Endocrinology

Androgen Receptor in Sertoli Cell Is Essential for Germ Cell Nursery and Junctional Complex Formation in Mouse Testes

Ruey-Sheng Wang, Shuyuan Yeh, Lu-Min Chen, Hung-Yun Lin, Caixia Zhang, Jing Ni, Cheng-Chia Wu, P. Anthony di Sant'Agnese, Karen L. deMesy-Bentley, Chii-Ruey Tzeng and Chawnshang Chang

Endocrinology 2006 147:5624-5633 originally published online Sep 14, 2006; , doi: 10.1210/en.2006-0138

0013-7227/06/$15.00/0
Printed in U.S.A.

Endocrinology 147(12):5624–5633

doi: 10.1210/en.2006-0138

Androgen Receptor in Sertoli Cell Is Essential for Germ Cell Nursery and Junctional Complex Formation in Mouse Testes

Ruey-Sheng Wang, Shuyuan Yeh, Lu-Min Chen, Hung-Yun Lin, Caixia Zhang, Jing Ni, Cheng-Chia Wu, P. Anthony di Sant'Agnese, Karen L. deMesy-Bentley, Chii-Ruey Tzeng, and Chawnshang Chang

George H. Whipple Lab for Cancer Research (R.-S.W., S.Y., L.-M.C., H.-Y.L., C.Z., J.N., C.-C.W., P.A.d.S., K.L.d.M.-B., C.C.), Departments of Urology and Pathology, University of Rochester, Rochester, New York 14642; Graduate Institute of Medical Sciences and Department of Obstetrics and Gynecology (R.-S.W., C.-R.T.), Taipei Medical University, Taipei 110, Taiwan; and Department of Obstetrics and Gynecology (L.-M.C.), China Medical University Hospital, Taichung, Taiwan

To examine the role of androgen receptor (AR) in Sertoli cells (SC), we used a SC-specific AR knockout (S-AR$^{-/y}$) mouse to further evaluate the chronological changes of seminiferous tubules and the molecular mechanisms of SC androgen/AR signals on spermatogenesis. Testes morphology began changing as early as postnatal day (PD) 10.5 in wild-type (WT), but not in S-AR$^{-/y}$ mice. After puberty (PD 50), the SC nuclei of WT testes migrated to the basal area of the seminiferous epithelium; however, in S-AR$^{-/y}$ testes, SC nuclei were disarranged and dislocated. Results from electron microscopy further showed an obvious duplication of basal lamina of the seminiferous epithelium in S-AR$^{-/y}$ testes at PD 50 compared with WT testes. Using quantitative RT-PCR analyses, the expression of SC gene profiles were compared in PD 10.5 testes. In S-AR$^{-/y}$ testes, the expression levels of 1) vimentin were significantly increased and laminin α5 was significantly decreased in PD 10.5, which contributed to functional defects in cytoskeletons and production of the basement membrane component of SC leading to cell morphology deterioration and thus affecting the integrity of seminiferous epithelium; 2) claudin-11, occludin, and gelsolin were significantly decreased in PD 10.5, which contributed to defects in intact junctional complex formation of SC leading to impairment of the integrity of the blood-testis barrier; 3) calcium channel, voltage-dependent, P/Q-type, α1A subunit; tissue-type plasminogen activator; transferrin; and epidermal fatty-acid-binding protein were significantly decreased in PD 10.5, which contributed to functional defects in production and/or secretion of specific proteases, transport proteins, and paracrine factors of SC, leading to impairment of its germ cells' nursery functions. (*Endocrinology* 147: 5624–5633, 2006)

WHEN ENRICO SERTOLI first described the Sertoli cell (SC) in 1865, these cells drew scientist's attention because of their close structural relationship with the spermatogenic cells in the seminiferous tubules. Presently, it is a well known fact that the SC plays a central role in fetal gonad development and postnatal spermatogenesis (1). The SC serves as the principal structural element of the seminiferous epithelium, providing physical support and creating an impermeable and immunological barrier, known as blood-testis barrier (BTB), in favor of normal germ cell development and maturation in adult testis (2).

Androgen and the androgen receptor (AR) (3–6) have been shown to play critical roles for normal spermatogenesis and fertility (7). AR expression has been detected in Sertoli, Leydig, and peritubular myoid cells (8–12), but the localization of AR in male germ cells remains controversial. Several studies indicated that AR is present in germ cells in different species (8, 10, 11, 13, 14); however, other reports show there is little or no AR staining in the germ cells (9, 15, 16). Earlier experiments showed that transplantation of spermatogonia from Tfm mice into recipient seminiferous tubules of wild-type (WT) mice results in qualitatively normal spermatogenesis (17). Despite the controversial results about whether AR exists in germ cells, the germ cell does not seem to need intrinsic AR. Nonetheless, recent publications (ours and others) clearly demonstrated that AR is essential for quantitative spermatogenesis in transgenic total AR knockout (18) and SC-specific AR knockout (S-AR$^{-/y}$) mouse models (19–21). These findings indicate that AR expression and function in testicular cells, other than germ cells, plays critical roles for normal spermatogenesis.

Spermatogenesis involves a series of synchronized cellular and molecular events. During spermatogenesis, basally located spermatogonia differentiate into the preleptotene/leptotene stage of primary spermatocytes and initiate meiosis. These primary spermatocytes must migrate from the basal compartment to the adluminal compartment traveling across the BTB in late stage VIII to early stage IX of the seminiferous epithelium cycle in mouse testis (22). This process involves the complex interaction of germ cells and SCs within the seminiferous tubules (23). Earlier studies show that AR has the highest immunopositive nuclear staining in SC during stages VII to VIII (24, 25), which coincides with the time point of primary spermatocyte movement through the BTB as they

First Published Online September 14, 2006

Abbreviations: AR, Androgen receptor; BTB, blood-testis barrier; Cacna1a, calcium channel, voltage-dependent, P/Q-type, α1A subunit; eFABP, epidermal fatty-acid-binding protein; EM, electron microscopy; PD, postnatal day; SC, Sertoli cell; T, testosterone; tPA, tissue-type plasminogen activator; WT, wild type.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

progress through the process of meiotic and postmeiotic development. S-AR$^{-/y}$ mice showed arrest before the completion of the first meiosis in spermatogenesis (19–21) that further emphasizes the importance of androgen/AR signal in the process of meiosis I during spermatogenesis.

An earlier study indicated that a majority of the mouse SC proliferation occurs before birth, and after birth, the proliferative activity gradually decreases (26). Upon puberty, the SC goes through morphological and functional transformation to enter a nonproliferative state with rapid maturation, leading to the establishment of the BTB (23, 27). Literary evidence indicates that mouse testis enter pubertal maturation at around postnatal day (PD) 8–10, which is defined as the period where germ cells enter meiosis and the first wave of spermatogenesis begins (28). In addition, other literature shows that AR starts to be expressed in SC around PD 7–10, and the expression increases in an age-dependent and seminiferous-tubule-stage-dependent manner (29, 30).

We hypothesized that AR plays a pivotal role in SC at this critical time point. A recently published paper using microarray analysis to examine prepubertal S-AR$^{-/y}$ mice also found that expression levels for protease inhibitors, cell adhesion molecules, cytoskeletal and extracellular matrix elements have diverse up- and down-regulated expression patterns (31). We would like to address the morphological analysis of the earliest changes in prepubertal S-AR$^{-/y}$ testis and ultrastructural changes in adult S-AR$^{-/y}$ testis as well as SC-specific functional gene expressions to further evaluate the mechanisms of androgen/AR acting in SC to affect spermatogenesis. We used AMH-Cre and floxed AR (FAR) mice to generate S-AR$^{-/y}$ mice as noted in our previous publication (19). Briefly, we mated FAR mice (18) with a transgenic line possessing the AMH promoter-driven expression of the Cre recombinase (32) to generate male S-AR$^{-/y}$ mice with the AR gene deleted only in SC. After serially analyzing different ages of mouse testes, we observed the earliest testicular structural changes at PD 10.5. Therefore, we used testes from PD 10.5 S-AR$^{-/y}$ (AMH Cre+ FAR/Y) and WT littermates (AMH Cre+ X/Y) to examine various SC-specific functional genes.

Materials and Methods

Generation of SC-specific AR knockout (S-AR$^{-/y}$) male mice

Protocols for use of animals were in accordance with National Institutes of Health standards. Transgenic AMH-Cre (C57-B6/SJL) male mice expressing Cre recombinase, under the control of the AMH gene promoter (32), were mated with FAR/AR (C57-B6/129/seve) female mice (18, 33). The expression of AMH promoter-driven Cre recombinase can efficiently and selectively delete the FAR gene in SC. S-AR$^{-/y}$ mice express FAR and Cre alleles in tail genomic DNA as shown in PCR genotyping described previously (18, 33).

