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ORIGINAL ARTICLE

Male Reproduction

Expression and function of HSP110 family in mouse testis after vasectomy

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HSP110 functions to protect cells, tissues, and organs from noxious conditions. Vasectomy induces apoptosis in the testis; however, little is known about the reason leading to this outcome. The aim of the present study was to evaluate the expression and function of HSP110 in mouse testis after vasectomy. Following bilateral vasectomy, we used fluorescent Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) to detect apoptosis, Western blotting and immunohistochemistry to examine HSP110 expression and localization. Serum antisperm antibody (AsAb) and testosterone were measured by Enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay, respectively. Expression of endoplasmic reticulum stress (ERS) sensors and downstream signaling components was measured by Reverse Transcription-Polymerase Chain Reaction (RT-PCR), and the phosphorylation of eIF2α and JNK was detected by Western blotting. Vasectomy induced morphologic changes, increased apoptosis in the testis, increased serum AsAb, and decreased testosterone levels. After vasectomy, ORP150 mRNA level was increased first and then decreased, Bcl-2 was decreased, and the expression of HSPA4l, GRP78, GADD153, PERK, ATF6, IRE-1, XBP-1s, Bax, Bak, and caspases and the phosphorylation of eIF2α and JNK were increased. We present that an ER stress-mediated pathway is activated and involved in apoptosis in the testis after vasectomy. HSPA4l and ORP150 may play important roles in maintaining the normal structure and function of testis.

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INTRODUCTION

Highly conserved and ubiquitous across species, most heat shock proteins (HSPs) are expressed strictly in response to stress such as hyperthermia, inflammation, and infection.[1] Based on their molecular weight, HSPs are divided into the HSP110, HSP90, HSP70, HSP60, and HSP27 families.[2] In mammals, the HSP110 family is composed of four members, namely HSPA4l/APG1, HSPA4/APG2, HSPH1/HSP105, and ORP150/HYOU1/GRP175. HSPA4l is highly expressed in spermatogenic cells of testis from late pachytene spermatocytes to postmeiotic spermatids[3] while HSPA4 is predominant in the testis and ovary.[4,5] ORP150 is expressed in the plasma membrane of mature mouse oocytes.[6] This expression pattern suggests a critical role for the HSP110 family proteins in reproduction.

Disturbances in the normal functions of the ER lead to an evolutionarily conserved cell stress response, the unfolded protein response (UPR), which was recently identified as a new pathophysiological paradigm that determines cell viability. Although the significance of UPR was initially to compensate for the damage, it eventually trigger the cell death if ER dysfunction is severe or prolonged, which was termed ERS.[7,8] Many disturbances, including those of cellular redox regulation, cause accumulation of unfolded proteins in the ER, triggering an evolutionarily conserved UPR.[9] The canonical UPR engages three distinct signaling cascades mediated by pancreatic ER kinase (PERK), inositol-requiring transmembrane kinase/endonuclease-1 (IRE-1), and activating transcription factor 6 (ATF6).[10,11] The combined action of these pathways leads to translation inhibition, protein degradation, and chaperone protein production, all of which promote either the recovery of ER function or cell death.[12]

Upon ERS induction, IRE-1 promotes the splicing of X-box-binding protein-1 (XBP-1) mRNA to its short form XBP-1s.[13] GRP78 binds misfolded proteins, thereby releasing activated ERS sensors to induce the UPR, which triggers adaptive responses to ER dysfunction,[9,14] including apoptosis during prolonged or severe ERS.[15,16] GADD153, a pro-apoptotic molecule, is expressed specifically under conditions of ER dysfunction[17] and is involved in ER stress-induced apoptosis by down-regulating Bcl-2 and up-regulating Bax.[18,19] However, during ERS, GADD153 is rapidly degraded, enabling cell survival. ORP150 expression is induced in response to ERS, whereas diminished levels of this protein could aggravate ERS and lead to cell death.[20] ERS also induces the phosphorylation of c-Jun N-terminal kinase (JNK) and eIF2α.[21,22] During ERS, caspase-12 is activated by cleavage and subsequently initiates downstream apoptotic pathways mediated by other caspases, including caspase-8 and caspase-9.[19]

