Abnormal expression of fibrosis markers, estrogen receptor α and stromal derived factor-1/chemokine (C-X-C motif) receptor-4 axis in intrauterine adhesions

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Abstract. Intrauterine adhesions (IUAs) are mainly derived from fibrous tissue formation following endometrial damage. The aim of the present study was to assess whether fibrosis markers, estrogen receptor (ER)α and the stromal derived factor (SDF)-1/C-X-C chemokine receptor type 4 (CXCR-4) axis are abnormally expressed in IUA endometrium. A total of 76 human endometrial biopsy samples (normal, n=20; mild-to-moderate IUAs, n=40; and severe IUAs, n=16) were employed, and Sprague-Dawley rat IUA models at different time points were constructed. Subsequently, the expression of transforming growth factor (TGF)-β1, matrix metalloproteinase (MMP)-9, ERα and the SDF-1/CXCR-4 axis was evaluated in human and rat IUAs using histology, immunohistochemistry, reverse transcription quantitative polymerase chain reaction and western blotting. In patients and rats with IUA formation, the expression of TGF-β1, MMP-9 and ERα was significantly higher compared with the control group at the mRNA and protein levels (P<0.05); in addition, in patients, the TGF-β1, MMP-9 and ERα levels were significantly higher in severe IUAs compared with those in mild-to-moderate IUA endometrium (P<0.05). Although the chemokine SDF-1 level in rats increased significantly during the early postoperative phase (reaching a peak at the second estrus phase) in rat endometrium (P<0.05), its special receptor CXCR-4 expression did not differ significantly compared with the control group in rats or patients (P>0.05). Our findings indicated that aberrant activation of fibrosis and expression of ERα may be involved in the pathology of IUA formation. The role of the SDF-1/CXCR-4 axis in IUAs as inflammatory medium in the short-term or special homing factors for bone marrow mesenchymal stem cells requires further verification in *in vivo* animal models.

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Introduction

Intrauterine adhesions (IUAs), also referred to as Asherman's syndrome, are mainly characterized by spanomenorrhea, amenorrhea, infertility, recurrent miscarriage, abdominal pain and other complications later in pregnancy (1). Those clinical symptoms are associated with major health concerns, particularly for women of childbearing age. As the pathogenesis of IUAs has not been fully elucidated, the successful pregnancy rate remains low, despite advances in therapeutic modalities.

IUAs usually develop following intrauterine surgery and infection. It has been made explicit that normal repair following trauma is regulated by a complex set of interactions in a network of pro- and anti-fibrotic cytokines. Once an insult has been delivered to the tissues, a fibrotic process is initiated with the activation of matrix-producing fibroblasts and accumulation of extracellular matrix (ECM) coupled with tissue regeneration. Any deregulation of the self-limited wound healing process and excessive accumulation of ECM may lead to abnormal formation of fibrous tissue (fibrosis) rather than normal tissue restoration (2). During this fibrous response, transforming growth factor (TGF)-β1, a multifunctional cytokine that regulates cell growth, adhesion, migration, apoptosis and differentiation, plays a crucial role in the canonical TGF-β/Smad signaling pathway (3,4). Furthermore, it may also regulate other downstream cellular responses, induce epithelial-to-mesenchymal transition (EMT) and mediate fibroblast activation, responses involved in facilitating fibrotic diseases (5,6). Matrix metalloproteinase (MMP)-9, the downstream target gene of TGF-β1, has been identified as an anti-fibrotic factor due to its proteolytic degradation of ECM that is usually downregulated in a number of fibrotic diseases.

By contrast, MMP-9 is occasionally significantly upregulated during EMT, promoting fibrous tissue proliferation, leading to chronic kidney disease and skin wound healing (7,8). However, the role of MMP-9 in the pathological process underlying the development of IUAs remains uncertain.

