National BioResource Project -Rat

The 19th Rat Resource Research Meeting

Lecture Abstracts

February 13, 2026　13:00 – 17:30

Program

Part 1

Chair　Chie Naruse (Kyoto University)
Masahide Asano (Kyoto University)

Rat Resource

1.　Introduction: NBRP-Rat Activities in This Fiscal Year　13:00-13:15
Chie Naruse
Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University

2.　Highly efficient production of transgenic rats using *piggyBac* transposase mRNA and piezo-assisted microinjection　13:15-13:35
Kohtaro Morita
RIKEN BioResource Research Center

3.　Advancing research infrastructure for mouse and rat resources using reproductive technologies　13:35-14:05
Toru Takeo
Division of Reproductive Engineering, Center for Animal Resources and Development (CARD), Kumamoto University

4.　Building up National-wide Infrastructure of Model Animal in Korea　14:05-14:30
Je Kyung Seong
College of Veterinary Medicine, Seoul National University

5. Introduction of Rat Resource Center in Korea　14:30-14:45
Jun-Won Yun
College of Veterinary Medicine, Seoul National University

Coffee Break　14:45-15:00

Part 2

Chair Kazuto Yoshimi (Tokyo University)
Toshiya Ihashi (Kyoto University)

Rat Research

6. A knockout rat model to elucidate the molecular mechanisms underlying the developmental origins of health and disease (DOHaD) theory — 15:00-15:30
Yasuhiro Uchimura
Division of Anatomy and Cell Biology, Department of Anatomy, Shiga University of Medical Science

7. Modeling human limb skeletal development using iPSCs, and applications in regenerative medicine — 15:30-16:00
Takeshi Takarada
Department of Regenerative Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

8. Recent Advancements in Skeletal Muscle Disease and Regeneration Research using Genetically Modified Rats as a Model System. — 16:00-16:30
Keitaro Yamanouchi
Laboratory of Veterinary Physiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo

9. Brain Mechanisms Regulating Mammalian Reproduction: — 16:30-17:00
The Central Role of Kisspeptin Neurons
Hiroko Tsukamura
Nagoya University

10. Discussion — 17:00-17:30
Chair Chie Naruse (Kyoto University)
Masahide Asano (Kyoto University)

Abstract

1. Introduction: NBRP-Rat Activities in This Fiscal Year

Chie Naruse
Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University

The National BioResource Project Rat (NBRP-Rat) was launched in 2002 and has been supported by MEXT and AMED.
As a five-year program, we have systematically collected, preserved, and distributed rat resources. As one of the largest rat resource centers in the world, we currently provide approximately 860 rat strains as of December 2025.

During the fifth phase of the project, one of our major goals has been the promotion of rat reproductive engineering technologies. To this end, we continue to organize training courses on rat reproductive engineering to expand the use of genetically modified rats and to broaden the rat research community.

A notable feature of this year has been a significant increase in new deposits. With the widespread adoption of genome editing technologies and increased awareness of our project, the number of deposited strains reached 51 by December, exceeding previous years.
We also introduced several new initiatives, including information dissemination through social media platforms such as LinkedIn, X, and Bluesky, and closer collaboration with the NBRP public relations team.
In addition, we renewed our website using supplementary funding to improve the inquiry system and database search functions. These efforts to enhance visibility and usability may have contributed to the increase in deposits.

Furthermore, analysis data for Cre-driver rat lines generated through our previous infrastructure development program have been made publicly available on the website of our collaborating institution, the University of Tokyo.
By increasing the availability of useful rat strains, we expect further growth in resource distribution in the coming years.

MEMO

2. Highly efficient production of transgenic rats using *piggyBac* transposase mRNA and piezo-assisted microinjection

Kohtaro Morita
RIKEN BioResource Research Center

We previously established an efficient method for integrating long DNA fragments into mice using *piggyBac* transposase (PBase) mRNA. In contrast, transgenic (Tg) rats produced via pronuclear injection of a PBase-encoding plasmid show low Tg-positive rates (14–25%). To improve the efficiency, we attempted Tg rat production using PBase mRNA and piezo-assisted microinjection.

[Methods]

We microinjected a mixture of a donor plasmid (11.7 kb), designed to express EGFP specifically in the heart and tdTomato ubiquitously (final conc. 5 ng/μL), and in vitro-transcribed PBase mRNA (final conc. 50 ng/μL) into the pronuclei of rat zygotes using a piezo-driven injection system. Resulting pups were screened for transgene insertion by PCR, and expression profiles and copy numbers were analyzed by RT-PCR and digital PCR, respectively.

