FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 59, No. 4, 2021
pp. 236–244

ORIGINAL PAPER

VIA MEDICA

Transcriptional activation and regulation of urokinase plasminogen activator inducted by LPS through MyD88 independent pathway in rat Sertoli cells

Yan Liu[1,2†], Kai Zhao[1†], Zhe Xiong[3], Yuanxiao Yi[1], Chengliang Xiong[1], Donghui Huang[1*], Hu Zhao[4*]

[1]Institute of Reproduction Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

[2]Puren Hospital Affiliated to Wuhan University of Science and Technology, Wuhan, China

[3]Department of Physiology, Medical School of Jianghan University, Wuhan, China

[4]Department of Human Anatomy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Abstract

Introduction. Urokinase plasminogen activator (uPA) is a serine protease and it also demonstrates proinflammatory properties. We thus hypothesized that uPA is involved in immunity of Sertoli cells in the rat testis.

Materials and methods. The uPA gene(*PLAU*) promoter was cloned by RT-PCR and the transcriptional activation of core domain was screened by using Dual-Luciferase Reporter Assay System. The Sertoli cells were harvested from 20-day-old Sprague-Dawley male rats and total RNA was isolated. The uPA mRNA levels and MyD88 pathway were tested by qPCR.

Results. We successfully cloned the 1517-bp rat uPA gene and screened the core domain (-455/+40) in five different fragments of uPA promoter. The uPA expression and uPA promoter activity were upregulated in lipopolysaccharide (LPS)-stimulated Sertoli cells. Furthermore, the uPA expression was regulated through the MyD88-independent pathway by interdicting IRF3 and interferon β.

Conclusion. uPA expression is likely induced by LPS through the core promoter domain of uPA. This finding implied that uPA played a role in the immune function of Sertoli cells in rat testis, which might provide the development of new treatments for male infertility. *(Folia Histochemica et Cytobiologica 2021, Vol. 59, No. 4, 236–244)*

Key words: rat; Sertoli cells; urokinase plasminogen activator; lipopolysaccharide; uPA promoter

Introduction

Plasmin is a serine protease that plays a crucial role in extracellular matrix (ECM) degradation under various physiological conditions [1]. Plasminogen is an inactive zymogen that can be activated by a limited proteolysis reaction catalyzed by two types of plasminogen activators (PA), namely, tissue-type PA (tPA) and urokinase-type PA (uPA) [2]. Circulating tPA is mainly involved in the activation of plasminogen during blood clotting in the degradation and dissolution of fibrin [3, 4] while uPA is involved in

Correspondence address: Hu Zhao
Department of Human Anatomy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China
phone: +86 13808674754
e-mail: zhaohu@hust.edu.cn
***Correspondence address:** Donghui Huang
Institute of Reproduction Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China
phone: +86 18872262607
e-mail: jhsyyjs@126.com; drjhsyyjs@163.com
†Yan Liu and Kai Zhao contributed equally to the work

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Folia Histochem Cytobiol. 2021
10.5603/FHC.a2021.0026
ISSN 0239-8508, e-ISSN 1897-5631

various aspects of cellular migration and regulation [5]. Urokinase-type plasminogen activator was proved to be closely related to male reproduction, such as sperm progressive motility, acrosomal reaction, capacitation, chemotactic response, and fertilization [6-7]. In monkey, uPA is produced mainly in Sertoli cells [6], and the highest uPA expression is found at stages VII and VIII of the seminiferous epithelium cycle in rat [8]. However, uPA receptor was localized in germ cells of mature testis, but not in spermatogonia or late spermatids [9]. Our previous study showed that the expression of *uPA* gene in rat was relatively high at postnatal Day 5 and Day 35, which indicated that uPA may be strongly linked to spermiation and spermatogenesis [10]. Downregulation of *uPA* could decrease the fertility of male mice, which may be caused by a reduction in sperm motility [11].

Although the plasmin system was mainly known as the system responsible for vascular fibrinolysis, recent study also showed that it is critically important as a mediator of inflammation and the innate immune system, which impacts upon a diverse set of mechanisms underlying the pathologies of many diseases [12]. In a recent review it was described that plasmin was involved in many immunological processes by directly interacting with various cell types including monocytes, macrophages, dendritic cells and vascular wall cells (endothelial cells, smooth muscle cells) as well as soluble factors of the immune system and components of the ECM [13]. Furthermore, uPA could become a new target for immunocontraception in developing male contraceptives [14]. Thus, the regulation mechanisms of uPA in Sertoli cells require further studies.