In addition, recent studies have demonstrated that endometrial stem cells are involved in endometrial regeneration, which may be crucial for the treatment of IUAs (9-11). Bone marrow stem cells (BMSCs) have been hypothesized to be important for endometrial regeneration and repair, but the underlying mechanism has not been reported in detail (12,13). Several lines of evidence indicate that the stromal derived factor (SDF)-1/C-X-C chemokine receptor type 4 (CXCR-4) axis plays a crucial role during BMSC homing (14,15). ERα is responsible for the estrogen involvement in cell growth and proliferation (16). Our previous cell study has demonstrated that ERα was able to effectively promote BMSC proliferation and migration via SDF-1/CXCR-4 signaling (17). The aim of the present study was to investigate the expressions of ERα and the SDF-1/CXCR-4 axis in human and rat endometrium with IUAs.

Materials and methods

Patient samples. A total of 76 patients were admitted to the First Affiliated Hospital of Chongqing Medical University between June 2015 and February 2016, and 56 cases (mild-moderate, n=40; and severe, n=16) were diagnosed with IUAs by hysteroscopy according to the standards of IUA diagnosis published by the American Fertility Society (AFS) (1,18). A total of 20 samples of normal endometrium were obtained following septate uterus excision and were used as the control group. All the endometrial biopsy samples were acquired from subjects during the luteal phase, in an attempt to avoid the influence of hormones, using disposable hysteroscopic endometrial biopsy catheters. The patients were aged 18-42 years, with a mean age of 27 years. Patients with additional endometrial complications, including dysfunctional uterine bleeding, polycystic ovary syndrome, adenomyosis, or other hormone-dependent conditions, were excluded. All the patients had regular menstrual cycles, with no hormone therapy at least 3 months prior to surgery; no pregnant or lactating patients were included. The study was approved by the Ethics Commission of Chongqing Medical University, and informed consent was obtained from all patients.

Experimental animals. A total of 100 female adult Sprague-Dawley rats, aged 6-8 weeks and weighing 220-280 g, provided by the Central Laboratory of Southwest Hospital, the First Affiliated Hospital of Third Military Medical University, were employed in this experiment. The rats were housed in a comfortable environment with controlled temperature, with free access to food and water. A 12-h dark and light cycle was maintained. Vaginal smears were collected at 8:00 a.m. and 3:00 p.m. daily to determine whether the animals had normal and regular estrous cycles. Through vaginal cytology assessment during two successive estrous cycles, a total of 80 rats that met the standards were selected and divided into 8 groups, including the control group (n=10) and 7 experimental groups (n=10 per group). The follow-up animal operations were all conducted during the estrus stage. All animal procedures in the subsequent experiments were approved by the Ethics Committee of the Third Military Medical University.

Animal model. Rat IUA models were constructed according to Jing *et al* and Hunter *et al* (12,19,20). Anesthesia was performed with 10% chloral hydrate (3 ml/kg, intramuscular injection) as previously described (21). All the animals were capable of breathing on their own during the entire procedure. The animals were then placed in a supine position and the lower abdomen was shaved and sterilized with 70% ethanol on the operating table. When the rats were confirmed to be sufficiently anesthetized, without righting or corneal reflexes, a vertical incision (2.5-3 cm) was performed until the bilateral uterine horns were exposed. After normal anatomy was verified, the junctions of the uterine horns and the proximal uterus were closed with clamps. Subsequently, ~0.5 ml of 95% ethanol was instilled into the lumen of the uterine horns for ~5 min using a 1-ml syringe. Before the abdomen was closed, the uterine cavity and peritoneal cavity were thoroughly rinsed with physiological saline. A comfortable environment was prepared for all rats postoperatively.

Animal specimen collection. To evaluate the IUA characteristics and development process, rat bilateral uterine horn tissues were collected from the control group (normal endometrium) and the experimental groups (at postoperative days 1 and 3 and the first, second, third, fourth and fifth estrus phase) and preserved in 10% paraformaldehyde and/or at -80˚C for the following experiments.

