

# Expression of Septin4 in Human Hepatic Stellate Cells LX-2 Stimulated by LPS

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**Abstract**—Septin4, a member of polymerizing GTP-binding proteins family, is reported to be involved in cytoskeletal organization in mitosis, apoptosis, fibrosis, and other cellular processes. Since various Septin4 expression patterns were reported in different diseases, this study aimed to investigate Septin4 expression in human LX-2 cell line stimulated by lipopolysaccharides (LPS) and attempted to clarify the relationship between Septin4 and hepatic inflammatory injury and fibrosis. In this subject, human stellate cell line LX-2 was stimulated by LPS. The expression of Septin4 was analyzed by Western blot and quantitative real-time PCR. To observe the relationship among Toll-like receptor 4 (TLR4), TGF- $\beta$ , and Septin4, proteins from the anti-TLR4 antibody blocked cells, as well as the TGF- $\beta$ -induced cells, were analyzed by the method of Western blot. As the results, LPS could induce the alteration of  $\alpha$ -smooth muscle actin and Septin4 expression in LX-2 cells. Septin4 expression was regulated by LPS stimulation through TLR4 and TGF- $\beta$  pathway. These results therefore suggest that Septin4 may be involved in the process of activation of hepatic stellate cells by LPS stimulation. Further work would focus on the function of Septin4 in hepatic inflammatory injury and fibrosis.

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**KEY WORDS:** Septin4; hepatic stellate cell; LPS;  $\alpha$ -SMA; toll-like receptor 4.

## INTRODUCTION

Hepatic fibrosis, a wound healing response, is the final common pathway for most chronic liver diseases, such as chronic hepatitis B and C, autoimmune hepatitis, schistosoma, and alcoholic liver disease [1, 2]. It is characterized by excessive deposition of extracellular matrix (ECM) proteins like type I, III collagen and exacerbated inflammatory response. Hepatic stellate cells (HSCs), which secrete ECM, are activated in the

process of hepatic fibrosis and become myofibroblastic cells that express representative marker as  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) [2, 3]. Initiation of HSC activation is associated with increases in several inflammatory cytokines.

Lipopolysaccharides (LPS), a cell-wall component of Gram-negative bacteria, often induces the production of several pro-inflammatory, antiviral and antibacterial cytokines through LPS/Toll-like receptor 4 (TLR4) signaling. LPS is widely used to stimulate some cells associated with inflammatory disorders, including chronic liver inflammation, which is a key prerequisite for triggering liver fibrosis [4]. Previous studies have shown that LPS signaling enhances hepatic fibrogenesis caused by experimental cholestasis in mice [5]. LPS could also promote fibronectin production from HSCs [6]. Besides these, LPS is also used to stimulate dendritic cells to produce some cytokines to analyze the Th1/Th2 responses in the progress of Schistosoma infection [7]; however, there is no report to research the direct role of LPS on hepatic stellate cells in the progress of schistosomiasis hepatic fibrosis.

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The Septins are a family of polymerizing GTP-binding proteins originally discovered in budding yeast. Septin4, which is reported to be involved in cytoskeletal organization in mitosis, apoptosis, fibrosis, and other cellular processes, belongs to the family [8]. Previous studies showed that Septin4 was upregulated in substantia nigra and amygdala in Parkinson's disease, and in tissues of colorectal cancer [9]. However, Septin4 was downregulated in some cancers like pulmonary adenocarcinoma [10]. Shen *et al* [8] found that highly expressed Septin4 protein exhibited in HepG2, HuH-7, and QGY-7703 in human hepatocellular carcinoma (HCC), but such expression was low or undetectable in SK-Hep1, SMMC-7721, and L02 cell lines of HCC [11]. Septin4 also expressed in human HSCs [12, 13]. Culture activation of HSCs upregulated the Septin4 expression compared with quiescent HSCs [14]. In our previous studies, the expression of Septin4 tend to upregulate to a peak at 12 weeks and then downregulate after being infected with *Schistosoma japonicum* [15]. Based on such interesting findings, we observed the expression of Septin4 in LX-2 cell line stimulated by LPS and attempted to clarify the relationship between Septin4 and hepatic inflammatory injury.

## MATERIALS AND METHODS

### Materials

LX-2 cell line was purchased from Nanfang Cell Technology Co., Ltd. (Guangzhou, China). LPS and transforming growth factor (TGF- $\beta$ 1) (Sigma, USA) were used to stimulate HSCs cells. Rabbit polyclonal to alpha smooth muscle actin antibody (Abcam, Hong Kong, China) and other antibodies (Santa Cruz, CA, USA) were prepared. HTA125 antibody (anti-TLR4) (Biolegend, CA, USA) was used as the blocking of LPS-induced cytokine production while mouse IgG2a ( $\kappa$  isotype, MOPC-173, Biolegend, CA, USA) was used as an isotype-matched control. RevertAid™ First Strand cDNA Synthesis Kits were offered by Fermentas of Thermo Fisher Scientific and SYBR® Premix Ex Taq™ RT-PCR Kits were purchased from Takara.

