Japanese Journal of Veterinary Research 57(2): 129–133, 2009

INFORMATION

Hokkaido University confered the degree of Doctor of Philosophy (Ph.D.) in Veterinary Medicine on June 30, 2009 to 3 recipients.
The titles of theses and other information are as follows:

Studies on characteristics of Japanese encephalitis virus and the serological survey on Okinawa Island

Mika Saito

Division of Molecular Virology and Oncology, Graduate School of Medicine, University of the Ryukyus, 903–0215, Japan

Recent changes in the antigenicity and genotype of JEV distributed throughout Okinawa Island were described and alternative surveillance to cope with these changes to JEV was proposed.

First, the appearance of genotype 1 JEV isolates on Okinawa Island was reported for the first time. All five JEV Okinawan isolates from swine in 2002 and 2003 belonged to genotype 1, while all of the studied Okinawan isolates between 1968 and 1992 in the region belonged to genotype 3. Genetically, these were similar to newly emerging genotype 1 in Asia but not in Australia. Sero-epidemiologic patterns of swine serum samples in 2002 are significantly different from those in the 1980s. Swine serum samples from 1985 to 1988 neutralized Nakayama (prototype, genotype 3), Naha Meat 54 (1985 Okinawan isolate, genotype 3) and Oki 431S (2002 Okinawan isolate, genotype 1) with high correlation, while those in 2002 neutralized Oki 431S and Nakayama, but not Naha Meat 54. These results showed that the antigenicity of JEV distributed throughout Okinawa Island has changed. The deduced amino acid sequences of domain III of the envelope (E) protein showed two genotype 1-specific amino acid substitutions in Oki 431S. To confirm that this substitution altered the antigenic profiles of Oki 431S, the sequences were compared with Naha Meat 54, and Nakayama. Nakayama and Naha Meat 54 shared identical amino acid sequences, demonstrating no correlation between the genotype 1-specific substitution of these two residues and the antigenic differences.

Next, the surveillance of Japanese encephalitis using captured invasive mongooses under an eradication project on Okinawa Island was proposed. Currently, there is no effective therapy to treat JE. Moreover, immunization with Japanese encephalitis vaccine is not recommended because of adverse events, and several more years are needed to develop a new vaccine; therefore, alternative surveillance and control are urgently required to cope with the genotype and antigenic changes of JEV occurring on Okinawa Island. A project to eradicate invasive small Asian mongooses is underway to conserve the unique ecosystem of Okinawa Island. A sero-survey clarified that mongooses are sensitive to JEV and could have an unidentified role in its transmission cycle. Analysis of the antibody prevalence and titers of individual mongooses with the capture site using GIS pinpointed high JEV activity areas in a narrow range. These results are consistent with ongoing surveillance of pigs that can show prevalence in a wide range, because pigs are farmed animals and so precise analysis is impossible. Sero-surveillance using GIS analysis enables the identification of high JEV activity areas precisely, and can highlight

priority areas for JEV control, such as vector control, education and vaccination with the current vaccine.

In order to control JEV on Okinawa Island, prospective studies are required to elucidate the antigenic, genetic, and pathologic characteristics of JEV and to follow JE cases without vaccination.

Original papers of this thesis appeared in *Am. J. Trop. Med. Hyg.*, **77**: 737–746 (2007) and *Vector Borne Zoonotic Dis.*, **9**: 259–266 (2009).

Age-related changes in the higher brain functions of Senescence-accelerated mouse prone 6 (SAMP6)

Kimie Niimi

Brain Science and Life Technology Research Foundation Tokyo, 175–0094, Japan

The senescence-accelerated mouse (SAM) was developed through selective breeding of the AKR/J strain based on a graded score for senescence, life span, and pathological phenotypes. There are nine senescence-prone (SAMP) strains and three senescence-resistant (SAMR) strains. SAMP strains have a shortened life span and show early manifestations of senescence, such as various skin lesions and increased lordokyphosis, after a period of normal development. Among SAMR strains, SAMR1 is long-lived, showing resistance to early senescence, and is used as a control. Among SAMP strains, SAMP6 is considered to be a model of senile osteoporosis with slow bone loss after 4 months of age. Recently, it was reported that SAMP6 exhibited increased expression of S100β in the brain compared to SAMR1, suggesting that SAMP6 is also useful as a model of age-related diseases related to central nervous system alterations. I performed a battery of behavioral analyses using 1- (juvenile stage), 4–6- (adult stage), and 8–12-month-old (old stage) SAMP6 and age-matched SAMR1 to investigate the age-related changes in behavioral features and the mechanisms involved.

