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In mammals, several social behaviors are dependent on sex. These sex-dependent patterns of behavior must be acquired through highly irreversible processes during development. We hypothesize that the long-term effects of sex steroids at the perinatal stage on behaviors after puberty are somehow marked at the genome level. We are currently investigating the epigenetic status of the discrete brain areas responsible for the sex difference of the structure and behaviors.

Ⅰ. DNA methylation of sex steroid receptor genes

In mammals, DNA methylation, mainly occurring on CG dinucleotides, is a fundamental mechanism that differentiates the gene expression pattern in respective cells. DNA methylation is a restraint of the pluripotency because once the pattern is established during development it is maintained through cell division. On the other hand, for example, some fish that contain much less methylation activity are found to easily and reversibly change their sex status according to the environmental context. In rodents, endocrine disturbance at the fetal and/or postnatal stage irreversibly changes behaviors such as the lordosis (in females) and the mounting (in males) after the pubertal stage that are normally dependent on genetic sex. In some cases, lordosis can be observed even in males and mounting in females. These clearly indicate that sex-dependent patterns of behavior are not directly dependent on "sex-specific" genes but rather established through epigenetic processes. We have found the sex- and brain area-dependent DNA methylation pattern of rodent steroid receptor genes (estrogen receptor α, *ERα*; androgen receptor, *AR;* progesterone receptor, *PR*) that can be modulated by the novel noncoding RNAs, named *ERαas*, *ARas* and *PRas*. Furthermore, we have generated *ERαas*- and *ARas*-transgenic (Tg) mice in which *ERαas* and *ARas* fragments are constitutively expressed, respectively. We report herein on the functional alteration of mouse brain by these noncoding RNAs.

1-1 Functional alteration of rodent brain by noncoding RNA for ER α promoter

ERα participates in the cellular, reproductive and behavioral mechanisms. We found novel endogenous antisense transcripts on *ERα* locus, named *ERαas*. In the brain, *ERαas* expression showed tissue-, development-, and sex- dependent pattern. *ERαas*-Tg mice gained weight faster than the wild type during prepubertal stages. *ERαas*-Tg females showed the significant reduction of lordorsis and the larger volume of ventromedial hypothalamus (VMH) which are rather reminiscent of the male-like patterns. The search for molecular targets of *ERαas* revealed that the *ERα* promoter was specifically demethylated, resulting in the *ERα* upregulation. It is to be noted that, of discrete brain areas, only the principal portion of the bed nucleus of the stria terminalis, but not the VMH, showed the sex-dependent DNA methylation pattern in mice and rats. Thus, *ERαas*, acting on the *ERα* promoter as a noncoding RNA, is a component that establishes its epigenetic status for gene expression. Spatiotemporal expression of *ERαas* may mediate the functional differentiation of the female and male brains with or without morphological alterations in rodents.

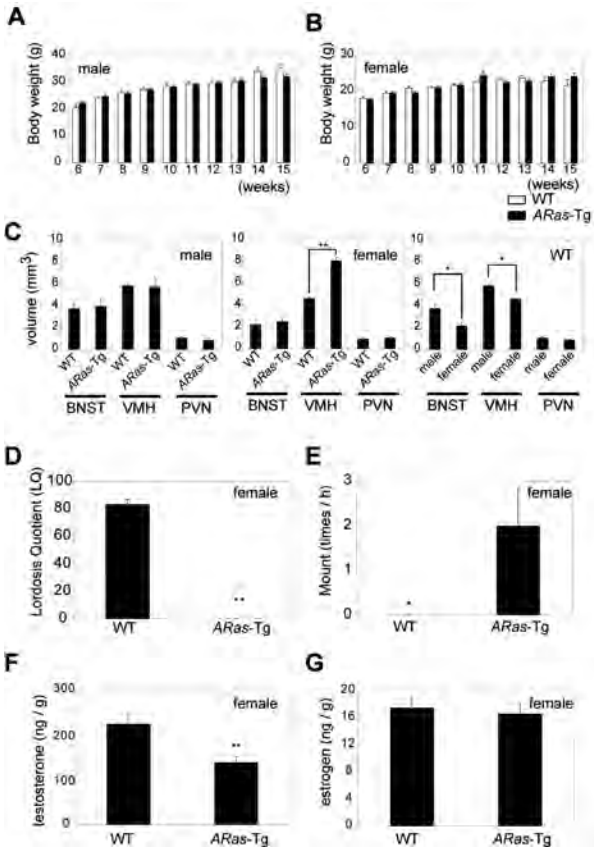

Figure 1. Physiological alterations in *ARas*-Tg mice. (A) Body weights of males. Growth graphs of the 15 weeks old *ARas*-Tg and the wild type. WT denotes wildtype. (B) Body weights of females. Growth graphs of the 15 weeks old *ARas*-Tg and the wild type. (C) Graphs showing the volume of each nucleus of the PVN (paraventriclar nucleus), BNST (principal portion of the bed nucleus of the stria terminalis), and VMH in *ARas*-Tg and wild type mice. (D) lordosis behavior test in *ARas*-Tg and wild type female mice (LQ scores). (E) Mount behavior tests in *ARas*-Tg and wild type female mice. Animals was measured with the times of mount behaviors. (F) Testosterone levels in excrement of *ARas*-Tg and wild type female mice. (G) Estrogen levels in the excrement of *ARas*-Tg and wild type female mice. Bars represent mean ±SE (**$p < .01$, $.01 <$ *$p < .05$; student t-test).

1-2 Functional alteration of rodent brain by noncoding RNA for AR promoter

In addition to the estrogen signaling, AR-mediated androgen signaling participates in the cellular, reproductive and behavioral mechanisms. We found novel endogenous antisense transcripts on *AR* locus, named *ARas*. In the brain, the expression showed tissue-, development-, and sex-dependent pattern. *ARas*-Tg female mice showed the significant reduction of lordorsis, occurrence of mount behavior, and larger volume of VMH which are rather reminiscent of the male-like patterns (Figure 1). The circulating testosterone level was lower in *ARas*-Tg females than in wild type females. The search for molecular targets of *ARas* revealed *AR* promoter to be specifically demethylated, resulting in the *AR* upregulation. Of the discrete brain areas, only VMH showed the sex-dependent DNA methylation pattern in mice and rats. Orchidoectomy and peripheral application of estradiol benzoate at the onset of birth increased and decreased the methylation level at several CG sites in males and females, respectively, suggesting that the sex-specific methylation of *AR* promoter was established/maintained by estrogen that was produced by the aromatization of testosterone derived from the testis. Thus, *ARas*, acting on the *AR* promoter, is a component that establishes its epigenetic status for gene expression in a spatiotemporal manner. It is likely that appropriate control of AR expression by *ARas* mediates the estrogen-induced sexual differentiation of the brain.

Publication List

〔Original papers〕

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〔Review article〕

- Maeda, K., Adachi, S., Inoue, K., Ohkura, S., and Tsukamura, H. (2007). Metastin/kisspeptin and control of estrous cycle in rats. Rev. Endocr. Metab. Disord. *8*, 21-29.