Original Article
Lipopolysaccharide activated TLR4/NF-κB signaling pathway of fibroblasts from uterine fibroids

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Abstract: Uterine fibroids (UF) are the most common benign tumor of the female reproductive tract. The aim of this study was to explore the role of lipopolysaccharide (LPS)-induced activation of TLR4/NF-κB signaling pathway on stromal fibroblasts in the pathogenesis of UF. Here, TLR4/NF-κB signaling pathway was more activated in UF, and UF cells (UFC) and UF derived fibroblasts (TAF) than in smooth muscle tissues, smooth muscle cell (SMC) and myometrial fibroblasts (fib) respectively. After lipopolysaccharide (LPS) stimulation, the activity of fib was enhanced, characterized by the increased expression of fibroblast activation protein (FAP), and increased secretion of collagen I and transforming growth factor-β (TGF-β). Moreover, TLR4 inhibitor (VIPER) and siTLR4 can represses LPS-activated fibroblasts and TLR4/NF-κB signaling transduction pathways in fib and UFC cells. Co-cultured with LPS-activated fibroblast enhanced fibroblast activation and TLR4/NF-κB signaling. In conclusion, LPS treatment activated TLR4/NF-κB signaling pathway on fibroblasts, which may involve in the development of UF. Our study indicated reproductive tract infection may be associated with fibroid pathogenesis through TLR4/NF-κB signaling. Targeting NF-κB with inhibitors may hold promises of treating uterine fibroid.

Keywords: Lipopolysaccharide, uterine fibroids, tumor associated fibroblasts, Toll-like receptors 4, NF-κB

Introduction

Uterine fibroids (UF), also known as leiomyomas, are the most common benign reproductive tract tumor in women and are the leading indications for hysterectomies [1]. The prevalence of UF is about 77% [2], while the exact pathogenesis underlying uterine leiomyomas is still unclear.

The surrounding stromal cells, various secreted growth factors, and the signal transduction between cells have been recognized as important factors in tumor growth, and these factors provide a complex tumor microenvironment, which promote the growth and invasion of tumor cells [3]. Under normal circumstances, fibroblasts are in a quiescent state but can be activated by injury, inflammatory, and tumor state [4]. It has been shown that about 80% of fibroblasts in tumor tissue keep a constant activation status [5]. Tumor tissue fibroblasts are also known as tumor-associated fibroblasts (TAF), and activated fibroblasts are involved in tumor development.

Uterine fibroid tissue is mainly composed of fibroid cells, fibroblasts, as well as a large number of extracellular matrix (ECM) components. Fibroblasts provide nutritional support and survival framework for fibroid cells, and ECM is primarily secreted from fibroblasts [6]. Previous studies on the pathogenesis of uterine fibroids have mainly focused on the differentiation and proliferation of fibroid cells. However, our previous study suggested that fibroid fibroblasts are in an activated state and activated fibroblasts may play an important role in the pathogenesis of uterine fibroids [7].

The idea that injury or reproductive tract infections might trigger fibroid development was introduced mentioned decades ago [8], but has never been adequately tested. In order to inves-

tigate the pathogenesis of uterine fibroid, we have reviewed a numbers of literature about chronic inflammation-associated tumor had been reported, which provided a suggestion that inflammation may involve in pathogenesis of uterine fibroid. Inflammation is now commonly considered a hallmark of cancer [9], of which NF-κB and STAT3 activation play a central role [10, 11]. Accumulating evidences indicate a key role for the bacterial in inflammation associated carcinogenesis [12-15]. Intriguingly, whether pathogen infection is directly involved in the initiation and development of fibroids has captured our attention. Our unpublished data on analysis of the samples of uterine and fallopian tube for leiomyoma indication. The immunohistochemical staining results showed that 222 samples of myoma uterine simultaneously occur with chronic cervicitis (48.64%), endometritis (0.91%) and salpingitis (0.91%). In addition, considerable published data also show the inflammation environments in leiomyoma [16-22]. Various growth factors and cytokines, such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), TGF-β and interleukin-6 (IL-6), perform regulatory actions in uterine myometrium and in leiomyomas.

Nevertheless, how does inflammation lead to tumorigenesis and regulate cell proliferation? As evidenced, the mechanism of microbiota-induced carcinogens is now attributed to pathogen-associated molecular patterns (PAMPs), of which toll like receptors (TLRs) is a cornerstone of innate immunity, one of the most powerful pro-inflammatory stimuli [23], and regulate epithelial carcinogenesis through epithelial cells, fibroblasts and MSCs [23, 24]. TLR4, the receptor for the Gram-negative bacterial cell wall component LPS, triggers and promotes carcinogenesis in the colon, liver, pancreas, lung and skin, and granulosa cell tumor (GCT) cell lines [13, 23, 25, 26], as shown by reduced tumor development in TLR4-deficient mice [27, 28]. Lipopolysaccharide (LPS) has been shown to bind directly to the TLR4/MD2 receptor complex that initiates the intracellular signaling cascade in a MyD88-dependant or MyD88-independent manner [29]. A key cancer-promoting downstream signals effect of TLR4 is mediated by activation of nuclear factor-κB (NF-κB), which produces large amounts of inflammatory cytokines(IL-6, TNF-α and COX2), regulating cell proliferation through autocrine and paracrine

pathway [30]. In the downstream, when IL-6 binding to IL-6 receptor, polypeptides stimulate membrane associated gp130 subunit, which triggers phosphorylation of Janus kinases (JAK) and its downstream effectors, signal transducer and activator of transcription 3 (stat3), promote cell survival, proliferation and yet inhibit apoptosis in the tumor stroma [30-32]. However, the mechanism of LPS induced cell signaling in fibroids is unclear.

Above all, in this study we suppose that pathogen infection be associated with the evolvement of fibroids. Therefore, aiming to explore and test this hypothesis, we establish the primary cell model, focus on fibroblasts and detect the expression of TLR4, MD2 and TLR4/NF-κB signaling pathway in uterine tissue or fibroblasts.