Tissue sampling and RNA analysis

Whole testes were removed from animals at different ages depending on the experiment. Immediately after removal, the testes were snap-frozen and stored in liquid nitrogen. Before RNA extraction, each testis was weighed and homogenized in an electronic homogenizer. To allow specific mRNA levels to be quantified per testis and to monitor for the efficiency of RNA extraction, RNA degradation, and the RT step, an external standard was used (34, 35), and 10 ng luciferase mRNA (Promega, Madison, WI) was added to each testis as external control at the start of the RNA extraction procedure. Total RNA was isolated with Promega RNAgents Mini Kit (Promega) according to the manufacturer's instructions, and 2 μg total RNA was reverse transcribed and subjected to real-time PCR using iCycle (Bio-Rad Laboratories, Hercules, CA), and the formulas and thermal cycling conditions used were described previously (36). In general, the real-time PCR was performed with SYBR Green PCR Master Mix (Bio-Rad). PCR was performed at 94 C for 3 min and 40 cycles of 94 C for 30 sec, 60 C for 30 sec, and 72 C for 30 sec on an iCycler iQ multicolor real-time PCR detection system. Each sample was run in triplicate. Data were analyzed by an iCycler iQ software (Bio-Rad).

Pair sequences used for studying gene expression changes were designed by Beacon Designer II software (Bio-Rad) and are shown in Table 1. The amount of each measured cDNA was compared with the external standard luciferase cDNA in the same sample. The mRNA levels of interested genes in PD 10.5 S-AR$^{-/y}$ testes were compared with those of their WT littermates. Our data showed that there are no differences in testis weight and seminiferous tubule diameter between S-AR$^{-/y}$ and WT mice at PD 10.5, with the exception of seminiferous tubule lumen formation, which is evident in WT but not in S-AR$^{-/y}$ testes. Therefore,

TABLE 1. Primer sequences for real-time PCR of testicular genes

Gene	GenBank		Primer sequence
CK 18	NM_010664	F	CCGCAAGGTGGTAGATGAC
		R	GCTGAGGTCCTGAGATTTGG
Vimentin	NM_011701	F	CACACGCACCTACAGTCTG
		R	GTCCACCGAGTCTTGAAGC
Laminin α5	BC020313	F	CAGAGCAACCACACCACAG
		R	TCCTCAAGCATCCTCGGTAG
Collagen IV α3	Z35166	F	GCGAGGCTTCATCTTCAC
		R	TTACAGAATAAGAACGGCATTG
Occludin	NM_008756	F	ATCCTGTCTATGCTCATTATTGTG
		R	CTGCTCTTGGGTCTGTATATCC
Claudin-11	NM_008770	F	CGTCATGGCCACTGGTCTCT
		R	GGCTCTACAAGCCTGCACGTA
Testin	X78989	F	TCAAGGATGCCACAATGCC
		R	CCTCTTACACTCCACGGAAC
Nectin-2	NM_008990	F	GGAGGTATTATCGCTGCCATC
		R	CCAAGGTGAAGAGTTGAGAGG
Zyxin	NM_011777	F	CTGAAGGAGGTAGAGGAGTTG
		R	CAAGTGAAGCAGGTGATGTG
Vinculin	NM_009502	F	GCACAGATAAGCGGATTAGAAC
		R	GGCATTATGAACCAGCATCTC
Laminin γ3	NM_011836	F	CGGCTTTGTGGGCTATAAATG
		R	GTGGTGACCTTGGTAGACATC
α-Catenins	NM_009818	F	AGAAGAAGCCGTTGGTGAAG
		R	GTGGAATAAGACAGTGTTCTCTAC
Gelsolin	J04953	F	CTACCTCTCCAGCCACATTG
		R	CTGTCGCCTCCATAGAACTG
N-cadherin	NM_007664	F	TGGCAATCAAGTGGAGAACC
		R	ATCCGCATCAATGGCAGTG
Cys-TE	AF440737	F	TGTGGAGCATGTCGTGTTC
		R	CTGGACCTTCCTGCAATGG
tPA	NM_008872	F	GCCACGGTAAGTCACACCTTTC
		R	GCACACCAGCTTGCCCTAAG
Transferrin	NM_133977	F	CAACCTCACGACTCCTGGAAG
		R	TAAGGCACAGCAGCGAAGAC
eFABP	NM_010634	F	CGATCATCTTCCCATCCTTCA
		R	CTGGTCCAGCACCAGCAAT
ABP	NM_011367	F	GCTTCCTTCTGCCTGAGTG
		R	GTCCCGATTCTCCCAACTTC
Dhh	NM_007857	F	CCACGTATCGGTCAAAGCTGA
		R	ATTTCCCGGAAAGCAGCCT
PDGF-A	NM_008808	F	TAACACCAGCAGCGTCAAG
		R	GGCTCATCTCACCTCACATC
AMH	NM_007445	F	TGGTGACAGTGAGAGGAGAG
		R	CAGCCAGATGTAGGACTAGC
Cacna1a	NM_007578	F	GAGGACAGCGACGAGGATG
		R	GCACAGGAAGATGAACGAGAC
Pem	NM_008818	F	CAAGGAAGACTCGGAAGAACAG
		R	CATAGGACCAGGAGCACCAG

F, Forward; R, reverse.

to avoid the effect from differences in testicular cell composition because of different testis size, we selected PD 10.5 as the time point to examine differentially expressed genes in S-AR$^{-/y}$ and WT testes. Each gene expression pattern was confirmed using at least three pairs of WT and S-AR$^{-/y}$ mice.

Immunohistochemistry

The testes from WT and S-AR$^{-/y}$ males at PD 10.5 and PD 50 were removed, weighed, and fixed overnight in 4% paraformaldehyde at room temperature. The tissues were dehydrated by passing through 70, 85, 95, and 100% ethanol, cleared in xylene and 1:1 xylene/paraffin for 45 min, and embedded in paraffin. Tissue sections were cut at a 5-μm thickness and mounted onto Probe-On Plus charged slides (Fisher Scientific, Pittsburgh, PA). For immunohistochemistry, sections were heated at 55 C for at least 2 h, deparaffinized in xylene, rehydrated, and washed in Tris-buffered saline (TBS, pH 8.0). For antigen retrieval, slides were microwaved in 0.01 M sodium citrate (pH 6.0), immersed with 1% hydrogen peroxide in methanol for 30 min, and blocked with 10% normal horse serum in TBS for 60 min. After washing with PBS, sections were incubated for 90 min with GATA-1 (sc-0266; Santa Cruz Biotechnology, Santa Cruz, CA) rat antimouse monoclonal antibody with 1:500 dilution in TBS containing 1% BSA, followed by horse antirat biotinylated secondary antibody diluted 1:300 in TBS containing 1% BSA. Sections were incubated with avidin-biotin-peroxidase complex solution for 30 min, followed by development with diaminobenzidine peroxidase substrate kit (Vector Laboratories, Burlingame, CA) for 2 min. Slides were counterstained with hematoxylin for 30 sec, dehydrated, cleaned in xylene, and mounted. As a negative control, some tissue sections were incubated with normal rat serum instead of with the specific primary antibody. Images were captured using an eclipse E800 microscope (Nikon) equipped with a camera (Nikon). Captured images were stored on computer and compiled using Photoshop 7.0 (Adobe).

Electron microscopy (EM) examination of ultrastructure of testes in adult WT and S-AR$^{-/y}$ mice

The testes from WT and S-AR$^{-/y}$ males at PD 50 were removed and fixed overnight in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The testes were washed in phosphate buffer (two changes), postfixed with 1.0% osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in EPON/Araldite resin. Thin sections were then cut, mounted on 200-mesh grids, stained with uranyl acetate and lead citrate, and examined using a H7100 Hitachi electron microscope. Digital images were captured using a MegaView III digital camera. The morphology of inter-SC tight junctions, nuclei position, and basement membrane were examined.

Assessment of serum testosterone (T) levels in adult WT and S-AR$^{-/y}$ mice

Serum T levels were estimated using solid-phase (antibody-coated tube) RIA, using materials and protocols provided by Diagnostic Systems Laboratories Inc. (Webster, TX). The volume of serum used for RIA was 50 μl, the minimal detectable concentration was 0.08 ng/ml, and the intra- and interassay coefficients of variation were 8.5 and 8.7%, respectively.

Statistical analysis

The Student's *t* test was used with the software SPSS to estimate the statistical significance of quantitative changes. Significance was set as $P < 0.05$. The values are presented as mean ± SEM.