Eukaryotic gene expression regulation can be divided into two major categories according to their nature: the first category is the instantaneous or reversible gene regulation, the equivalent of prokaryotic cells in response to different environmental conditions; the other is developmental or irreversible gene regulation, is the essence of eukaryotic gene regulation, which determines the whole process of growth, differentiation and development of eukaryotic cells [15]. Transcription regulation is the most important control point in the regulation of eukaryotic gene expression, while a promoter is a region of DNA that initiates transcription of a particular gene [16]. Therefore, studying the functional motif of promoters is of great significance for the research of regulation mechanisms of gene expression. Although uPA plays an important role in male reproduction, the transcription regulation mechanism of uPA remains unclear.

To study the transcription regulation mechanism of *uPA* gene expression in rat Sertoli cell, we carried out the cloning and analysis of the promoter region of the rat uPA gene, and subcloned the *Plau* core promoter nucleotide sequence containing approximately 1.6-kb 5'-flanking region [17]. In addition, uPA has been shown to demonstrate potent proinflammatory properties [18, 19]. The domains responsible in the uPA molecule for proinflammatory activity involved in neutrophil activation are distinct from those responsible for fibrinolytic or chemotactic properties [20]. Furthermore, uPA induction by inflammatory stimuli, such as IL-1, TNF, and lipopolysaccharide (LPS), has been reported in a number of human cells [21]. The activation of TLR signaling pathways plays an essential role in the detection of invading pathogens. After LPS stimulation, the response may be mediated by MyD88-dependent or MyD88-independent pathways, which leads to the activation of MAP kinases and NF-κB [22].

This study examined the trans-acting factors involved in LPS regulation of *uPA* gene expression and the related pathway to explore the role of uPA in the male reproductive system.

Materials and methods

Animals. Twelve male Sprague–Dawley rats (20-day-old) were purchased from the Animal Center of Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). The rats were reared under a 12 h light/ /dark cycle and provided with free access to food and water. All experiments were conducted in accordance with formal approval from the Animal Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (Approval No. 2012-0917). In addition, the animals were handled in accordance with the Guide for Laboratory Animals of the National Institutes of Health.

Isolation and culture of rat Sertoli cells. Sertoli cells were harvested from 20-day-old Sprague-Dawley male rats. The primary Sertoli cell culture was prepared as described previously [23]. Testis fragments were subjected to sequential enzymatic digestion using 0.25% trypsin (Gibco, Grand Island, NY, USA), 1 mg/mL hyaluronidase (SigmaAldrich, Louis, St. MO, USA), and 0.1% collagenase I (SigmaAldrich) at 37°C for 15–30, 30, and 60 min, respectively. The Sertoli cells were allowed to settle between enzymatic treatments and washes through sediment gravity flow. The final cell suspension was resuspended in DMEM/F12 (Gibco) supplemented with 10% fetal calf serum (FCS), 10 μg/mL insulin, 5 μg/mL transferrin, and 5 g/L EGF (SigmaAldrich) in a final cell concentration of 1×10^6 viable cells/mL. The Sertoli cell suspension (2 mL) was subsequently seeded in each well of six-well plates and then cultured at 34°C in a highly humidified incubator with

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10.5603/FHC.a2021.0026
ISSN 0239-8508, e-ISSN 1897-5631

5% CO_2. After 48 h, the cells were treated with a hypotonic solution (20 mM Tris-HCl, pH 7.4) to remove the contaminating germ cells.

Immunofluorescence. The cultured rat Sertoli cells were fixed in 4% paraformaldehyde for 15 min. The cells was then processed through blocking steps using 0.25% Triton-X, anti-uPA antibody incubation at 1:1000 (Abcam, Cambridge, MA, USA) for 12 hours at 4°C, rinse/blocking steps, followed by incubation with FITC fluorophore-conjugated secondary antibodies (Thermo Fisher Scientific, Waltham, MA, USA), and DAPI (Invitrogen, Carlsbad, CA, USA) nuclear staining. Cell climbing piece were imaged on an Olympus IX70 (Olympus, Tokyo, Japan) fluorescence microscope.

Renilla luciferase reporter assays. In the transient transfection experiments, the Sertoli cells were seeded into six-well plates at a density of 2×10^6 cells per well. The cells were transfected with 350 ng of plasmid, which was prepared using endotoxin-free plasmid isolation kits (Qiagen, Valencia, CA, USA) containing the uPA promoter. The thymidine kinase *Renilla* luciferase reporter vector (PGL3-Enhancer, Promega, Madison, WI, USA, 40 ng/well) was co-transfected to normalize data and ensure transfection efficiency. Plasmids were transfected using Lipofectamine 2000 (Invitrogen). After 24 h, total cell lysates were prepared and analyzed for luciferase activity by using Dual-Luciferase Reporter Assay System Kit (Promega) according to the manufacturer's protocol.