Hematoxylin and eosin (H&E) and Masson's trichrome staining. After fixation in 10% paraformaldehyde, endometrial samples from patients and uterine sections from rats were dehydrated in graded ethanol solutions, embedded in paraffin and cut into 6-μm transverse sections. H&E staining was applied to observe the morphological variations of the endometrium and confirm whether the thin endometrium of IUAs was successfully formed: First, the thickness of the endometrium was measured using a light microscope (XS-71; Leica Microsystems GmbH, Wetzlar, Germany) and an imaging analysis system (AxioVision rel.4.8; Carl Zeiss, Jena, Germany). In addition, endometrial gland count, gland density and other morphological variations were observed and evaluated under a light microscope at a magnification of x200 and/or x400, and the differences between the control and IUA groups were evaluated and compared with the Student's t-test and/or analysis of variance. Masson's trichrome staining was employed to confirm the degree of endometrial fibrosis in animal samples.

Immunohistochemistry. Slices were prepared by H&E staining. After the sections were dewaxed in xylene and hydrated with descending ethanol concentrations, the sections were heated in citrate buffer (pH 6.0; ZL1-9065; Zhongshan Jinqiao, Beijing, China) in a microwave oven for 20 min for antigen retrieval and then cooled naturally to room temperature. Washing the sections was performed in PBS for 3 min x 3 cycles prior to incubation in 3% H_2O_2 for 15 min at room temperature; then washing was performed with PBS for 5 min x 3 cycles. After the sections were blocked in 10% rabbit

serum for 30 min at room temperature, they were incubated overnight at 4˚C with rabbit anti-vimentin (dilution, 1:300; bs-8533R, Bioss, Beijing, China), rabbit anti-cytokeratin (dilution, 1:50; ab41825, Abcam, Shanghai, China), rabbit anti-CD34 (dilution, 1:300; Abcam), rabbit anti-ERα (dilution, 1:100; MAB57151, R&D Systems, Shanghai, China), mouse anti-TGF-β1 (dilution, 1:200; ab92486, Abcam), rabbit anti-MMP-9 (dilution, 1:100; ab76003, Abcam), and rabbit anti-CXCR-4 (dilution, 1:100; ab124824, Abcam) antibodies. Negative control included omission of the primary antibody and use of irrelevant primary antibodies. The sections were then incubated with the corresponding-secondary antibodies for 30 min at 37˚C. The slides were washed in PBS for 5 min for 3 cycles prior to incubation in horseradish enzyme labeled chain avidin solution for 30 min at 37˚C and washed in PBS for 5 min x 3 cycles. The sections were visualized by diaminobenzidine followed by counterstaining, dehydration, clearing and sealing. The slides were evaluated independently by 3 pathologists for distribution and intensity of signal. Intensity was scored from 0 to 3 as follows: 0, absent immunopositivity; 1, low immunopositivity; 2, moderate immunopositivity; and 3, intense immunopositivity. A mean of 22 fields was observed for each tissue. All values are represented as the mean ± standard error of the mean (SEM) (3,22).

Reverse transcription quantitative-polymerase chain reaction (RT-qPCR) assay. Total RNA was extracted using a high-purity total RNA rapid extraction kit (RP1201, BioTeke, Beijing, China) according to the manufacturer's instructions. cDNA was synthesized using the Rever Tre Ace-a kit (Toyobo Co., Ltd., Shanghai, China). The primers used for amplifying ERα, TGF-β1, MMP-9, SDF-1 and CXCR-4 were purchased from Jinmai Co., Ltd. (Chongqing, China). qPCR was performed using the ABI 7500 Real-Time PCR System (Applied Biosystems, Shanghai, China) according to the manufacturer's instructions using the SYBR-Green Premix Ex Taq kit (Toyobo Co., Ltd.). The PCR conditions were 96˚C for 30 sec, 57˚C for 30 sec, and 72˚C for 30 sec. The experiments were performed in triplicate for each sample. Relative quantification of mRNA was performed using the comparative threshold cycles (CT) method. This value was used to plot the gene expression employing the formula $2^{-\Delta\Delta Cq}$.

ERα, F: 5'-AACCACCTTTGATCTATTC-3' and R: 5'-GCGCCAGACCAGACCAATCATC-3'; TGF-β1, F: 5'-GACCGCAACAACGCAATCTATG-3' and R: 5'-CTCCACAGTTGACTTGAATC-3'; MMP-9, F: 5'-CCCTACTGCTGGTCCTTCTG-3' and R: 5'-GACCGTCCTTGAAGAAATGCAG-3'; SDF-1, F: 5'-TGCACAATGGAGCTTTTATAAC-3' and R: 5'-AAAGCAAACCGAATACAGAC-3'; and CXCR-4, F: 5'-AGGCCGTCTATGTGGGTGTCTGG-3' and R: 5'-GAGGGCCTTGCGCTTCTGG-3'.