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Vasectomy is a common surgical procedure and permanent method for family planning.[23] Although vasectomy is regarded as a safe method for male fertility control, several unwanted outcomes have been reported, including obstruction in the duct, inflammation, and degeneration of the seminiferous tubules coinciding with a decrease in spermatogenic cell number.[24] Vasectomy has also been shown to induce apoptosis in the testis.[25–27] Because the family of HSP110 plays a crucial role in protecting cells, tissues, organs, and organisms from various noxious conditions, we investigated the relationship between vasectomy-induced apoptosis and HSP110 family. To accomplish this, we generated a vasectomized mouse model and examined HSP110 family expression and localization in the testis at different stages after vasectomy. We also assessed the expression of ERS and apoptosis-related signaling molecules in the testis after vasectomy. Our research demonstrates that ERS, through modulation of UPR signaling, may be involved in vasectomy-induced apoptosis in the mouse testis, and the family of HSP110 may play an important role in this process.

MATERIALS AND METHODS

Ethics statement

All animal husbandry and animal experiments were approved by the Animal Experiment Ethics Committee of the Stem Cell Research Center of Shandong Province. This study was conducted in strict accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All surgeries were performed under anesthesia, and all efforts were made to minimize suffering.

RNA isolation and semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted with RNAiso Plus reagent (TaKaRa, Dalian, China) according to the manufacturer's instructions. RNA concentration was determined by spectrophotometry (Nanodrop 2000/2000C, Thermo Scientific, Wilmington, USA). Total RNA (3 μg) was reverse transcribed using AMV Reverse Transcriptase (Promega, Madison, WI, USA). PCR reaction conditions were: denaturation at 94°C for 3 min, followed by cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 30 s, and final extension at 72°C for 10 min. All primer sequences and Tm values are presented in **Supplementary Table 1**. Forward and reverse primers were designed to span several introns to eliminate contaminating genomic DNA. Distilled water in place of cDNA was used as a negative control for all PCR reactions. PCR products were separated on 1.5% (w/v) agarose gel and visualized by ethidium bromide staining.

Western blot analysis

Tissue proteins were extracted into RIPA buffer (20 mmol l^{-1} Tris-HCl, 150 mmol l^{-1} NaCl, 5 mmol l^{-1} EDTA, 1% (v/v) NP-40, 5 mmol l^{-1} NaPPi, 1 mmol l^{-1} Na_3VO_4, 1 mmol l^{-1} β-glycerophosphate and 1 mmol l^{-1} PMSF). Protein concentration was determined using the Bradford assay (Bio-Rad, Hercules, CA, USA). Proteins (40 μg per lane) separated on a 12% (w/v) sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and transferred to polyvinylidene difluoride membranes that were subsequently blocked with 5% (w/v) nonfat milk for 1 h at room temperature. Membranes were incubated with primary antibody overnight at 4°C, washed with TBST (TBS with 0.05% (v/v) Tween-20), and incubated with HRP-conjugated IgG (ZB-2306: Zhongshan Biotechnology, Beijing, China) for 1 h at room temperature. The horseradish peroxidase was visualized using DAB (Zhongshan Biotechnology, Beijing, China) for HSP110, and ECL for GRP78, GADD153, phospho-JNK, phospho-eIF2α, JNK, and eIF2α. The primary antibodies used were: GAPDH (sc-25778: Santa Cruz Biotechnology, Santa Cruz, CA, USA); HSPA4 (sc-365366: Santa Cruz Biotechnology, Santa Cruz, CA, USA); HSPA4l, ORP150, and HSPH1 (ARP53693, ARP46716, ARP53693: Aviva Systems Biology, Beijing, China); phospho-JNK, phospho-eIF2α, JNK, eIF2α, GRP78, and GADD153 (Cell Signaling Technology, Danvers, MA, USA).