### Cell Culture and Treatment

LX-2 cells are immortalized human stellate cells and cultured in Dulbecco's modified Eagle's medium supplemented with 10 % (v/v) FBS, 100 units/ml penicillin and 100  $\mu$ g/ml streptomycin at 37 °C under

5 % CO<sub>2</sub> in humidified air. LX-2 cell line is most similar to that of "activated" cells *in vivo*. It showed strong similarity in gene expression between primary HSCs and LX-2 (98.7 %) by microarray analysis. It provides valuable new tools in the study of liver disease [16]. LPS from *Escherichia coli* 0127:B8 or TGF- $\beta$ 1 was reconstituted according to the protocols from Sigma kit. In the concentration gradient experiments, LX-2 cells were pretreated with LPS at various concentrations (0.001, 0.01, 0.1, and 1  $\mu$ g/ml) for 6 h, and the cells cultured for 6 h with no LPS stimulation were harvested as the control group. In the time gradient experiments, LX-2 cells were pretreated with LPS at 0.1  $\mu$ g/ml concentration for various times (1, 3, 6, 9, and 12 h), and the cells harvested before LPS stimulation were as the control (0 h). HTA125 antibody (anti-TLR4) was added to block LPS-induced cytokine production [17] at the concentration of 10  $\mu$ g/ml as the blocking antibody, and Mouse IgG2a ( $\kappa$  isotype, MOPC-173) was used as an isotype-matched control for 2 h pretreatment before LPS.

### RNA Isolation and Quantitative Real-time PCR

Total RNA was extracted using Trizol reagent according to the kit's instruction. cDNA was synthesized following RevertAid™ First Strand cDNA Synthesis Kit with Oligo (dT) 18 primers (0.5 mg/ml). Quantitative real-time PCR (qRT-PCR) was performed according to the protocol of SYBR® Premix Ex Taq™ RT-PCR Kit in the machine Eco Real-time PCR system (Illumina, USA). The primers were synthesized (Invitrogen, China) as follows: human Septin4 sense: 5'-AAGGATGTGACGC GGGAGAC-3' and antisense: 5'-GGTGGACAGCAG GGATGG-3'; human  $\alpha$ -SMA sense: 5'-CGCATCCTCAT CCTCCCT-3' and antisense: 5'-GGCCGTGA TCTCCTTCTG-3'; human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sense: 5'-TCAACGGATTT GGTCGTATTG-3' and antisense: 5'-TGGAAGATGGT GATGGGATT-3'. PCR amplification was carried out with an initial polymerase activation step at 95 °C for 30 s, then 40 PCR cycles: 95 °C for 5 s, 58 °C for 30 s, and 72 °C for 30 s, followed by next program for melt curve: 95 °C for 15 s, 55 °C for 30 s, and 95 °C for 15 s. The GAPDH gene was used as an internal control for standardization. The target gene values were normalized to the values of GAPDH and expressed as relative fold increase  $2^{-\Delta\Delta Ct}$  over the non-stimulated samples. All experiments were repeated in triplicate using different HSCs isolations each time, and the data were represented as mean  $\pm$  SD.

### Western Blot

LX-2 cells were harvested and resuspended in protein lysis buffer to extract protein. The concentration of the protein was measured and quantified by the Bradford method. The protein solution was heat-denatured with an equal volume of 2×SDS loading buffer for 5 min, separated on 10 % SDS-PAGE and then electrotransferred onto polyvinylidene fluoride membrane. After blocked with 5 % nonfat milk, the membrane was incubated with primary and secondary antibody diluted in Tris-buffered saline Tween-20 subsequently. Then revelation was obtained by enhanced chemiluminescence.

Band quantification analysis was performed by GeneTools software of Syngene, and expression of target proteins was normalized to GAPDH. The control samples without stimulation were referred to acquire relative intensity for bands.

### Statistical Analysis

All the experiments were performed in triplicate, and all data were presented as mean ± SD. Data were analyzed by one-way ANOVA (LSD) or independent-samples *t* test in SPSS 15.0 to determine significant differences. Data were considered to be statistically significant at  $P < 0.05$ .

## RESULTS

### LPS Induced the Alteration of $\alpha$ -SMA Expression in LX-2 Cells

After HSCs stimulated with LPS at various concentrations (0, 0.001, 0.01, 0.1, 1  $\mu\text{g/ml}$ ) or time points (0, 1, 3, 6, 9, 12 h), qRT-PCR showed that  $\alpha$ -SMA expression increased after LPS treatment in a concentration or time-dependent manner ( $*P < 0.05$ , Fig. 1 a, b).

Western blot analysis on LX-2 cells showed the activation state of HSCs. After treated with various concentrations of LPS (0.001, 0.01, 0.1, and 1  $\mu\text{g/ml}$ ) for 6 h, the expression of  $\alpha$ -SMA increased at the concentration of 0.001, 0.01, 0.1, and 1  $\mu\text{g/ml}$  ( $P < 0.05$ , Fig. 1c). However, there was no significant difference among the groups of cells stimulated by LPS with the concentration of 0.001, 0.01, 0.1, or 1  $\mu\text{g/ml}$ . Then LX-2 cells were stimulated with LPS for 0, 1, 3, 6, 9, and 12 h at the concentration of 0.1  $\mu\text{g/ml}$ . The expression of  $\alpha$ -SMA increased in a time-dependent manner ( $P < 0.05$ , Fig. 1d).

