Blackwell Publishing

Asian J Androl 2007; 9 (5): 679–683
DOI: 10.1111/j.1745-7262.2007.00272.x

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·*Original Article*·

Gene expression changes of urokinase plasminogen activator and urokinase receptor in rat testes at postnatal stages

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Abstract

Aim: To investigate the gene expression changes of urokinase plasminogen activator (uPA)/urokinase receptor (uPAR) in rat testes at postnatal stages and explore the effects of uPA/uPAR system on the rat spermatogenesis. **Methods:** The mRNAs of uPA and uPAR in rat testes were measured by using real-time quantitative polymerase chain reaction (PCR) at postnatal days 0, 5, 10, 15, 21, 28, 35, 42, 49 and 56, respectively. **Results:** The tendencies of uPA and uPAR mRNA expression were similar at most postnatal stages except for D_0. The expression of uPAR mRNA in rats testes was relatively higher than that of uPA at postnatal D_0, and both were decreased until D_{21}, increased obviously at postnatal D_{28}, reached a peak at postnatal D_{35}, then declined sharply at postnatal D_{42} and retained at a low level afterwards. **Conclusion:** The uPA/uPAR system may be strongly linked to spermiation and spermatogenesis via regulating germ cell migration and proliferation, as well as promoting the spermiation and detached residual bodies from the mature spermatids. *(Asian J Androl 2007 Sep; 9: 679–683)*

Keywords: rats; spermatogenesis; urokinase plasminogen activator; urokinase receptor; quantitative polymerase chain reaction

1 Introduction

The urokinase plasminogen activator (uPA)/urokinase receptor (uPAR) system plays an important role in fibrinolysis, cell migration and invasion (e.g. cancer invasion and metastasis), tissue remodeling, angiogenesis and so on [1, 2]. As a serine stretch protein hydratase, uPA induces matrix degradation by activating the plasminogen into plasmin. It is regarded as the critical trigger for plasminogen activation during cell migration and invasion under some physiological and pathological conditions such as cancer metastasis [1]. The specific cellular receptor of uPA, uPAR, can combine with urokinase on the cell membrane. The combination not only reduces fibronolysis but also leads to signal transduction, which can enhance cell migration and modulate cell adhesion [3]. It has been reported in the recent decade that uPA/uPAR system may also be essential for cell proliferation, growth, apoptosis and differentiation through cellular signal transduction pathways [3, 4].

It has been proved that the uPA/uPAR system has a

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Received 2006-09-02 Accepted 2007-02-08

direct relation to the male genital system. The effects of the uPA/uPAR system on the sperm function were various, such as regulation of spermiation, maturation, motility activation, capacitation, chemiotaxis, liquefaction and fertilization [5]. Therefore, dysfunction of the uPA/uPAR system is presumably one important factor that results in male infertility. However, there are few studies about the specific role of the uPA/uPAR system on spermatogenesis. In our study, gene expression of uPA/uPAR in rat testes at postnatal stages was assessed by real-time quantitative reverse transcription- polymerase chain reaction (RT-PCR) and the effects of uPA/uPAR system on the rat spermatogenesis were studied.

2 Materials and methods

2.1 Materials

Twenty-six male Sprague-Dawley rats (D_0, D_5, D_{10}, D_{15}, D_{21}, D_{28}, D_{35}, D_{42}, D_{49}, D_{56}, the day at birth defined as D_0, n = 2–4) were obtained from the Laboratory Animal Centre of Tongji Medical College (Wuhan, China). They were maintained under standard conditions (12 h light: 12 h dark cycle; 25 ± 3ºC; 35%–60% relative humidity). Rat feed and tap water were available *ad libitum*. The rats were killed by cervical dislocation and the testes were removed and decapsulated at the time specified.

2.2 Methods

2.2.1 RNA extraction and reverse transcription

Total RNA was extracted with Trizol reagent (Invitrogen, Breda, the Netherlands) according to the manufacturer's protocol. Briefly, approximately 50 mg testes tissue were pipetted in 1 mL Trizol reagent. The concentration and purity of RNA were determined spectrophotometrically at 260 nm and 280 nm. Total RNA was reversely transcribed into first-strand cDNA by using the First Strand cDNA Synthesis kit (Toyobo, Tokyo, Japan). Each RT reaction mixture contained 3 μg total RNA, 1× RT buffer, 0.5 mmol/L of dNTP, 1 μg Oligo d(T)20, 400 IU moloney murine leukemia virus reverse transcriptase, 40 IU Rnasin and H_2O to a final volume of 40 μL; and was incubated at 42ºC for 20 min, then at 99ºC for 5 min.