Immunohistochemistry of mouse testis

Mouse testicular tissues were fixed for immunohistochemistry in Bouin's solution immediately after dissection. Twenty-four hours after fixation, the tissues were embedded in paraffin and 4-μm-thick sections were deparaffinized in dimethylbenzene and rehydrated in a graded ethanol series. Antigen retrieval was accomplished by boiling sections for 20 min in a microwave oven (Haier, Qingdao, China) in 0.1 mol l^{-1} citrate buffer, followed by treatment with 3% (v/v) H_2O_2. Subsequently, sections were blocked for 1 h with 3% (w/v) bovine serum albumin in TBS at room temperature and incubated overnight at 4°C with HSP110 antibodies. After washing in TBS, the sections were incubated for 2 h at 37°C with HRP-conjugated IgG (diluted 1:200 in TBS). Sections were counterstained with hematoxylin and eosin (H and E), dehydrated, and mounted for bright-field microscopy (DM LB2, Leica, Nussloch, Germany). Normal IgG was used instead of primary antibody as a negative control. All images were taken at 100× amplification. The positive area was yellow stained, and Image-Pro Plus software (Media Cybernetics, USA) was used to quantify the integrated optical density. The mean density was used to present the relative amount of the substance expression in the testis.

Vasectomy

Adult male mice (10 weeks, 30–40 g) were vasectomized. The animals were housed under standard laboratory conditions and maintained on pelleted food and water. After recording initial body weight, they were divided into 10 groups, bilaterally vasectomized, and sacrificed on days 2, 4, 6, 8, 10, 15, 30, 45, 60, and 110 (10 groups of 5 each). The operations were performed under ketamine/xylazine (80/10 mg kg^{-1}, i.p.) anesthesia via a lower mid-abdominal incision with a segment of vas deferens excised and the cut ends ligated with silk threads. Sham-operated controls (10 groups of 3 each) underwent the same surgical procedure without actual vasectomy. Following sacrifice, blood samples were collected, and testes were dissected. Plasma was immediately obtained by centrifugation of the blood samples at 2000 ×*g* for 20 min at 4°C. Plasma samples were stored at −80°C until assayed for testosterone and antisperm antibody.

Detection of apoptotic cells in testis

Fluorescent TUNEL staining was performed according to the manufacturer's instructions. Paraffin sections (4 μm) from experimental mice were cut onto silane-coated glass slides, dewaxed with dimethylbenzene, and rehydrated in a graded ethanol series. After washing with phosphate-buffered saline (PBS), the sections were treated with 20 μg ml^{-1} of proteinase K in PBS at 37°C for 20 min. The sections were rinsed with PBS, stained with 50 μl of TUNEL detection solution (Beyotime, Shanghai, China), and incubated in a lucifugal humidified chamber at 37°C for 1 h. After washing, the sections were stained with propidium iodide (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. The sections were analyzed, and images were captured by confocal laser scanning microscopy (LSM-510 META, Carl Zeiss, Jena, Germany).

ELISA kit (IBL, America) for AsAb

The ELISA-plate is coated with spermatozoa proteins of mouse that are recognized by anti-spermatoza antibody. The samples and standards are pipetted into the wells and then incubated. During this incubation,

anti-spermatoza antibody binds to the spermatozoa proteins and thus immobilized on the plate. After washing, the enzyme conjugate, consisting of anti-mouse globulin antibodies covalently coupled to horseradish peroxidase, is added. After removal of the unbound conjugate by washing, the horseradish peroxidase oxidizes the then added substrate TMB (3,3',5,5'-tetramethylbenzidine) yielding a color reaction that is stopped with 0.25 mol l^{-1} sulphuric acid (H_2SO_4). The extinction is measured at a wavelength of 450 nm with a microplate reader. The use of a reference measurement with a wavelength 550 nm is recommended.

RIA for testosterone
Concentrations of testosterone in serum were determined by a double-antibody RIA system with ^{125}I-labeled radioligands as described previously.[28]

Statistical analysis
The results were presented as mean ± s.d. The unpaired *t*-test was used to test for differences between groups (vasectomy and sham-operation), and $P < 0.05$ was considered to be statistically significant. All statistical analyses were performed using a commercial software package (SPSS 18.0; SPSS Inc., Chicago, IL, USA).