Materials and methods

Reagents

Antibodies for CD90, TLR4, MD2, Myd88, IκBα, c-myc and p-c-myc were purchased from Abcam (Cambridge, MA, USA). FAP antibody was from Santa Cruz (Santa Cruz, CA, USA). Antibodies for NF-κB p65, STAT3 and p-STAT3 were purchased from cell signaling technologies (Danvers, MA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was obtained from Fermentas (Andingmen East Street, Beijing, China). Secondary antibodies of goat anti-mouse FITC, goat anti-rabbit HRP, and goat anti-mouse HRP were purchased from Beyotime Institute of Technology, China.

Patients and samples

The research was carried out according to the principles of the Declaration of Helsinki, and informed consent was obtained from all patients. This study was approved by the ethics committee of the Yang-Pu Hospital, Shanghai, China. Patients were randomly selected from the Department of Obstetrics and Gynecology, Yang-Pu Center Hospital between May 2013 and July 2013, 6 uterine fibroid cases were studied. The age for these individuals ranged from 48 to 51 years (mean ± SD, 49.33±1.21 years), all of them with no hormonal treatment within three months. The removal criteria included patients who were subsequently diagnosed with uterine adenomyosis and patients

with a history of coronary artery disease, hypertension, or hematologic disorders. Fibroid (subserous and intramural fibroids) and myometrial tissue were obtained after operation of hysterectomy. Fresh tissue specimens 1.5 × 1.0 × 1.0 cm in size were collected either in phosphate-buffered saline (PBS) buffer (100 U/mL penicillin, 100 μg/mL streptomycin added), stored at 4°C, and processed within 2-18 h, or frozen on liquid nitrogen and stored at -80°C for RNA and protein extraction.

Western blot analysis

Protein were extracted with radio-immunoprecipitation assay (RIPA) buffer containing protease inhibitors cocktail and centrifuged at 12,000 g for 15 minutes at 4°C. The supernatant protein was quantified by bicinchoninic acid assay (BCA, Thermo Fisher Scientific, Rockford, USA) and stored at -80°C. Total lysates were resolved in SDS-PAGE. Proteins were blotted onto a nitrocellulose membrane and incubated with primary antibodies and the corresponding secondary antibodies. Immune complexes were visualized by the use of an enhanced chemiluminescence Western blotting system (BioRad, Richmond, CA).

Primary cell isolation and two-dimensional co-culture systems

Primary fibroid and myometrial cells were isolated and cultured as previously described [33]. The tissues were immersed in PBS buffer (100 U/mL penicillin, 100 μg/mL streptomycin) and then digested, using 0.4% collagenase type 3 (Imgenex, USA) at 37°C for 2.5 hours on a shaker. Cells were cultured in DMEM/F12 (Gibco®life technology, Carlsbad, CA) supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin/ streptomycin and 2 mM glutamine at 37°C in a 5% CO_2 atmosphere. Uterine fibroid cells (UFC), smooth muscle cells (SMC), tumor associated cells (TAF) and fibroblasts were isolated through magnetic cell separation (MACS, Miltenyi Biotec, Inc., Auburn, CA). UFC and SMC were then co-cultured with CAF (fibroblasts activated by LPS) for 72 hours.

Real-time polymerase chain reaction

Real-time polymerase chain reaction (qRT-PCR) for FAP and TLR4 was performed with SYBR-Green Master Mix on an ABI 7300/7500 plat-

form (Applied Biosystems, Foster City, CA) [39]. GAPDH was used as an internal control. The following primer sets were used: TLR4, forward primer: 5'-CCGCTTTCACTTCCTCTCAC-3', reverse primer: 5'-CATCCTGGCATCATCCTCAC-3'; FAP, forward primer: 5'-GAATGTTTCGGTCCTGTC-3', reverse primer: 5'-CCATCCAGTTCTGCTTTC-3', GAPDH, forward primer: 5'-CACCCACTCCTCCA-CCTTTG-3', reverse primer: 5'-CCACCACCCTGT-TGCTGTAG-3'. Total RNA was extracted with Trizol reagent from frozen tissue samples. Complementary DNA was synthesized by reverse transcriptase at 42°C for 1 hour and 75°C for 5 minutes. PCR cycling conditions were as follows: 94°C for 7 min, followed by 40 cycles of 15 s at 94°C and 60°C for 45 s. The comparative Ct method was used and the amount of target was normalized to the GAPDH control ($2-\Delta\Delta Ct$) assuming an efficiency of 2.

Cell treatment

UFC, SMC, TAF and fibroblasts were treated with lipopolysaccharide (LPS, 100 ng/ml) derived from Escherichia coli (serotype 0111:b4; Sigma, St Louis, MO, USA) for 0.5 hour, 2 hours, 12 hours and 48 hours. For TLR4 antagonist, 500 nM TLR4 inhibitor VIPER (Imgenex, USA) and LPS was added simultaneously to the fibroblasts and UFC. The shRNAs targeting TLR4 mRNA were designed online: 5-GGACCTCTCT-CAGTGTCAA-3. A scramble sequence that shared no homology with mammalian genome was use as control. Lentiviral constructs were made and packaged into the recombinant lentivirus Lv-TLR4-shRNA in 293T cells. Fibroblasts and UFC were then infected with the lentivirus for 72 hours, and then treated with LPS for 0.5 hour, 2 hours, 12 hours and 48 hours for further analysis.

Enzyme-linked immunosorbent assay (ELISA) assay

Cell culture supernatant was collected for the detection of the concentration of TGF-β and collage I by commercial ELISA kit (Bioswamp). Microplate reader (Lab systems Multiskan MS) was used to detect the 450 nm absorbance of each well to compare experimental groups.

