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Immunoexpression of androgen receptor in genital tissues of male BALB/c mice

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Abstract

Aim: Androgens are hormones that are essential for the differentiation and development of male genital organs, preserving their structural features and maintaining their functions. These hormones exert their effects on target tissues mainly through androgen receptors (AR). The aim of this study was to determine the AR immunoexpression pattern in the genital tissues of male mice.

Materials and Methods: A total of 6 BALB/c male mice were anesthetized with ether, and the genital tissues, which were quickly removed from the body, were fixed in Bouin's solution for 18 hours. Tissues that underwent routine histological procedures after fixation were embedded in paraffin. Tissue sections cut with a thickness of 5 µm from paraffin blocks were taken on a slide and examined by staining with immunohistochemical methods.

Results: AR-positive immunostaining in the testis was observed only in somatic cells such as Sertoli, peritubular myoid cells, and Leydig cells. Germ cells were AR-negative. In the caput epididymis, ductus deferens, and seminal vesicle, AR positive immunoexpression was observed in stromal cells, especially epithelial cells. Although AR-positive staining was observed in some of the epithelial and stromal cells in these organs, some of them were AR-negative.

Conclusion: In the current study, it was determined that the AR expression pattern in the genital tissues of male BALB/c mice was similar to that in other species, although there were minor differences. The findings support the hypothesis that androgens exert their effects in testis mainly through somatic cells, and their effects in other genital tissues through epithelial cells, and the fact that androgens/ARs are essential for maintaining the structures and functions of male genital organs.

Introduction

The male reproductive system consists of organs such as testicles, genital excurrent ducts, accessory sex glands, and penis. The main tasks of the testicles are spermatogenesis and androgen synthesis [1]. Sperm produced in the testis as a result of spermatogenesis has not yet developed the ability to move forward and fertilize the oocyte. Sperm acquire this ability in the genital excurrent ducts, especially in the epididymis [2]. Accessory sex glands, on the other hand, transport and nourish sperm through the secretions they produce [1, 3]. Androgens, particularly testosterone and dihydrotestosterone, regulate the prenatal and postnatal development of internal and external genital organs, male-specific brain differentiation, secondary sex character development, spermatogenesis, and the preservation of the normal structures and functions of the genital organs in adults [4-6]. Androgens such as testosterone and dihydrotestosterone exert their effects on target tissues mainly through the androgen receptor. The androgen receptor, a receptor belonging to the steroid hormone nuclear receptor family, is localized in the cytoplasm in the absence of ligand (androgen). In the presence of androgen, the androgen-binding receptor undergoes a conformational change and the androgen-receptor complex is translocated to the nucleus. Reaching the nucleus, the androgen receptor complex binds to DNA, stimulating transcription of target genes [5, 7]. Many studies have been conducted on the immunohistochemical distribution of AR in male reproductive tract organs in mice, rats, and other species [6, 8-16]. Although some studies [8, 14, 17, 18] have reported that AR is found only in somatic cells in the testis and spermatogenesis is regulated by somatic cells, other studies [6, 11, 19] have reported that AR is localized in both somatic and germ cells. While most of the studies on AR localization in the epididymis reported that all principal

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cells were stained AR-positive [11, 18, 20, 21], the presence of AR-negative principal cells was also reported [13]. In addition, the presence of AR in basal cells in seminal vesicle epithelium is controversial. While some investigators reported that AR was found in basal cells [22-25], others reported that basal cells were AR-negative [21, 26]. As it can be understood from the information given above, although there are many studies on AR receptor localization in the male reproductive system, there is no complete consensus regarding AR localization in various organs of this system. It has been shown that androgen/AR signaling has important effects on the initiation and progression of many cancer types [5, 27]. Defining the precise localization of AR in the male reproductive system is important for understanding the biological effects and mechanisms of action of androgens in the reproductive system. Determination of localization is also important in understanding the pathogenesis of diseases such as hormone-related cancers, androgen deficiency, and androgen insensitivity syndrome, and in developing treatment strategies against these diseases.

Materials and Methods

Animals

Experimental procedures during this study were carried out according to the permission of Nigde Omer Halis Demir University Animal Experiments Local Ethics Committee (Decision dated 12.04.2022 and numbered 2022/08). After 6 BALB/c male mice obtained from Omer Halis Demir University Experimental Animal Production and Research Laboratories were anesthetized with ether, the extirpated tissues were quickly removed from the body and fixed in Bouin's solution for 18 hours. Afterward, the tissues were washed in tap water overnight, passed through alcohol and xylene series, and embedded in paraffin. Paraffin tissue blocks were cut with a thickness of 5 µm using a microtome and transferred to poly-l-lysine-coated slides.

