Shiroishi Research Collaborative Group
Functional Genomics Research Group

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Goal

After whole genome sequence of the human and mouse were accomplished, the next challenge of Genome Science is to elucidate the function of every gene in the genome. Understanding of the function of genes involved in the pathogenesis of human diseases is essential to develop therapies and diagnosis of human diseases. Mouse mutants have become ideal models because of the close similarity of their genomes, developmental pathways and physiology to those of human. In our project, we have aimed to develop a large number of mouse mutants by genome-wide screening for various aspects of phenotypes to provide resources for studying the functions of genes, and to establish animal models for human diseases, in collaboration with many experts in the relevant research fields.

Activities

1. To develop bioresources for systematic analysis of gene functions, we will produce a large number of mouse mutants by means of genome-wide screen.
2. We will establish a comprehensive screening platform for late-onset phenotypes, and develop animal models for common human diseases.
3. We will construct an integrated database of mutant resources, which contains information concerning in-depth phenotype characterization and chromosomal locations of the mutant genes.

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Specific aim

We started ENU-mutagenesis program in 1999, with the aim to develop mouse models for human diseases. More than ten thousands mice have been subjected to dominant phenotype assays of the Modified-SHIRPA, hematological, biochemical, urinary, behavioral, sensory, cardiovascular and histological screens. Inheritance tests have been done for almost half of the phenodeviants (mutant candidates), and about half of the tested phenodeviants have been proved to be mutants. The tests for remaining of them are now in progress.We also started recessive phenotype assays for G3 mice generated by systematic IVF procedure. Achievements in each research section are described below.

Infrastructure of the mutagenesis program

Animal facility

Mouse facility of our group was established in January 2004 in RIKEN Tsukuba Institute. The facility has four floors, and total area is ca. 3,580 square meters, consisting of mouse housing areas of about 2,220 square meters, and cage washing rooms, storage rooms, locker rooms and others. There are 41 mouse rooms in this facility, and the capacity is ca. 38,000 mice in 297 mouse racks in total. This facility allows us to keep mice for long-term life up to 78 weeks of age, and to transfer mice frequently between the areas.

Production mice for dominant and recessive screening

To expand a spectrum of mutant phenotypes to be detected, we have used C3H/HeJ as well as DBA/2J, as a partner strain in the cross with ENU-mutagenized C57BL/6J for dominant screening. Approximately 2,000 C3B6F1 mice have been screened so far, and the inheritance testing and characterizations of the phenodeviants are now in progress. In our recessive screening, the mutagenized G0 males are crossed with C57BL/6J females. Then, males of the generated G1 founders are crossed again with C57BL/6J females. In production of the G2, G3 and G4 progeny, we perform IVF and/or natural mating. All G4 progeny is produced from a single G1 founder male.

Archives of mutant mice

To resurrect mutant mice, in vitro fertilization (IVF) is performed. Some mutants obtained in ENU-mutagenesis show reduced viability and fecundity. In order to resurrect such mutant mice, partial zona

dissection (PZD) and intra-cytoplasmic sperm injection (ICSI) have been used in our project.

High-Speed Gene Mapping

To improve gene mapping, we developed high-speed SNP(s)-base genotyping. We newly added TaqMan MGB probes that detects C57BL/6 vs. C3H and C57BL/6 vs. MSM SNP polymorphisms. This mapping system works routinely in our program. Thus far, we have mapped 109 lines out of 224 inherited mutant lines.

Improvement of central database

We improved MUSDB (Mutagenesis Universal Support Database) that supports the whole mutagenesis program. This year, we expanded various systems. For example, client software that supports pathological screening, blood tests, genetic mapping, mating with IVF technique and management of recessive screening have been improved and newly added.