[Results]

Our results showed that 83% of the offspring carried the transgene. Fluorescence imaging and RT-PCR showed heart-specific EGFP and ubiquitous tdTomato expression. The average copy number was 4.74 (range: 0.19–22.2), with many individuals harboring multiple insertions. Furthermore, germline transmission of the transgene was also verified.

We next applied this method to generate Tg rats carrying a 9.9 kb sequence encoding a gene with five mutations found in human familial Alzheimer's disease (5xFAD). Using the same injection protocol, we achieved a 96% Tg-positive rate, with an average copy number of 13.3 (range: 0.69–58.2). However, despite detection of transgene-derived mRNA, no detectable transgene-derived protein was observed. Although establishment of an Alzheimer's disease rat model remains a challenge, our method enables high-efficiency integration of long DNA (>10 kb) sequences into the rat genome, providing a powerful tool for disease modeling and functional genomics in rats.

The detailed protocol for generating transgenic rats has been deposited in protocols.io (DOI: 10.17504/protocols.io.dm6gpmnwdgzp/v1).

MEMO

3. Advancing research infrastructure for mouse and rat resources using reproductive technologies

Toru Takeo[1], Satohiro Nakao[1], Yoshiko Nakagawa[1], Naomi Nakagata[2]

1. Division of Reproductive Engineering, Center for Animal Resources and Development (CARD), Kumamoto University
2. Division of Reproductive Biotechnology and Innovation, Center for Animal Resources and Development (CARD), Kumamoto University

Robust research infrastructure in laboratory animal science is fundamental to advancing discovery and innovation in biomedical research. To date, numerous genetically modified mouse and rat strains have been generated and preserved in bioresource repositories worldwide. To ensure their efficient and sustainable management, we have developed and optimized reproductive technologies. In mice, we established a comprehensive platform for embryo and sperm cryopreservation combined with IVF using frozen–thawed sperm at the CARD Mouse Bank. An advanced superovulation protocol employing inhibin antiserum and equine chorionic gonadotropin (IASe) markedly increased oocyte yield while reducing the number of donor females required. In addition, efficient cold storage and transportation of embryos and sperm have enabled the formation of a seamless inter-institutional network, providing a sustainable and globally connected infrastructure for mouse resources. Building on this experience, we expanded our efforts to rats, where fertilization efficiency of cryopreserved rat sperm remains a significant technical challenge. We reported a reliable method for rat sperm cryopreservation in 2020 and subsequently improved fertilization rates through optimization of IVF conditions, including treatment with high concentrations of bovine serum albumin and removal of cumulus cells. More recently, we established an optimized DAP213-based vitrification protocol for rat zygotes that enhances cryosurvival and developmental competence. A cold-transport system for rat sperm is also under development to facilitate inter-institutional distribution. Collectively, these advances establish a comprehensive reproductive technology platform at the CARD Mouse Bank and CARD Rat Bank, strengthening sustainable resource management. Through continued technology transfer and international collaboration, we strive to advance a globally integrated research infrastructure that promotes research integrity, sustainability, animal welfare, and drives scientific discovery and innovation.

MEMO

4. Building up National-wide Infrastructure of Model Animal in Korea

Je Kyung Seong
College of Veterinary Medicine, Seoul National University

MEMO

5. Introduction of Rat Resource Center in Korea

Jun-Won Yun
College of Veterinary Medicine, Seoul National University

Rats are essential animal models in diverse life science and preclinical research fields, including toxicology, pharmacology, and cardiovascular and neuroscience research, due to their physiological similarity to humans, larger body size, and complex behavioral traits. Leveraging these advantages, we proposed an integrated activation strategy centered on a Rat Resource Center, encompassing the generation of genetically engineered rat models and the establishment of standardized systems for resource management and distribution. A sustainable rat resource banking system is developing through genetically engineered rat production, standardized quality control, and the advancement of assisted reproductive technologies, including sperm and embryo cryopreservation and in vitro fertilization. In addition, researcher accessibility is enhanced via an integrated portal for rat resource registration, distribution, and information sharing within the model animal cluster, complemented by educational programs to strengthen research capacity. Collectively, active collaborative research based on these strategies, together with strengthened domestic and international cooperation, will enhance the efficiency and reproducibility of rat-based research and further reinforce our competitiveness in biotechnology and novel drug development.

