## LABORATORY OF BIORESOURCES

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Medaka is a small egg-laying "secondary" fresh water fish found in brooks and rice paddies in Eastern Asia. This species has a long history as an experimental animal, especially in Japan. Our laboratory has conducted studies on evolution of the sex determination system using medaka and relatives, identification of the causal gene of mutants for primordial germ cell (PGC) migration and pigment cell development, and the gonadal development of medaka. In addition to these activities, our laboratory is stepping forward to lead the National BioResource Project Medaka (NBRP Medaka).

### I. Evolution of the sex chromosome and sex determination genes in *Oryzias* fish

Recent studies have demonstrated that *Oryzias* species have different genetic sex-determination systems (XX/XY and ZZ/ZW) (Figure 1). Furthermore, the sex chromosomes differ in their origin and degree of differentiation. These findings suggest the repeated creation of new sex chromosomes from autosomes during evolution of *Oryzias* fishes, possibly in association with the formation of new sex-determining genes. We are now trying to positionally clone the novel sex-determining genes in these species. Identification of these genes would provide a clue to understand the evolutionary process underlying frequent turnover of sex determination mechanisms.

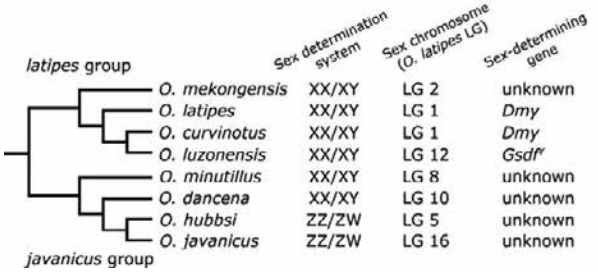


Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

### II. Genetic dissection of migration of primordial germ cells in medaka

Germ cells are responsible for the sustainability of life over generations in many multicellular animal species. To elucidate the mechanisms underlying the development of PGCs, we identified multiple mutations affecting the migration and development of the primordial germ cells in medaka in a prior large-scale mutagenesis screening project, and have analyzed a set of them to date. We focused on three mutants that have defects in primordial germ cell migration, *kamigamo*, *shimogamo*, and *naruto* that were isolated in the screening project. Positional cloning and analysis of the genes carrying the mutations are now in progress. In addition, two mutations, *kamigamo* and *shimogamo*, cause cystic pronephric ducts simultaneously with abnormal positioning of the primordial germ cells. Therefore, the analysis of these mutations will be important in giving basal knowledge underlying the mechanisms of human cystic kidney diseases.

### III. The study of type 2 diabetes using leptin receptor knockout medaka

Leptin in mammals is a peptide hormone secreted by adipose tissue. It has been shown to play a key role in the maintenance of energy homeostasis through the regulation of food intake and a range of physiological functions. Mice with a deficiency of leptin or its receptor exhibit hyperphagia (an increase in food intake). The hyperphagia causes obesity leading to type 2 diabetes-like symptoms, which is consistent with Caucasian patients. Leptin has also been isolated from fish, including medaka, however, the amino acid sequence is poorly conserved between fish and mammals (11-30%), and fish leptins are expressed mainly in the livers. To clarify the function of leptin on fish, we generated leptin receptor knockout (LepRKO) medaka by the TILLING method. The phenotypic analyses allowed us to reveal an appetite suppressive function of leptin signaling on medaka as well as mammals, and to find new value in medaka as a novel animal model for studying type 2 diabetes. As for appetite suppressive functions; LepRKO medaka showed high expression of the mRNA of NPY (3.5-fold) and AgRP (6-fold), which are known to be orexigenic peptides, and an increase in food intake (1.7-fold). Next, as for glucose metabolism; adult mutants showed signs of diabetes, such as fasting hyperglycemia and impaired insulin secretion, which is a late-onset disorder caused by excessive feeding during post-juvenile stages. Furthermore, they showed hyperglycemia even with the same fat level in the blood, muscle, and liver as WT medaka. The symptom is consistent with those of Asian patients, not but Caucasian patients and mice with leptin signaling deficiencies. Now, we are investigating the gene expression associated with dysfunction of pancreatic tissues under various feeding conditions. This will allow us to identify the factors of diabetes that are sensitive to food intake, regardless of obesity.

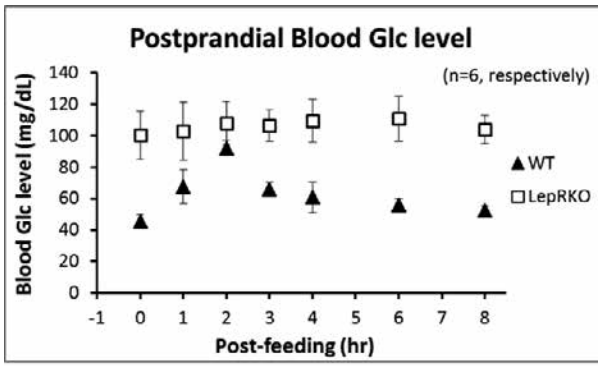


Figure 1. Postbrandial blood glucose levels of WT and LepRKO medaka.

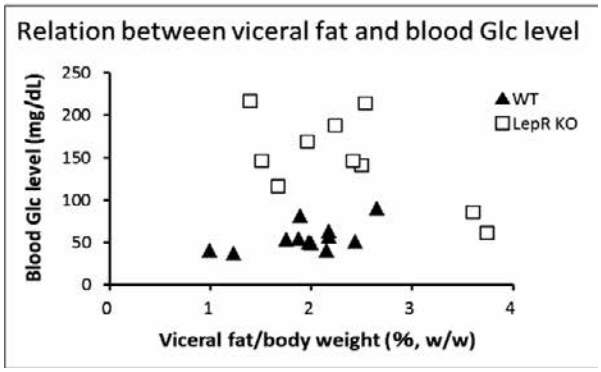


Figure 2. Relation between visceral fat and blood glucose levels of WT and LepRKO medaka.

## V. National BioResource Project Medaka (NBRP Medaka) (http://www.shigen.nig.ac.jp/medaka/)


Figure 3. NBRP Medaka website.

In 2007, NIBB was selected as the core facility of NBRP Medaka. Our laboratory is taking an active part in this project. With the goal of facilitating and enhancing the use of medaka as a model organism, we provide, maintain and collect living resources such as standard strains, inbred strains, and mutants in addition to frozen resources such as EST/cDNA, BAC/Fosmid clones, and hatching enzymes, as well as integrated information on medaka (Figure 3). We have been providing BAC clones of medaka related species, a library screening system employing a 3D PCR strategy for evolutionary studies, and the TILLING screening system for promoting the reverse genetic approach. NBRP Medaka aims to establish a first rate biological resource with the highest possible levels of accessibility and ease of use.

## Publication List

〔Original papers〕


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