Results

Testes morphology began changing as early as PD 10.5 in WT but not in S-AR$^{-/y}$ mice

In S-AR$^{-/y}$ mice, testis weights on PD 7.5 and 10.5 were comparable to those of WT but were reduced to 65% of WT value on PD 18.5 (Fig. 1C; Table 2; n = 5 in each different age group of WT or S-AR$^{-/y}$ mice) and to 23–25% of WT value on PD 50 (Fig. 1D; Table 3; n = 5 in each group). The S-AR$^{-/y}$ mice epididymis weights were reduced to 63% of WT value on PD 50, but the seminal vesicle weights were comparable to WT (Table 3). To identify the earliest onset of testis morphology changes in S-AR$^{-/y}$ mice, we examined a series of mouse testes at different ages. The morphology of seminiferous tubules from PD 7.5 WT and S-AR$^{-/y}$ mice were comparable, containing mainly SCs and spermatogonia with large round nuclei (Fig. 2, A and B). Some of the seminiferous tubules began generating a central lumen in PD 10.5 WT mice (Fig. 2, C and I), but this did not occur in PD 10.5 S-AR$^{-/y}$ mice (Fig. 2, D and J). We did not observe seminiferous tubules morphological changes at PD 9.5 WT testis (data not shown). By PD 18.5, the seminiferous tubules of WT had enlarged and formed a well defined central lumen (Fig. 2, E and K). However, the seminiferous tubules in PD 18.5 S-AR$^{-/y}$ mice (Fig. 2, F and L) still lacked lumen formation and the diameters of tubules were smaller than WT. By PD 50, all stages of spermatogenesis were seen in WT (Fig. 2G), but in testis of S-AR$^{-/y}$, the tubular morphology was similar to PD 18.5 and a majority of spermatogenesis did not go beyond the pachytene primary spermatocyte stage (Fig. 2H). An early report by Tan *et al.* (30) discussed the defects in maturational development of SC in S-AR$^{-/y}$ but did not identify the earliest onset of testis morphology change. Our data clearly indicated that as early as PD 10.5 we can observe the seminiferous tubule structural defects in S-AR$^{-/y}$ testes.

Early reports by our lab and others (19, 20, 30) indicated a discrepancy in detecting the changes in T level in adult S-AR$^{-/y}$ mice. Therefore, we carefully reexamined the serum T levels in PD 50, 90, and 120 S-AR$^{-/y}$ and WT mice. Serum T levels in WT mice showed wide variations between animals, and S-AR$^{-/y}$ mice consistently showed 60–70% lower levels of serum T compared with WT at various ages (Fig. 1F; T levels from PD 50 mice are shown).

Disorganization and dislocation of SC nuclei as well as increase of vimentin mRNA levels in PD 10.5 S-AR$^{-/y}$ testes

GATA-1 is a zinc finger transcription factor that is produced by the SC coinciding with the first wave of spermatogenesis in prepubertal mouse (37). GATA-1 is immunoexpressed in the nuclei of SC, and the levels of its expression in the adult depend on the stage of the spermatogenic cycle (37). SCs are irregularly shaped tall columnar epithelial cells that extend from the basal to the adluminal compartment of the seminiferous tubules (38). Our data showed that the locations of GATA-1-immunopositive SC nuclei were similar between WT (Fig. 3A; n = 5) and S-AR$^{-/y}$ (Fig. 3B; n = 5) testes by PD 10.5. After puberty, the SC nuclei migrated to the basal area of the seminiferous epithelium in WT testes (Fig. 3C; n = 5), yet the location of SC nuclei in S-AR$^{-/y}$ testes (Fig. 3D; n = 5) showed disorganization and dislocalization.

Moreover, we checked the SC structure support components (such as cytokeratin 18 and vimentin) and found that the mRNA level of vimentin was significantly increased in PD 10.5 S-AR$^{-/y}$ testes compared with WT testes (Fig. 3E; n = 3 in each group). The result of altered vimentin mRNA ex-

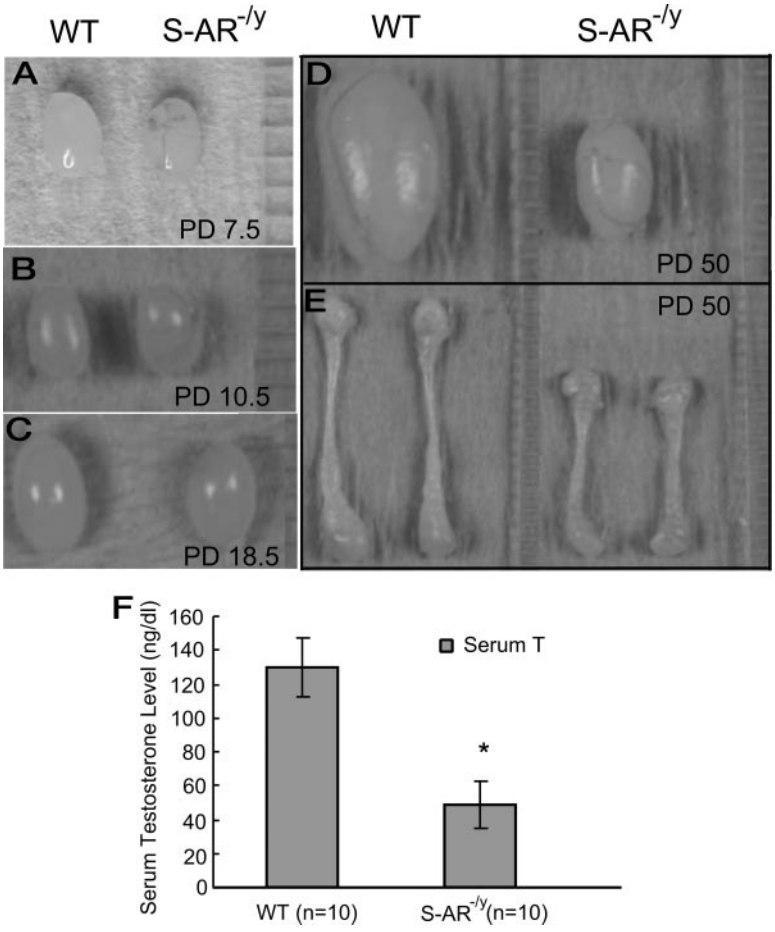

FIG. 1. A–C, Gross appearance comparison of testes obtained from PD 7.5 (A), PD 10.5 (B), and PD 18.5 (C) WT and S-AR$^{-/y}$ male mice; D and E, gross appearance of testis (D) and epididymis (E) obtained from PD 50 WT and S-AR$^{-/y}$ male mice; F, serum testosterone levels of PD 50 S-AR$^{-/y}$ compared with WT mice. S-AR$^{-/y}$ mice have lower serum testosterone levels than WT mice. *, Significant difference at $P < 0.05$ (t test).

pression, together with GATA-1 immunoexpression data in S-AR$^{-/y}$ testes (Fig. 3D), clearly indicated SCs require androgen/AR signal to maintain normal cell structure and morphology.

Abnormal duplication of basal lamina in PD 50 S-AR$^{-/y}$ testes as well as decreased laminin α5 mRNA levels in PD 10.5 S-AR$^{-/y}$ testes

In addition to GATA-1 immunoexpression data, we performed EM analysis to examine the structural changes in seminiferous tubules of S-AR$^{-/y}$ testes. Compared with WT testes (Fig. 4A; n = 5), results from EM showed an obvious and abnormal duplication of basal lamina of seminiferous tubules in S-AR$^{-/y}$ testes (Fig. 4B; n = 5) at PD 50. There were no marked differences in SC nuclei morphology between PD 50 WT and S-AR$^{-/y}$ testes (data not shown). Meanwhile, we checked the components of seminiferous tubule basement membrane (such as laminin α5 and collagen IV α3) and found that laminin α5 mRNA levels were significantly reduced in PD 10.5 S-AR$^{-/y}$ testes compared with WT (Fig. 4C; n = 3 in each group). This result indicated that SCs required androgen/AR signals to maintain basement membrane development of seminiferous tubules.

Differential expressions of components responsible for junctional dynamics in S-AR$^{-/y}$ testes: decreased mRNA levels of occludin, claudin-11, and gelsolin

One of the major functions of the SC is to create the BTB, which is located in the basal third of seminiferous epithelium. The BTB functions as a natural barrier to regulate the passage of various molecules into and out of the adluminal compartment of the seminiferous epithelia and an immunological barrier to create a specialized environment for the differentiation and movement of developing germ cells (39). Thus, BTB segregates seminiferous tubules into the basal compartment (containing spermatogonia and preleptotene

TABLE 2. Testis weight (mean ± SEM) at PD 7.5, 10.5, and 18.5 in WT and S-AR$^{-/y}$ male mice (n = 5 in each different age group of WT or S-AR$^{-/y}$ mice)

Group	Body weight (g)			Testis weight (mg)		
	PD 7.5	PD 10.5	PD 18.5	PD 7.5	PD 10.5	PD 18.5
WT	3.26 ± 0.18	6.40 ± 0.08	8.64 ± 0.09	2.45 ± 0.08	5.61 ± 0.10	14.25 ± 0.32
S-AR$^{-/y}$	3.32 ± 0.08	6.10 ± 0.14	8.81 ± 0.12	2.40 ± 0.10	5.66 ± 0.10	10.13 ± 0.43[a]

[a] Significant difference at $p < 0.05$ (t test).

