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Review Article

TRANSCRIPTOME ANALYSIS OF BRAIN FROM STEROIDOGENIC FACTOR 1 KNOCKOUT MICE

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Summary: The brain begins its life as neither male nor female and waits to be impacted by differences of genetic and hormonal actions that continue throughout the lifetime of an organism. Many differences have been described in the brain between sexes of a variety of species, ranging from amphibians and reptiles through birds and mammals, including humans. In our study the genomic-base array revealed six genes differentially expressed in brains from *SF-1* knockout males and females and their WT controls. All of these genes are sex chromosome-linked genes.

Key words: brain; sex difference; mouse; DNA microarray

Sex differences in body and brain

An origin of differences between sexes in mammals is in sex chromosomes with males having one X- and one Y-chromosome and females having two X-chromosomes. On the Y-chromosome there is a sex-determining region – *Sry* gene, which induces an undifferentiated gonads to form as testes rather than ovaries [1]. Testes then secrete hormones, testosterone and anti-Müllerian hormone, and the body develops in masculine fashion. If *Sry* is absent, gonads develop as ovaries, and the body forms in a feminine way [2]. In the last decade several studies in model organisms revealed that some sex differences in non-gonadal tissues are a consequence of differential effects of X-linked and Y-linked genes acting within non-gonadal cells [3; 4; 5; 6]. From these studies arises the question whether genes on sex chromosomes, which are present in different quantities in male and female genomes, might be expressed in the brain and might be partially responsible for a sex-specific model of development and/or function [7]. The non-recombinant region of the Y-chromosome (NRY) in males contains genes that are not present in females, and might act in the brain to cause masculine neural development. On the other hand, genes in the non-pseudoautosomal region of the X-chromosome (NPX), which are present in two copies in females but only in a single copy in males, could cause female-specific neuronal development [8]. Although one X chromosome is inactivated during development, a significant number of NPX genes escape inactivation, so the amount in the two sexes may not be equal [9].

The study of Xu *et al.* [8] with four core genotypes (FCG) mice (four genotypes are XY gonadal males (XYM), XX gonadal females (XXF), XX gonadal males (XXM) and XY gonadal females (XYF)) showed that some of the Y-linked genes (Usp9y, Ube1y, Smcy, Eif2s3y, Uty and Dby) do not require testicular secretion for sexual dimorphic expression in the brain as they were expressed in XYM and in XYF mice. In the same study they showed that six X-linked homologues (Usp9x, Ube1x, Smcx, Eif2s3x, Utx and Dbx) were also expressed in the brain, and in adulthood all of these transcripts were expressed at notably higher levels in female brains in comparison to male brains, regardless of their X-inactivation status. Several other studies revealed similar findings [10; 11].

SF-1 KO mice as a model for brain sexual differentiation

Steroidogenic factor 1 (SF-1, NR5A1) is a member of the nuclear receptor superfamily of transcription

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factors with important roles in the development and function of endocrine organs [12]. Mice lacking *SF-1* (*SF-1* knockout mice, *SF-1* KO) are born without adrenal glands and gonads; they have non-functional gonadotrope cells in the pituitary and ventromedial hypothalamus is not developed as a compact nucleus [13; 14]. Due to adrenal insufficiency they die shortly after birth. Early corticosteroid injections followed by adrenal transplantation can maintain *SF-1* KO mice into adulthood [15]. Because of early regression of gonads and adrenals, these mice are not exposed to endogenous sex steroids during development and are consequently an important model for studying hormone independent development of brain sex differentiation. Gonadal deficiency does not necessarily prevent exposure to sex steroids from other resource such as placenta, mother, or nearest siblings during fetal development, but these influence should be the same for all *SF-1* KO offspring [16].

For our studies, heterozygous *SF-1* KO mice were mated to obtain homozygous *SF-1* KO animals and wild type (WT) littermate controls. All newborn mice were treated with daily corticosteroid injections. After genotyping, a transplantation of adrenals to *SF-1* KO mice is performed on postnatal day 7 as described before [16]. All control WT mice are gonadectomized before puberty.

Current work

In our study we used the central part of an adult mouse brain, which involved the preoptic area, hypothalamus, amygdala, hippocampus and part of the cortex from WT control and SF-1 KO mice. After isolating the total RNA (mRNA), we performed a microarray experiment using mouse genomic-based array Affymetrix GeneChip®.

Data mining for differentially expressed genes revealed several genes that were statistically significantly different between sexes but not between genotypes. Interestingly, all candidate genes were sex chromosome linked, but validation of these results by quantitative RT PCR and/or in situ hybridization will be needed and is currently underway.

Conclusions

Since *SF-1* KO mice are not exposed to endogenous sex steroid hormones during development and after birth, changes in gene expression found in the microarray experiment must be sex hormone independent, as the same expression is present in WT and SF-1 KO animals.

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ANALIZA TRANSKRIPTOMA MOŽGANOV MIŠI BREZ GENA SF-1

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Povzetek: Možgani sprva niso niti moški niti ženski, temveč se diferencirajo pod vplivom različnih genetskih in hormonskih dejavnikov, ki so jim izpostavljeni skozi celotno življenje organizma. Razlike v možganih med spoloma so opisane že pri mnogih vrstah, od dvoživk in plazilcev preko ptic in sesalcev pa vse do človeka. V raziskavi s pomočjo DNK mikromrež smo odkrili šest genov, ki so različno izraženi v možganih samcev in samic miši brez gena *SF-1* in njihovimi kontrolnimi skupinami divjega tipa. Vseh šest genov se nahaja na spolnih kromosomih.

Ključne besede: možgani; spolne razlike; miš; DNK mikromreže