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AN ANIMAL MODEL OF LUTEOLYSIS: ABNORMAL ACCUMULA-TION OF LUTEAL BODIES IN OVARIES OF THE SENESCENCE ACCELERATED MOUSE (SAM)

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Introduction

The senescence accelerated mouse (SAM), which is an inbred strain of AKR mice, was bred and established in Kyoto University by Takeda *et al.* and is a useful animal model of accelerated senescence [1]. SAM consists of two related strains: SAM prone (SAMP) and SAM resistant (SAMR). SAMP mice show accelerated changes in many of the signs of aging including moderate to severe loss of activity, hair loss and lack of hair glossiness, skin coarseness, periophthalmic lesions, increased lordokyphosis of the spine and a shortened life span as compared with the control strain SAMR that ages normally [1, 2]. These model animals are considered to be very useful for studies of the mechanisms of senescence, but are very difficult to breed [2]. Although physiological, pathological and biochemical changes associated with aging in SAMP mice with advancing age have been studied by many investigators, insufficient data are available reading the on reproductive properties of SAM mice [3].

Previously, we compared the morphometric parameters of spermatogenic cells in male SAMP mice with those of SAMR mice after birth until 40 weeks old (approximately the mean life span of the SAMP strain). Our results indicated that testicular maturation begins at an earlier age in SAMP than SAMR mice, and signs of testicular deterioration we evident only in SAMP mice at the age of 40 weeks [4, 5]. Moreover, we previously compared female reproductive properties and early embryonic development of SAMP mice with those of SAMR mice [6, 7]. The reproductive senescence of SAMP mice is more accelerated than that of SAMR mice. The reproductive life span of SAMP mice was shorter than that of SAMR mice, and the total number of SAMP pups was 41.7% less than from SAMR. Cell cleavage was delayed in embryos of SAMP as compared to SAMR mice.

During these investigations of the reproductive properties of SAM mice, we found the abnormal accumulation of luteal bodies (LBs) in the ovaries of SAMP mice. However, the mechanism of such abnormal accumulation is not yet understood.

To determine the mechanism of the abnormalities in luteal cell regression in the ovaries of SAMP mice, we examined the changes in serum progesterone and 17ß-estradiol levels in SAM mice during the estrus cycle, enzymehistochemically demonstrated the activities of 17ß-hydroxysteroid dehydrogenase (17ß-HSD) and 20a-hydroxysteroid dehydrogenase (20a-HSD) to determine steroid hormone production in the ovaries, and histochemically determined the apoptotic cells in the LBs by the terminal deoxynucleotidyl transferase-mediated biotinylated deoxyuridine triphosphate nick end-labeling (TUNEL) method.

Materials and Methods

Histopathology and histochemistry for 17β-HSD and 20a-HSD

Female SAMP (SAMP1//FAP) and SAMR (SAMR1/FAP) mice (20 to 30 weeks old) were used. The mean life span of the SAMP mice (42 weeks old) was shortened by about 26% relative to that of the SAMR mice (57 weeks old) [1, 2, 4-7]. SAM mice were checked daily, and only those without severe changes (severe loss of activity, hair loss and lack of hair glossiness, skin coarseness, periophthalmic lesions, and increased lordokyphosis of the spine) estimated according to Takeda *et al.* were used [1, 6, 7]. Estrus cycle stages were determined by daily examination of vaginal cytology according to Pedersen, and animals showing more than two consecutive 4-day-estrus cycles were used [8]. They were sacrificed under ether anesthesia in the afternoon (approximately 14:00) at proestrus, estrus, metestrus and diestrus stages. Ovaries were rapidly removed, weighed and sectioned.

For conventional histopathology and TUNEL staining, a part of the ovary was fixed with 10% phosphate buffered formalin (pH 7.4). The tissue samples were dehydrated through a graded ethanol series and embedded in paraffin, and then serial sections (3 µm thick) were cut from the median part of each ovary on a microtome, mounted on glass slides, deparaffinized in xylene, and rehydrated through a graded ethanol series. For enzymehistochemistry, the remaining tissue was frozen in dry ice and isopentane mixture, and then serial frozen sections (5 µm thick) were cut on a cryostat and mounted on glass slides.

In situ detection of fragmented DNA and DNA electrophoresis

Paraffin sections were stained by the TUNEL method to allow visualization of the apoptotic cells [10, 11]. LBs were isolated under a surgical dissecting microscope, and then DNA samples prepared from LBs were electrophoresed [10].

Results and Discussion

The life span of SAMP mice is shorter than that of other mouse models used for aging research (CBA, C57BL, RFM and NZB strains), and age-dependent changes in reproductive functions are more severe in SAMP mice than in other murine models [3-7]. Female reproductive parameters in aged SAMP mice, i.e. shorter reproductive life span, a reduction in parturition number, less live pups, and lower incidence of regular estrus cycles in comparison with age-matched SAMR mice, in which these reproductive parameters are within the normal ranges, indicate that age-related severe deterioration of ability of reproduction occurs only in SAMP mice [6, 7]. Even in young SAMP mice (15 weeks old), reduced litter size and lower incidence of regular estrus cycles were noted as compared with normal/SAMR mice [6, 7]. Interestingly, we found abnormally accumulated LBs in the ovaries of SAMP mice with regular estrus cycle.