MEMO

6. A knockout rat model to elucidate the molecular mechanisms underlying the developmental origins of health and disease (DOHaD) theory

Yasuhiro Uchimura
Division of Anatomy and Cell Biology, Department of Anatomy, Shiga University of Medical Science

Epidemiological studies have demonstrated a significant association between nutritional stress during fetal development and the onset of lifestyle-related non-communicable diseases (NCDs), such as obesity and type 2 diabetes, as well as psychiatric disorders, including schizophrenia, in adulthood. These observations led to the formulation of the developmental origins of health and disease (DOHaD) theory. However, the molecular mechanisms responsible for the phenotypic changes associated with DOHaD theory remain largely unclear.

To elucidate the molecular mechanisms, we conducted studies using rats as a mammalian model organism. We found that fetal undernutrition during early development (gestational days 5.5 – 10.5) irreversibly upregulates expression of the solute carrier 22 family member 23 (*Slc22a23*) gene in the rat brain. *Slc22a23* encodes a transporter expressed on the plasma membrane. However, its substrates have not been identified, and its physiological functions remain poorly understood. To investigate *Slc22a23* function in vivo, we generated *Slc22a23* knockout rats and analyzed their phenotypes. We then performed mass spectrometric analysis of plasma to identify substrates of the SLC22A23 transporter. Among the metabolites examined, lysophosphatidylcholine C20:4 (LPC 20:4) was the most markedly decreased in the plasma of knockout rats compared to wild-type rats. We therefore consider LPC20:4 a candidate substrate of the SLC22A23 transporter and compared its effects following intravenous administration. In wild-type rats, LPC 20:4 administration caused a more pronounced decrease in spontaneous locomotor activity in the open-field test and a greater reduction in target-reaching time in the Morris water maze test compared to *Slc22a23* knockout rats.

These results suggest that although LPC 20:4 administration increases its plasma concentration in both wild-type and *Slc22a23* knockout rats, wild-type rats expressing the SLC22A23 transporter can deliver LPC20:4 to brain neurons more efficiently than knockout rats. We interpret these results to indicate that efficient uptake of arachidonic acid within the LPC 20:4 moiety by brain neurons enhanced learning performance. As a next step, it is essential to determine whether the SLC22A23 transporter directly mediates the delivery of LPC 20:4 to the brain and its uptake into neuronal cells. To address this, we are conducting experiments using primary cultured neurons derived from both knockout and wild-type rats. LPC 20:4 is added to the culture medium, and we are examining whether there are differences in the efficiency of LPC 20:4 uptake between these primary neurons.

MEMO

7. Modeling human limb skeletal development using iPSCs, and applications in regenerative medicine

Takeshi Takarada
Department of Regenerative Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

Advances in human pluripotent stem cell (ES/iPS cell) technologies have enabled the recapitulation of human developmental processes and the establishment of disease models, thereby opening new avenues in regenerative medicine research. Among these, limb-bud mesenchymal cells (LBM), which originate from the lateral plate mesoderm of the embryo and give rise to the limb skeleton, therefore, Recapitulation of human limb-bud mesenchymal cells using human pluripotent stem cells represents a central challenge in bone and cartilage regenerative research.

We established a PRRX1-tdTomato reporter human iPS cell line and efficiently induced PRRX1-positive limb-bud–like mesenchymal cells (LBM) by precise modulation of WNT, BMP, TGF-β, and Hedgehog signaling (Nat. Biomed. Eng., 2021; STAR Protocols, 2022). By maintaining FGF and WNT signaling, we further generated long-term expandable LBM (ExpLBM), creating a stem-cell engineering platform that recapitulates the developmental progression from lateral plate mesoderm to limb formation and chondrogenesis.

ExpLBM displays strong chondrogenic capacity and forms hyaline cartilage–like extracellular matrix, enabling large-scale production of cartilage spheroids in stirred bioreactors (Biochem. Biophys. Res. Commun., 2023), shape-engineered cartilage constructs using CAT polymers (Biomed. Mater., 2023), and cartilage cell sheets on temperature-responsive substrates (Stem Cell Res. Ther., 2023). In our latest study, ExpLBM-derived cartilage particles and plates were xeno-transplanted into minipig articular cartilage defects, showing robust engraftment and expression of human cartilage markers (SOX9, COL2A1, ACAN) within two weeks, irrespective of graft morphology (NPJ Regenerative Medicine, 2025).

These findings represent an important step toward the clinical application of ExpLBM-based cartilage regenerative therapies. The ExpLBM technology established in this study enables faithful modeling of human limb skeletal development and holds promise for applications in cartilage regeneration, skeletal disease modeling, and the development of broadly applicable iPS cell–based cellular products. In this presentation, we will review the developmental trajectory and recent advances of this research and discuss the future potential of orthopedic medicine through the integration of human developmental biology and regenerative medicine.