Mutants detected from phenotype screens and their characterization Modified SHIRPA

We had screened over 10,923 G1 animals using Modified-SHIRPA for dominant phenotypes, which had yield 555 phenodeviants. They were subsequently subjected to inheritance test by crossing with wild-type mice. We confirmed that 155 mouse lines out of tested 300 were heritable mutants. We have also screened totally 1,436 G3 mice from April of 2004 to January of 2005 by the Modified-SHIRPA assay for recessive phenotypes. Eighteen new mouse lines showed transmission of the phenotypes to progeny in inheritance test.

Blood test

We have screened about 10,000 mice for early onset dominant screening, and 2,000 for late dominant screening. In early dominant screening, we identified 85 mutant lines including hematology and clinical biochemistry. In the case of late-onset screening, we found 135 phenodeviants. Inheritance test for the late-onset screening is in progress. In recessive screening, 22 pedigrees, consisting of about 300 mice in total, were screened. Thirteen and twelve abnormalities were found in early- and late-screening respectively. Inheritance tests are in progress.

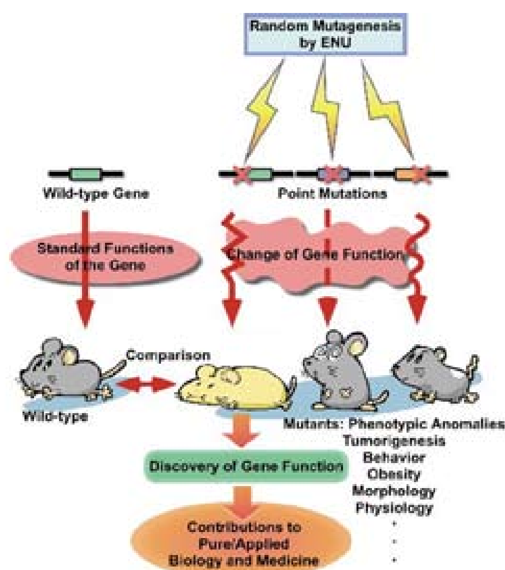

Figure 1 Mouse ENU mutagenetics project in RIKEN GSC.

Behavior

We had established high-throughput behavioral screening system to monitor home-cage activity, open-field activity and passive avoidance learning. By December 2003, we had screened about 2,000 G1 animals in total and obtained 16 dominant mutants with abnormal behavioral phenotypes. Initial characterization and fine mapping are underway in the three lines with high-activity and one line with learning deficit.

Sensory

By observing startle response evoked by click box stimulation at 9-weeks-of-age, 11,874 G1 mice have been screened so far, and 49 mutant candidates were detected. Then they were

subjected to the auditory brainstem response (ABR) measurement to analyze early-phases of impairment of auditory functions. Forty G1 mice out of the 49 candidates showed abnormality in ABR, and 27 mouse lines clearly indicated heritability of the phenotype. Funduscopic screening was carried out. The G1 mice with ocular abnormalities were subjected to inheritance test by backcrossing to DBA/2J strain. The seventeen lines were established as dominant mutants with ocular abnormalities such as cataract, microphthalmia, conus, shiny reflex of retinal arterioles and retinal degeneration.

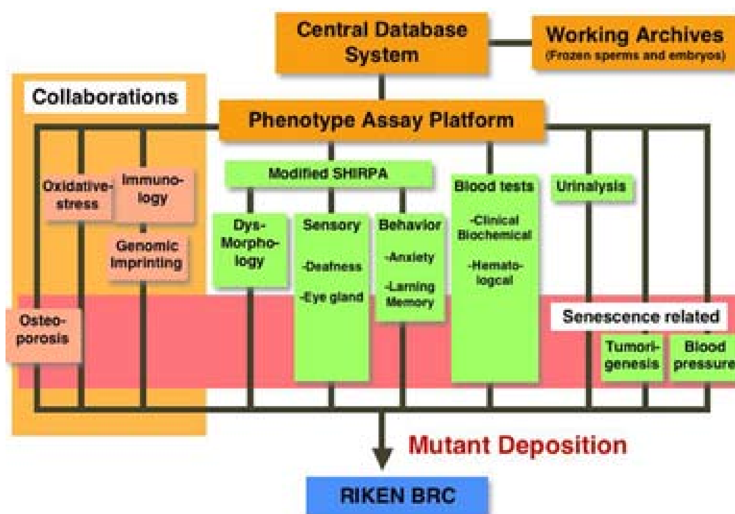

Figure 2 Mouse phenotyping platform of mutagenesis.