The battery of behavioral analyses revealed innate behavioral alterations in SAMP6, including higher motor activity, lower anxiety, increased short-term memory, a motor coordination deficit, and antidepressant activity. The higher motor activity of SAMP6 was observed until the adult stage, and then the motor activity began to decline, and lower motor activity was observed at the old stage, indicating that the motor activity of SAMP6 exhibited the same pattern of age-related changes as seen in the bone mass of SAMP6. The marked motor coordination deficit of SAMP6 was observed at the juvenile and old stages, whereas amelioration in the motor coordination deficit was seen in the adult stage, suggesting that the motor coordination of SAMP6 exhibited a pattern of age-related changes similar to that of the bone mass of SAMP6. On the other hand, the differences in anxiety and antidepressant activity between SAMP6 and SAMR1 decreased gradually with age, indicating that the lower anxiety and antidepressant activity of SAMP6 showed another pattern of age-related change. No apparent age-related change was observed in the increased short-term memory of SAMP6. Accordingly, the behavioral features of SAMP6 were divided into three categories based on the pattern of age-related changes: (1) accelerated-senescence-like behaviors; (2) behaviors with age-related changes; and (3) behaviors with no age-related changes.

The expression of tyrosine hydroxylase, an enzyme involved in the biosynthesis of dopamine, and its phosphorylated form was increased in the striatum and nucleus accumbens (NAc) of juvenile SAMP6, suggesting an increase in the concentration of dopamine in the juvenile SAMP6 brain. This was thought to be one of the innate alterations related to the higher motor activity of SAMP6. Increased expression of D1 in the striatum, an over-activated D1 signal cascade, and an increased dopamine concentration in the NAc were seen in adult SAMP6, which seemed to explain the higher activity of adult SAMP6. An apparent decrease in the sensitivity of D1 of old SAMP6 compared to adult SAMP6 was observed, which was thought to be involved in the decreased motor activity of old SAMP6. These results suggest that an age-related alteration in the D1 sensitivity of SAMP6 is one of the mechanisms altering motor activity, one of the accelerated-senescence-like behaviors observed in this strain. On the other hand, the increased D3 expression in the cerebellum of adult SAMP6 was thought to be one of the mechanisms related to the motor coordination deficit, another accelerated-senescence-like behavior observed in this strain. However, further examinations of the D3 expression levels in juvenile and old SAMP6 cerebellum are needed to evaluate whether the altered D3 expression is involved in the accelerated-senescence-like alteration of this behavior.

The serotonin system was studied to examine the mechanism of the lower anxiety and antidepressant activity, behaviors with age-related changes, of SAMP6. The expression of tryptophan hydroxylase, a serotonin biosynthesis enzyme, and its phosphorylated form were increased in the brainstem of juvenile SAMP6, suggesting an increase in the serotonin concentration in the juvenile SAMP6 brain. This was thought to be one of the innate mechanisms related to the lower anxiety and antidepressant activity of SAMP6. Serotonin concentrations were increased the cortex and NAc of adult SAMP6, which likely explained these behavioral patterns in adult SAMP6. However, further examination of the serotonin concentration of juvenile and old SAMP6 brains is needed to evaluate whether the altered serotonin concentration is involved in the age-related change of these behaviors.

The dopamine and serotonin systems and *N*-methyl-D-aspartate (NMDA) receptors were studied to examine the mechanisms for increased short-term memory, a behavior with no age-related changes, of SAMP6. As mentioned above, the increased dopamine and serotonin concentrations in the juvenile SAMP6 brain were also thought to be one of the innate changes related to the increased short-term memory of SAMP6. In addition, expression of the NMDA receptor subunit 2B (NR2B) was increased in the forebrain of adult SAMP6, and this appeared to be involved in the increased short-term memory of adult SAMP6. Further examination of the mechanisms involved in this behavioral property of old SAMP6 is needed.

In this study, a battery of behavioral analyses using animals at three different ages showed various behavioral alterations with aging. In addition, biochemical and pharmacological approaches revealed the involvement of several different mechanisms in the behavioral alterations. These results suggest that the higher brain functions are controlled by variable thresholds of the respective neurons and complex neuronal networks. Studies using SAMP6 might elucidate the influences of aging on higher brain functions and related mechanisms, resulting in the specification of the signal cascades that activate higher brain function and the development of new drugs that act on these cascades. These could increase the healthy longevity and quality of life of humans.