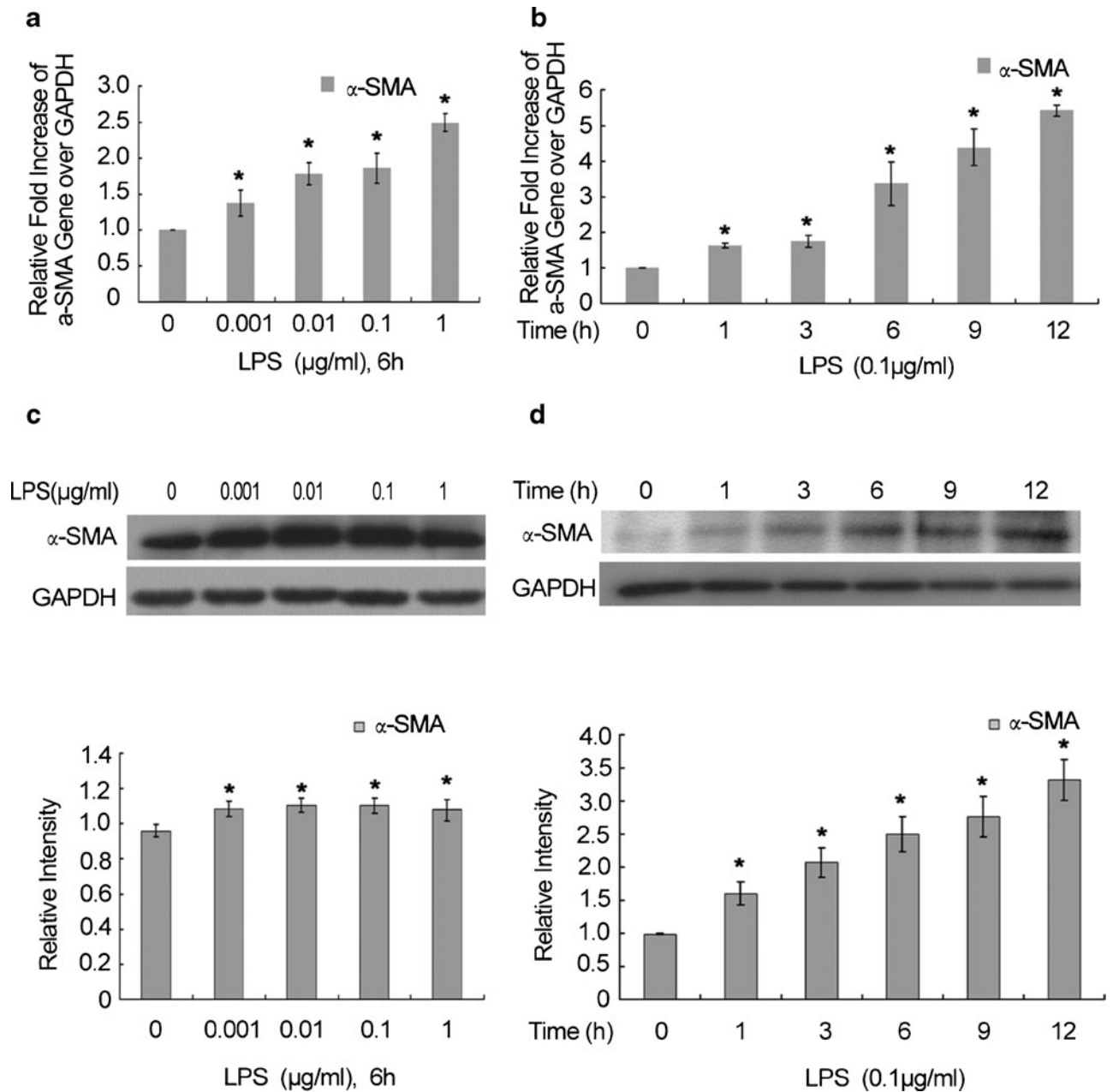
### The Expression of Septin4 Altered in LX-2 Cells Stimulated by LPS

After HSCs stimulated with LPS at various concentrations (0, 0.001, 0.01, 0.1, and 1  $\mu\text{g/ml}$ ) or time points (0, 1, 3, 6, 9, and 12 h), qRT-PCR showed that Septin4 expressed at low level in normal LX-2 cells, but increased after LPS treatment at the concentration of 0.01, 0.1, and 1  $\mu\text{g/ml}$  for 6 h ( $*P < 0.05$ , Fig. 2a). The expression level of Septin4 mRNA was upregulated to a peak at 6 h but then downregulated after LPS stimulation (0.1  $\mu\text{g/ml}$ ) ( $*P < 0.05$ , Fig. 2b).