TABLE 3. Testis weight, epididymis weight, and seminal vesicle weight (mean ± SEM) of PD 50 WT and S-AR$^{-/y}$ male mice

Group	n	Body weight (g)	Testis weight (mg)	Epididymis weight (mg), both sides	Seminal vesicle weight (mg), both sides
WT	5	24.5 ± 0.3	93.8 ± 1.9	53.0 ± 1.6	104.6 ± 7.9
S-AR$^{-/y}$	5	23.8 ± 0.2	21.8 ± 1.3[a]	33.4 ± 1.1[a]	109.5 ± 2.2

[a] Significant difference at $P < 0.05$ (t test).

and leptotene spermatocytes) and the adluminal compartment (containing different stages of meiotic spermatocytes, round spermatids, elongated spermatids, and spermatozoa) (39). To examine the AR role in SC junctional dynamics, we used quantitative RT-PCR to check the expression of genes responsible for the formation of the tight junction and the anchoring junction (39) in PD 10.5 testes. These functional genes were grouped as 1) components of the tight junction complex located between SC at the site of the BTB, including claudin-11 and occludin, and 2) components of the anchoring junction complex located between SCs and Sertoli-germ cells, including gelsolin, testin, nectin-2, zyxin, N-cadherin, vinculin, laminin γ3, and α-catenins (39).

Among the components of the tight junction and anchoring junction complex, we found the claudin-11, occludin, and gelsolin mRNA levels consistently reduced in PD 10.5 S-AR$^{-/y}$ testes compared with WT (Fig. 5A; n = 3 in each group). Although the claudin-11 mRNA expression levels in S-AR$^{-/y}$ mice has been reported by other groups (30, 31), the mRNA expression changes for occludin and gelsolin have not been described previously. Taken together, these results indicate that SCs require androgen/AR signals to maintain normal functions and/or structure of tight junctions as well as anchoring junctions, leading to the maintenance of the intact BTB.

Tissue remodeling factors, transport proteins, and endocrine and paracrine factors were altered in S-AR$^{-/y}$ testes

In SCs, the production and secretion of diverse functional glycoproteins and peptides occurred in a seminiferous-epithelium-stage-dependent manner and were involved in germ cell development (40). To examine the role of AR on SC protein production and secretion, we used quantitative RT-PCR to check the expression levels of several SC-specific genes in PD 10.5 testes. These functional genes were grouped as 1) tissue remodeling factors including cystatin-TE (cys-TE) and tissue-type plasminogen activator (tPA); 2) transport proteins for nourishment of germ cells including androgen-binding protein (ABP), epidermal fatty-acid-binding protein (eFABP), and transferrin; 3) endocrine and paracrine factors for nourishment of germ cells and interstitial cell differentiation, including desert hedgehog (Dhh), anti-Müllerian hormone (AMH), and platelet-derived growth factor-A (PDGF-A); 4) a cell membrane calcium channel gene, such as calcium channel, voltage-dependent, P/Q type, α1A subunit (Cacna1a); and 5) a well-defined androgen target gene, such as Pem.

For the selected genes expressed in SCs, we found the Pem, Cacna1a, tPA, transferrin, and eFABP mRNA levels were reduced in PD 10.5 S-AR$^{-/y}$ testes compared with WT (Fig. 5B; n = 3 in each group). Although the mRNA levels of tPA, transferrin, eFABP, and Pem in S-AR$^{-/y}$ mice have been reported by other groups (30, 31), the gene expression changes for Cacna1a have not been described previously. Taken together, these results indicate that loss of AR in the SC results in multiple functional deteriorations as early as PD 10.5, the age in which the mouse testis enters pubertal maturation and begins the first wave of spermatogenesis (28). This diverse up- and down-regulation of various genes indicate that loss of functional AR in the SC can cause a broad spectrum of functional defects in SCs, which finally leads to the arrest of the first meiosis of spermatogenesis and stimulates apoptosis of growth-arrested germ cells (19–21).

Discussion

Testes have two major functions, synthesis of steroid hormones (steroidogenesis) and production of mature sperm (spermatogenesis), which are achieved through coordination among various cell types (SCs, Leydig cells, peritubular myoid cells, and germ cells) within the testes (41). Results from transgenic total AR knockout (18) mouse studies show that functional AR is required for proper development and function of testes. Results from S-AR$^{-/y}$ mouse studies further prove that functional AR in SCs is required for normal germ cell differentiation, especially for meiosis progression of primary spermatocytes and haploid spermatid differentiation in the progression of spermiation (19–21).

Several important functions of SCs have been proposed: 1) maintenance of the BTB and secretion of seminiferous tubular fluid (42), 2) providing structural support for development and maturation of germ cells (43, 44), 3) cooperating with germ cells in germ cell movement and spermiation (38, 39), and 4) secreting diverse functional glycoproteins and peptides to nourish germ cells (45, 46). In this study, our data demonstrate that loss of functional AR in SCs will cause a broad spectrum of functional defects and finally lead to the arrest of spermatogenesis before the second wave of meiosis (19–21).

AR is a crucial regulator for SC function to provide cell structure support and maintain the BTB and secretion of seminiferous tubular fluid

Earlier studies showed that mouse testes enter puberty maturation around PD 8–10 (28). It is generally believed that seminiferous tubule fluid is produced by SCs and the onset of fluid production is just after the BTB formation when testes enter pubertal maturation (47, 48). The immunohistochemistry staining data also showed that SC AR starts to express around PD 8–10 with age-dependent increasing levels. After maturation, the expression of SC AR is presented in a seminiferous-tubule-stage-specific manner (29, 30). Our data showed that the seminiferous tubule fluid accumulation and lumen formation occurred as early as PD 10.5 in WT testes

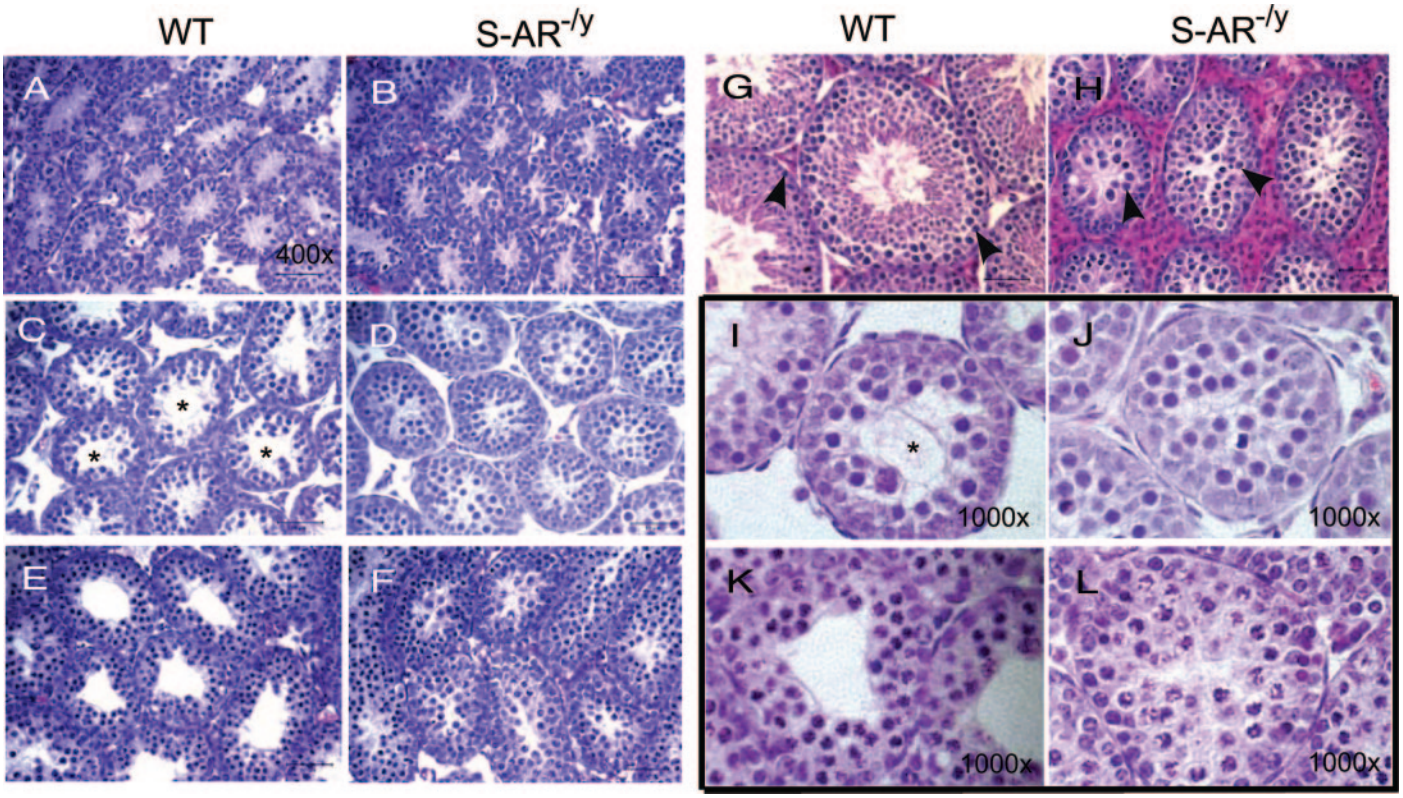

FIG. 2. Earliest morphological changes are observed in PD 10.5 WT testis. Testes obtained from PD 7.5 (A and B), PD 10.5 (C, D, I, and J), and PD 18.5 (E, F, K, and L) WT and S-AR$^{-/y}$ mice were subject to histological analyses using hematoxylin-eosin staining (n = 5 in each different age group of WT or S-AR$^{-/y}$ mice). In some areas of PD 10.5 WT testis, the seminiferous tubules began generating central lumen (*asterisk*) (C and I) but not in PD 10.5 S-AR$^{-/y}$ testis (D and J). By PD 18, S-AR$^{-/y}$ testes (F and L) still lacked lumen formation, and the diameter of tubules was smaller than WT (E and K). By PD 50 (n = 5 in each group), all stages of spermatogenesis were seen in WT testes (G), but in testes of S-AR$^{-/y}$ mice (H), the tubular morphology was similar to PD 18 and a majority of spermatogenesis did not go beyond the pachytene primary spermatocyte stage as indicated by the *arrowheads*. Magnification, ×400 (A–H) and ×1000 (I–L).

