Androgen Receptor Expressed in Three Types of Testicular Cells and Played an Important Role in Reproductive System

Xiao-Yan WANG¹,²,³, Peng YE¹, Chang-Jun ZHANG¹,* and Kai DENG¹,³,a,*

¹Reproductive Medicine Center, Renmin Hospital, Hubei University of Medicine, Shiyan, China
²Department of clinical Oncology, Taihe Hospital, Hubei University of Medicine, Shiyan, China
³Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom

a dkeanig@163.com
* Corresponding author

Keywords: Androgen Receptor, Ar Knockout, Feminization of Male.

Abstract. Aims: To investigate the expression and distribution of Androgen Receptor in all kinds of testicular tissue cells, as well as its effect to reproductive system. Methods: Using immunofluorescence method to detect the mice testicular tissue after the paraffin section and then find out AR expression and distribution in testis cells. By constructing Ar knockout mice and observing its reproductive system phenotype to identify how AR affect the reproductive system. Results: AR have a strong signal in Sertoli cells, Peritubul myoid cells and Leydig cells but without any specific signal in Germ cells. Ar knockout mice changed penis position compared to control and the distance of its perineum became shorter, just seems like fake clitoris. The testicular volume of Ar knockout mice was significantly smaller and the cell species, distribution and proportion within the seminiferous tubules changed significantly. Conclusion: AR expressed in testis Sertoli cells, Peritubul myoid cells and Leydig cells except in germ cells. Ar knockout mice appeared feminization of male and its sperm was blocked during the early phase of the first meiosis.

Introduction

Spermatogenesis is a highly complicated and tightly controlled process of germ cell proliferation and differentiation. Spermatogenesis can be roughly divided into three stages from spermatogonial stem cells proliferation to eventually become the mature sperm[1]. The first stage is the spermatogonial stem cells mitosis into spermatocyte, and then the spermatocytes become haploid round sperm after meiosis. The final stage is sperm cells further deformation, and circular specialized into mature sperm[2]. Spermatogenesis is highly dependent on autocrine and paracrine regulation of all kinds of testicular tissue cells, it is mainly included the sertoli cells(SCs), peritubul myoid cells(PM), leydig cells(LCs) and germ cells(GCs)[3]. The first wave of sperm production cycle is about four to five weeks after the mouse was born, and its three phases respectively need 11, 10, 14 days or so[4]. Androgen plays an important role in spermatogenesis regulation, and testosterone is one of the most important steroid hormones in androgen that secreted by the interstitial cells. SCs is the mainly target cells of testosterone signals[5]. Testosterone plays an important role in regulating meiosis and other stage of spermatogenesis[6]. As a intermediate for adjust the androgen effects, Ar mainly exist in the SCs, PM and LCs, but there is a controversy whether it also exists in GCs[7]. Our experiment
will determine the distribution of AR in testicular tissue cells by immunofluorescence, and then discuss its function in the reproductive system.

Ar gene located on the X chromosome q11-12 districts, composed by eight exons and seven introns, and encoded 919 amino acids[8]. The Ar gene knockout mice model of the compared system, is based on the zinc finger nuclease’s (ZFN-Zinc Finger Nuclease) gene knockout technology which can make the fixed point breakdown on the target genes and significantly improve the efficiency of homologous recombination. It is a new type of high efficient gene targeting technology[9,10]. In this experiment, we will use ZFN technology to knockout Ar gene in mice, then investigate the effects on the reproductive system by observing the changes of Ar gene knockout mice’s phenotype.

Materials and Methods

Experimental Animal

The Ar-null male mice birth from Ar-null female mice mated with adult C57BL/6J male mice

The Establishment of the Ar Knockout Mice and its Efficiency Detection

Ar gene knockout female mice was made by zinc-finger nucleases(ZFN) mediated gene targeting recombinant technology[8,9]. Due to missing 4 base pairs of the first exon, the coding sequence occurs the frame shift mutation. Therefore, it can neither produce normal mRNA, nor translate AR protein.

Authenticating method: The DNA extracted from cracking rat tail can be used for genotype identification. Due to the knocking out mice’s Aval restriction enzyme was destroyed, there exist only a complete fragment of PCR products. But in the wild type, two short clips will appear after enzyme digestion.

Tissue paraffin embedding, Immunofluorescence Colony Staining, Protein immunoblotting, Chromosome immunofluorescence and In vitro cultivation of testicular tissue are discribed as Su-Ren Chen, et al. Ongotarget 2016 [10].

Statistical Analysis

Each set of data are from three independent experiment, and was reported as the mean ±SE with computer analyzed using SPSS 19.0 software. Comparison between multiple sets of data using one-way variance statistic analysis. Statistical comparisons between the two groups were accomplished by Independent sample T -test. P < 0.05 was considered statistically significant[11].