RESULTS
Histological changes in testis following vasectomy
First, we examined the testis of vasectomized mice for any morphological changes that may have been incurred by vasectomy. Overall, the animals maintained a healthy appearance throughout the study with no significant difference observed between the initial and final body weights of the vasectomized and control animals (data not shown). Histology revealed that the control mice exhibited normal morphology with regular seminiferous tubules and spermatogenic cell lines (**Figure 1a**). Moreover, successive stages of spermatogonia differentiating into spermatozoa and an orderly arrangement of multilayered epithelial cells within the seminiferous tubules were observed. The reproductive epithelium was also tightly linked to the basement membrane of the tubules, which were rich in spermatozoa and surrounded by interstitial cells. Following vasectomy, however, the seminiferous epithelium contained only spermatogonia and Sertoli cells. The spermatogenic cells, of which fewer could be seen, were arranged loosely with some degeneration observed in these cells.

Increased apoptosis following vasectomy
The total number of apoptotic cells throughout the testis was significantly increased in all postvasectomy groups. Apoptosis first appeared on day 4 in the early spermatids, primary spermatocytes, and spermatogonia (**Figure 1b**). Between days 6 and 10, the number of apoptotic spermatids increased gradually, and apoptosis was observed predominantly in spermatozoa and elongating spermatids. By day 15, apoptosis was dramatically up-regulated in spermatogonia and primary spermatocytes. The apoptotic signal was gradually weakened between days 30 and 45. From days 60 to 110, fewer apoptotic cells could be observed. No positive signals were observed in negative control (data not shown).

Expression of HSP110 family members in vasectomized mouse testis
Our analysis revealed that all HSP110 family members are expressed in mouse testis. While HSPA4 and HSPH1 expression remained unaltered after vasectomy, HSPA4l expression was higher compared to the control. The highest level of ORP150 expression was detected on day 8, and decreased gradually between days 10 and 45 to a level below that of the control group. Interestingly, the level of ORP150 expression was restored between days 60 and 110 (**Figure 2a** and **2b**). Western blot analysis demonstrated that HSP110 protein levels were consistent with mRNA expression (**Figure 2c**).

Localization of HSP110 family members in mouse testis
We performed immunohistochemistry to determine the localization of HSP110 family members within the testis. HSPA4l, which was absent in spermatogonia, exhibited high expression in spermatocytes, Sertoli cells, and spermatids (round and elongating) with greater intensity in the vasectomized groups (**Supplementary Figure 1a**). ORP150