LPS-induced uPA expression in Sertoli cells. The Sertoli cells were seeded into six-well plates at a density of 2×10^6 cells per well. The cells were transfected with 380 ng of PGL3-499 plasmid containing the uPA promoter or with PGL3-control (Promega) luciferase reporter gene plasmid containing the SV40 promoter by using Lipofectamine 2000. On the following day, the cells were incubated with LPS (20 μg/mL) (SigmaAldrich) for 0, 12, 24, or 48 h. The LPS concentration (20 μg/ml) was selected according to the data presented by Cudicini *et al.* [23] and Wendy *et al.* [24]. Cell lysates were prepared and analyzed for luciferase activity by using the Dual-Luciferase Reporter Assay System Kit (Promega) according to the manufacturer's protocol. Data are expressed as the means of relative stimulation ± SD.

Real-time PCR analysis. Real-time PCR analysis was performed using Power SYBR® Green PCR Mix Kit on ABI PRISM 7300 real-time cycler (Applied Biosystems, Foster City, CA, USA). Total RNA was extracted from 1×10^6 Sertoli cells by using TRIZOL reagent (Invitrogen) according to the manufacturer's instruction. cDNA synthesis was performed with the purified RNA by using the SuperScript III First-strand Synthesis System (Invitrogen). The reaction system of 20 μL of reaction mixture, including 2 μL of cDNA, 1 μL of the forward primers, and 1 μL of reverse primers, and 10 μL of SYBR Green PCR MasterMix (Takara, Tokyo, Japan). The steps were as follows: (1) 95°C for 30 s and (2) amplification over 40 cycles at 95°C for 5 s and 58°C for 30 s. All samples were normalized against β-actin by using the comparative CT method (ΔΔCT). Table 1 shows the primer sequences. In addition, mRNA expression was determined using the $2^{-\Delta\Delta Cq}$ method.

The intervening experiment in Sertoli cells. Sertoli cells were treated *in vitro* by blank control, LPS (20 μg/mL, SigmaAldrich), anti-IRF3 (10 nM, Amersco, Solon, OH, USA), LPS+anti-IRF3, LPS+IFN-β (20 nM, SigmaAldrich) and IFN-β groups, and the induction time was 0,12, 24 or 48 hours respectively. The levels of uPA mRNA and protein were detected by Real-time PCR and Western-blot with β-Actin as internal reference.

Western blot analysis. Sertoli cells were washed with ice-cold buffered saline (PBS) and lysed using lysis buffer (10% glycine, 150 mM NaCl, 1.0% NP-40, 10 mM EDTA, 20 mM NaF, 1 mM vandate, 1 mM benzamidine) on ice for 30 min. Protein concentration in the lysates was determined using BCA Kit (Life Technologies, Carlsbad, CA, USA). The lysates were boiled for 4 min and then subjected to electrophoretic separation on 4–20% SDS-polyacrylamide gel (Life Technologies, USA). The proteins were transferred onto Immobilon-P membrane (Millipore, Darmstadt, Germany). The blots were incubated in a blocking buffer (PBS containing 0.05% Tween and 5% nonfat dry milk) for 30 min and probed with primary antibody, anti-uPA (Abcam, Cambridge, MA, USA), or anti-β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After three 10-min washes with PBS containing 0.05% Tween 20, the membranes were incubated for 1 h with goat anti-rabbit immunoglobulin G (1:5000, Thermo Fisher Scientific) in blocking buffer. The immunoreactive proteins were then visualized using Supersignal West Pico Chemiluminescence Kit (Thermo Fisher Scientific). Band density was quantified using the software Gel-Pro Analyzer 4.

Statistical analysis. All experiments were performed in triplicate, and numerical data are presented as means ± SD. The statistical differences between groups were tested by one-way ANOVA. All data were analyzed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Values were significantly different if two-tailed P value was < 0.05.

Results

Identification of core promoter of the rat uPA gene by luciferase assay

Five fragments of the uPA promoter region, namely, 211 (–167/+40), 499 (–455/+40), 802 (–758/+40), 1220 (–1156/+40), and 1588 bp (–1544/+40), were amplified from different regions and verified by electropho-

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Folia Histochem Cytobiol. 2021
10.5603/FHC.a2021.0026
ISSN 0239-8508, e-ISSN 1897-5631