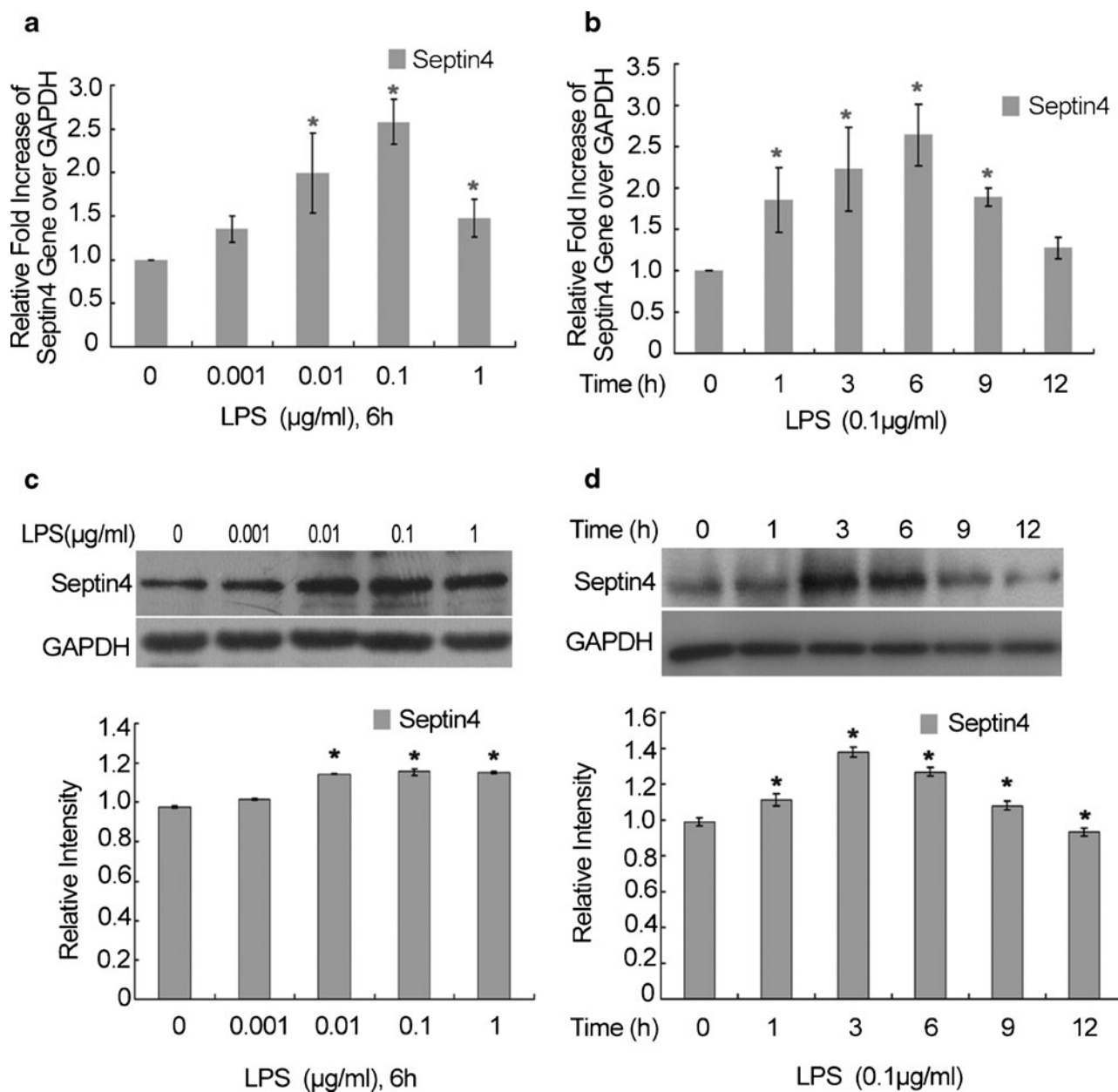
To investigate whether Septin4 protein expression was different in LX-2 cells stimulated by LPS, the cultured HSCs were stimulated with LPS at various concentrations (0, 0.001, 0.01, 0.1, and 1  $\mu\text{g/ml}$ ) or time points (0, 1, 3, 6, 9, and 12 h). Consistent with the expression level of Septin4 mRNA, the results of Western blot indicated that Septin4 expressed at low level in normal LX-2 cells but increased after LPS treatment for 6 h at the concentration of 0.01, 0.1, and 1  $\mu\text{g/ml}$  (Fig. 2c). After cells were stimulated at various time points, the expression of Septin4 protein also demonstrated a similar significant tendency as Septin4 mRNA expression (Fig. 2d).

