This study also suggests that the KO hamster system would be a useful model for identifying new gene functions or analyzing human and animal disorders because of its technical facility and reproducibility

Plan until Mar. 2025 we will identify how and where acrosin is involved in sperm penetration through the zona pellucida, because no one knows where acrosin is distributed after the acrosome reaction. This will lead to add an important basic information on the sperm-oocyte interaction, which will also help identification of new male-factors that may affect the fertility of animals including humans.

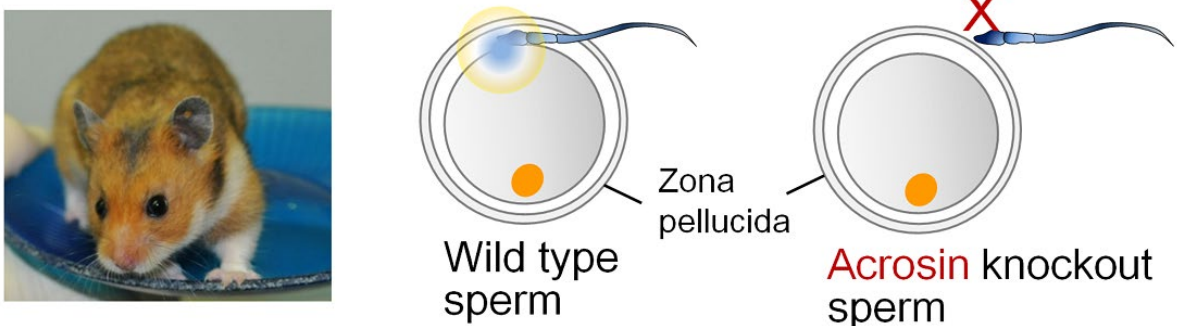

Fig. 2. Acrosin KO hamsters revealed an indispensable role of acrosin in fertilization. The fertilizing spermatozoa must penetrate the zona pellucida to reach the oocyte plasma membrane. Our result indicates that sperm penetration through the zona requires not only the strong movement of the sperm, but also the enzymatic activity provided by the sperm.

(3) Members as of March, 2020

(Chief Scientist)

Atsusho Ogura

(4) Representative research achievements

1. “Birth of a marmoset following injection of elongated spermatid from a prepubertal male.”, Ogonuki N, Abe Y, Kurotaki YK, Nakao K, Aiba A, Sasaki E, Ogura A., Mol Reprod Dev 86 (2019) 928-930.
2. “Paternal knockout of Slc38a4/SNAT4 causes placental hypoplasia associated with intrauterine growth restriction in mice.”, Matoba S, Miura K, Hirose M, Shiura H, Kohda T, Nakamuta N, Ogura A., Proc Natl Acad Sci USA 116 (2019) 21047-21053.
3. “Early production of offspring by in vitro fertilization using first-wave spermatozoa from prepubertal male mice.”, Mochida K, Hasegawa A, Ogonuki N, Inoue K, Ogura A., J Reprod Dev 65 (2019) 433-441.
4. “How to improve mouse cloning.”, Ogura A., Theriogenology 150 (2020) 215-220.
5. “Acrosin is essential for sperm penetration through the zona pellucida in hamsters.”, Hirose M, Honda A, Fulka H, Tamura-Nakano M, Matoba S, Tomishima T, Mochida K, Hasegawa A, Nagashima K, Inoue K, Ohtsuka M, Baba T, Yanagimachi R, Ogura A., Proc Natl Acad Sci USA 117 (2019) 2513-2518.

Laboratory Homepage

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