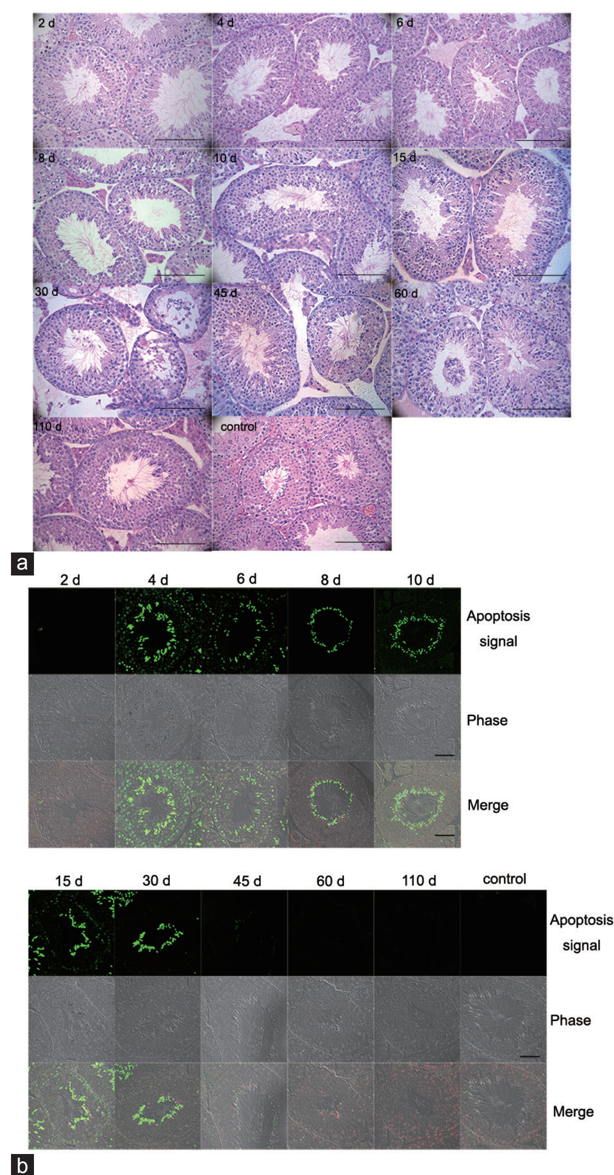

Figure 1: Vasectomy induces morphological changes and increased apoptosis in the testis. (**a**) Hematoxylin and eosin staining of testis from vasectomized and sham-operated. Control, one representative of sham-operated control (note that sham-operated controls at each time point postvasectomy had the same apoptotic pattern). Mice isolated on the indicated day after surgery. The scale bar represents 50 μm; (**b**) Analysis of apoptotic status of mouse testes at different time points postvasectomy by TUNEL. Apoptotic cells with fragmented DNA display green signal. Control, one representative of sham-operated control (note that sham-operated controls at each time point postvasectomy had the same apoptotic pattern). The scale bar represents 50 μm.

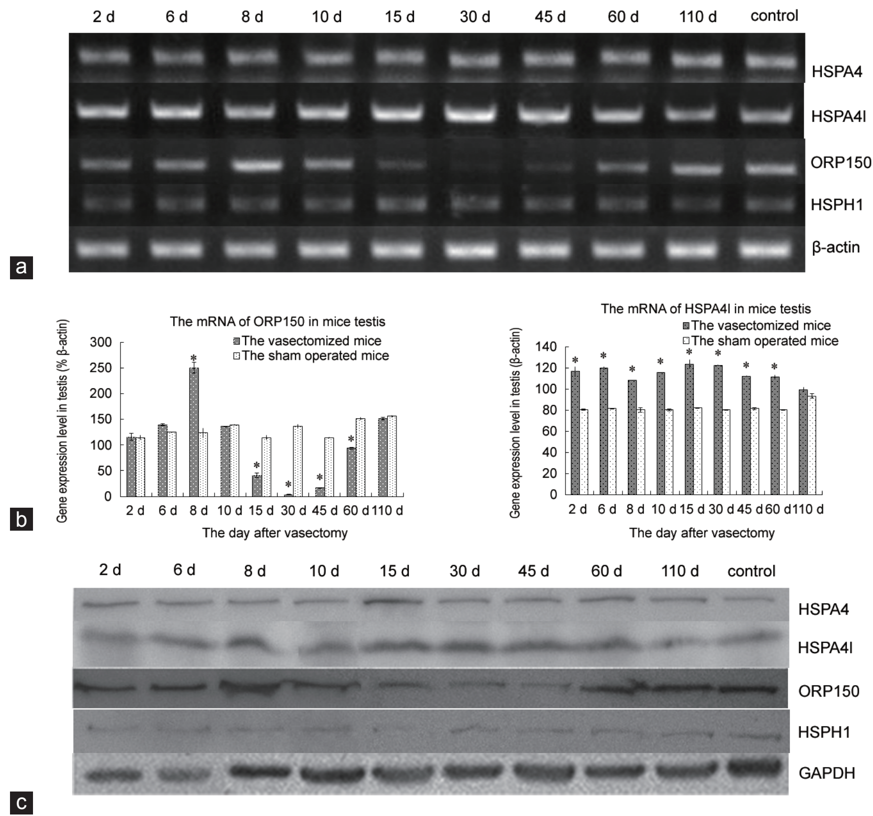

Figure 2: Vasectomy induces changes in mRNA and protein levels of HSP110 family members. (**a**) The level of HSP110 mRNA in the mouse testis was determined by RT-PCR. (**b**) Quantification of ORP150 and HSPA4l mRNA in vasectomized and control mice relative to β-actin. Control, one representative of sham-operated control (note that sham-operated controls at each time point postvasectomy had the same expression level for each HSP110 gene). *Statistically significant difference for vasectomy versus sham operation of the same day ($P < 0.05$). Results of one representative experiment of two others. (**c**) Expression of HSP110 in mouse testes at different time points postvasectomy detected by Western blot. GAPDH was assessed as an internal loading control. Control, one representative of sham-operated control (note that sham-operated controls at each time point postvasectomy had the same expression level for each HSP110 protein). Results of one representative experiment of two others.