Statistical analysis

Analysis of variance (ANOVA) was used to compare the differences in the mean among the
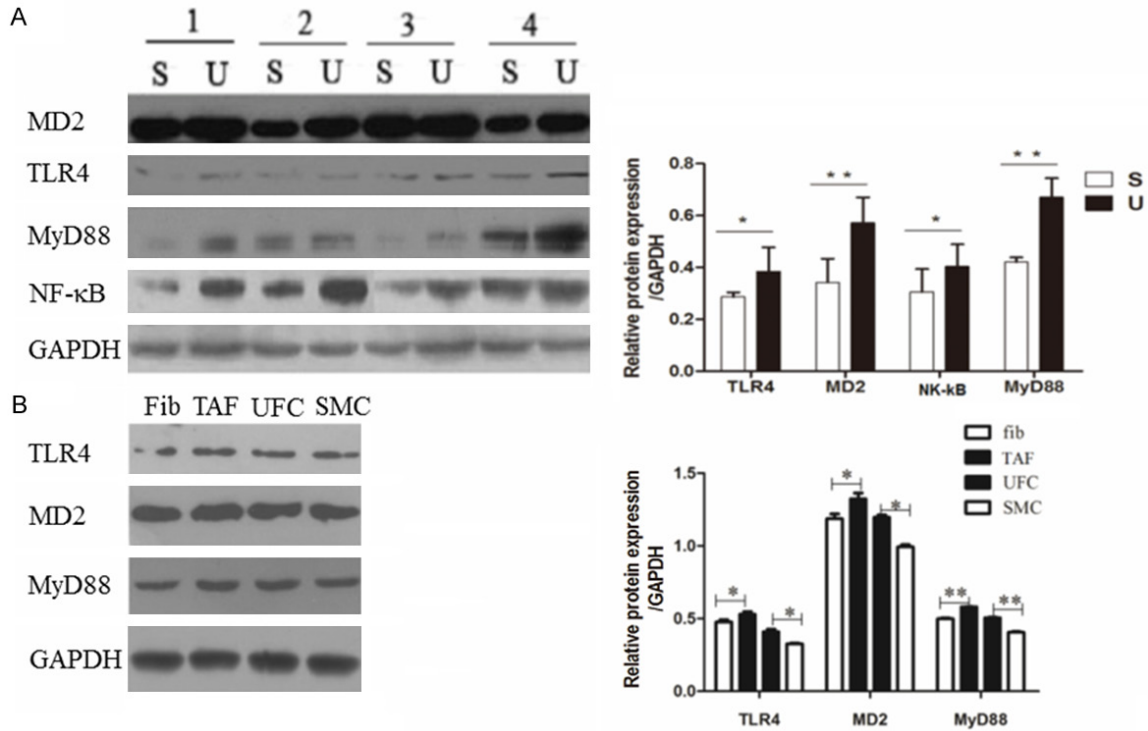
Figure 1. Expression of TLR4, MD2, MyD88 and NF-κB was detected by Western blot. A. Six pairs of uterine fibroid (U) tissues and controlled uterine smooth muscle tissues (S) were collected for Western blot. B. CD90+ fibroid fibroblasts (TAF), CD90+ myometrial fibroblasts (fib), uterine fibroid cells (UFC) and smooth muscle cell (SMC) were obtained from six patients for Western blot. Representative images (left panel) and quantitative data (right panel) were shown (*P<0.05, **P<0.01). MD2:25kDa; TLR4:93kDa; GAPDH:35kDa; NF-κB p65:65kDa; MyD88:35kDa.