Immunohistochemistry

In order to melt the paraffin and ensure that the tissues adhere well to the slide, they were kept in an oven at 59 °C for 1 hour, and then the antigen retrieval technique was applied to the tissue sections as detailed in previous studies [17, 28, 29]. Then, these sections were stained with immunohistochemical methods using the Avidin-Biotin-Peroxidase Complex (ABC) technique. A rabbit anti-rat polyclonal antibody (Millipore) diluted 1/50 with PBS was used to detect AR localization. PBS was applied instead of primary antibody to tissue sections used as a negative control (Figure 1N). Following the completion of the immunohistochemical staining, the tissue sections covered with water-based adhesive after counterstaining with hematoxylin for 30 seconds were examined with an Olympus BX-53 microscope and photographed with the camera of this microscope (DP 80). Examined tissues were scored in terms of AR-positivity and staining intensity by semi-quantitative analysis (analysis of 10 microscopic fields at 400X magnification) as intense (++), weak (+), negative (-) or variable [22, 23]

Results

The expression pattern and intensity of AR in testis, epididymis, ductus deferens, and seminal vesicle tissues are shown in Table 1. In all examined tissues, AR positive staining was observed only in the nuclei of the cells, while the cell cytoplasm was AR negative (Figure 1).

AR-positive staining in the testis; somatic cells such as Sertoli, peritubular myoid cells, and Leydig cells were also observed. Germ cells were AR-negative. Positive immunostaining was observed in all peritubular myoid cells, while the staining characteristics of Sertoli cells differed between tubules. These cells were AR-positive in some of the tubules and negative in others. The intensity of AR expression in Leydig cells ranged from negative to intense (Figure 1A).

AR-positive immunoexpressing was observed in the caput region of the epididymis, especially in the epithelial layer. Almost all of the peritubular smooth muscle cells were AR negative, although there were very few cells with positive staining, albeit at low intensity. While most of the principal cells in the epithelial layer showed positive staining, some of them were negative. Positive staining intensity was greater in principal cells, basal cells, and apical cells in the epithelial layer than in peritubular smooth muscle cells (Figure 1B).

AR-positive immunoexpressing in the ductus deferens was particularly evident in the epithelial layer. Most of the principal cells were AR-positive, while AR-negative ones were present. In basal cells, the ratio of AR-positive and AR-negative cells was almost equal. AR-positive staining

Table 1. Androgen receptor localization in adult male mouse reproductive tissues.

	AR
Testis	
Germ cell	-
Sertoli cell	++/-
Leydig cell	++/-
Peritubuler myoid cell	++
Caput epididymis	
Principal cell	++/-
Apical cell	++/-
Basal cell	++/-
Peritubular smooth muscle cell	+/-
Ductus deferens	
Principal cell	++/-
Basal cell	++/-
Stromal cells	++/-
Seminal vesicle	
Luminal cell	++/-
Basal cell	++/-
Periacinar smooth muscle cell	++/-
Stromal smooth muscle cell	+/-

*Symbols are as follows: ++ = intense, + = weak, – = negative, ++/–, +/– = variable.

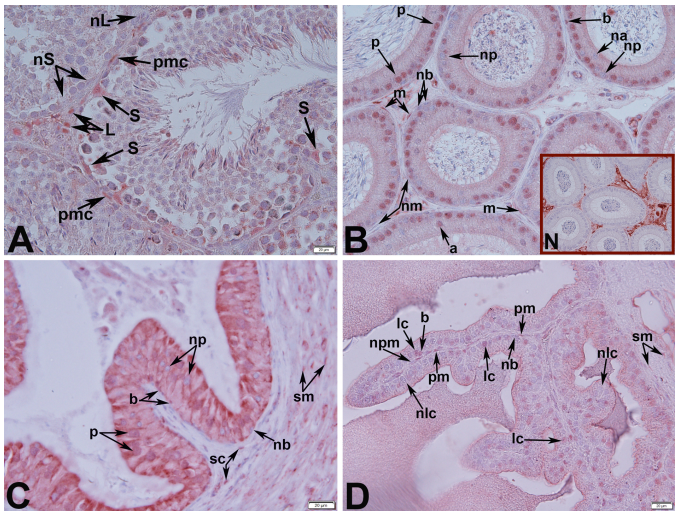

Figure 1. Immunohistochemical expression of AR in BALB/c mice's testis (A), epididymis (B), ductus deferens (C), and seminal vesicle (D). S: positive Sertoli cell, nS: negative Sertoli cell, L: positive Leydig cell, nL: negative Leydig cell, pmc: positive peritubular myoid cell, p: positive principal cell, np: negative principal cell, b: positive basal cell, nb: negative basal cell, a: positive apical cell, na: negative apical cell, m: positive peritubular smooth muscle cell, nm: negative peritubular smooth muscle cell, sm: positive smooth muscle cell, sc: positive stromal cell, lc: positive luminal cell, nlc: negative luminal cell, pm: positive periacinar smooth muscle cell, npm: negative positive periacinar smooth muscle cell, N: Negative control (A, B, C, D ×400).

in the vas deferens was also observed in smooth muscle cells forming the tunica muscularis layer and other stromal cells (Figure 1C).