was found in all spermatogenic and Sertoli cells, in the cytoplasm of Leydig cells, and spermatids (round and elongating). On day 8, ORP150 expression in the vasectomized mice was higher than the control animals; however, this level of expression decreased dramatically between days 15 and 45 (**Supplementary Figure 1b**). HSPA4 resided mainly in the cytoplasm of Sertoli cells, spermatocytes, and round spermatids but was only moderately expressed in spermatogonia and poorly expressed in Leydig cells (**Supplementary Figure 1c**). However, HSPH1 was detected only in the cytoplasm of Leydig cells (**Supplementary Figure 1c**) and, like HSPA4, did not change significantly after vasectomy. A summary of these results is presented in **Supplementary Table 2**. The quantification of immunostaining is presented in **Supplementary Figure 2**.

Effects of vasectomy on levels of antisperm antibody and testosterone
To begin understanding the reasons involved in these changes in the testis, we examined the levels of serum antisperm antibody (AsAb) and testosterone by ELISA and radioimmunoassay, respectively. All animals demonstrated a high AsAb level following vasectomy. The AsAb serum titer was increased by day 6 and peaked on day 15. Between days 45 and 60, the serum AsAb level was higher in vasectomized animals. By day 110, the AsAb level had returned to the baseline (**Table 1**).

The testosterone level in vasectomized mice also decreased between days 6 and 45 before recovering to baseline levels (**Figure 3**).

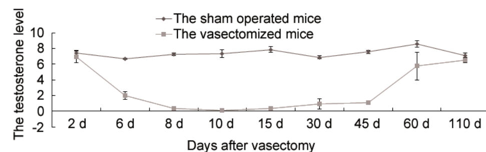

Figure 3: Changes in serum testosterone level after vasectomy.

Table 1: AsAb levels after vasectomy

	2 days (n=5)	*6 days (n=5)*	*8 days (n=5)*	*10 days (n=5)*	*15 days (n=5)*	*30 days (n=5)*	*45 days (n=5)*	*60 days (n=5)*	*110 days (n=5)*
Experimental group/Sham group (≥2.1 is positive)	1.020	1.021	1.171	2.112	2.428	1.872	1.405	1.205	1.074
	0.988	1.440	1.163	1.788	2.239	1.044	1.078	1.338	1.181
	0.966	1.022	1.325	1.493	1.969	2.106	1.514	1.136	0.994
	0.997	1.389	1.438	1.611	2.111	1.983	1.707	1.152	1.018
	0.998	1.165	1.231	1.939	2.178	2.145	1.832	1.225	1.083
Positive rate (%)	0	0	0	20	80	40	0	0	0

ELISA kit was used for the detection of the serum AsAb, the numbers in the table are the ratio of the experimental measurements and controls (≥2.1 is positive). AsAb: antisperm antibody; ELISA: enzyme-linked immunosorbent assay

Role of ER stress in vasectomy-induced apoptosis in the testis
Because ORP150 is a marker for ER stress, we hypothesized that changes in its expression after vasectomy may be caused by induction

of this biological response. To confirm this, we assessed the expression of GRP78 and GADD153, major markers of the ER stress response, by RT-PCR and Western blot analysis. Compared to control mice, GRP78 and GADD153 mRNA and protein were higher in the testis of vasectomized mice and the phosphorylation of eIF2α and JNK was also increased (**Figure 4a–4c**), suggesting induction of the ER stress response following vasectomy.

Further examination revealed that PERK, ATF6, and IRE-1 expression was also higher after vasectomy (**Figure 4d** and **Supplementary Figure 3**). Moreover, we detected increased expression of caspase-12, caspase-9, and caspase-8 in vasectomized mice. The expression of other pro-apoptotic genes, *Bax* and *Bak*, was also higher in these animals while expression of the anti-apoptotic gene *Bcl-2* was lower, the mRNA expression level of spliced XBP-1 (XBP-1s) increased at 15d, 30d, 45d, and 60d. These results indicate that vasectomy induces a general ER stress response during apoptosis and the UPR signaling branches were activated after vasectomy.