The present study was performed to determine the mechanism of the abnormal accumulation of LBs in SAMP mice. During the estrus cycle, no differences were observed in the kinetics of 17ß-estradiol or progesterone in peripheral blood between SAMP and SAMR mice, indicating that there are no differences in female hormonal regulation between the two strains. In luteal cells of abnormally accumulated LBs of SAMP mice, high levels of 20a-HSD activities were demonstrated histochemically. Moreover, high levels of 17ß-HSD activities were also demonstrated in these cells by an enzyme histochemical method.

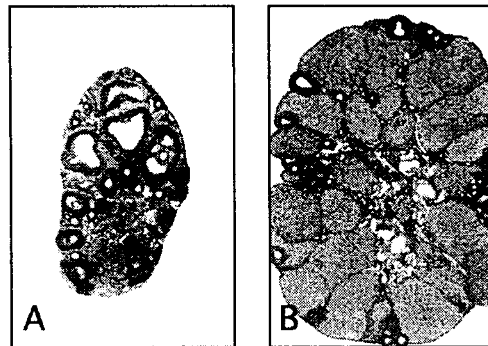

Fig. 1. Representative tissue sections of the ovaries of SAMR and SAMP mice (A and B, respectively). For conventional histopathological evaluation, paraffin sections were stained with hematoxylin and eosin. Many luteal bodies (asterisks) were accumulated only in SAMP ovaries (B), but not in those of SAMR ovaries (A). x 40.

Steroid hormones synthesized and secreted from the ovaries coordinate the function of the entire female reproductive system, and increased 17ß-estradiol secretion characterizes the follicular phase of the estrus cycle, reflecting the specialized endocrine function of the preovulatory follicle, and the steroid hormone balance in peripheral blood reflects folliculogenesis and ovulation [7]. In luteal cells of rodents, progesterone is catabolized to 20a-hydroxyprogesterone, the physiologically inactive form, by 20a-HSD. Thus, the present results indicated that progesterone is synthesized and then rapidly catabolized into an inactive steroid in luteal cells of abnormally accumulated LBs in SAMP ovaries, that such catabolism causes normal progesterone level in peripheral blood in SAMP mice, and that SAMP mice show normal consecutive 4-day estrus cycles.

In our preliminary study, extremely low concentrations of serum 17ß-estradiol were detected in SAMP mice with irregular estrus cycles (unpublished data), and such irregular secretion of 17ß-estradiol is considered to reflect the age-related deterioration of reproductive functions in the ovaries of SAMP mice [6, 7]. These findings indicated that functional regression, i.e. a decrease in progesterone production, occurs in the luteal cells of abnormally accumulated LBs in SAMP mice.

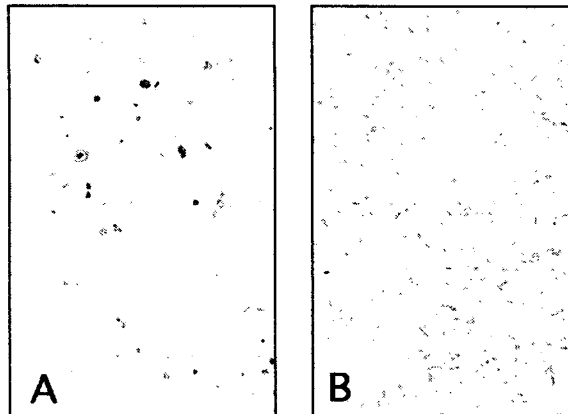

Fig. 2. Representative sections of luteal bodies of SAMR (A) and SAMP (B) ovaries were histochemically stained to detect the apoptotic cells by the TUNEL method. During regression of the luteal bodies, many TUNEL-positive cells (arrows) were detected among the luteal cells in the ovaries of SAMR mice (A). No TUNEL-positive apoptotic cells were seen in abnormally accumulated LBs in the ovaries of SAMP mice (B). x 400.

No TUNEL-positive (apoptotic) cells were histochemically demonstrated in abnormally accumulated LBs of SAMP ovaries, but many positive cells were detected in the LBs of SAMR ovaries. Moreover, no DNA ladder was seen in DNA samples prepared from abnormally accumulated LBs of SAMP ovaries, while clear DNA laddering was seen in DNA samples prepared from the LBs of SAMR ovaries. These histochemical and biochemical findings confirmed that arrest of morphological regression, i.e. deficiency of apoptosis, occurs in the luteal cells of abnormally accumulated LBs in SAMP mice.

Functional regression occurs but structural regression is inhibited by blockage of apoptosis in luteal cells of abnormally accumulated LBs in SAMP mice [12]. These findings suggested the SAMP mouse is a useful animal model in which to study the local mechanism of regulation of LB regression in murine ovaries.

Acknowledgments

The authors wish to thank Y. Akiyama-Sasagawa, Y. Kurihara, S. Maeda, J. Matsumoto and M. Sugimoto for excellent assistance. This work was supported by a Grant-in-Aid for Creative Scientific Research to N. M. from the Japan Society for the Promotion of Science (13GS0008), by a

Grant-in-Aid to N. M. from the Ministry of Education, Science, Sports and Culture of Japan (A2-13027241), and by a Grant to N. M. from the Itoh Memorial Foundation.

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