but not in S-AR$^{-/y}$ testes. Although the testes weight and germ cell composition are similar between WT and S-AR$^{-/y}$ testes at PD 10.5, our data indicate that AR in SC plays an essential role during this time when testes enter pubertal maturation and form functionally mature BTB. To clearly illustrate the SC location in seminiferous tubules, we performed GATA-1 immunostaining to identify the SC nuclei. Our results showed that the locations of GATA-1-immunopositive SC nuclei were similar in WT and S-AR$^{-/y}$ testes at PD 10.5. After puberty (PD 50), the comparison of WT testes, in which SC nuclei migrated to the basal area of the seminiferous epithelium, the location of SC nuclei in S-AR$^{-/y}$ testes were disarranged and dislocated. Furthermore, results from EM showed an obvious duplication of basal lamina of seminiferous tubules in PD 50 S-AR$^{-/y}$ testes compared with WT. These findings indicate that androgen/AR signals in SCs are essential for maintaining cell morphology, basement membrane development, and seminiferous epithelial integrity.

In addition to morphological changes, we used quantitative RT-PCR to examine various gene expressions of cell structure support, tight junction, and anchoring junction components in PD 10.5 S-AR$^{-/y}$ and WT testes.

Cell structure support components. SCs have abundant and well-developed cytoskeletons, which have been shown to be involved in 1) maintaining cell shape, positions, and transport of organelles within the cell and 2) stabilizing the cell membrane at sites of cell-cell contact, adheres and aids in the movement of developing germ cells and in the release of mature spermatids during spermiation (38, 39). In SCs, there are three major cytoskeletal protein families, which include intermediate filaments, microfilaments, and microtubules (49). Cytokeratins are members of the intermediate filament protein family and are specifically expressed in epithelial cells and their appendages. Our data showed that the vimentin mRNA level was increased in PD 10.5 S-AR$^{-/y}$ testes compared with WT. The result of altered cytokeratin expression correlated well with the finding in Fig. 3D, in which the location of GATA-1-immunopositive SC nuclei in S-AR$^{-/y}$ testes were disarranged and dislocated after puberty. A recently published paper using microarray studies also revealed that cytoskeletal-related genes (tubulin β3 and actinin α3) significantly decreased in S-AR$^{-/y}$ testes compared with WT (31), yet there is no additional study to address how lack of AR in SC will result in these defects. Taken together, results from our group and others indicate loss of functional AR in SCs might alter the production of those cytoskeletons, consequently leading to cell morphology deterioration and affecting the integrity of the seminiferous epithelium after puberty.

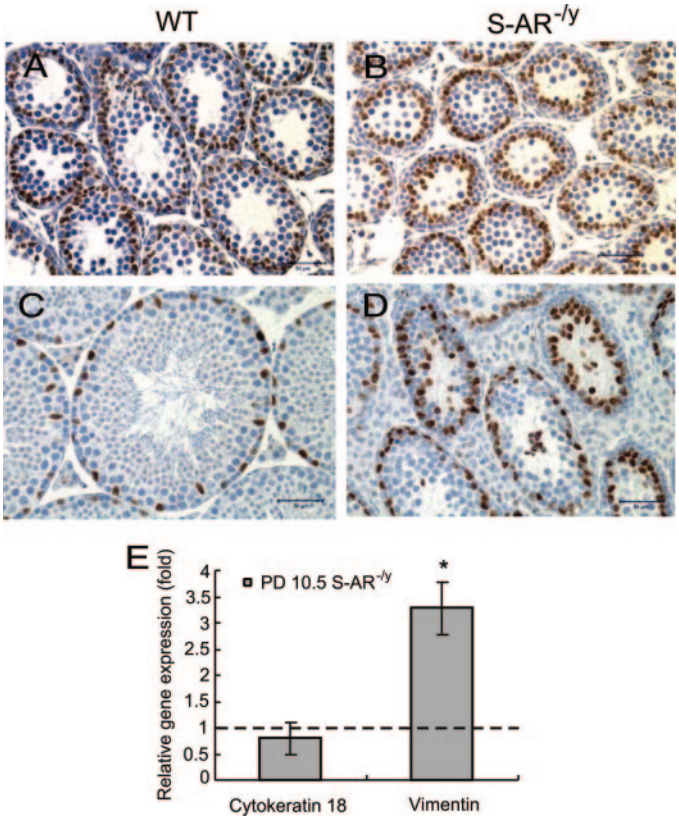

FIG. 3. Cell location and structure component gene expression of SCs in WT and S-AR$^{-/y}$ mouse testes using immunohistochemical staining (n = 5 in each different age group; magnification, ×400). Photomicrographs illustrate the distribution of SC (GATA-1 immunopositive; *brown* staining) in WT (A and C) and S-AR$^{-/y}$ (B and D) seminiferous tubules at PD 10.5 (A and B) and PD 50 (C and D). GATA-1-immunopositive SC nuclei locations were similar in PD 10.5 WT and S-AR$^{-/y}$ testis (A and B). After puberty, the SC nuclei migrated to the basal area of the seminiferous epithelium in PD 50 WT testis (C), but the locations of GATA-1-immunopositive SC nuclei in PD 50 S-AR$^{-/y}$ testis were disorganized (D). E, Changes in expressions of cell structure component genes cytokeratin 18 and vimentin in PD 10.5 S-AR$^{-/y}$ testis compared with the WT (n = 3 in each group). RNA was extracted from testes and cDNA was prepared as described in *Materials and Methods*. Real-time PCR was used to measure cDNA levels of cytokeratin 18 and vimentin relative to external control luciferase. *, Significant difference at $P < 0.05$ (*t* test).

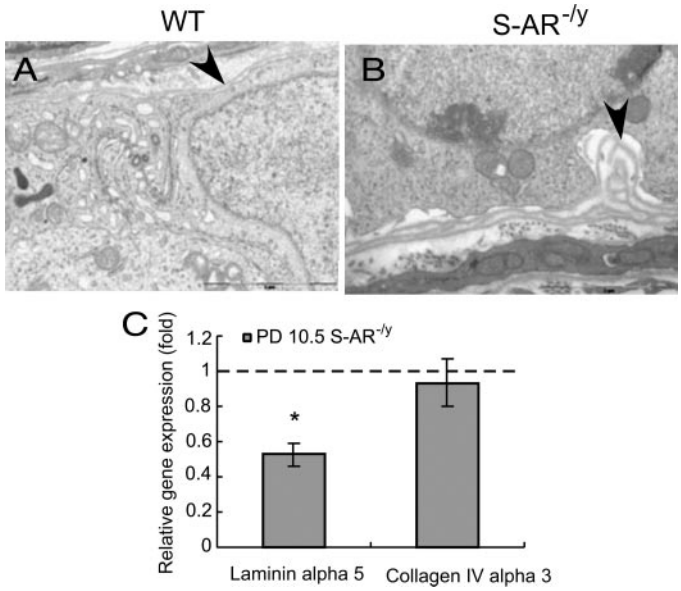

FIG. 4. Structural examination by EM and gene expression of basement membrane components in S-AR$^{-/y}$ and WT mouse testes (n = 5 in each different age group). An electron micrograph (magnification, ×20,000) showed the structure of seminiferous tubule basement membrane (*arrowhead*) in PD 50 WT testes (A) and a definite duplication of seminiferous tubule basement membrane (*arrowhead*) in PD 50 S-AR$^{-/y}$ (B) testes. C, Changes in expression of basement membrane component gene in PD 10.5 S-AR$^{-/y}$ testes compared with the WT (n = 3 in each group). Testicular RNA extraction and cDNA preparation were described in *Materials and Methods*. Real-time PCR was used to measure cDNA levels relative to external control luciferase. *, Significant difference at $P < 0.05$ (*t* test).