Western blot analysis. Tissues were lysed with RIPA buffer containing protease inhibitors (Roche, Shanghai, China) following grinding in a mortar. The cell lysates were centrifuged at 16,000 x g for 15 min at 4˚C and the supernatants were collected and the protein contents were determined using a bicinchoninic acid protein assay kit. Protein extracts were loaded on a 10% sodium dodecyl sulfate-polyacrylamide gel for electrophoresis and transferred onto polyvinylidene fluoride membrane. The blots were blocked in 5% skimmed milk in Tris-buffered saline containing 0.1% Tween-20 for 2 h at room temperature, and then incubated with the primary antibody at 4˚C overnight. Anti-ERα mouse polyclonal antibody (1:1,000), anti-TGF-β1 mouse polyclonal (1:1,000); anti-CXCR4 rabbit polyclonal antibody (1:500), anti-SDF-1 rabbit polyclonal antibody (1:1,000) or anti-MMP-9 rabbit polyclonal antibody (1:1,000) was incubated with the membrane for 2 h at 37˚C. Secondary antibodies that were conjugated to horseradish peroxidase were incubated with the membrane for 1 h at 37˚C. The proteins that were revealed by western blot were visualized using chemiluminescence (Biyuntian Company, Shanghai, China). The densities of the bands were analyzed using a gel imaging system and calculated compared with the internal control.

Statistical analysis. All data were analyzed with SPSS software version 22.0 (IBM Corp., Armonk, NY, USA). Histological and immunohistochemical data are presented as mean ± SEM. Other data are reported as mean ± standard deviation. Each experiment was performed in triplicate. Student's t-test was employed for within-group comparisons and analysis of variance for multiple groups. Statistical significance was defined as a P-value of <0.05.

Results

Immunohistochemical findings. Hematoxylin and eosin staining revealed the following: Compared with the control group, the thickness and number of glands of the IUA endometrium were significantly reduced, whereas the cuboid epithelial cells of the endometrium were gradually replaced by low columnar cells or were absent ($P<0.05$; Fig. 1A and B). The IUA endometrium was thinner and less continuous, with irregularly structured, sparse glands. Endometrial epithelial cells and glandular epithelial cells were transformed into low columnar or even flat cells (Fig. 1A).

In order to determine the fibrotic characteristics in rat IUAs, Masson's trichrome staining was employed to detect fibrosis. It was observed that, as the time progressed postoperatively, the endometrium was gradually replaced or covered by fibrous scar tissue (Fig. 1C).

The protein expression levels of keratin, vimentin and CD34 in rats were detected by immunohistochemistry. Owing to reduction of cells lining the endometrial cavity/glandular epithelial cells, keratin protein expression in rat experimental groups was significantly decreased compared with the control group ($P<0.05$). Due to the reduction of stromal cells and capillaries, vimentin and CD34 protein expression were significantly decreased in rat experimental groups compared with the control group ($P<0.05$; Fig. 2).

The expression levels of ERα, TGF-β1 and MMP-9 were significantly increased in patients with IUAs. To investigate the association among fibrosis, ERα and IUA endometrium development and progression, TGF-β1 and MMP-9 expression was detected in patient endometrium with different degrees of IUAs. As shown in Fig. 3, the results of protein and mRNA analysis revealed that TGF-β_1 and MMP-9 were significantly increased in the IUA groups compared with the control group ($P<0.05$) (Fig. 3). Furthermore, TGF-β1 and MMP-9 in severe