In the seminal vesicle, some of the luminal cells were AR-negative while others were positive. The proportions of AR-positive and negative-staining luminal cells were almost equal. AR-positive staining in the seminal vesicle was observed in basal cells, periacinar smooth muscle cells, and stromal smooth muscle cells as well as luminal cells (Figure 1D).

Discussion

In this study, which aimed to determine the localization and expression intensity of AR in the tissues of the male reproductive system using immunohistochemical techniques, it was observed that AR-positive immunostaining was found only in the cell nuclei in all tissues examined. The cytoplasm of cells was AR negative. This result, which shows that the AR receptor is mainly localized in the cell nucleus, is in line with the literature [13, 16, 22, 30]. It supports the hypothesis by stating that ligand-dependent regulators are mainly localized in the nucleus of steroid-sensitive target cells [31].

Although it has been stated in some studies that AR expression in the testis is observed only in somatic cells [8, 14, 17, 32], there are also studies reporting that it is seen in germ cells [6, 11, 33]. In the current study, it was determined that AR-positive immunostaining was formed only in testicular somatic cells. Germ cells were AR-negative. This result supports the hypothesis that androgens regulate spermatogenesis only through somatic cells [14]. In the present study, Sertoli cells were found to be AR-negative in some of the seminiferous tubules and AR-positive in some. This result is parallel with studies [8, 14] reporting that AR expression in Sertoli cells may vary depending on the cycle of the seminiferous epithelium.

In the examinations performed in the caput epididymis, it was determined that the number of AR-positive staining cells in the epithelial layer and the staining intensity in these cells were considerably higher than in the peritubular smooth muscle cells. This result, which is consistent with what has been reported in previous studies, can be interpreted as that androgens exert their effects in the epididymis mainly through epithelial cells. In addition, this finding confirms the studies reporting that the synthesis and secretion of proteins that are effective in gaining the fertilization ability of sperm in the epididymis are regulated by androgens [34-38]. Findings regarding AR expression patterns of principal cells and peritubular smooth muscle cells in this study are consistent with the results of previous studies [13, 36].

Although some studies have reported that the intensity of AR-positive staining in the ductus deferens epithelium in humans [15, 39], mice [15], and rats [40] is quite weak, it has been reported that the intensity of AR-positive staining is strong in other studies conducted in goats, rats [18], and humans [41]. The intensity of AR staining observed in the ductus deferens epithelium in this study was quite strong and was consistent with some of the above literature [18, 41]. Although it was reported in previous studies [15, 16, 23] that all of the principal cells in the ductus deferens epithelium were AR-positive, it was observed in this study that some of the main cells were stained AR-positive and some AR-negative. In addition, the AR-positive immunostaining finding observed in the smooth muscle cells forming the tunica muscularis layer of the vas deferens in this study is consistent with the results of previous studies [16, 23]. In previous studies, it has been reported that AR-positive staining in seminal vesicles is observed in both epithelial and stromal cells, and the intensity of AR-staining in epithelial cells is stronger than in stromal cells [22-24]. In the current study, the AR pattern in the seminal vesicle was found to be similar to the results of the studies reported above. Although it was reported in a study performed in rats [22] that AR-positive stained luminal cells were significantly higher than AR-negative stained ones, it was observed that the ratio of AR-positive and negatively stained luminal cells was almost equal in this study. It can be said that this difference between the results may be due to the structural and functional differences between the species. There is no consensus regarding AR expression in basal cells in the seminal vesicle epithelium. Although some studies reported that these cells were AR-negative [21, 25], some of the cells were AR-negative and some AR-positive in this and other studies [6, 22].

Conclusion

This study showed that AR distributions in the genital tissues of male BALB/c mice were generally similar to those in other species, with minor differences. AR-positive immunoexpressing was detected in somatic cells in the testis and in epithelial and stromal cells in other organs. This result confirms that androgens and AR are essential for maintaining the normal structures and functions of the genital organs in adults, and especially for the function of spermatogenesis.

Ethics approval

Animal Experiments Local Ethics Committee of Nigde Omer Halisdemir University (Decision no: 2022/08 dated 12.04.2022).

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