MEMO

8. Recent Advancements in Skeletal Muscle Disease and Regeneration Research using Genetically Modified Rats as a Model System.

Keitaro Yamanouchi
Laboratory of Veterinary Physiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo

The advent of genome editing technology, facilitated by the CRISPR-Cas method, has led to significant advancements in the field of genetic modification, extending beyond mice to encompass a broader array of animal species.

This method was applied to rat genetic modification, resulting in the creation of the world's first rat model for human muscular dystrophy in 2014 (Nakamura et al., 2014). In human muscular dystrophy, mutations occur in the dystrophin gene, which maintains skeletal muscle strength. Duchenne muscular dystrophy (DMD) results from an out-of-frame mutation that causes complete loss of dystrophin protein. Becker muscular dystrophy (BMD) arises when an in-frame mutation produces an incomplete dystrophin protein. We have successfully generated both DMD-type and BMD-type rat models (Sugihara et al., 2020a; Teramoto et al., 2020). The DMD model rat (DMD rats) shows progressive muscle weakness and cardiac dysfunction (Sugihara et al., 2020b), similar to human DMD, and resolves the discrepancy between mdx mouse pathology and human disease, effectively bridging the gap caused by the mild phenotype of conventional models.

Using DMD rats, we ascertained that a specific mechanism contributing to the exacerbation of muscle pathology in DMD is the occurrence of premature cellular senescence, accompanied by increased expression of the senescence-associated factor p16 in skeletal muscle (Sugihara et al., 2020a). In addition, we have generated p16-deficient DMD rats and have found that muscle pathology is significantly alleviated (Sugihara et al., 2020a). More recently, we discovered that in the skeletal muscle of DMD rats, p16 is mainly expressed in multinucleated non-dividing muscle fibers. While p16 is recognized for its role in inducing cellular senescence in dividing cells through the arrest of the cell cycle, its expression in non-dividing cells indicates the potential for p16 to fulfill functions beyond the scope of cell division.

In this lecture, I will present the research progress from the generation of DMD rats to the present, along with several examples of discrepancies between the skeletal muscle phenotypes observed in genetically modified rats and mice.

References: Nakamura et al., Sci Rep. 4:5635 (2014), Sugihara et al., Sci Rep. 10:16385 (2020a), Sugihara et al., Int Heart J. 61:1279 (2020b), Teramoto et al., Dis Model Mech. 13:dmm044701 (2020).

MEMO

9. Brain Mechanisms Regulating Mammalian Reproduction: The Central Role of Kisspeptin Neurons

Hiroko Tsukamura
Nagoya University

Accumulating evidence indicates that hypothalamic kisspeptin neurons are the master regulators of mammalian reproduction by governing the hypothalamus–pituitary–gonadal (HPG) axis. Mutations or deletions of the kisspeptin gene (*Kiss1*) or its receptor gene (*Gpr54*) cause hypogonadotropic hypogonadism, resulting in pubertal failure and infertility in both humans and rodent models. Kisspeptin neurons are primarily located in two distinct hypothalamic regions—the anteroventral periventricular/preoptic area (AVPV/POA) in the anterior hypothalamus and the arcuate nucleus (ARC) in the posterior hypothalamus.

This presentation focuses on the role of AVPV/POA kisspeptin neurons as the gonadotropin-releasing hormone (GnRH) surge generator that triggers ovulation, and of ARC kisspeptin neurons as the GnRH pulse generator that drives folliculogenesis and steroidogenesis. The AVPV neurons in rodents and POA neurons in ruminants, primates, and other mammals, serve as the estrogen positive feedback site, as estrogen upregulates *Kiss1* expression and activates these neurons to induce the GnRH surge and subsequent luteinizing hormone (LH) surge. In contrast, ARC kisspeptin neurons—also known as KNDy neurons due to their co-expression of neurokinin B (NKB) and dynorphin A (Dyn)—are widely recognized as the source of GnRH/gonadotropin pulse generation. This presentation will discuss how stimulatory NKB and inhibitory Dyn organize the pulsatile activity of KNDy neurons to produce GnRH/LH pulses, drawing on evidence from our gene-modified rat models. The ARC kisspeptin neurons also function as a major site of estrogen negative feedback, as estrogen suppresses *Kiss1* expression in this region across multiple species. This presentation will further highlight the epigenetic mechanisms by which estrogen differentially regulates *Kiss1* expression in the AVPV/POA and ARC populations. Finally, I will discuss the neuroendocrine pathways through which malnutrition and lactation inhibit ARC kisspeptin neurons, thereby suppressing GnRH/LH pulses and contributing to malnutritional and lactational anestrus in mammals.

MEMO