The seminiferous tubule basement membrane components. The seminiferous tubule basement membrane is a sheet-like extracellular structure in contact with the basal surface of epithelial cells and composed of different extracellular matrix proteins, such as collagen, laminin, and proteoglycans (50). In the testes, the relative morphological relationship between tight junctions and anchoring junctions is remarkably different from other epithelia. Instead of locating at apical portion in other epithelia (51), testis tight junctions locate at the basolateral region of the SC and are very close to the basement membrane (52). The morphological relationship between tight junctions and the basement membrane, associated with the findings that abnormal basement membrane structures are detected in infertile patients with defects of spermatogenesis (53, 54), clearly illustrates the significant role of basement membrane in BTB dynamics and spermatogenesis. Earlier studies also demonstrated that laminins, such as laminin α5, and collagens, two major components of the basement membrane of the seminiferous tubule, are pivotal regulators of the tight-junction dynamics of SCs (55, 56). Our data showed that the laminin α5 mRNA levels were significantly reduced in PD 10.5 S-AR$^{-/y}$ testes. This result, associated with EM findings of duplication of seminiferous tubules basement membrane in PD 50 S-AR$^{-/y}$ testes, suggests that the SC requires functional AR to maintain the development of seminiferous tubule basement membrane.

The tight junction complex components. There are three types of junctions in the testis, which are known as occludin inter-SC tight junction, anchoring junctions (including ectoplasmic specializations and tubulobulbar complex), and gap junctions (39). The occluding inter-SC tight junctions are the major constituents of the BTB at the basal compartment of the seminiferous epithelia. In addition, basal ectoplasmic specializations and basal tubulobulbar complex are also found at the BTB site. Both caludin-11 and occludin are integral components of tight junctions between SCs (39). Earlier studies reported that *in vivo* administration of flutamide (a nonsteroidal AR antagonist) can induce down-regulated expression of occludin mRNA in rat testes (57), and the T treatment of *in vitro*-cultured SCs can induce claudin-11 expression (58). Consistently, both claudin-11 knockout male mice and occludin knockout male mice are sterile (59, 60). Claudin-11 expression in mice testis is age dependent, starting to increase from PD 3, reaching a plateau around PD 6–16, and then gradually declining to lower levels in adulthood (61). A

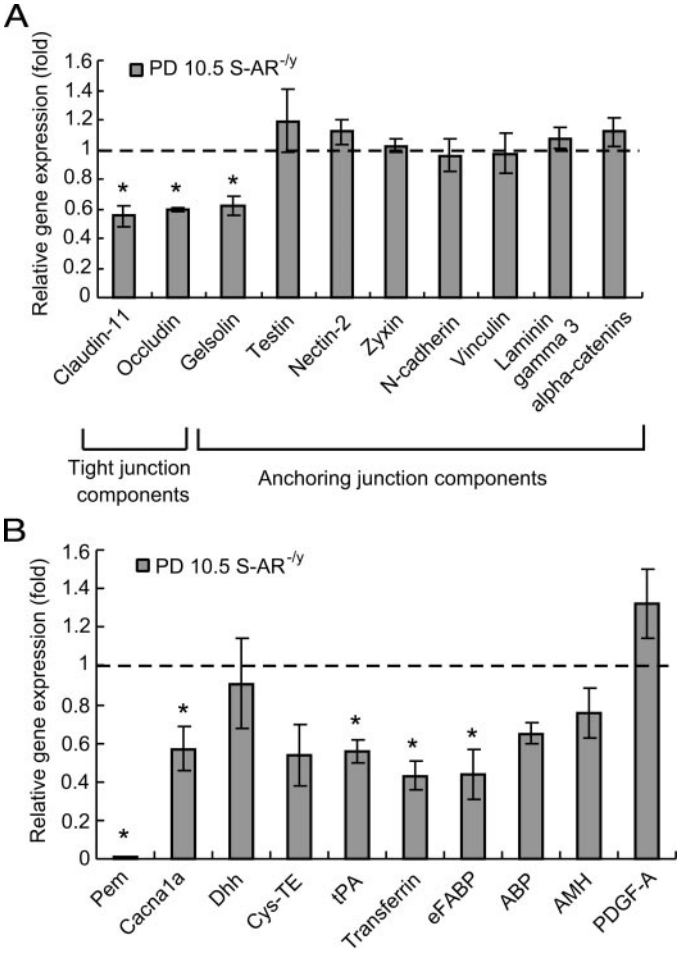

FIG. 5. A, Changes in expression of tight junction and anchoring junction components genes in PD 10.5 S-AR$^{-/y}$ testis; B, changes in expression of tissue remodeling factors, transport proteins, endocrine and paracrine factors, and calcium channel genes in PD 10.5 S-AR$^{-/y}$ testis compared with the WT control (n = 3 in each group). RNA extraction and cDNA preparation were described in *Materials and Methods*. Real-time PCR was used to measure cDNA levels relative to external control luciferase. *, Significant difference at $P < 0.05$ (*t* test).

recently published paper (62) also indicated that the permeability of the BTB to biotin was increased in adult S-AR$^{-/y}$ testes compared with WT testes. In our studies, the claudin-11 and occludin mRNA expressions were significantly reduced in PD 10.5 S-AR$^{-/y}$ testis. These data indicated that loss of AR in the SC might impair functional tight junction formation and integrity from the earlier time point when the SC goes through functional maturation and establishment of the BTB.

The anchoring junction complex components. Ectoplasmic specializations are actin-filament-containing testis-specific adhesion complexes found at the site of intercellular attachment between SCs at the basal compartment and between SCs and germ cells at the adluminal compartment of seminiferous epithelium in rodent testes. Ectoplasmic specializations are morphologically characterized by the triplet structures containing the SC plasma membrane, a submembrane bundle of actin filaments, and an attached cistern of endoplasmic reticulum. The intercellular space between two apposing SCs at the basal ectoplasmic specializations is sealed by tight junctions to form the BTB (63). Testin, nectin-2, zyxin, vinculin, laminin γ3, α-catenins, gelsolin, and N-cadherin are components of ectoplasmic specializations (39). Our data showed that gelsolin mRNA expression was significantly reduced in PD 10.5 S-AR$^{-/y}$ testes compared with WT testes. The above evidence clearly indicates that functional AR in SCs is required for morphogenesis and functional junction complex formation of SCs.

AR plays a pivotal role in SC secretion of functional glycoproteins and peptides for nourishing germ cells, and to cooperate with germ cells in germ cell movement and spermiation

Tissue remodeling factors. The SC synthesizes and secretes proteases and protease inhibitors, which might participate in the events of germ cell movement and spermiation. Cystatin-TE (64), tPA, and urokinase-type plasminogen activator are identified in mammalian testes and proposed to involve the migration of developing germ cells from the basal compartment to the lumen of the seminiferous tubule and in the release of mature spermatids during spermiation (65). Our data showed that the mRNA levels of tPA were significantly decreased in PD 10.5 S-AR$^{-/y}$ testes compared with WT. Our result indicates that loss of AR in the SC might impair the production and secretion of these proteases.

Transport proteins. Transferrin is an iron transport glycoprotein, which is secreted by differentiated SCs and is proposed to transport iron to the developing germ cells within the adluminal compartment of seminiferous tubules (66). Iron is necessary for cell proliferation, differentiation, and metabolism (67). Our data showed that transferrin mRNA levels were significantly decreased in PD 10.5 S-AR$^{-/y}$ testes compared with WT. This result indicated that loss of functional AR in the SC might impair the synthesis and secretion of transferrin.

Expression of eFABP was found in SCs and proposed to involve transport of essential fatty acids for growth and function of the surrounding germ cells (68). Our data showed that eFABP mRNA expression levels were significantly reduced in PD 10.5 S-AR$^{-/y}$ testes compared with WT, which indicated that loss of functional AR in SCs will impair eFABP production and further lead to deterioration of the SC nursery functions for surrounding germ cells.

The critical function of SCs in the regulation of normal spermatogenesis is secretion of a complex fluid into the seminiferous tubule lumen behind the BTB. The SC-secreted fluid contains many essential proteins that are necessary for maintenance of normal development and differentiation of germ cells in the adluminal compartment. Earlier studies have shown that SCs possess different subtypes of voltage-operated calcium channels (69), which mediate Ca^{2+} influx in rat SCs and have roles in SC junction dynamics (70) as well as in SC secretory processes (71). Our data revealed that the Cacna1a mRNA expression levels were significantly decreased in PD 10.5 S-AR$^{-/y}$ testes compared with WT. This result indicated that SC loss of functional AR might impair

the function of voltage-operated calcium channels, and this might further affect the protein secretory function of SC.

Pem is a member of the homeobox transcription factor family, and its expression is regulated by androgen in the testis and epididymis (72, 73). In the testis, Pem expression is directly regulated by androgen/AR in the cultured SC (74). The defined role of Pem in spermatogenesis is still unclear. We used this gene as a positive control to examine mRNA expression pattern in S-AR$^{-/y}$ testes. As expected, our data showed that the Pem mRNA expression levels were significantly decreased in PD 10.5 S-AR$^{-/y}$ testes compared with WT.