Table 1. The list of primers in this study

Target	Primer	Sequences	Region(bp)
Primers for gene clone			
uPA	Sense	5'-AGGCTAGGCTCACCACCAAAG-3'	
	Antisense	5'-CGCAAGGACTGGATTGATGAC-3'	
1588bp	Sense	5'-ctcgagATGATTGGCAGCAGGAGAGAC-3'	–1544/+40
1220bp	Sense	5'-ctcgagATCCGATGCCCTCTTCTGGT-3'	–1156/+40
802bp	Sense	5'-ctcgagTGGATTGAAGGGTATGCCATT-3'	–758/+40
499bp	Sense	5'-ctcgagCAGCATTTGACATGTGGGAAC-3'	–455/+40
211bp	Sense	5'-ctcgagTGGAAATCCCATGACTTCGTC-3'	–167/–40
common	Antisense	5'-aagcttAGACACGCAAGGACTGGATTG-3'	
Primers for real-time PCR			
IL-1	Sense	5'-GGACAGAACATAAGCCAACA-3'	
	Antisense	5'-TTAGGAATAGTGCAGCCATC-3'	
IL-6	Sense	5'-GTATGAACAGCGATGATGC-3'	
	Antisense	5'-AAACGGAACTCCAGAAGACC-3'	
uPA	Sense	5'-TGGTGGGAGCCTCATCAGT-3'	
	Antisense	5'-CCCGTGCTGGTACGTATCTT-3'	
β-actin[a]	Sense	5'-CACCCGGAGTACAACCTTC-3'	for uPA
	Antisense	5'-CCCATACCCACCATCACACC-3'	
β-actin[b]	Sense	5'-GTCAGGTCATCACTATCGGCAAT-3'	for IL-1,IL-6
	Antisense	5'-AGAGGTCTTTACGGATGTCAACGT-3'	

[a]β-actin is the internal reference of uPA; [b]β-actin is the internal reference of IL-1 and IL-6.

resis (Fig. 1A). These fragments were independently constructed into the PGL3-enhancer flu reporter vector. The plasmids were successfully identified *via* double digestion (Fig. 1B) and named as PGL3-211, PGL3-499, PGL3-802, PGL3-1220, and PGL3-1588, respectively. This series of fragments demonstrated significantly different promoter activities when their recombinant vectors were transfected into Sertoli cells. Among them, PGL3-499 showed the highest promoter activity (Fig. 1C).

Upregulation of uPA expression and uPA promoter activity in LPS-stimulated Sertoli cells

To explore the potential immunologic function of uPA in Sertoli cells, we assessed uPA expression after adding LPS. The results showed that LPS-stimulated Sertoli cells in which the uPA protein was labeled with red fluorescence antibody by using immunofluorescence technology, displayed a significantly upregulated uPA expression after 12 h of incubation (Fig. 2A). When LPS was added into the cultured Sertoli cells, the proportion of uPA-positive cells significantly increased, as revealed by statistical analysis, and then peaked after 12 h (Fig. 2B, 62.3 ± 4.5% *vs.* 23.6 ± 2.1%, $P < 0.05$). To further investigate the cause of the upregulated uPA expression, we detected the promoter activity of uPA, especially the screened region, through *Renilla* luciferase reporter assays. The result showed that the promoter activity of PGL3-control containing the SV40 promoter was not obviously affected by LPS after 48 h. However, in the core promoter of uPA, the plasmid PGL3-499 was interfered by addition of LPS (20 μg/mL) to Sertoli cell cultures (Fig. 2C) after 12 h; moreover, uPA promoter activity was significantly upregulated and peaked at 24 h (Fig. 2C, $P < 0.05$).

Downregulation of IL-1 and IL-6 by anti-IRF3 in LPS-stimulated Sertoli cells

Numerous studies confirmed that LPS stimulates IL-1 and IL-6 mRNA. The present results showed that IL-1 and IL-6 mRNA expression levels in Sertoli cells were both significantly upregulated 12 h after adding LPS (Fig. 3). TLR4 signaling occurs along into two pathways, the MyD88-dependent and MyD88-independent. In the latter, the transcription factor IRF3 is crucial for the activation of the TIR-containing adaptor protein. To explore whether the process involves the MyD88-independent NF-κB//JNK pathway, we added 10 nM anti-IRF3 to Sertoli

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10.5603/FHC.a2021.0026
ISSN 0239-8508, e-ISSN 1897-5631