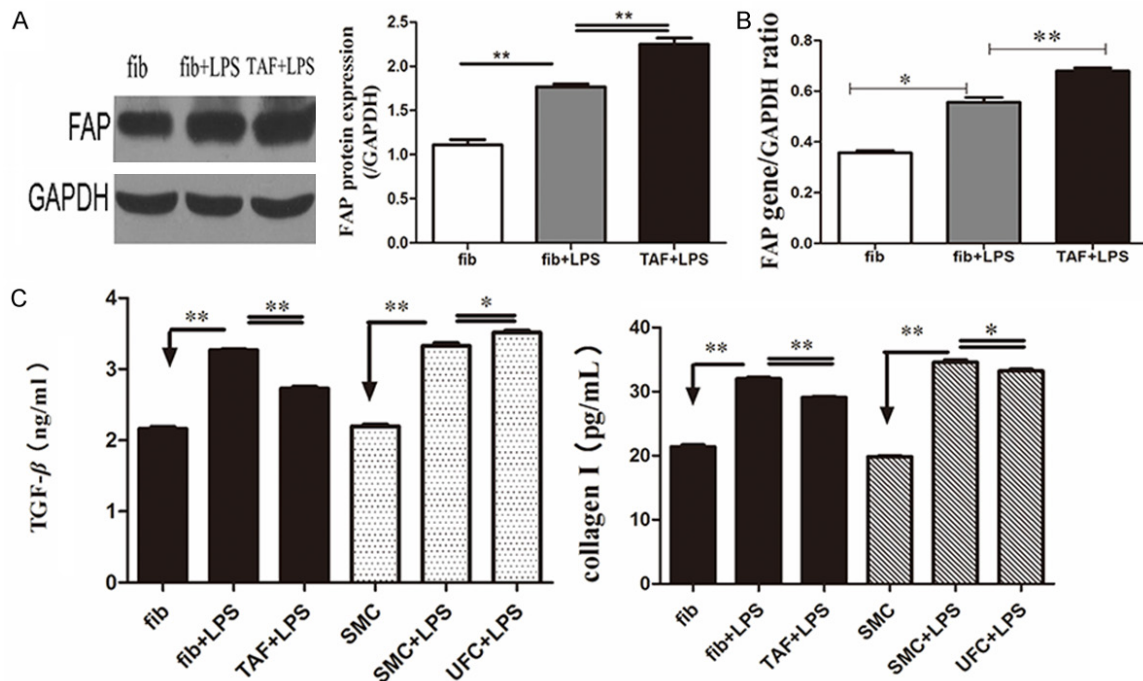
Figure 2. LPS (100 ng/ml) activates CD90+ fibroblasts. Expression of fibroblast activation protein (FAP) in CD90+ myometrial fibroblasts (fib) and CD90+ fibroid fibroblasts (TAF) was detected by Western blot (A) and real time PCR (B). (C) Collagen I and TGF-β was significantly increased after 48 h treatment with LPS (*P<0.05, **P<0.01).
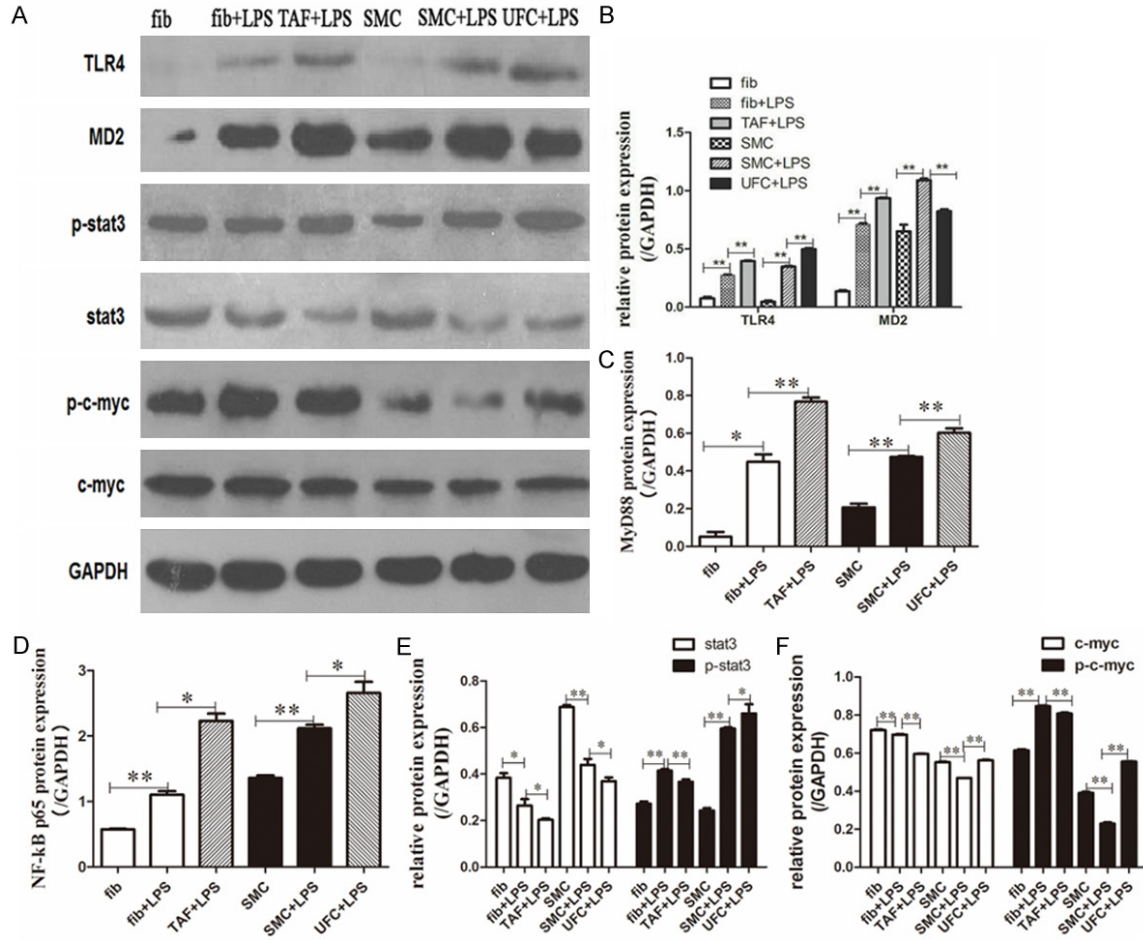
Figure 3. LPS activated TLR4/MyD88/NF-κB signaling in vitro. Western blotting was performed in CD90+ fibroid fibroblasts (TAF), CD90+ myometrial fibroblasts (fib), uterine fibroid cells (UFC) and smooth muscle cell (SMC). Representative images (A) and quantitative data (B-F) were shown (*P<0.05, **P<0.01).