2.2.2 Real-time quantitative PCR

Real-time quantitative PCR was based on the high-affinity, double-stranded DNA-binding dye SYBR green using an Mx3000PT detection system (Stratagene Inc., La Jolla, CA, USA). The specific primers used for amplification of uPA, uPAR and β-actin were designed and synthesized by TaKaRa Biotechnology (Dalian, China). Co-amplification reactions were carried out in a final volume of 25 μL containing 150 ng reverse-transcribed RNAs, 1 U Taq polymerase, 1× PCR buffer, 3 mmol/L $MgCl_2$, 2 mmol/L dNTPs, 0.25 μL SYBR Green I (20 ×), 100 pmol each of the 5' and 3' sequence-specific primers for uPA (5'-ACA GAT TCC TGC TCG GGA GAT-3', 5'-CCA ATG TGG GAC TGA ATC CAG-3', length of product is 167 bp) or uPAR (5'-GGA CCA ATG AAT CAG TGC TTG-3', 5'-CCA CAG TCT GAG GGT CAG GAG-3', length of product is 252 bp) or β-actin (5'-TCC TCC CTG GAG AAG AGC TA-3', 5'-TCA GGA GGA GCA ATG ATC TTG-3', length of product is 302 bp).

The amplification program was consisted of the following three steps. The first step was an initial heating for 10 min at 95ºC to denature the cDNA. In the second step, DNA was amplified for 35 cycles of denaturation at 95ºC for 30 s, annealing at 62ºC for 40 s, elongation at 72ºC for 30 s, detecting fluorescence at 84ºC for 8 s. Finally, the temperature was raised gradually (0.2ºC/s) from 55ºC to 95ºC for the melting curve analysis.

2.2.3 Establishment of standard curve

The amplified products were recovered from gel by using gel extraction kit (Tiangen, Beijing, China), and were used as a template for amplification at a range of 10^3 to 10^8 copies to make standard curve.

2.2.4 Specificity evaluation of real time quantitative PCR

Specific PCR products were confirmed using the melting curve analysis where the presence of different PCR products was reflected in the number of first-derivative melting peaks. To verify the melting curve results, 10 μL of each PCR product were electrophoresed in parallel with size markers on 2% agarose gels.

2.2.5 Statistical analysis

The numbers of copies of samples were calculated by setting their crossing points to the standard curve. Relative quantitation of uPA/uPAR was showed as the ratio of target cDNA concentration/β-actin cDNA concentration. Each experiment was tested for three times to estimate expression stability. The data were presented as mean ± SD. Statistical analyses were performed using the SPSS version 11.5 (SPSS In., Chicago,

IL, USA) by one-way ANOVA.

3 Results

3.1 Specific amplification and standard curve

Melting curve analysis demonstrated that each of the PCR products amplified a single predominant product with a distinct melting-out temperature (Tm) as shown in Figure 1A. The predicted length of each product had been confirmed by agarose gel electrophoresis.

The standard curve of uPA/uPAR and β-actin shows a linear relation from 10^3 to 10^8 copies with coefficient correlation > 0.99 as shown in Figure 1B and 1C.

3.2 uPA/uPAR gene expression levels in rat testes at postnatal stages

The uPA mRNA level in the testes of rats was low from birth to postnatal D_{21}, increased sharply at postnatal D_{28}, arrived at a peak at postnatal D_{35}, then declined sharply and remained low from postnatal D_{42} to postnatal D_{56} (Figure 2A). The uPAR mRNA level in testes of rats was relatively high at postnatal D_0 and declined after birth, was low from postnatal D_{10} to postnatal D_{21}, increased sharply after postnatal D_{28}, arrived at a peak at postnatal D_{35}, then declined sharply and remained low from postnatal D_{42} to postnatal D_{56} (Figure 2B). The express tendencies of uPA and uPAR were similar at mostly postnatal stages except at postnatal D_0.

Expression of uPA mRNA in testes of rats was low from postnatal D_0 to D_{21}. It increased obviously at postnatal D_{28}, arrived at peak at postnatal D_{35}, declined sharply at postnatal D_{42} and retained at a low level afterwards (Figure 2A). The expression of uPAR mRNA in testes of rats was relatively high at postnatal D_0. Then it declined to a low level at postnatal D_{10}, increased obviously at postnatal D_{28}, arrived at peak at postnatal D_{35}, and declined sharply at postnatal D_{42} and remained low afterwards (Figure 2B). The express tendencies of uPA and uPAR mRNA were similar at most postnatal stages, except at postnatal D_0.