DISCUSSION

The vasectomy-induced morphological changes we observed in the mouse testis suggest damage to the germinal epithelium. The frequency of apoptotic cells in the seminiferous tubules was dependent on the elapsed time after vasectomy. The most intriguing phenomenon in our study is that germ cell apoptosis occurred mainly in spermatids and elongating spermatids, but not in spermatogonia and spermatocytes. These results are contrary to early research, in which apoptotic spermatogonia, spermatocytes were exited after vasectomy.[29–31] More recent studies have reported the occurrence of spermatozoa apoptosis,[32,33] which is consistent with our results. And from days 45 to 110, fewer apoptotic cells was observed that may be associated with the gradually vanish of trauma and acute inflammation, which indicated that long-term after vasectomy, the effects on the testis can be recovered in the end.

Vasectomy may result in dysfunctional spermatogenesis due to increased pressure in the seminiferous tubules and autoimmunity.[34] Recently, vasectomy was reported to induce germ cell apoptosis;[25–27] however, the exact mechanism remains unclear. Alexander and Tung[35] reported that 40%–100% of the vasectomy patients produced AsAb, which interferes with spermatogenesis. Ball *et al.*[36] proposed that the release of spermatozoa, or spermatozoan antigens, into the interstitium could lead to a granulomatous inflammatory reaction. Under normal circumstances, the blood-testosterone barrier prevents autoimmunity caused by AsAb. However, if this barrier is disrupted by inflammation, injury, or blockage, AsAb is produced. Serum AsAb recognizing spermatozoan antigens can lead to autoimmune orchitis and interfere with spermatogenesis.[37] Although Zhao *et al.*[38] hypothesized that this process is regulated by testosterone, other studies have demonstrated no significant effect on the testis by serum testosterone.[39]

In this study, we measured serum AsAb and testosterone levels after vasectomy. Our results showed that mean AsAb levels were higher in mice that exhibited testicular changes than in those with normal testes, suggesting that AsAb production may play a role in the development of testicular lesions after vasectomy. However, further work is necessary to define the nature of the association between AsAb and testicular alterations in this model.

Vasectomy-induced stress adversely affects the testis by reducing spermatogenesis, which is associated with lower testosterone levels.[40,41] Lue *et al.*[42] reported that vasectomy did not affect testosterone levels. However, other studies have reported a significant reduction in testosterone during the early stages after vasectomy.[43] Our data demonstrate that testosterone decreases significantly after vasectomy and gradually returns to baseline levels. This temporary decline in testosterone may lead to damage in the testis and increased apoptosis in spermatozoa cells.[44] The main secretory cells of testosterone are

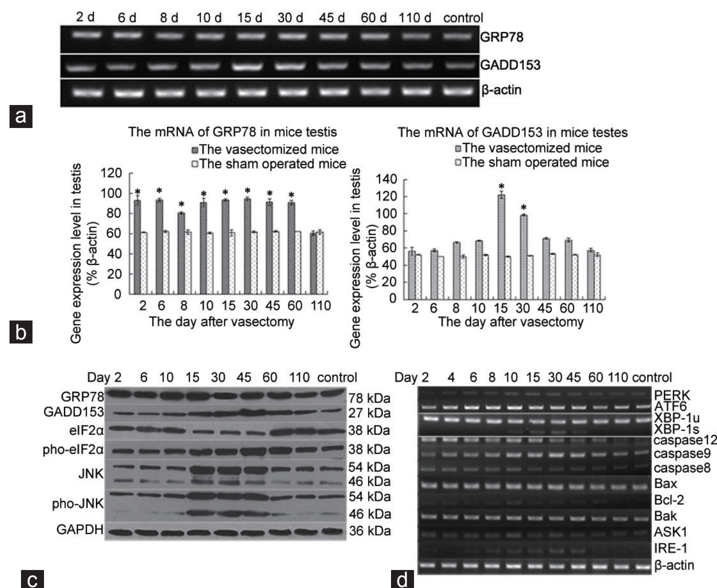

Figure 4: Vasectomy induces apoptosis and ER stress responses. (**a**) The expression levels of GRP78 and GADD153 mRNA in vasectomized and control mice was determined by RT-PCR. (**b**) Quantification of GRP78 and GADD153 mRNA in vasectomized and control mice relative to β-actin. Control, one representative of sham-operated control (note that sham-operated controls at each time point postvasectomy had the same expression level for each HSP110 gene). *Statistically significant difference for vasectomy versus sham operation of the same day ($P < 0.05$). (**c**) The testes extracts were analyzed for phosphorylation of JNK and eIF2α, expression of GADD153 and GRP78 by Western blot analysis using antibodies against phospho-JNK, total JNK, phospho-eIF2α, total eIF2α, GADD153, GRP78, and GAPDH. (**d**) The level of ER stress sensors and downstream signaling components mRNA in the mouse testis after vasectomy was determined by RT-PCR.