### The Modulation of Septin4 and $\alpha$ -SMA in LX-2 Cells was Mediated by TLR4

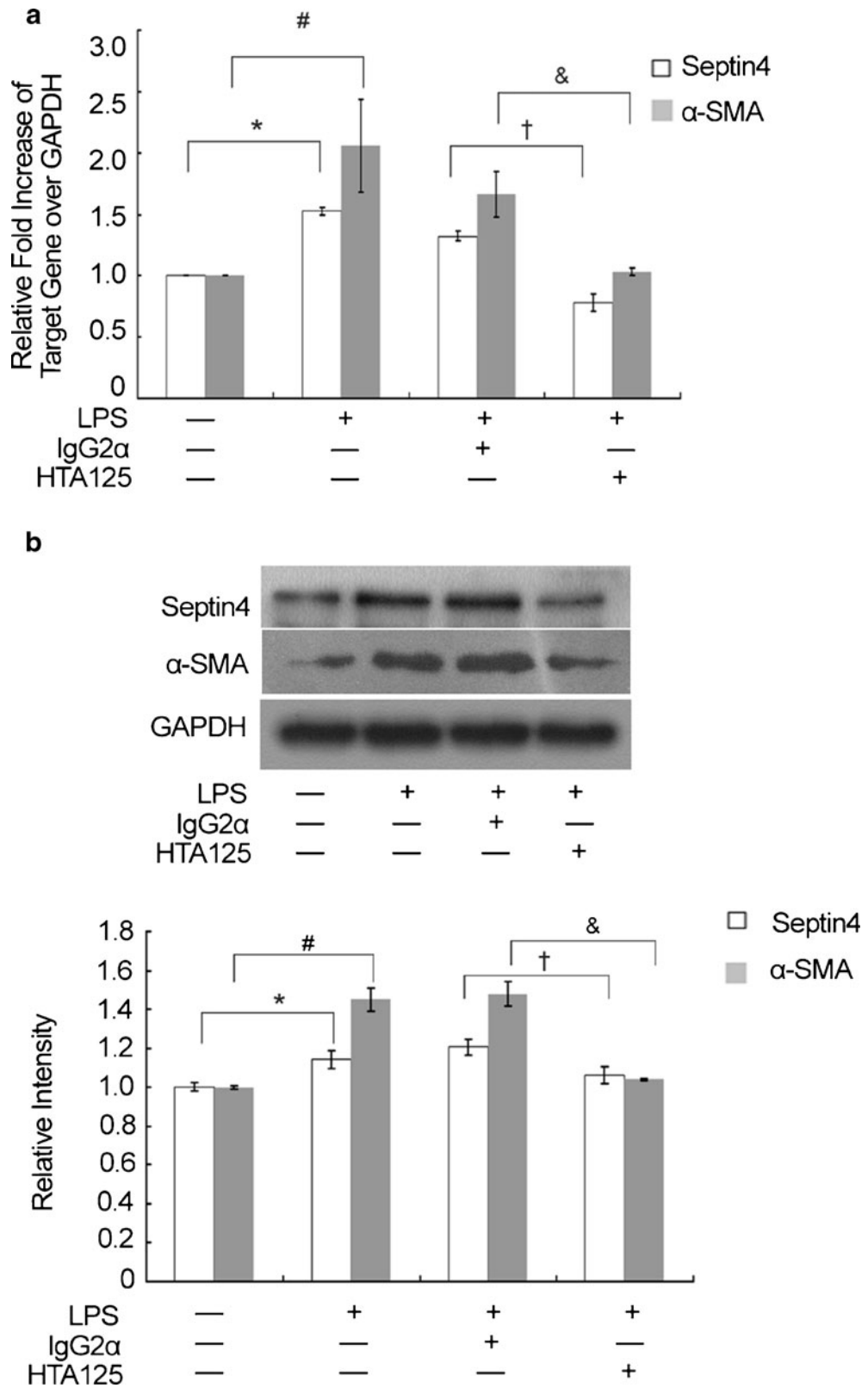
To examine whether TLR4 was involved in the effect of LPS on Septin4 and  $\alpha$ -SMA expression, HTA125 antibody (anti-TLR4 antibody) was used to interrupt LPS-induced activation of LX-2 cells. HTA125 antibody (anti-TLR4) could block LPS-induced cytokine production [17], while mouse IgG2a ( $\kappa$ isotype, MOPC-173) was used as an isotype-matched control. Expression of target gene was normalized to GAPDH, and the results displayed as the relative fold over the reference group (LPS-HTA125-IgG2a-). Significant differences were found from values between the LPS+HTA125-IgG2a-group and LPS-HTA125-IgG2a-group (Fig. 3). The expression of Septin4 and  $\alpha$ -SMA gene decreased significantly after blocking of LPS-induced cytokine production (LPS+HTA125+IgG2a-) compared to the cells pretreated with LPS and IgG2a (LPS+HTA125-IgG2a+) (Fig. 3a). The same results were found in Fig. 3b, which represented the expression level of Septin4 and  $\alpha$ -SMA protein after blocking of LPS-induced cytokine production by Western blot. The results suggested that the regulation of Septin4 and  $\alpha$ -SMA expression by LPS was mediated by TLR4.