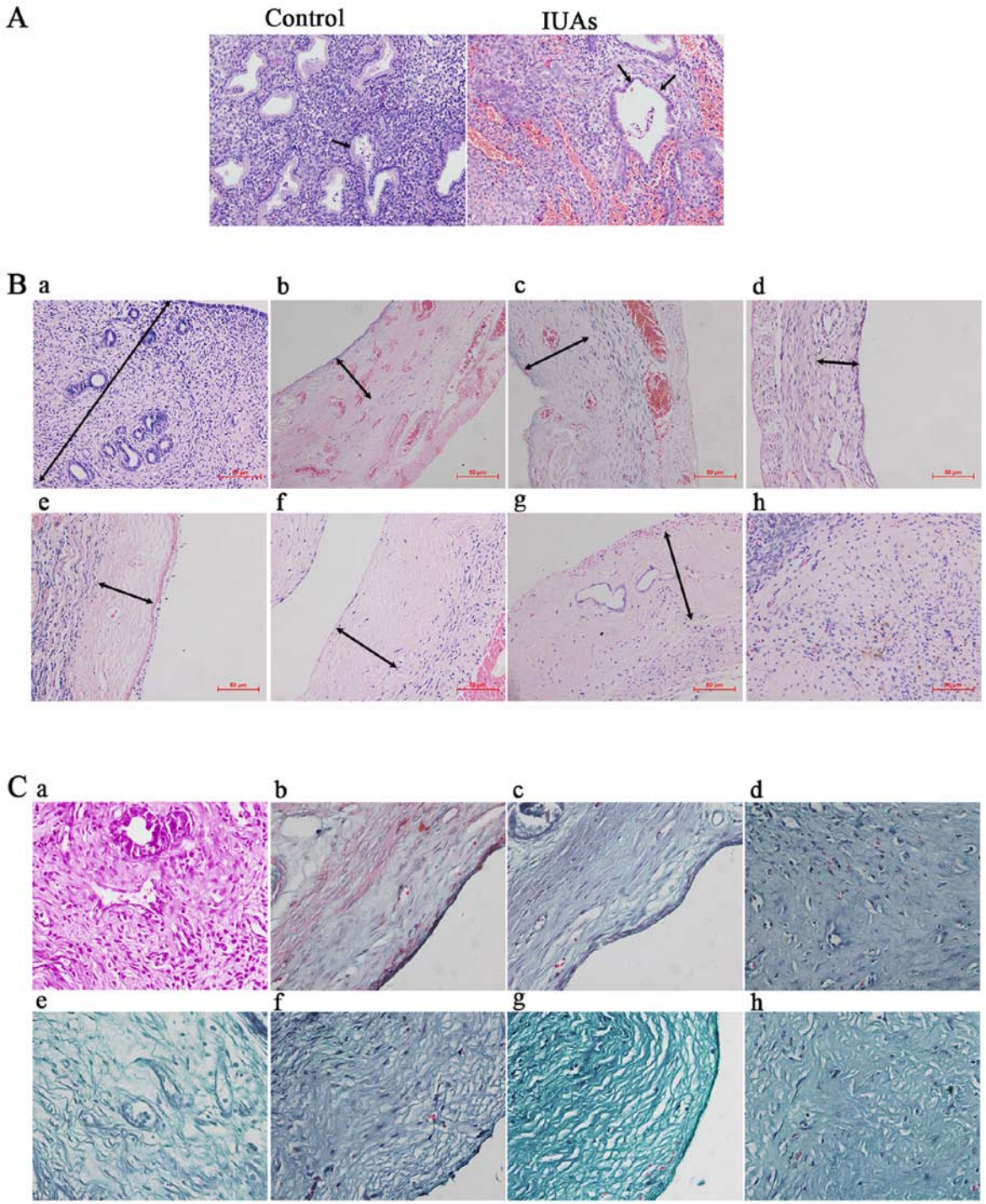

Figure 1. Hematoxylin and eosin staining (H&E) and Masson's trichrome staining for patients and rats with intrauterine adhesions (IUAs). (A) H&E staining of patient endometrium with IUAs (x200). Black arrow in control group indicates cuboid epithelial cells; black arrows in the IUAs group indicate low columnar and flat glandular epithelial cells. (B) H&E staining of rat endometrium (x200). a, Control group endometrium. Black arrow indicates the thickness of normal endometrium; b→h, experimental groups on postoperative days 1 and 3, and in the first, second, third, fourth and fifth estrous cycles, respectively. Black arrows indicate the reduced thickness of the endometrium. (C) Masson's trichrome staining for endometrial fibrosis of rat IUAs (magnification, x400). a, Control group endometrium; b→h, representative fibrotic endometrium on days 1 and 3, and at the first, second, third, fourth and fifth estrous cycles after the operation, respectively.