In summary, androgen binding to AR might activate a transcriptional reaction in SCs leading to changes in target gene expression and subsequent signaling transduction. However, how androgen/AR acts in SCs and consequently affects germ cell differentiation is largely unknown. Our results showed clear and novel evidence that androgen, acting through SC AR, might regulate the microenvironment of seminiferous epithelium by influencing a broad spectrum of gene changes in the SC. Loss of AR specifically in the SC could affect 1) structure support elements of the SC leading to impaired normal supportive function for movement of developing germ cells; 2) junction complex formation and basement membrane development of SC leading to impaired functional integrity of the BTB; and 3) SC-specific protease, transport protein, and paracrine factor production and/or secretion, leading to impaired SC nursery functions for developing germ cells.

Acknowledgments

Received February 3, 2006. Accepted August 24, 2006.

Address all correspondence and requests for reprints to: Chawnshang Chang, George H. Whipple Lab for Cancer Research, Departments of Urology and Pathology, University of Rochester, Rochester, New York 14642. E-mail: chang@urmc.rochester.edu.

This work was supported by National Institutes of Health Grants CA60948 and DK60912 and the George H. Whipple Professorship Endowment.

Disclosure statement: None of the authors have anything to declare.

References

1. **Griswold MD** 1998 The central role of Sertoli cells in spermatogenesis. Semin Cell Dev Biol 9:411–416
2. **Tindall DJ, Rowley DR, Murthy L, Lipshultz LI, Chang CH** 1985 Structure and biochemistry of the Sertoli cell. Int Rev Cytol 94:127–149
3. **Chang CS, Kokontis J, Liao ST** 1988 Molecular cloning of human and rat complementary DNA encoding androgen receptors. Science 240:324–326
4. **Chang CS, Kokontis J, Liao ST** 1988 Structural analysis of complementary DNA and amino acid sequences of human and rat androgen receptors. Proc Natl Acad Sci USA 85:7211–7215
5. **Heinlein CA, Chang C** 2002 Androgen receptor (AR) coregulators: an overview. Endocr Rev 23:175–200
6. **Heinlein CA, Chang C** 2004 Androgen receptor in prostate cancer. Endocr Rev 25:276–308
7. **Sharpe R** 1994 Regulation of spermatogenesis. In: Knobil E, Niell JD, eds. The physiology of reproduction. 2nd ed. New York: Raven Press; 1363–1434
8. **Kimura N, Mizokami A, Oonuma T, Sasano H, Nagura H** 1993 Immunocytochemical localization of androgen receptor with polyclonal antibody in paraffin-embedded human tissues. J Histochem Cytochem 41:671–678
9. **Bremner WJ, Millar MR, Sharpe RM, Saunders PT** 1994 Immunohistochemical localization of androgen receptors in the rat testis: evidence for stage-dependent expression and regulation by androgens. Endocrinology 135:1227–1234
10. **Vornberger W, Prins G, Musto NA, Suarez-Quian CA** 1994 Androgen receptor distribution in rat testis: new implications for androgen regulation of spermatogenesis. Endocrinology 134:2307–2316
11. **Zhou X, Kudo A, Kawakami H, Hirano H** 1996 Immunohistochemical localization of androgen receptor in mouse testicular germ cells during fetal and postnatal development. Anat Rec 245:509–518
12. **Shan LX, Bardin CW, Hardy MP** 1997 Immunohistochemical analysis of androgen effects on androgen receptor expression in developing Leydig and Sertoli cells. Endocrinology 138:1259–1266
13. **Janssen PJ, Brinkmann AO, Boersma WJ, Van der Kwast TH** 1994 Immunohistochemical detection of the androgen receptor with monoclonal antibody F39.4 in routinely processed, paraffin-embedded human tissues after microwave pre-treatment. J Histochem Cytochem 42:1169–1175
14. **Arenas MI, Royuela M, Lobo MV, Alfaro JM, Fraile B, Paniagua R** 2001 Androgen receptor (AR), estrogen receptor-α (ER-α) and estrogen receptor-β (ER-β) expression in the testis of the newt, *Triturus marmoratus marmoratus* during the annual cycle. J Anat 199:465–472
15. **Suarez-Quian CA, Martinez-Garcia F, Nistal M, Regadera J** 1999 Androgen receptor distribution in adult human testis. J Clin Endocrinol Metab 84:350–358
16. **Pelletier G, Labrie C, Labrie F** 2000 Localization of oestrogen receptor α, oestrogen receptor β and androgen receptors in the rat reproductive organs. J Endocrinol 165:359–370
17. **Johnston DS, Russell LD, Friel PJ, Griswold MD** 2001 Murine germ cells do not require functional androgen receptors to complete spermatogenesis following spermatogonial stem cell transplantation. Endocrinology 142:2405–2408
18. **Yeh S, Tsai MY, Xu Q, Mu XM, Lardy H, Huang KE, Lin H, Yeh SD, Altuwaijri S, Zhou X, Xing L, Boyce BF, Hung MC, Zhang S, Gan L, Chang C** 2002 Generation and characterization of androgen receptor knockout (ARKO) mice: an in vivo model for the study of androgen functions in selective tissues. Proc Natl Acad Sci USA 99:13498–134503
19. **Chang C, Chen YT, Yeh SD, Xu Q, Wang RS, Guillou F, Lardy H, Yeh S** 2004 Infertility with defective spermatogenesis and hypotestosteronemia in male mice lacking the androgen receptor in Sertoli cells. Proc Natl Acad Sci USA 101:6876–6881
20. **De Gendt K, Swinnen JV, Saunders PT, Schoonjans L, Dewerchin M, Devos A, Tan K, Atanassova N, Claessens F, Lecureuil C, Heyns W, Carmeliet P, Guillou F, Sharpe RM, Verhoeven G** 2004 A Sertoli cell-selective knockout of the androgen receptor causes spermatogenic arrest in meiosis. Proc Natl Acad Sci USA 101:1327–1332
21. **Holdcraft RW, Braun RE** 2004 Androgen receptor function is required in Sertoli cells for the terminal differentiation of haploid spermatids. Development 131:459–467
22. **Russell L** 1977 Movement of spermatocytes from the basal to the adluminal compartment of the rat testis. Am J Anat 148:313–328
23. **Jegou B** 1993 The Sertoli-germ cell communication network in mammals. Int Rev Cytol 147:25–96
24. **Sharpe RM, Maddocks S, Millar M, Kerr JB, Saunders PT, McKinnell C** 1992 Testosterone and spermatogenesis. Identification of stage-specific, androgen-regulated proteins secreted by adult rat seminiferous tubules. J Androl 13:172–184
25. **Hill CM, Anway MD, Zirkin BR, Brown TR** 2004 Intratesticular androgen levels, androgen receptor localization, and androgen receptor expression in adult rat Sertoli cells. Biol Reprod 71:1348–1358
26. **Vergouwen RP, Jacobs SG, Huiskamp R, Davids JA, de Rooij DG** 1991 Proliferative activity of gonocytes, Sertoli cells and interstitial cells during testicular development in mice. J Reprod Fertil 93:233–243
27. **Pelliniemi LJ, Fröjdman K, Paranko J** 1993 Cell biology of testicular development. In: de Kretser D, ed. Molecular biology of the male reproductive system. San Diego: Academic Press; 21–65
28. **McCarrey J** 1993 Development of the germ cell. In: Desjardins C, Ewing LL, eds. Cell and molecular biology of the testis. Oxford, UK: Oxford University Press; 58–89
29. **Al-Attar L, Noel K, Dutertre M, Belville C, Forest MG, Burgoyne PS, Josso N, Rey R** 1997 Hormonal and cellular regulation of Sertoli cell anti-Mullerian hormone production in the postnatal mouse. J Clin Invest 100:1335–1343
30. **Tan KA, De Gendt K, Atanassova N, Walker M, Sharpe RM, Saunders PT, Denolet E, Verhoeven G** 2005 The role of androgens in Sertoli cell proliferation and functional maturation: studies in mice with total or Sertoli cell-selective ablation of the androgen receptor. Endocrinology 146:2674–2683
31. **Denolet E, De Gendt K, Allemeersch J, Engelen K, Marchal K, Van Hummelen P, Tan KA, Sharpe RM, Saunders PT, Swinnen JV, Verhoeven G** 2006 The effect of a Sertoli cell-selective knockout of the androgen receptor on testicular gene expression in prepubertal mice. Mol Endocrinol 20:321–334
32. **Lecureuil C, Fontaine I, Crepieux P, Guillou F** 2002 Sertoli and granulosa cell-specific Cre recombinase activity in transgenic mice. Genesis 33:114–118
33. **Yeh S, Hu YC, Wang PH, Xie C, Xu Q, Tsai MY, Dong Z, Wang RS, Lee TH, Chang C** 2003 Abnormal mammary gland development and growth retardation in female mice and MCF7 breast cancer cells lacking androgen receptor. J Exp Med 198:1899–1908
34. **Baker PJ, O'Shaughnessy PJ** 2001 Expression of prostaglandin D synthetase during development in the mouse testis. Reproduction 122:553–559
35. **Johnston H, Baker PJ, Abel M, Charlton HM, Jackson G, Fleming L, Kumar TR, O'Shaughnessy PJ** 2004 Regulation of Sertoli cell number and activity by