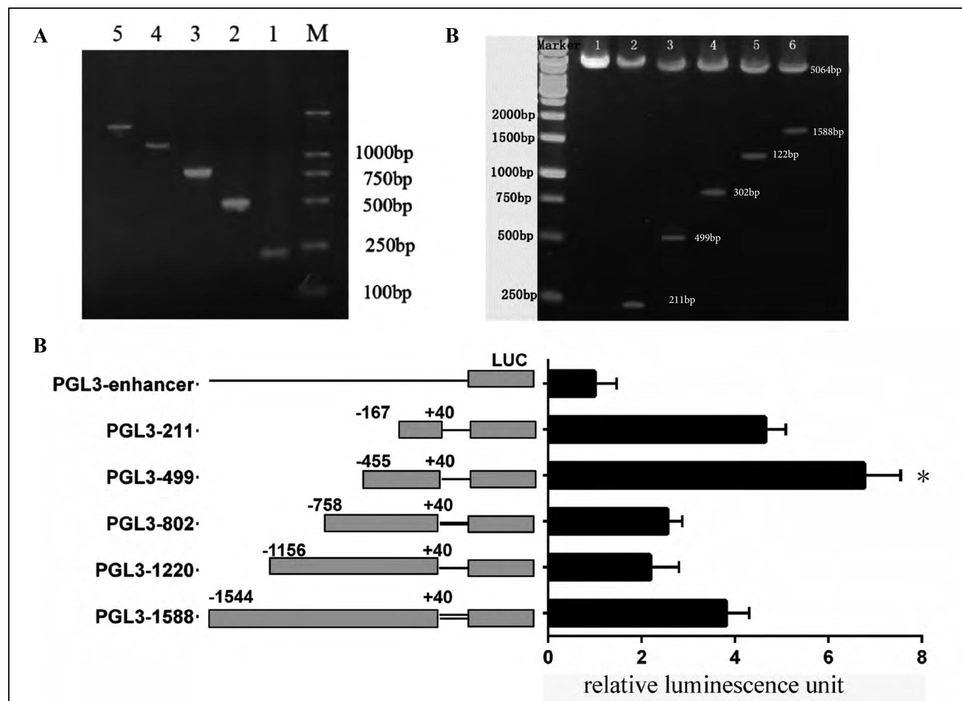

Figure 1. Core promoter of rat urokinase plasminogen activator (uPA) gene was identified by luciferases assay. **A.** Different length of the uPA promoter regions (211bp, 499bp, 802bp, 1220bp and 1588bp) were amplified and verified by electrophoresis. Marker: DL2000 (TaKaRa, Japan). **B.** Plasmid was verified by enzyme digest. Marker: 1KB DNA marker (Fermtas, USA), PGL3-enhancer; PGL3-211; PGL3-499; PGL3-802; PGL3-1220; PGL3-1588. **C.** Renilla luciferases (RL) density was examined in different region of promoter transfected into Sertoli cell. PGL3-499 showed the highest promoter activity compared with other groups. *P < 0.05 compared with PGL3-enhancer group.

cells cultured with or without LPS. The results showed that the IL-1 and IL-6 mRNA levels of the anti-IRF3 group were unchanged compared with those in the blank group but were significantly downregulated compared with those in the LPS+anti-IRF3 group (Fig. 3). This finding confirmed that IRF3 is a key factor in the signaling pathway of IL-1 and IL-6 in Sertoli cells stimulated with LPS.

uPA pathway in LPS-stimulated Sertoli cells

To further study the candidate pathway of uPA regulation, we tested the *uPA* mRNA expression in different experimental groups. The *uPA* mRNA level was significantly lower in the blank group than those in the LPS, interferon β (IFN-β), LPS+IFN-β, and LPS+anti-IRF3 groups ($P < 0.01$, $P < 0.05$, $P < 0.001$, and $P = 0.028$, respectively) (Fig. 4A). In addition, *uPA* mRNA expression was significantly higher in the LPS+anti-IRF3 group than that in the anti-IRF3 group ($P = 0.001$). The *uPA* mRNA expression was also significantly higher in the LPS + IFN-β group than that in the IFN-β group ($P < 0.01$). Furthermore, the uPA protein was higher in the LPS + IFN-β group than those in the other groups (Fig. 4B). Moreover, uPA protein expression in the LPS group stimulated for 12 h was significantly different from those in groups stimulated for 24 h and 48 h, which is consistent with the previous immunocytochemical results. The protein expression of uPA in the IFN-β group was also not significantly different from that in the LPS+anti-IRF3 group. These results suggested that LPS induced Sertoli cell activation through the IRF3-independent mechanism.

Discussion

In this study, we identified the rat *uPA* gene and examined its promoter and activity. Sequence analysis revealed that the *uPA* genomic gene is composed of the 5'-flanking region (approximately 1.6 kb) containing a typical GC/TATA box. In addition, the mammalian *uPA* promoter contains enhancer elements, such as AP-1 and SP-1; the binding of these enhancers to the corresponding transcription factors will enhance gene transcription [25, 26]. In the present study, we demonstrated a protein-binding site overlapping the GC-box element (GGGCGG) between -61 and -56. We examined the *uPA* promoter activity by using Dual-Luciferase assay techniques, which are useful for rapid and accurate determination of the activity of a given promoter in a cell line [27]. The results showed that all of the fragments displayed a promoter activ-