Blood pressure

To measure systolic and diastolic blood pressure levels of mice, we have adopted non-invasive system (BP2000) with coordinated 4 combined tail-cuffs, which is monitored by personal computer. We have screened 3,099 G1 mice of 17 weeks of age by this system, and 48 mutant candidates were subjected to the inheritance test. Twenty candidates were proved to be mutants, and subjected to gene mapping to identify the causative genes.

Genome imprinting analysis

We have developed a screening procedure for genomic imprinting. So far, we have screened 1,016 of G1 male, 187 of G3 male, and 1,114 of G3 female. As a result, some of the G1 and G3 individuals showed abnormal methylation patterns of target imprinted genes.

Pathological analysis

G1 male mice of 78 week old age were screened for late-onset tumor development as well as other late-onset abnormalities. Anatomical, histological, and hematological analyses are performed to discover neoplastic lesions in different organs and the hematic system. Fifty five candidates for tumor prone mutants and other late-onset abnormalities were subjected to the heritability tests using IVF-produced G2 progeny derived from the G1 frozen sperm.

Modifier gene mutants of the Apc^{Min}

Familial adenomatous polyposis coli (FAP) is caused by mutation of a tumor suppressor gene *Apc*. The multiple intestinal neoplasia (Min) mutant mice provide an excellent model for FAP, since Min mice carry a nonsense mutation in their *Apc* gene, and heterozygous Min mutants ($Apc^{Min/+}$) develop numerous intestinal adenomas. It is known that he wild-derived strains bear “modifier gene(s)”, which may suppress the carcinogenesis by the Apc^{Min} mutation. To identify such modifier genes, we have started a new ENU-mutagenesis screening using the Min mutant in the B6 background in combination with wild-derived inbred strains, MSM/Ms (*Mus musculus molossinus*; Japanese wild-derived inbred strain) and KJR/Ms (*Mus musculus yamasinai;* Korean wild-derived inbred strain). We have initiated phenotyping and finished

screening for the six pedigrees. Several G2 mice in a few pedigrees showed noticeably increased number of polyps. The assessment of the reproducibility and the inheritance of these phenotypes are in progress.

Establishment of diabetes model mice

We identified 12 mutations in the glucokinase (*Gck*) gene that were associated with hyperglycemia. Heterozygous mutations in human *Gck* cause maturity onset diabetes of the young, type 2 (MODY2). The mutations in our 12 mutant lines are scattered in the mouse *Gck* gene, and four lines bear identical mutations to those found in human MODY2 patients. Other than the *Gck* mutants, we have finished rough mapping for several diabetic mutant lines that are associated with obesity, insulin resistance, and destruction of pancreatic beta-cells.

Screening for mitochondrial disorders

We have developed a platform for screening dominant and recessive nuclear-encoded mitochondrial disorders. Mutants detected from the assay will provide good animal models for human mitochondrial disorders.

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Oral Presentations

International

1. Masuya H., Shimizu K., Sezutsu H., Sakuraba Y., Nagano J., Shimizu A., Fujimoto N., Ishijima J., Kaneda H., Kobayashi K., Maeda T., Gondo Y., Noda T., Wakana S., and Shiroishi T.: ENU-induced mouse enamelin (Enam) mutants as models for different clinical subtypes of human monogenesis imperfecta (AI), 18th International Mouse Genome Conference, Seattle, USA, Oct. (2004)

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