**Fig. 1.** The expression of  $\alpha$ -SMA was regulated by LPS. **a, b** qRT-PCR showed that  $\alpha$ -SMA expression increased after LPS treatment at various concentrations (0, 0.001, 0.01, 0.1, and 1  $\mu\text{g/ml}$ ) for 6 h. It also showed that  $\alpha$ -SMA expression increased after LPS treatment at 0.1  $\mu\text{g/ml}$  concentration for various times (0, 1, 3, 6, 9, and 12 h). (The *asterisks* indicate  $P < 0.05$ , significantly different from the control group). **c, d** Western blot analysis showed  $\alpha$ -SMA levels in LX-2 before (control) and after LPS stimulus. **c**  $\alpha$ -SMA levels increased at 0.001, 0.01, 0.1, and 1  $\mu\text{g/ml}$  of LPS at 6 h. **d** Also  $\alpha$ -SMA levels increased in a time-dependent manner. Histograms showed the relative intensities of  $\alpha$ -SMA bands (averages of three experiments), in which each density was normalized as to GAPDH. (The *asterisks* indicate  $P < 0.05$ , significantly different from the control group).



**Fig. 2.** Septin4 expression was regulated by LPS in LX-2 cells. **a** The cultured HSCs were stimulated without or with LPS at various concentrations for 6 h. As the results of qRT-PCR, Septin4 was expressed at low level in non-stimulated samples (control) and increased after LPS treatment at the concentration of 0.01, 0.1, and 1 µg/ml to a peak at 0.1 µg/ml. **b** Also, the cultured HSCs were stimulated with LPS (0.1 µg/ml) for 0 h (control), 1, 3, 6, 9, and 12 h. qRT-PCR results indicated that Septin4 increased after LPS treatment for 1, 3, 6, and 9 h to a peak at 6 h. The results are the mean±SD of three independent sets of analyses. (The asterisks indicate  $P < 0.05$ , significantly different from the control group). **c, d** Western blot analysis showed Septin4 levels in LX-2 before (control) and after LPS stimulus. Histograms showed the relative intensities of Septin4 bands (averages of three experiments), in which each density was normalized as to GAPDH. **c** Septin4 levels increased at 0.01, 0.1, and 1 µg/ml of LPS for 6 h. **d** Also, Septin4 increased at 1 h in 0.1 µg/ml and reached peak at 3 h, followed by downregulation at 6, 9, and 12 h. (The asterisks indicate  $P < 0.05$ , significantly different from the control group).



**Fig. 3.** The modulation of Septin4 and  $\alpha$ -SMA in LX-2 was mediated by TLR4. The cells were pretreated with HTA125 antibody or mouse IgG2a. Expression of target gene was normalized to GAPDH and the results displayed as the relative intensities over the reference samples (LPS-HTA125-IgG2a<sup>-</sup>). All the experiments were performed in triplicates. **a** As the results of qRT-PCR, significant differences ( $P < 0.05$ ) existed from values for LPS+HTA125-IgG2a<sup>-</sup> group and LPS-HTA125-IgG2a<sup>+</sup> group. The expression of Septin4 (*left panel*) and  $\alpha$ -SMA (*right panel*) gene were decreased significantly after blocking of LPS-induced cytokine production (LPS+HTA125+IgG2a<sup>-</sup>) compared to the cells pretreated with LPS and IgG2a (LPS+HTA125-IgG2a<sup>+</sup>). **b** The same results were found in Fig. 3b, the data in which were obtained and analyzed by Western blot. (The *asterisks* indicate  $P < 0.05$ , Septin4, compared to the group of LPS-HTA125-IgG2a<sup>-</sup>. The *number sign* indicates  $P < 0.05$ ,  $\alpha$ -SMA, compared to the group of LPS-HTA125-IgG2a<sup>-</sup>. The *dagger sign* indicates  $P < 0.05$ , Septin4, compared to the group of LPS+HTA125-IgG2a<sup>+</sup>. The *ampersand sign* indicates  $P < 0.05$ ,  $\alpha$ -SMA, compared to the group of LPS+HTA125-IgG2a<sup>+</sup>).

### The Alteration of Septin4 and $\alpha$ -SMA Expression in LX-2 Stimulated by LPS was Associated with TGF- $\beta$ Signaling

Bambi was a pseudoreceptor of TGF- $\beta$ . Some studies suggested that TLR4 activation downregulated the expression of Bambi and sensitized HSCs to TGF- $\beta$ -induced hepatic fibrosis signals in HSCs [18, 19]. In our experiments, significant difference was detected between the LPS+HTA125-IgG2a<sup>-</sup> group and LPS-HTA125-IgG2a<sup>+</sup> group (Fig. 4a, \* $P < 0.05$ ). Smad4 was the common mediator smad (Co-Smad) of TGF- $\beta$  signaling pathway through TLR4. It decreased significantly in the LPS+HTA125+IgG2a<sup>-</sup> group compared to the group pretreated with LPS only or LPS with mouse IgG2a (Fig. 4a, # $P < 0.05$ ).