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10.5603/FHC.a2021.0026
ISSN 0239-8508, e-ISSN 1897-5631

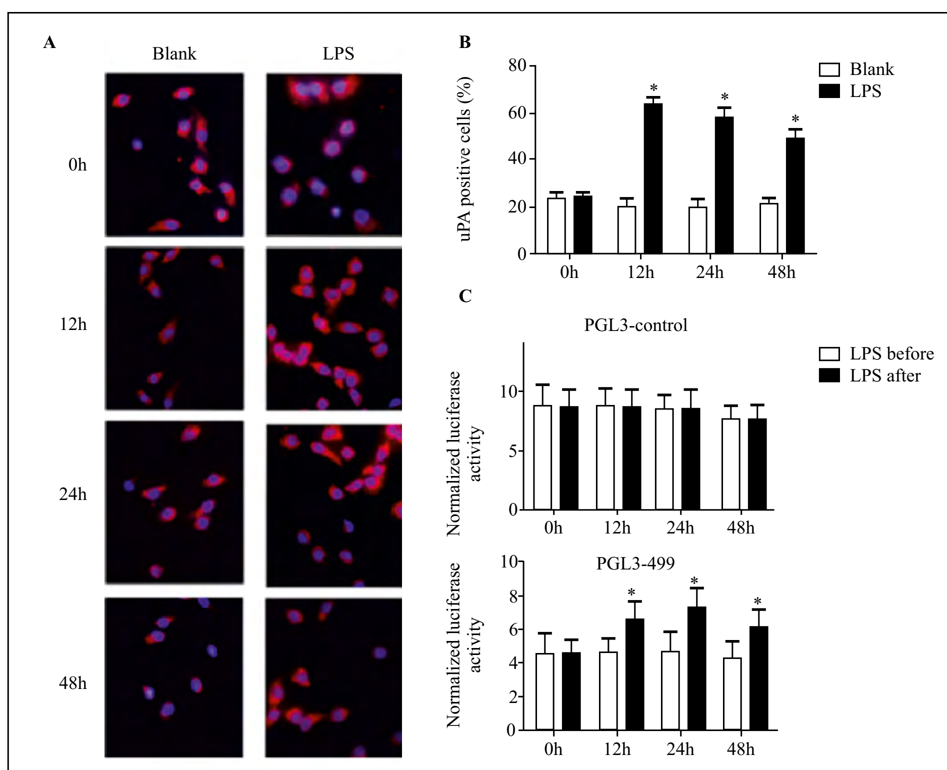

Figure 2. Urokinase plasminogen activator (uPA) expression in rat Sertoli cells stimulated with LPS (20 μg/ml). **A.** Representative microphotographs of the expression of uPA in Sertoli cells in a time-dependent manner with or without LPS-stimulation visualized by immunofluorescence. uPA protein was labelled with red fluorescence antibody, and cell nucleus was stained with DAPI as described in Methods. **B.** Proportion of uPA-positive cells in Sertoli cell in blank or LPS group. The uPA expression was significantly upregulated compared with blank group after 12 h. **C.** *Renilla* luciferases activity after incubation with LPS (20 μg/mL) with PGL3-enhancer or PGL3-499 in Sertoli cells as incubated for the indicated time point. $^{*}P < 0.05$ compared with blank group.

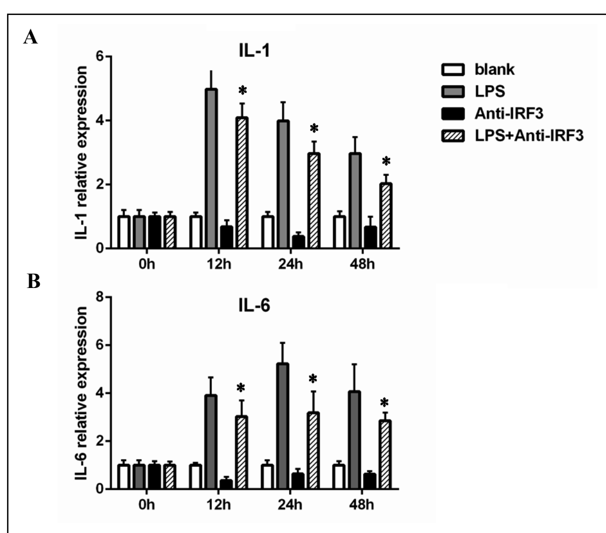

Figure 3. The IL-1 and IL-6 expression in rat Sertoli cells in different groups at various times. Sertoli cells were incubated with or without LPS and anti-IRF3 (10 nM), the IL-1 (**A**) and IL-6 (**B**) mRNA levels were determined by real-time PCR, and normalized to β-actin mRNA. The IL-1 and IL-6 mRNA levels in the LPS+anti-IRF3 group were significantly downregulated compared with those in the LPS group. $^{*}P <$ 0.05 compared with LPS group.

ity when the recombinant vectors were transfected into Sertoli cells [28]. PGL3-499 showed the highest promoter activity (Fig. 1C), indicating that an important element functioning in negative regulation was deleted in the pGL3-499 plasmid. PGL3-499, which has a 499-bp core promoter containing GC/TATA box upstream the translation initiation codon, showed a significantly high promoter activity. The data also suggested that the regulatory promoters or enhancers and other c/s-elements present from -1156 to -1544 are necessary to maintain uPA-specific expression.