groups when the data approximately represented a normal distribution. A *P* value less than 0.05 was considered significant.

Results

Expression of TLR4, MD2, MyD88 and NF-κB p65 in uterine fibroid and CD90+ fibroblasts derived from uterine fibroid

To explore the relationship between reproductive tract infection and uterine fibroid, we firstly examined the expression of TLR4, the receptor of LPS, in uterine fibroid (U) and smooth muscle tissues (S) from four patients admitted to Department of Gynecology and Obstetrics, Yangpu Hospital by western blotting (**Figure 1A**). The TLR4 protein level was significantly higher in uterine fibroid than that in smooth muscle tissue. Moreover, the protein levels of

co-receptor MD2, adaptor protein MyD88 and downstream effector NF-κB p65 were also increased in uterine fibroid compared to smooth muscle tissue. These results indicated that TLR4/MyD88/NF-κB signaling was activated in uterine fibroid.

Tumor tissue fibroblasts are known as tumor-associated fibroblasts (TAF), whose activation are involved in tumor development. Fibroblasts were then separated from uterine fibroid and matched myometrium by using immune magnetic beads labeled with fibroblast specific marker CD90. 7 A high-purity of CD90+ fibroid fibroblasts (TAF) and CD90+ myometrial fibroblasts (fib) were obtained. The expression of TLR4, MD2 and MyD88 were notably up-regulated in uterine fibroid cells (UFC) and TAF (**Figure 1C**), compared with those in smooth
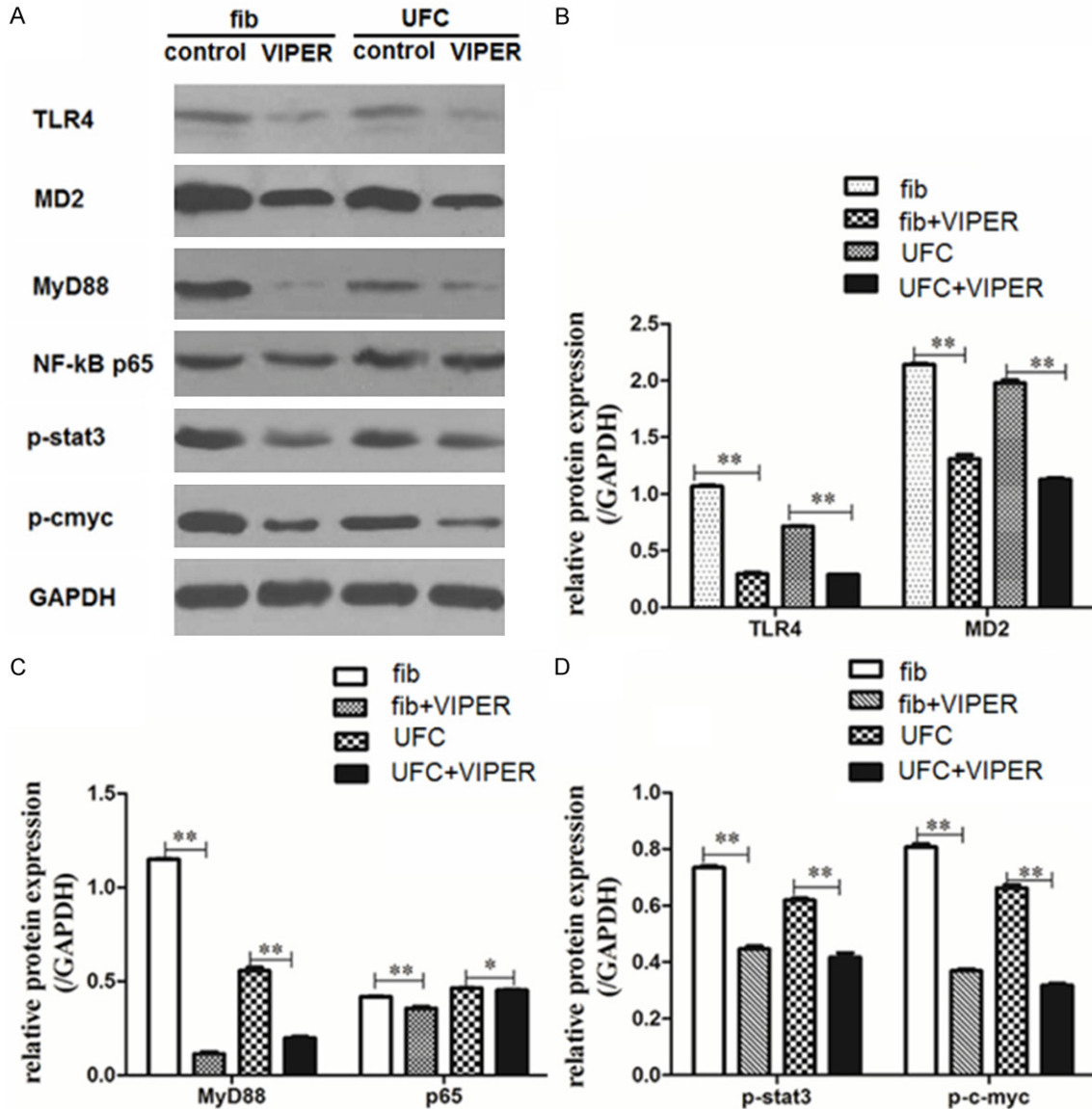
Figure 4. TLR4 inhibitor inhibited the effects of LPS on TLR4/MyD88/NF-κB signaling. TLR4 inhibitor VIPER, 500 nmol, was added to CD90⁺ myometrial fibroblasts (fib) and uterine fibroid cells (UFC) at the same time as LPS (100 ng/ml). Representative Western blotting images (A) and quantitative data (B-D) were shown (*$P<0.05$, **$P<0.01$).

muscle cell (SMC) and myometrium fibroblasts (fib), respectively.

E. coli LPS L4391 induced the expression of FAP and the secretion of collagen I and TGF-β in CD90⁺ fibroblasts

Our previous study demonstrated that estrogen could stimulate CD90⁺ fibroblast activation and promote the expression of fibroblast activation protein (FAP), a specific marker of active fibroblasts in tumor [9]. As an initial inflammatory mediator, bacterial endotoxin or lipopolysac-