4 Discussion

Spermatogenesis is a complex differentiation process that consists of three major phases: a proliferation phase of spermatogonial cells by mitosis; a meiotic phase of spermatocytes, in which recombination of genetic materials and reductive division occur; and a transformation

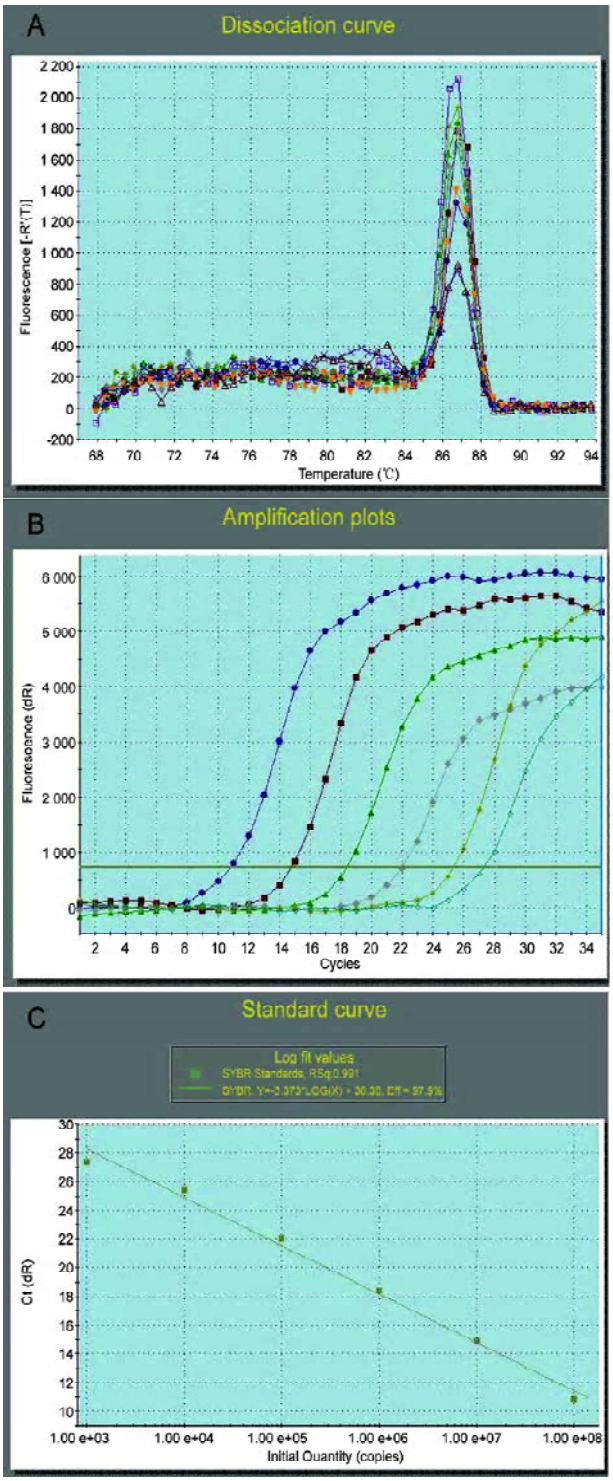

Figure 1. Specific amplification of urokinase plasminogen activator (uPA). (A): A melt-curve analysis of a uPA amplification reaction showing the sharp and high peak of temperature (86.8°C) indicates the presence of a specific product that melts at this temperature. (B): A plot of fluorescence against cycle number. (C): A linear standard curve from 10^3 to 10^8 copies of uPA.