Sertoli cells play an important role in spermatogenesis by creating microenvironment that protects germ cells in the wall of seminiferous tubules. Sertoli cells are part of the blood–testis barrier (BTB), which separates the seminiferous epithelium into two distinct compartments. Therefore, Sertoli cells mechanically segregate germ cell autoantigens by means of the BTB and create an effective immune-privileged environment that protects germ cells from invading pathogens [28]. Sertoli cells can phagocytose the apoptotic spermatogenic cells and apoptotic bodies to prevent the autoimmune reaction from the latent antigen of the spermatogenic cell. Sertoli cells also

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10.5603/FHC.a2021.0026
ISSN 0239-8508, e-ISSN 1897-5631

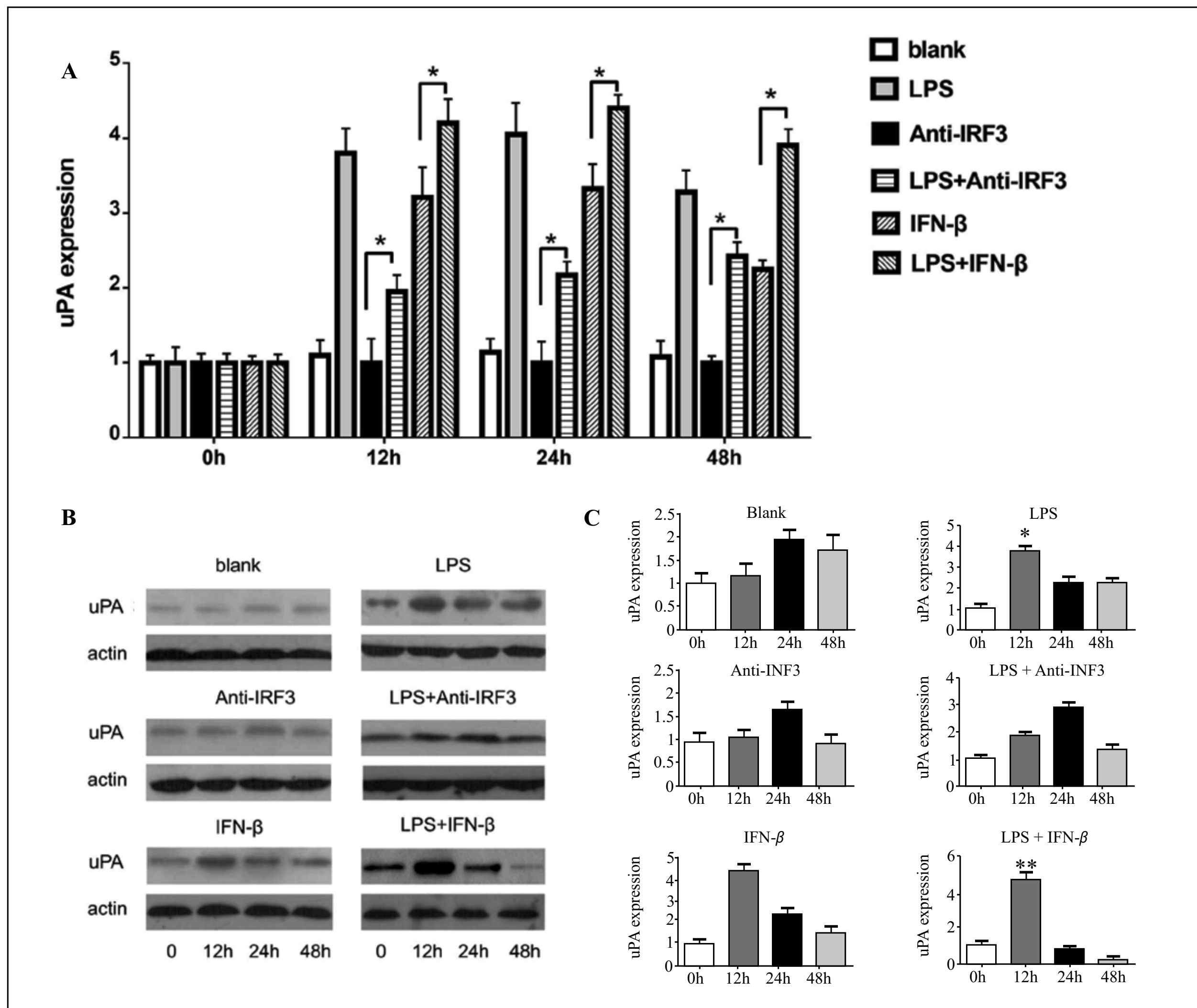

Figure 4. Urokinase plasminogen activator (uPA) mRNA and protein expression in rat Sertoli cells in the different experimental groups at various time points. **A.** uPA mRNA level of a different group at various time points was assessed by real-time PCR. uPA mRNA level was significantly higher in the LPS+anti-IRF3 group than that in the anti-IRF3 group ($P < 0.05$). **B.** uPA protein expression of different groups at various time points was assessed by Western blotting. **C.** Statistical analysis of the mean light density of picture. uPA protein expression in the LPS group stimulated for 12 h was significantly upregulated compared with those in cells stimulated for 24 or 48 h. uPA protein levels were also higher in the LPS + IFN-β group than those in the other groups $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the LPS group.

contain an immune-privileged site, where both allo- and autoantigens can be tolerated [29]. As a result, Sertoli cells have been identified as key players for conferring this immune privilege [30].

LPS are found in the outer membrane of Gram-negative bacteria, and elicit strong immune responses. When LPS particles released from the bacterial wall invade the tissues, they cause the release of inflammatory mediators through the TLR signaling pathways [31, 32]. In this study, we isolated Sertoli cells from 20-d-old Sprague-Dawley male rats because Sertoli cells become mature and are not easily contaminated by germ cells at this time. The present study clearly demonstrated that uPA gene expression was upregulated by LPS in cultured rat Sertoli cells, which indicated that LPS may stimulates the degradation of the ECM by increasing of uPA expression and interfere with the immune defense mechanism of Sertoli cells.

Several lines of evidence have shown that LPS can activate the transcriptional factors NF-κB and cAMP-response element binding protein (CREBP) [33, 34]. LPS can induce systemic inflammatory response *via* MyD88-dependent or MyD88 independent pathway [35]. In MyD88-dependent pathway, MyD88 is recruited to the Toll-like receptor 4 (TLR4) through interaction with the TIR domain of TLR4 whereas MyD88-deficient cells failed to produce any pro-inflammatory cytokines such as interleukin-1β (IL-1β),