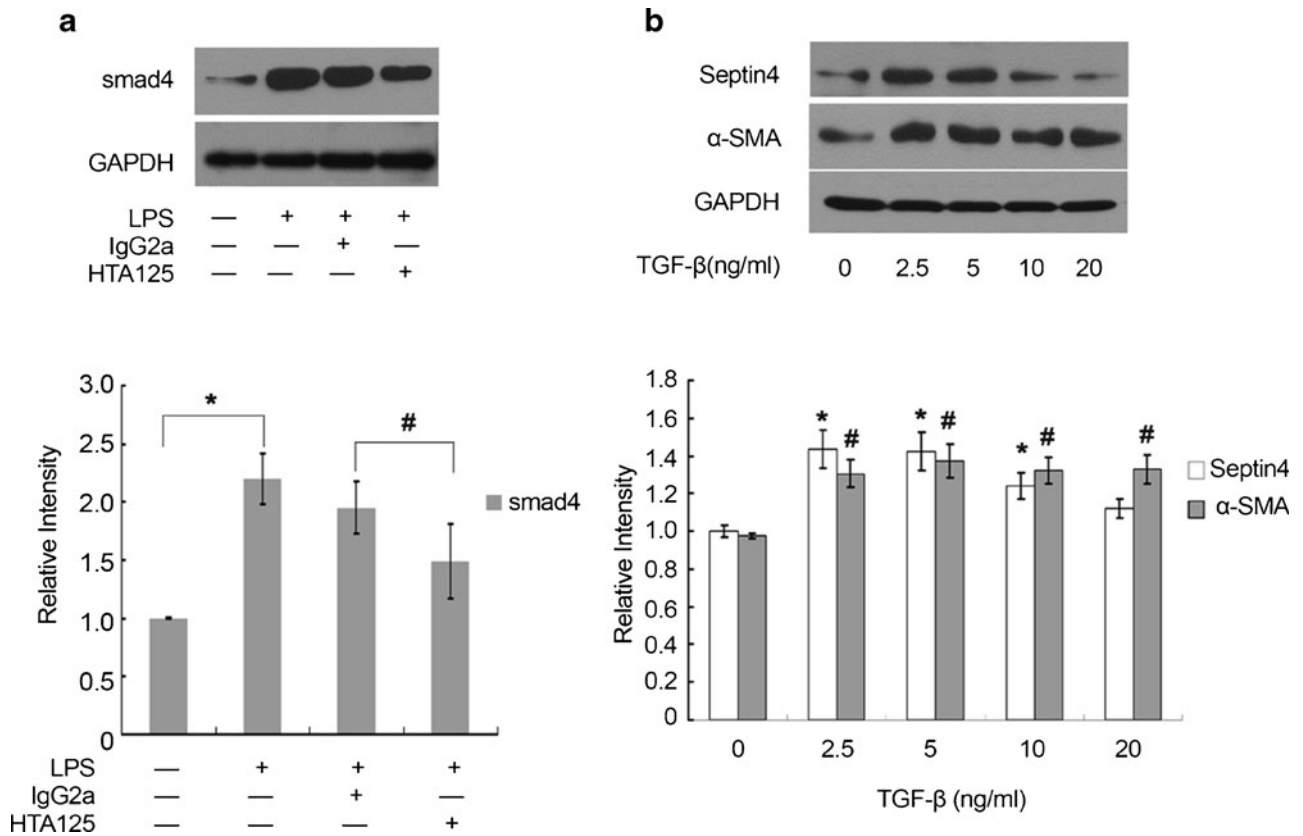
Moreover, we also verified that TGF- $\beta$  signaling played an important role in the activation of HSCs. Septin4 was upregulated by low concentration of TGF- $\beta$ 1 stimulation, followed by downregulation by high concentration of TGF- $\beta$ 1.  $\alpha$ -SMA expression was upregulated by TGF- $\beta$ 1 stimulation, with no significant difference among the groups of cells stimulated by TGF- $\beta$ 1 with the concentration of 2.5, 5, 10, and 20 ng/ml (Fig. 4b). The expression of these two proteins in the process of HSCs activation may be associated with TLR4-TGF- $\beta$ -smad4 pathway.

## DISCUSSION

Increased and sustained inflammation as a result of liver injury/disease states often perpetuates activation of

the stellate cell ultimately leading to increased hepatic injury and hepatic fibrosis. During the activation and fibrosis process, HSCs will lose vitamin A, become highly proliferative, and synthesize fibrotic matrix rich in type I and III collagen [2, 20]. Activation of HSCs is the central event in hepatic inflammatory injury and fibrosis [20]. Although hepatic fibrosis due to viral, parasitic, or alcohol-induced injury is contributing to death worldwide, it is still out of curative treatment [21, 22]. Since more and more evidences have suggested that hepatic fibrosis is a reversible wound healing response and the activated HSCs decrease due to apoptosis in the recovery process, the latest studies on hepatic fibrosis will emphasize the promotion of apoptosis and inhibition of proliferation as well as activation of HSCs [20, 23, 24].