charide (LPS) has been recently reported to regulate TLR4-mediated growth of endometriotic cells. The appropriate concentration of LPS was determined as 100 ng/ml by our preliminary experiment (data not shown). Western blotting and real-time PCR were performed to quantify FAP expression in fib and TAF in response to LPS challenge (**Figure 2A** and **2B**). The effect of LPS on cell growth factors and extracellular matrix was further analyzed. TGF-β and extracellular matrix collagen I, which play an important role in fibroblast proliferation,
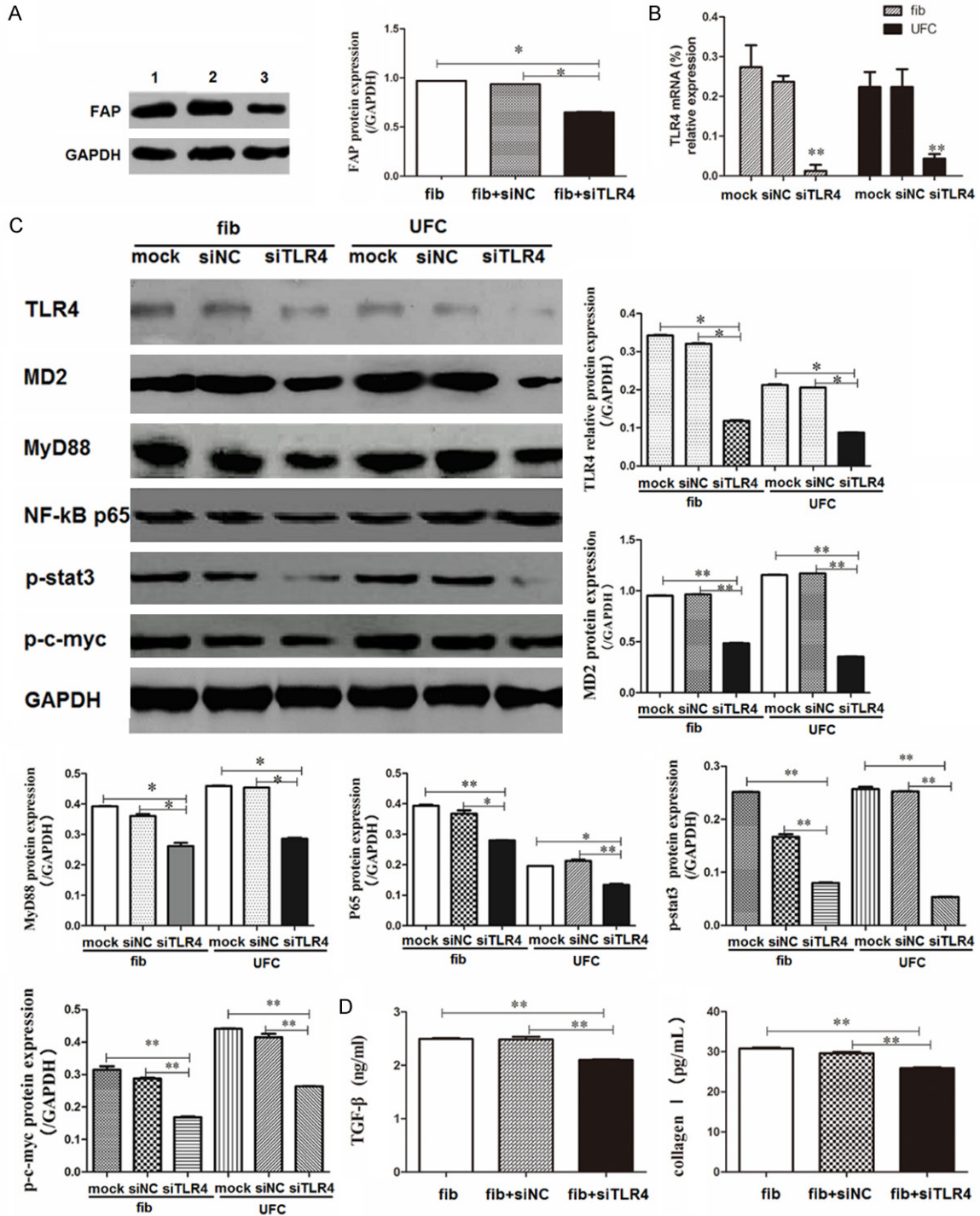
Figure 5. TLR4 siRNA inhibited the effects of LPS on TLR4/MyD88/NF-κB signaling. A, B. Expression of fibroblast activation protein (FAP). C. TLR4/MyD88/NF-κB signaling was detected. Fib: CD90⁺ myometrial fibroblasts, UFC: uterine fibroid cells. D. Concentrations of TGF-β and collagen were detected by ELISA.

were detected by ELISA assay (**Figure 2C**). Compared to the control group, the expression of FAP, collagen I and TGF-β secretion were increased significantly in fib and TAF after treat-ed with LPS for 48 hours (*P*<0.05) (**Figure 2**). TAF presented significantly higher level of FAP than fib after LPS stimulation (*P*<0.01) (**Figure 2**). These results suggested that LPS can stim-
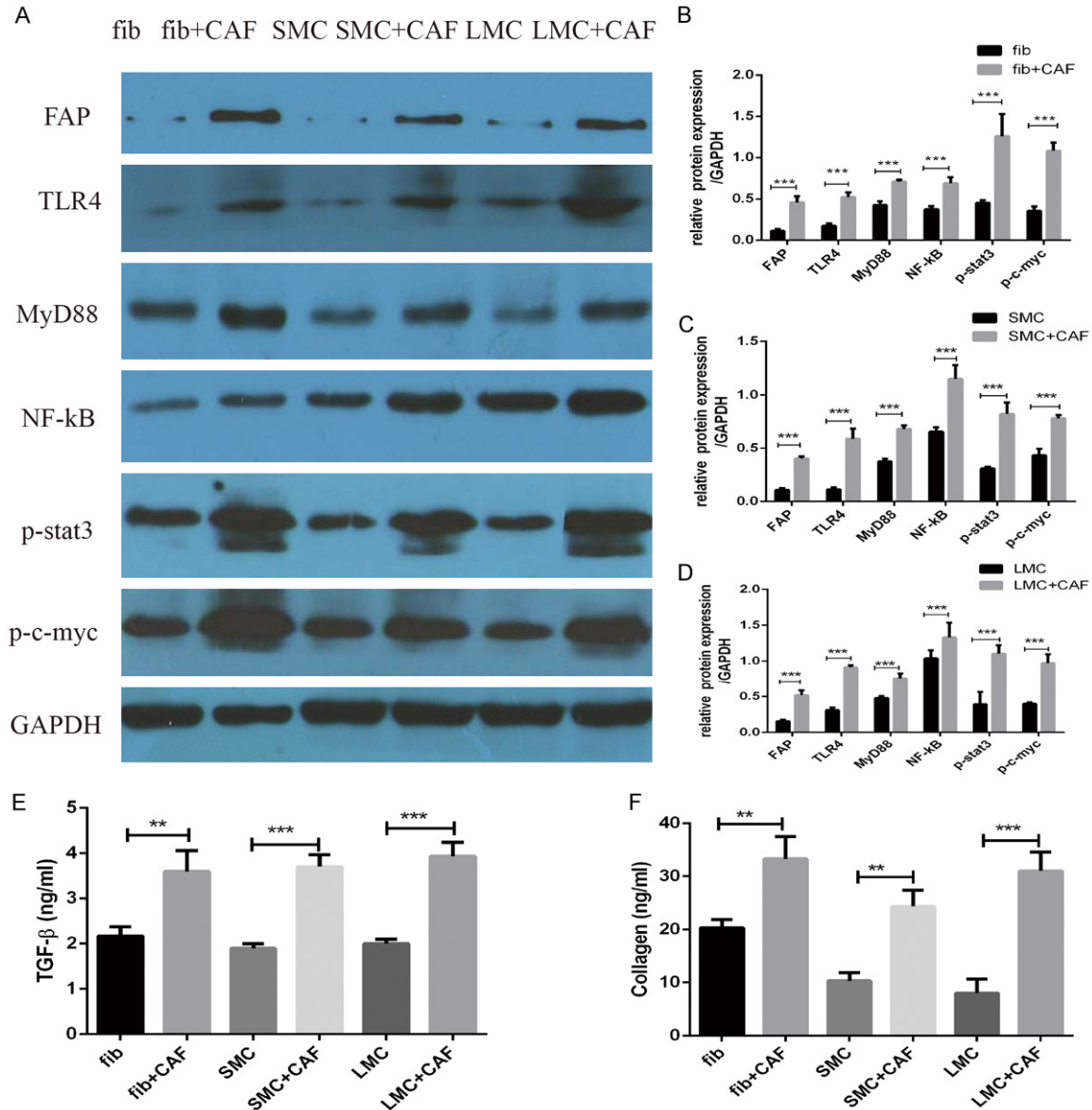
Figure 6. Activation of fibroblast, TLR4/MyD88/NF-κB signaling, Collagen I and TGF-β in two-dimensional (2D) co-culture systems. A-D. FAP expression and TLR4/MyD88/NF-κB signaling was assessed by Western blotting. E, F. Collagen I and TGF-β in the supernatant was evaluated (*P<0.05, **P<0.01). CAF: LPS-activated fibroblast, Fib: CD90^{+} myometrial fibroblasts, UFC: uterine fibroid cells and SMC: smooth muscle cell.

