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Morphology and gene expression profile of the submandibular gland of androgen-receptor-deficient mice

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ABSTRACT

Objectives: In the submandibular gland (SMG) of mice, the granular convoluted tubule (GCT) develops preferentially in males dependent on androgens. To clarify the molecular mechanism of androgen action in SMG, we examined the SMG of mice deficient for the androgen receptor (ARKO).

Design: The morphological features and gene expression in the SMG of control and ARKO mice with or without hormone treatments were analysed by immunohistochemistry, DNA microarray, and RT-PCR.

Results: The development of GCT and expression of GCT-specific products such as NGF were even lower in ARKO male SMG than in control female SMG. The administration of androgens to ARKO males had no effect on SMG, whereas the administration of thyroid hormone (T4) caused the extensive conversion of striated duct cells to GCT cells with the increase of NGF mRNA. Gene expression profiles in control and ARKO male SMG were analysed by DNA microarrays, and genes with higher or lower expression in ARKO male SMG were determined. They were then classified into groups according to their responsiveness to the administration of dihydotestosterone (DHT) or T4 to ARKO males. RT-PCR revealed that, while no gene was responsive to DHT, expression of many genes was up- or down-regulated by T4.

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Abbreviations: SMG, submandibular gland; SD, striated duct; GCT, granular convoluted tubule; NGF, nerve growth factor; EGF, epidermal growth factor; AR, androgen receptor; ARE, androgen response element; T3, triiodethyronine; T4, L-thyroxine; Tfm, testicular feminization mutation; CREB, cyclic AMP response element-binding protein; ARKO, androgen receptor-knockout; DHT, dihydrotestosterone; IHC, immunohistochemistry; RT, reverse transcriptase; PCR, polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HE, haematoxylin–eosin; MAPK, mitogen-activated protein kinase; ATF-1, activating transcription factor 1; PI3K, phosphatidylinositol-3 kinase.

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Conclusions: These results confirmed that GCT cell differentiation induced by androgens is dependent on the classical androgen receptor (AR), whereas that by T4 is independent of AR. Differential reactivity of genes to androgens and thyroid hormone in ARKO mice may shed light on the mechanism of androgen action in the SMG.

1. Introduction

The extensive development of both acinar and ductal components takes place postnatally in the submandibular gland (SMG) of rodents under the control of neuronal and hormonal mechanisms.[1–3] The duct system of the rodent SMG is composed of an intercalated duct, striated duct (SD), granular convoluted tubule (GCT), and excretory duct.[4] At approximately 3–5 postnatal weeks (W) in mice, most of the SD in the male gland undergoes differentiation to the GCT, resulting in a marked sexual dimorphism in the morphology and function of the duct system, with the GCT developing preferentially in the male gland.[1,3,5] The epithelial cells of the GCT have abundant secretory granules that contain various biologically active peptides, including nerve growth factor (NGF), epidermal growth factor (EGF), renin, and kallikrein,[6,7] as well as the typical salivary digestive enzyme α-amylase.[8,9] The castration of adult males causes extensive involution of the GCT due to the conversion of the phenotype of GCT cells to that of SD cells, whereas the administration of androgens to females or castrated males has the opposite effect.[2,10] Such androgen-dependent GCT cell differentiation is accompanied by the up-regulation of gene expression for GCT-specific products[11]; however, the molecular mechanisms underlying this differentiation remain largely unknown. In addition, the administration of thyroid hormone causes stimulatory effects that are similar to those of androgens on the development of GCT cells and production of GCT-specific factors in normal females or castrated males.[12–14]

Androgens, similar to other steroid hormones, exert their biological functions by binding to the androgen receptor (AR), a member of the cytoplasmic/nuclear receptor family.[15] AR is a 110 kDa protein coded by the gene located on the X-chromosome and has four functional domains: the NH2-terminal transactivation domain, DNA-binding domain, hinge region, and ligand-binding domain. Upon binding with androgens, the AR forms a homodimer, translocates to the nucleus, and acts as a transcription factor in itself by binding to the androgen response element (ARE) of DNA located upstream of androgen-regulated genes.[16–18]

The AR is expressed in both acinar and duct cells in the rodent SMG.[19,20] The involvement of the AR in the sexual dimorphism of the mouse SMG was investigated in mice with the testicular feminization mutation (Tfm).[21] The Tfm mouse has a single base deletion in the coding region of the AR gene, which causes a frame-shift in the translation of AR mRNA, resulting in the premature termination of AR synthesis before the DNA-binding and androgen-binding domains are reached.[22] In the SMG of adult male Tfm mice, both the number of GCT granules and production of GCT-specific proteins such as NGF and EGF were markedly lower than in the normal male SMG and were similar to those in the normal female SMG.[3,23] Such hypoplasia and hypofunction of GCT cells was not ameliorated by the administration of androgens, whereas the administration of thyroid hormone (triiodothyronine, T3 or L-thyroxine, T4) to Tfm mice caused a marked increase in the number of GCT cell granules and production of GCT-specific factors in the SMG.[24–26] These findings suggested that the function of androgens on the GCT phenotype may be mediated by the AR, and that the function of thyroid hormone on the GCT phenotype may be independent of the AR. However, the Tfm mouse produces a small amount of the truncated form of the AR with intact DNA- and androgen-binding domains by translation from an internal initiation codon downstream of the stop codon and, therefore, is not completely deficient of the AR.[27]

A growing body of evidence has suggested that androgens, as well as other steroid hormones, can exert rapid, non-classical, or non-genomic cellular effects.[28–30] Several mechanisms have been postulated for the non-classical, non-genomic actions of androgens, some of which involve the classical AR while some do not, but all of which involve the activation of membrane-derived second messenger cascades and transcription factors distinct from the classical AR. In our previous studies, we demonstrated that some transcription factors in the mouse SMG such as JunD and activated cyclic AMP response element-binding protein (CREB) increased with the administration of testosterone, which suggested that non-classical pathways of androgen action also exist in the SMG.[31–33]

In the present study, using the AR-knockout mouse (ARKO) that is completely deficient of the classical AR due to targeted gene disruption, we first aimed to clarify if androgen-dependent GCT cell differentiation was mediated by the classical AR by confirming the phenomena reported in the SMG of Tfm mice in the SMG of ARKO mice. We then aimed to reveal the genes that are critical in the phenotype of GCT cells, and distinguish genes that are regulated by the classical AR from those that are not, by analyzing the genes that are up- or down-regulated in ARKO SMG and their responsiveness to the administration of androgens and thyroid hormones.

2. Materials and methods

2.1. *Animals and tissue preparation*

The floxed AR mouse line with a C57BL6 background, which carries loxP sites in the first exon of the AR gene including the transcription initiation site located on the X chromosome, was generated as described previously[34] and kindly provided by Dr. Shigeaki Kato, former Tokyo University. Mating female homozygous floxed AR mice with male CAG-Cre transgenic