It is well recognized that HSCs are the potential mediator of liver injury induced by LPS. HSCs stimulated by LPS express LPS-recognizable receptors, such as TLR4, CD14, and MD2 [19, 25]. TLR4, but not TLR2, is required for the activation of HSCs and enhances hepatic inflammation and fibrogenesis. Some researches have shown that TLR4 activation downregulated the TGF- $\beta$  pseudoreceptor Bambi and enhanced TGF- $\beta$ -induced hepatic fibrosis signals in HSCs through the MyD88-NF-KB pathway [19, 26]. TLR4 siRNA could abrogate the downregulation of Bambi, TGF- $\beta$ 1 mRNA expression, and NF-KB activation induced by LPS in HSCs [26]. Moreover, some inflammatory chemokines (IL-8, MCP-1, etc.) and cell surface adhesion molecules (ICAM-1, VCAM-1, etc.) are induced and upregulated in activated human HSCs stimulated by LPS depending on NF-KB and JNK activation [25]. In our studies, we found that the expression of  $\alpha$ -SMA could be regulated in LX-2 cell line after LPS stimulation, and the results were consistent with that of the previous studies [4, 14]. LPS-induced inflammatory chemokines secretion (IL-8, data not shown) could be blocked by HTA125 through TLR4. Blocking of TLR4 with HTA125 could also inhibit the LPS-induced hepatic fibrosis as the downregulation of  $\alpha$ -SMA expression. Furthermore, we also found the downregulation of smad4 expression, which was the Co-Smad of TGF- $\beta$  signaling pathway, after blocking with HTA125. Therefore, we confirmed that TLR4 was indeed involved in HSCs activation induced by LPS, which had been approved by other researchers [4]. It provided a novel linkage between pro-inflammatory and profibrogenic signals through TGF- $\beta$  signaling pathway.



**Fig. 4.** The expression of Septin4 and  $\alpha$ -SMA in LX-2 was associated with TGF- $\beta$ 1. **a** Expression of target proteins was normalized to GAPDH, and the results displayed as the relative fold over the reference samples (LPS-HTA125-IgG2a-). All the experiments were performed in triplicates. As the results of Western blot, the expression of smad4 protein increased in LPS+HTA125-IgG2a- group, compared with the group of LPS-HTA125-IgG2a- (The asterisks indicate  $P < 0.05$ ). It decreased significantly (the number sign indicates  $P < 0.05$ ) after blocking of LPS-induced cytokine production (LPS+HTA125+IgG2a-) compared to the cells pretreated with LPS and IgG2a (LPS+HTA125-IgG2a+). **b** The expressions of Septin4 from the cells pretreated with 2.5, 5, and 10 ng/ml of TGF- $\beta$ 1 (left panel, the asterisks indicate  $P < 0.05$ ) were all increased significantly, compared to the cells pretreated without TGF- $\beta$ 1.  $\alpha$ -SMA (right panel, number sign indicates  $P < 0.05$ ) protein expression was also upregulated by TGF- $\beta$ 1 stimulation. However, it had no significant difference in  $\alpha$ -SMA protein expression among the groups of cells pretreated with TGF- $\beta$ 1 at the concentration of 2.5, 5, 10, or 20 ng/ml.

As a family of polymerizing GTP-binding proteins, Septins are reported to be required for apoptosis, cytokinesis, and exocytosis [8]. Septin4 gene is also named H5, PNUTL2, or CDCrREL-2, and one of its transcript variants encodes apoptosis-related protein in the TGF- $\beta$  signaling (ARTS) pathway in many cells. ARTS can aggravate cell death induced by TGF- $\beta$ , etoposide, and staurosporine. Some researches have shown that Septin4 is involved in the suppressive modulation of myofibroblastic transformation and hepatic fibrosis due to CCl<sub>4</sub> and BDL [27]. Yanagida *et al* [28] found that Septin4<sup>-/-</sup> mice were prone to hepatic fibrosis with downregulated DKK2 and blocked canonical Wnt pathway, and they believed that Septin4 expressed exclusively in quiescent HSCs and

downregulated in human HSCs with hepatic fibrosis. However, it was inconsistent with some researches that Septin4 mRNA was upregulated in the culture-activated HSCs [14]. As we previously reported, the expression of Septin4 in the liver of mice increased to peak at 12 weeks after infected with *S. japonicum* and then decreased [15]. It is commonly believed that *S. japonicum*-induced egg granuloma and subsequent hepatic fibrosis is a complex immunopathogenic process which is different from the hepatic fibrosis process induced by CCl<sub>4</sub> and BDL [29]. The different expression of Septin4 in these different models of fibrosis is elusive. In this study, we selected LX-2 cell line [16], the best hepatic fibrosis cell model, to study the alteration of Septin4 expression in LX-2 cells. We



detected that the expression of Septin4 first upregulated to peak at 6 h then downregulated, while the expression of  $\alpha$ -SMA increased in a time-dependent manner. We also found the expression of Septin4 increased when cells pretreated with various concentration of LPS. We concluded that Septin4 was regulated in LX-2 cells stimulated by LPS. It was upregulated by LPS with low concentration or short-acting time, while downregulated with high concentration or long time. Besides, we found that the LPS-mediated Septin4 protein expression was regulated through TLR4 and TGF- $\beta$  signaling pathway.

In conclusion, our study provides the evidence that Septin4 may be involved in the process of activation of HSCs by LPS stimulation, which is associated with TLR4 and TGF- $\beta$  signaling. Our further work would focus on primary HSCs isolated from the normal animals or the animals with hepatic inflammatory injury and fibrosis to analyze the expression and function of Septin4 in hepatic fibrosis.

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**Conflict of Interest.** None.

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