Original papers of this thesis appeared in *Behav. Brain Res.*, **188**: 329–336 (2008), *Exp. Gerontol.*, **43**: 847–852 (2008), *Brain Res.*, **1250**: 175–182 (2009) and *Physiol. Behav.*, **96**: 464–469 (2009).

Studies on developmental dynamics and causing factors of testicular oocyte in MRL/MpJ mice

Saori Otsuka

Laboratory of Anatomy, Department of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060–0818, Japan

Gonad is unique among all organs because of its bipotential nature, a testis or an ovary. In most mammals, sex determination is genetically controlled by the presence or absence of the Y chromosome. Germ cells also have the plasticity to develop either oogonia or prospermatogonia. The sex of primordial germ cell is determined by the time points of meiosis initiation which regulated by gonadal somatic cells. In mice, this phenomenon occurs at E13.5, when ovarian germ cells enter meiosis and testicular germ cells are arrested in the G0/G1 of the mitotic cell cycle. These sex differentiation mechanisms advocate a universal rule 'mammalian males produce only sperms in their testes and females produce only oocytes in their ovaries'. However, as an exception to this rule, the author found oocytes in the testes of newborn MRL/MPJ (MRL) male mice. In this thesis, the author attempted to reveal the mechanism of initiating oogenesis in testis and verify the possibility of production of offspring using testicular oocyte.

At first, the author proved the existence of oocytes in testis by their morphological characteristics and the expression of some oocyte-specific genes in the testis of MRL mice. The testicular oocytes had a diameter of 50 to 70 μm, and were surrounded by zona pellucida observed between oocytes and follicular epithelial cells. Although the follicular epithelial cells formed a multilayer similar to that observed in early stage secondary follicles, they never formed a follicular antrum or a polar body-like structure. Ultrastructurally, the testicular oocytes contained numerous microvilli and cortical granules, receiving cytoplasmic projections from follicular epithelial cells. The testicular oocytes appeared from 0 to 30 days afterbirth and the largest number was found around day 14. The expression of the oocyte-specific genes zona pellucida 1–3 (*Zp1–3*) and oocyte maturation, alpha (*Omt2a*) was detected in testes from MRL mice. These morphological characteristics as an oocyte and the expression of oocyte-specific genes indicate that newborn MRL male mice evidently have the ability to produce oocytes in their testes.

The author next examined the derivation of testicular oocyte and verified the characteristics as an oocyte. In MRL fetal testes, some germ cells underwent meiosis and oogenesis just like fetal ovarian germ cells at the same stages. Additionally, the zona pellucida of testicular oocyte contained ZP3, while follicular epithelial cells lacked forkhead box L2 (FOXL2). Furthermore, the testicular oocyte had the ability to fuse with sperms. These results suggest that testicular oocytes are derived from primordial germ cells at about E13.5 under the same process with ovarian oocytes and they contain the characteristics as so-called oocytes such as the ability to fuse with sperms. However, follicular epithelial cells lacking FOXL2 might be involved in the limitation of testicular oocyte development.

Finally, the author attempted to identify the causing genes of appearance of testicular oocyte. As a result of examination of testes from several inbred strains and F1 produced between MRL and C57BL/6, testicular oocytes were also found within newborn AKR mice and B6MRLF1 other than MRL mice. Based on the observation of F1, one of the genes causing the appearance of testicular oocyte existed on the Y chromosome. Then

the author analyzed the sex determining region on Y (*Sry*) genes from several inbred mouse strains and identified a shortened glutamine repeat near the C-terminal region that was unique to MRL and AKR. These findings suggest that polymorphism of glutamine repeat within SRY correlates with the appearance of testicular oocyte and this phenotype is derived from AKR, one of the original strains of MRL mice.

As conclusion, testicular oocytes found in newborn male MRL mice are derived from primordial germ cells during embryonic period and their appearance correlates with polymorphism of SRY glutamine repeat. Although it is difficult to expect normal development of oocytes within testicular environment at this point, there is still a possibility of healthy growth by rescue and culture *in vitro*. Thus testicular oocytes contain potential keys to open new doors in reproductive biology and provide more clues concerning the development of the reproductive system in mice.

Original papers of this thesis appeared in *Biol. Repod.*, **79**: 9–16 (2008) and *Jpn. J. Vet. Res.*, **56**: 129–138 (2008).