the Leydig cells. After vasectomy, the blood-testosterone barrier was damaged due to pressure, Leydig cells are affected by the spillover sperm and the pressure in the test is that influence the secretion of hormones, this may be the main reason of reducing of testosterone.

We also systematically investigated the expression and localization of HSP110 family members in the mouse testis after vasectomy to elucidate a possible mechanism for the observed testicular damage. HAPA4l is required for normal spermatogenesis and may play a role in spermatozoa apoptosis.[45] Consistent with this, our data showed that HSPA41 expression was higher in vasectomized animals. By assisting in protein synthesis, repair and positioning, and inhibition of injury, HSPA41 up-regulation may protect the testis and ensure the normal cell cycle of spermatogenic cells and allow spermatogenesis to return to normal. Thus, HSPA4l may play important roles in maintaining the normal structure and function of testis.

HSPH1 was detected only in Leydig cells which are distributed in the loose connective tissue surrounding seminiferous tubules. Leydig cells function to synthesize and secrete testosterone.[46] Although HSPH1 mRNA was not altered significantly by vasectomy, the protein level was slightly lower. Thus, it is possible that vasectomy damages Leydig cells, which results in a decrease in testosterone and, therefore, HSPH1 protein.

ORP150 is an anti-apoptotic ER resident chaperone that exerts a protective effect against ER stress-dependent apoptosis. This protein is induced by stress, such as oxygen or glucose deprivation, and functions by limiting ER stress.[47,48] Our results demonstrate that ORP150 mRNA levels rise soon after vasectomy and decrease dramatically. These changes in ORP150 expression were accompanied by a significant increase in GRP78 and GADD153 expression, indicating that ER stress may be involved in the increase in testicular apoptosis following vasectomy. Our data also revealed that expression of the UPR-related components, PERK, ATF6, and IRE-1, was increased in vasectomized mice. We also detected increased *Bax* and *Bak* mRNA along with a decreased level of *Bcl-2* mRNA, the mRNA expression level of spliced XBP-1s increased, as well as the phosphorylation of eIF2α and JNK was increased. Moreover, the levels of caspase-12, caspase-9, and caspase-8 mRNA were significantly higher in the testis of vasectomized mice. These data suggest that factors involved in the ER stress response (GADD153, GRP78, and ORP150), UPR signaling branches (PERK, ATF6, and IRE-1), *Bcl-2* family members, and caspases, and the early phosphorylation of eIF2α and JNK are involved in mediating apoptosis in the testis after vasectomy. Each of these signaling molecules has been linked to ER stress and apoptosis in previous studies.

CONCLUSION

Vasectomy induced morphologic changes and increased apoptosis in the testis. HSPA4l and ORP150 may play important roles in maintaining the normal structure and function of testis. We present the first evidence of apoptosis induced by ER stress, as well as the pathways involved in mediating apoptosis after vasectomy. Further investigation of the apoptotic mechanism is needed to provide the theoretical basis on the safety of vasectomy.

AUTHOR CONTRIBUTIONS

CTR carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. SJL, SHJ carried out the immunoassays. ZP participated in the sequence alignment. HYW participated in the design of the study and performed the statistical analysis. ZND, SH, JYL conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing financial interests.

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Supplementary information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Supplementary Table 2: Summary of the relative expression levels of HSP110 family members in mouse testis

	HSPA4	*HSPA4l*	*ORP150*	*HSPH1*
Leydig cell	–	+	+	+
Sertoli cell	+++	+++	+	–
Spermatogonia	+	–	+	–
Spermatocyte	+++	+++	+	–
Round and elongating spermatids	+++	+++	+	–

+++: strong staining; +: moderate staining; –: no staining