follicle-stimulating hormone and androgen during postnatal development in the mouse. Endocrinology 145:318–329
36. **Ni J, Wen X, Yao J, Chang HC, Yin Y, Zhang M, Xie S, Chen M, Simons B, Chang P, di Sant'Agnese A, Messing EM, Yeh S** 2005 Tocopherol-associated protein suppresses prostate cancer cell growth by inhibition of the phosphoinositide 3-kinase pathway. Cancer Res 65:9807–9816
37. **Yomogida K, Ohtani H, Harigae H, Ito E, Nishimune Y, Engel JD, Yamamoto M** 1994 Developmental stage- and spermatogenic cycle-specific expression of transcription factor GATA-1 in mouse Sertoli cells. Development 120:1759–1766
38. **de Kretser DM, Kerr JB** 1988 The cytology of the testis. In: Knobil E, Neill JB, Ewing LL, Greenwald GS, Markert CL, Pfaff DW, eds. The physiology of reproduction. New York: Raven Press; 837–932
39. **Mruk DD, Cheng CY** 2004 Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. Endocr Rev 25:747–806
40. **Parvinen M** 1982 Regulation of the seminiferous epithelium. Endocr Rev 3:404–417
41. **Anderson RA, Martin CW, Kung AW, Everington D, Pun TC, Tan KC, Bancroft J, Sundaram K, Moo-Young AJ, Baird DT** 1999 7α-Methyl-19-nortestosterone maintains sexual behavior and mood in hypogonadal men. J Clin Endocrinol Metab 84:3556–3562
42. **Waites GM, Gladwell RT** 1982 Physiological significance of fluid secretion in the testis and blood-testis barrier. Physiol Rev 62:624–671
43. **Vogl AW, Pfeiffer DC, Mulholland D, Kimel G, Guttman J** 2000 Unique and multifunctional adhesion junctions in the testis: ectoplasmic specializations. Arch Histol Cytol 63:1–15
44. **Russell L** 1993 Morphological and functional evidence for Sertoli-germ cell relationships. In: Russell LD, Griswold MD, eds. The Sertoli cell. Clearwater, FL: Cache River Press; 365–390
45. **Griswold MD** 1988 Protein secretions of Sertoli cells. Int Rev Cytol 110:133–156
46. **Skinner M** 1993 Secretion of growth factors and other regulatory factors. In: Russell LD, Griswold MD, eds. The Sertoli cell. Clearwater, FL: Cache River Press; 237–247
47. **Jegou B, Le Gac F, de Kretser DM** 1982 Seminiferous tubule fluid and interstitial fluid production. I. Effects of age and hormonal regulation in immature rats. Biol Reprod 27:590–595
48. **Jegou B, Le Gac F, Irby DC, de Kretser DM** 1983 Studies on seminiferous tubule fluid production in the adult rat: effect of hypophysectomy and treatment with FSH, LH and testosterone. Int J Androl 6:249–260
49. **Amlani S, Vogl AW** 1988 Changes in the distribution of microtubules and intermediate filaments in mammalian Sertoli cells during spermatogenesis. Anat Rec 220:143–160
50. **Junqueira LC, Carneiro J, Kelley RO** 1998 Basic Histology. In: Junqueira LC, Carneiro J, Kelley RO, eds. Basic Histology. Stamford, CT: Appleton, Lange; 63–88
51. **Siu MK, Cheng CY** 2004 Dynamic cross-talk between cells and the extracellular matrix in the testis. Bioessays 26:978–992
52. **Dym M** 1994 Spermatogonial stem cells of the testis. Proc Natl Acad Sci USA 91:11287–11289
53. **Salomon F, Saremaslani P, Jakob M, Hedinger CE** 1982 Immune complex orchitis in infertile men. Immunoelectron microscopy of abnormal basement membrane structures. Lab Invest 47:555–567
54. **Lehmann D, Temminck B, Da Rugna D, Leibundgut B, Sulmoni A, Muller H** 1987 Role of immunological factors in male infertility. Immunohistochemical and serological evidence. Lab Invest 57:21–28
55. **Tung PS, Fritz IB** 1993 Interactions of Sertoli cells with laminin are essential to maintain integrity of the cytoskeleton and barrier functions of cells in culture in the two-chambered assembly. J Cell Physiol 156:1–11
56. **Siu MK, Lee WM, Cheng CY** 2003 The interplay of collagen IV, tumor necrosis factor-α, gelatinase B (matrix metalloprotease-9), and tissue inhibitor of metalloproteases-1 in the basal lamina regulates Sertoli cell-tight junction dynamics in the rat testis. Endocrinology 144:371–387
57. **Gye MC, Ohsako S** 2003 Effects of flutamide in the rat testis on the expression of occludin, an integral member of the tight junctions. Toxicol Lett 143:217–222
58. **Florin A, Maire M, Bozec A, Hellani A, Chater S, Bars R, Chuzel F, Benahmed M** 2005 Androgens and postmeiotic germ cells regulate claudin-11 expression in rat Sertoli cells. Endocrinology 146:1532–1540
59. **Gow A, Southwood CM, Li JS, Pariali M, Riordan GP, Brodie SE, Danias J, Bronstein JM, Kachar B, Lazzarini RA** 1999 CNS myelin and Sertoli cell tight junction strands are absent in Osp/claudin-11 null mice. Cell 99:649–659
60. **Saitou M, Furuse M, Sasaki H, Schulzke JD, Fromm M, Takano H, Noda T, Tsukita S** 2000 Complex phenotype of mice lacking occludin, a component of tight junction strands. Mol Biol Cell 11:4131–4142
61. **Hellani A, Ji J, Mauduit C, Deschildre C, Tabone E, Benahmed M** 2000 Developmental and hormonal regulation of the expression of oligodendrocyte-specific protein/claudin 11 in mouse testis. Endocrinology 141:3012–3019
62. **Meng J, Holdcraft RW, Shima JE, Griswold MD, Braun RE** 2005 Androgens regulate the permeability of the blood-testis barrier. Proc Natl Acad Sci USA 102:16696–16700
63. **Vogl AW, Pfeiffer DC, Redenbach DM, Grove BD** 1993 Sertoli cell cytoskeleton. In: Russell LD, Griswold MD, eds. The Sertoli cell. Clearwater, FL: Cache River Press; 39–86
64. **Li Y, Friel PJ, Robinson MO, McLean DJ, Griswold MD** 2002 Identification and characterization of testis- and epididymis-specific genes: cystatin SC and cystatin TE-1. Biol Reprod 67:1872–1880
65. **Monsees TK, Schill WB, Miska W** 1997 Protease-protease inhibitor interactions in Sertoli cell-germ cell crosstalk. Adv Exp Med Biol 424:111–123
66. **Skinner MK, Schlitz SM, Anthony CT** 1989 Regulation of Sertoli cell differentiated function: testicular transferrin and androgen-binding protein expression. Endocrinology 124:3015–3024
67. **Kahn A, Levin MJ, Zakin M, Bloch B** 1987 The transferring gene. In: Guroff G, ed. Oncogenes, genes, and growth factors. New York: John Wiley and Sons; 277–309
68. **Kingma PB, Bok D, Ong DE** 1998 Bovine epidermal fatty acid-binding protein: determination of ligand specificity and cellular localization in retina and testis. Biochemistry 37:3250–3257
69. **D'Agostino A, Mene P, Stefanini M** 1992 Voltage-gated calcium channels in rat Sertoli cells. Biol Reprod 46:414–418
70. **Cheng CY, Mruk DD** 2002 Cell junction dynamics in the testis: Sertoli-germ cell interactions and male contraceptive development. Physiol Rev 82:825–874
71. **Taranta A, Morena AR, Barbacci E, D'Agostino A** 1997 ω-Conotoxin-sensitive Ca^{2+} voltage-gated channels modulate protein secretion in cultured rat Sertoli cells. Mol Cell Endocrinol 126:117–123
72. **Lindsey JS, Wilkinson MF** 1996 An androgen-regulated homeobox gene expressed in rat testis and epididymis. Biol Reprod 55:975–983
73. **Rao MK, Wayne CM, Meistrich ML, Wilkinson MF** 2003 Pem homeobox gene promoter sequences that direct transcription in a Sertoli cell-specific, stage-specific, and androgen-dependent manner in the testis *in vivo*. Mol Endocrinol 17:223–233
74. **Barbulescu K, Geserick C, Schuttke I, Schleuning WD, Haendler B** 2001 New androgen response elements in the murine pem promoter mediate selective transactivation. Mol Endocrinol 15:1803–1816

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**