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IL-6, and TNF in response to LPS [36]. The cytokines IL-1α and IL-6 were found in the testis at high levels, which were closely related to immune defense response of Sertoli cells [37, 38]. Interferon regulatory factor 3 (IRF-3) plays a key role in MyD88-independent signaling pathway [39]. We showed that LPS stimulated the expression of IL-1 and IL-6 in Sertoli cells, while IRF3 antibody(anti-IRF3) antagonized this response, indicating that LPS induced the immune response of Sertoli cells through MyD88-independent pathway. Anti-IRF3 can significantly decrease the LPS-induced increase of uPA expression. Thus, our experiments demonstrated that LPS could increase the expression of uPA through MyD88-independent pathway.

In summary, we found that uPA gene contains the 5'-flanking region (approximately 1.6 kb) with a typical GC/TATA box. PGL3-499, which has a 499-bp core promoter contains GC/TATA box upstream the translation initiation codon. uPA expression in Sertoli cells is regulated through MyD88-independent pathway induced by LPS stimulation. This finding implied that uPA plays a role in the immune function of Sertoli cells in rat testis, and may provide a new target for the treatment of male immune-related infertility.

Funding

This work was supported by the National Natural Science Foundation of China (NO: 81771575, 81701539) and Wuhan Municipal Health scientific research project (EX20E16). The authors declare that there is no conflict of interest that would prejudice the impartiality of this work.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YL and KZ conducted laboratory studies, wrote the article, and performed all necessary literature searches and data compilation. ZX, CL X, YY X performed the necessary literature searches and data compilation. HZ and DH H designed the review, reviewed, and approved the submitted manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All experiments were conducted in accordance with formal approval from the Animal Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (Approval No. 2012-0917). In addition, the animals were handled in accordance with the Guide for Laboratory Animals of the National Institutes of Health.

Conflicts of interest

The authors declare that they have no conflicts of interest to report regarding the present study.

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10.5603/FHC.a2021.0026
ISSN 0239-8508, e-ISSN 1897-5631

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Submitted: 7 January, 2021
Accepted after reviews: 6 November, 2021
Available as AoP: 30 November, 2021

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