Results

Effect Appraisal of the Ar Gene Knockout Mice

The Ar knockout female mice mated with adult male C57BL/6J, access to offspring and extract the protein from their tail. Then detect the proteins by the method of immunoblot (Fig. 1), Ar gene was knockout(Ar-null). AR was detected in WT while nothing was detected in Ar-null, and this proved that Ar knockout is effective.
The Expression of The Ar Gene In Testis

The wild type mice testicular tissue paraffin section immunity fluorescence with 20 days of age, shows the AR have strong signals in three types of cells, such as the SCs, PM and LCs cells, but there is no specific markable signals of AR protein in reproductive cells (Fig. 2), which suggests that AR specifically expressed in the SCs, PM and LCs in the cell of testicular tissue, with no expression in reproductive cells.

Figure 2. Immunofluorescence analysis of adult mice testicular tissue. The AR protein(red) was specific dyeing in LY(3), SCs(2)and PM(1),but there wasn’t have an AR signal in the germ cells (taillessness arrows)that had staining by Scp3(green).

The Reproductive System of Phenotypes in The Ar Gene Knockout Mice

The Ar - null mice’s external genitalia obviously altered at the age of 21 in days. Ar-null mice appears the phenomenon of male feminisation, the penis position change and perineum distance become shorter, similar with fake clitoris. Dissection of the Ar-null mice and found the testicular volume decreased significantly(Fig. 3).
Seminiferous Tubule Cell Phenotype of the Ar-null Mice

The types, distribution and proportion of the seminiferous tubule cell has changed obviously in the Ar-null mice. The SCs, PM, and LCs of seminiferous tubule, the spermatogonium and each phase of the spermatocyte of the wild type showing the radial distribution. The SCs in the seminiferous tubule of Ar-null mice was abnormal, and some of them protruding from the base to the luminal. Spermatogonia was located at the base of normal, with a little similar pachytene spermatocytes, but there exists no haploid round spermatids (Figure 4). The results suggest that Ar-null mice spermatogenesis process was blocked during early in the first reductional division.

Discussion

The Biological function of androgen is mediated by specific androgen receptor (AR), to study the distribution of androgen receptors in the testicles has an important significance in understanding the ways and means to maintain and adjust sperm production process.
According to the results of our this study, AR expressed in the Sertoli cells(SCs), Peritubulmyoid cells(PM) and Leydig cells(LCs). That the AR being positive expressed in sertoli cells and myoid cells around the seminiferous tubule indicating the androgen participate in spermatogenesis. The AR specially expressed in the sertoli cells suggests the sertoli cells specificity reaction of androgen on the process, and peritubular cells may be involved in maintain the process of sperm production[12]. But the specific regulatory mechanism remains to be further confirmed. Some researchers study with androgen receptor-deplete mouse and found that the fetal rat mesenchymal cells developed normally. As the growth of the age, it shown abnormal, which only has the partial function of natural mesenchymal cells of normal mice[13]. What is said above provides exquisite evidence that the androgen which mediated by AR are essential for mesenchymal cells development.

Spermatogenesis is dependent on androgen regulation, however, we have some controversy for whether AR is expressed in germ cells (sperm cells) in previous studies . But our experimental shows that Ar gene do not express in the germ cells.

In this study, we found the impacts of AR for the reproductive system by the Ar knockout mouse model .The Systemic Ar knockout males comes up characteristics of the male feminization of the external. The main performance is the penis get shorter as the clitoris, and the perineum is shortened, with a caecum vagina in. We can see the ectopic testis rise to abdomen after anatomical, it’s volume is smaller than the normal one. Spermatogenesis was blocked in the early of the first reductional division and caused infertility. Some researchers believe that the loss of the gene AR is not the major reason that result in the stagnation of sperm, because by gene AR knockout at the level of stem ,the location of animal testicles descend revealed abnormal, which is similar to human undesceded testicle. It is probably because that the testicles at higher temperature cause spermatogenesis stagnation[14]. So it is necessary to do experiments by gene AR specific knockout to explore the biological effect of the receptor. For example, we can selectively knock out the gene Ar of SCS. PM and LCS, and then observe the corresponding period that spermatogenesis are blocked.

In recent years the incidence of male infertility is increasing around the world ,according to the world health organization[15]. The result of in vitro cell level research show that the AR gene is closely related to spermatogenesis, the in-depth fundamental research about AR can give a diagnosis and treatment of male infertility with new perspective. Previous studies indicated that the serum testosterone level is differ with the levels that our body available to used, sperm production obstacle may be due to the AR changed[16], therefore, the detection of AR gene polymorphism can be a diagnostic indicator to determine the cause of the infertility in clinical. Moreover, the patients with testicular biopsy that have been proven sperm production retardation, can determine the stage of spermatogenesis block and the specific reasons through AR immunohistochemistry.

Acknowledgments

This research were financially supported by grants from the National Natural Science Foundation of China (81401200), Research Project of Hubei Provincial Department of Education (B2015478, B2015493) and Scientific Research Fund for National Project, Hubei University of Medicine(2013GPy04, 2013GPY10)
References