ulate fibroblast activation and promote the expressions of ECM components.

LPS activated TLR4/MyD88/NF-κB signaling

In order to investigate the effects of LPS on primary cultured cells, the expression levels of TLR4, MD2, MyD88 and the p65 subunit of NF-κB were analyzed by western blotting. TLR4 protein level was significantly increased in fib after treated with LPS for 120 min (*P*<0.01, **Figure 3**). In addition, after LPS treatment, TAFs

expressed more TLR4 than fib; while UFC expressed more TLR4 than SMC (*P*<0.01, **Figure 3B**). Stimulation with E. coli LPS led to the up regulation of co-receptor MD2, adapter protein MyD88, and the p65 subunit of NF-κB (**Figure 3C** and **3D**).

Stat3 and c-myc, two downstream molecules of TLR4/MyD88/NF-κB signal pathway, are recently reported phosphorylated in inflammation associated cancer. We then tested the phosphorylation of stat3 and c-myc. After 12

hours of LPS treatment, p-stat3 and p-c-myc were up regulated in all primary cultured cells, while stat3 and c-myc expression were decreased significantly ($P<0.05$, **Figure 3E** and **3F**). Our results indicated that LPS exposure stimulates the activation of TLR4/MyD88/NF-κB signaling in vitro.

TLR4 inhibitor suppressed LPS-induced activation of CD90⁺ fibroblast and TLR4/NF-κB signaling

Since TLR4 play an important role in the activation of fibroblast and NF-κB signaling, we explored its role in the LPS-mediated fibroblast activation by using TLR4 inhibitor, VIPER. VIPER treatment resulted in a decreased expression of FAP, collagen I and TGF-β in LPS-treated CD90⁺ fibroblasts ($P<0.05$) (**Figure 4**). These results clearly demonstrated that LPS executed its effects on CD90⁺ fibroblasts activation through TLR4.

The NF-κB signal pathway was then examined in LPS-treated CD90⁺ fibroblasts pretreated with VIPER. MyD88, NF-κB p65, p-stat3 and p-c-myc were significantly decreased by the pretreatment of VIPER ($P<0.01$, **Figure 4**). Similar results were obtained for UFC treated with VIPER and LPS. These data implied that LPS can activate CD90⁺ fibroblasts and regulate NF-κB pathway through TLR4.

TLR4 siRNA repressed LPS-induced activation of CD90⁺ fibroblast and TLR4/NF-κB signaling

We then explored the role of TLR4 in the LPS-mediated fibroblast activation by using shRNA method. TLR4 was knocked down by lentiviral carrying TLR4 siRNA, the control lentiviral group carrying the scramble siRNA. The silencing effect was validated by real-time PCR (**Figure 5B**) and Western blotting (**Figure 5A**). A significant decrease of TLR4 mRNA and protein was observed in fib and UFC cells infected with TLR4 shRNA when compared with that in control cells. The most significantly silencing effect was obtained at 72 hours after infection, which was then chosen for subsequent experiments.

Cells were infected with lentiviral carrying TLR4 siRNA or the scramble siRNA (siNC) for 72 hours and then treated with LPS for 120 min. As shown in **Figure 4**, FAP protein level, collagen I

and TGF-β secretion were significantly decreased in siTLR4 group compared with control group. To examine the effects of siTLR4 on NF-κB signaling, we measured the expression of MD2, MyD88, NF-κB p65, p-stat3 and p-c-myc in response to LPS. As shown in **Figure 5**, TLR4 blockage led to a significant decrease in MD2, MyD88, NF-κB p65, p-stat3 and p-c-myc expression in LPS-activated cells. These findings were consistent with the observations in cells with TLR4 inhibitor treatment, indicating that the activation of fibroblasts and MyD88-NF-κB signaling by LPS was through TLR4.