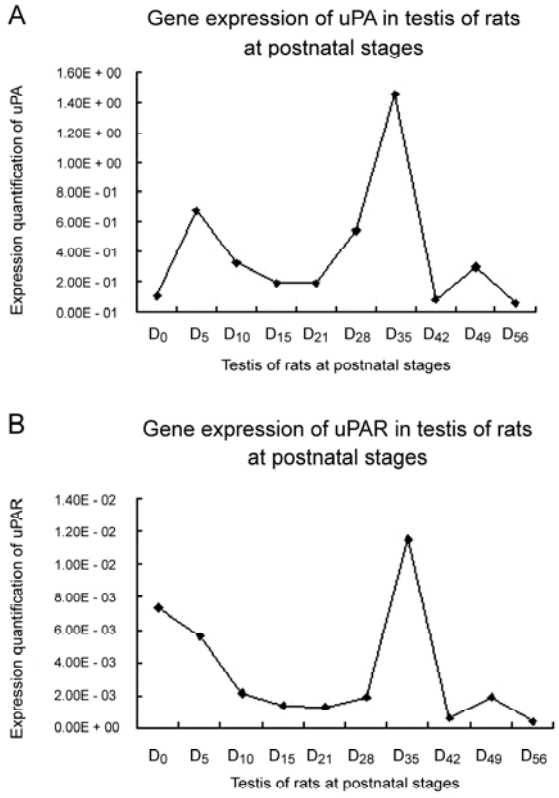

Figure2. Gene expression of urokinase plasminogen activator (uPA)/ urokinase receptor (uPAR) in testes of rats at postnatal stages. (A): Gene expression of uPA in testes of rats at postnatal stages. (B): Gene expression of uPAR in testes of rats at postnatal stages.

phase, in which mature spermatozoa from haploid spermatids are produced. The durations of complete spermatogenesis cycles, the days required for a type A spermatogonium transformed into an elongate spermatid, vary among species, such as 52–54 days for rats and 72 days for human. The spermatogenesis cycle occurs in different fashions in rats and humans. In rats, each stage of the spermatogenesis cycle occupies a significant length of the tubule, and the stages appear to occur sequentially along the length of the tubule, forming waves of the seminiferous epithelium. In contrast, there are no classic waves in the human seminiferous tubules [6]. In rats, there are only male germ cells (gonocytes) and Sertoli cells in the seminiferous tubules at postnatal D_0. The gonocytes would gradually proliferate and move towards the seminiferous tubule basal lamina and adequate proliferation and migration ensure gonocyte progeny to become type A spermatogonia [7]. The first 3–5 days of postnatal life are crucial for the successful start of spermatogenesis and future fertility [8]. From postnatal D_7 some of these stem cells cease division and undergo further differentiation through various intermediate spermatogonial stages. After Sertoli cell division has ceased at postnatal D_{15}, the first wave of meiotic and postmeiotic germ cell development in rats occurs [9]. The round postmeiotic spermatids appear at postnatal D_{28}, which means the start of spermiogenesis. Then they transform to elongate spermatids at postnatal D_{40} [10]. With the release of the first mature spermatids from the testes around postnatal D_{44} [9], the first wave of spermatogenesis is completed.

Although the morphological changes of germ cells in spermatogenesis have been well described, the molecular mechanisms of gene regulation involved in these important reproductive events are rarely known [11]. As an important system in many physiologic and pathological functions, the expression of uPA/uPAR in seminiferous tubules was recently investigated. The expression of uPA is stage-specifically in Sertoli cells of adult rat testes and reaches its peak during stages VII–VIII of the cycle [12]. uPAR is synthesized by mouse germ cells during spermatogenesis, and is present on spermatids and mature spermatozoa [13]. In the monkey, uPAR mRNA was localized in germ cells of mature testes except for spermatogonia or late spermatids [14]. Recently a new urokinase receptor gene named spermatogenesis-related gene (SGRG) was found in spermatogonia in rat and human testes, but not in spermatocytes, and it was conjectured to regulate spermatocyte migration through breaking down of extracellular matrix protein barriers during spermatogenesis [15].

Real-time quantitative RT-PCR is an easily performed technique with high sensitivity that allows quantification of rare transcripts and small changes in gene expression. It provides the necessary accuracy and produces reliable and rapid quantification results. In this study, mRNAs of uPA and uPAR in rat testes at postnatal stages were measured by using real-time quantitative RT-PCR, and the results indicated that gene expression of uPA and uPAR in rat testes exhibited obvious regular patterns during the first wave of spermatogenesis. The uPA mRNA expression was low in rat testes, while the expression of uPAR mRNA was relatively higher from postnatal D_0 to D_5, which indicated uPAR had an intimate relationship with the onset of spermatogenesis. We presumed that uPAR participated in spermatogenesis shortly after birth

by signal conduction in addition to regulating germ-cell migration through breakdown of extracellular matrix protein barriers [15]. The highest gene expressions of uPA and uPAR were observed at postnatal D_{35}, when round spermatids transformed to elongate spermatids and began to spermiation. The mechanisms may be related to tissue remodeling of the uPA/uPAR system during spermiation and spermiogenesis, such as the detachment of residual bodies from the mature spermatids [16].

Acknowledgment

This investigation was supported by the National Key Technologies R&D Program of China During the 10th Five-year Plan Period (No. 2004BA720A33-1).

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Edited by Dr Ralf Henkel