LPS-activated fibroblast enhanced fibroblast activation and TLR4/NF-κB signaling in two-dimensional (2D) co-culture systems

Fibroblasts were activated by LPS (CAF), and then co-cultured with fib, SMC and UFC for 72 hours. In 2D co-culture experiments, CAF strongly stimulated the activation of fibroblast by increasing of 2-fold compared with fib alone (**Figure 6**) ($P<0.05$). This effect was also obtained more remarkable in the presence of co-culture with SMC (2-fold increase as compared with SMC alone, $P<0.05$; n=3) (**Figure 6**). Interestingly the similar promotion consequence can be observed in TLR4, MD2, MyD88 and NF-κB dependent phosphorylation of sata3 and c-myc. These combined results indicated that co-culture with TAF can stimulate the activation behavior of CD90⁺ fibroblasts derived from leiomyoma.

Discussion

Our previous studies have shown that the fibroblasts in fibroid were activated and fibroid cells, fibroblasts, and signal transduction between these cells may be involved in the pathogenesis of uterine fibroids [7]. Yet the exact mechanism of fibroblasts activation is poorly understood. However, our experiment indicates that E. coli LPS can activate CD90⁺ fib in myoma microenvironment and enhance fibroid TAF activation. Activated fibroblasts can produce more large amounts of FAP, TGF-β and collagen I than the control group. Activated fibroblasts in tumor also named tumor associated fibroblasts, and its biological function are directly involved in the regulation of tumorigenesis [34]. For further understanding the principle of activation, we first aim to investigate the expression of LPS-induced TLR4/NF-κB signaling in

myometrial fib and fibroid TAF. Interestingly, we found that TLR4/NF-κB signaling may be crosstalk with the mechanism of LPS-induced fibroblast activation, which need further exploration in future. Combined with blocking assays (VIPER and siTLR4), the results implied that VIPER and siTLR4 could inhibit LPS-induced fibroblast activation and repress the TLR4/NF-κB signaling. He Z et al. demonstrated that LPS could stimulate lung fibroblast activation via a TLR4 signaling mechanism that involved the expression of PTEN downregulation and PI3K-Akt pathway activation [35], while He ZW inferred that lipopolysaccharide maybe activate rheumatoid synovial fibroblasts via p38 MAPK and NF-κB signaling pathways [36]. For simulating the microenvironment in body, fibroblasts activated by LPS were co-culture with UFC and SMC, results of which showed that activated-fibroblasts promoted the expression of TLR4/NF-κB signaling and enhanced activation markers (FAP, TGF-β and collagen I) in UFC and SMC. TAF enhanced effect should be associated with autocrine and paracrine interactions, even the cell-cell communication mechanism.

To explore the relationship between reproductive tract infection and fibroid, we first detect one receptor of pathogen-associated molecular patterns (PAMPs), TLR4. Both immunofluorescence analysis and western blot results in myoma tissue and primary cells excitingly revealed that TLR4 was expressed in uterine fibroid and UFC, and showed significantly higher level compared to smooth muscle tissue and SMC respectively. TLR-4, a LPS-specific receptor has been identified in chronic inflammation related gallbladder carcinoma and hepatocellular carcinoma and recently reported involved in adenomyosis and human ovarian granulose tumor development [37, 38]. Lipopolysaccharide (LPS) is a cell wall component of Escherichia coli, a Gram-negative bacterium that is most prevalent in the bowel [39]. TLR4 can mediate LPS-induced NF-κB signaling as reported. Functional TLR4 protein, which functions in complex with MD-2, has been attached primarily to the recognition of LPS and transduces signaling across the plasma membrane [40]. TLR4 is only one of TLRs that uses all four adaptor proteins and activates both the MyD88- and TRIF-dependent pathways. What's more, TLR4 activates the MyD88-dependent pathway earli-

er than the TRIF-dependent pathway [28]. TLR4 initially recruits TIRAP at the plasma membrane and subsequently facilitates the recruitment of MyD88 to trigger the initial activation of NF-κB [41], which is necessary for the induction of inflammatory cytokines (IL-6/8, COX-2) via TLR4 signaling. IL-6 links inflammation and carcinogenesis through stat3 phosphorylation, which was demonstrated by many previous study [42]. There is considerable evidences for NF-κB pathway in the progression of wound repair, fibrosis and tumor [30-32]. Our investigation shows that LPS-induced TLR4 activation and MyD88 dependent NF-κB signaling were significantly increased in myoma compared with myometrium. While blocking TLR4, the effect was obviously reduced, which referred the important role of TLR4/NF-κB signaling in the development of uterine fibroid.

The idea that injury or reproductive tract infections might trigger fibroid development was introduced decades ago [43], but has never been adequately tested. Our study indicates reproductive tract infections maybe have relationship with fibroid development. Although Shannon K et al. suggest that certain pathogens do not remain latent in fibroid tissue. He believes they could still have an acute "hit and run" effect on tumor initiation or tumor growth [43]. Moreover, the recent new concept of "endometrial subendometrial myometrium unit disruption disease" has been proposed in uterine myoma [43]. Andrea Ciavattini et al. indicate the interaction of endometrium and subendometrial myometrium in uterine fibroid pathogenesis [44]. All indications impressed that reproductive tract infections possibly associate tumorigenesis of fibroids. Nevertheless, the mechanism of uterine fibroid need further investigation.

In conclusion, the present study suggests that uterine fibroids and CD90+ fibroblasts express endotoxin-receptor TLR4. E. coli LPS can activate CD90+ fibroblasts and enhance TLR4/NF-κB signal transduction pathways. The modulation of TLR4/NF-κB signaling may be crosstalk with the mechanism of LPS-induced fibroblast activation. Fibroblast activated by LPS can promote peripheral cell biology effect, which maybe via autocrine and paracrine communication. In the end, TLR4 ligand mediated NF-κB signaling may involve in fibroid pathogen-

esis, thereby provide a potential new therapeutic target.

Acknowledgements

Disclosure of conflict of interest

None.

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