IUA endometrium were significantly higher compared with those in mild-to-moderate IUA endometrium ($P<0.05$; Fig. 3). For ERα, the expression levels detected by western blotting, immunohistochemistry and RT-qPCR in the IUA groups were significantly higher compared with those in the control group; similar to TGF-β1 and MMP-9, ERα expression in the severe IUA group was significantly higher compared with that in the mild-to-moderate group. ($P<0.05$; Fig. 3).

The expression of ERα, TGF-β1 and MMP-9 was significantly increased in rat endometrium with IUAs. To identify the association of ERα, TGF-β1 or MMP-9 with IUA formation and degree of progression, rat uterine tissues were collected

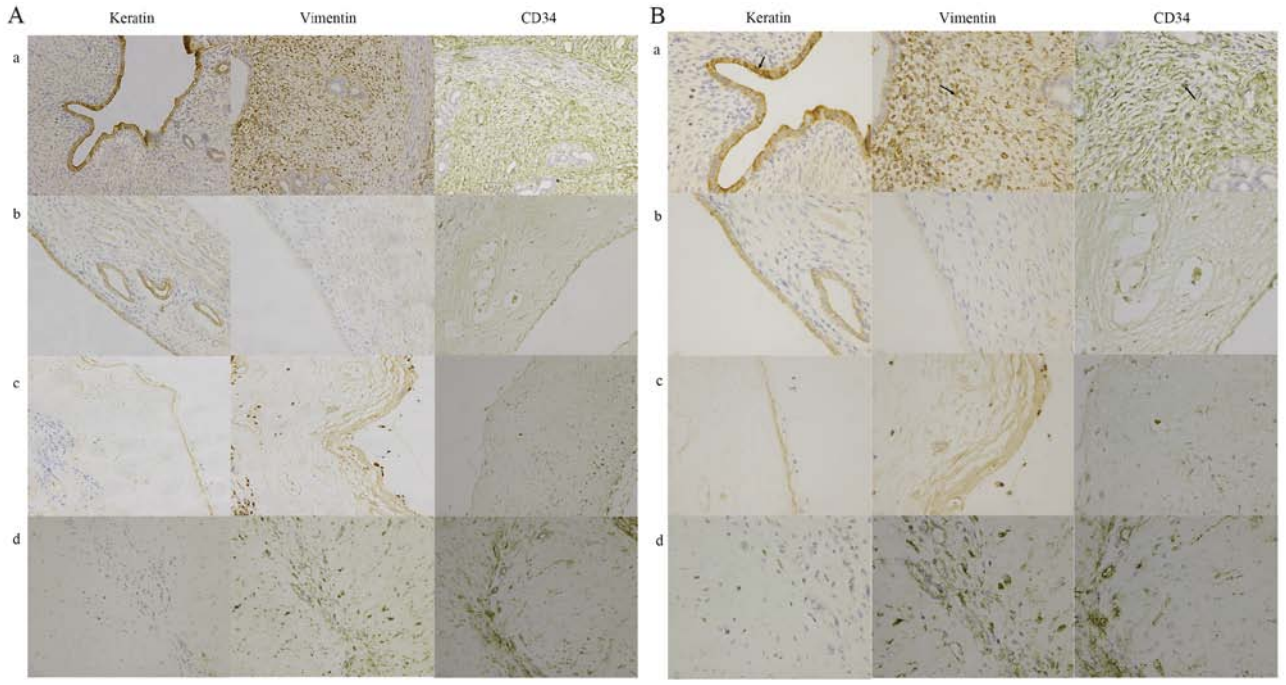

Figure 2. Immunohistochemistry for keratin, vimentin and CD34 of rat endometrial tissues (magnification, A: x200 and B: x400). a, Control group endometrium; b→d, compared with the control group, the expression of keratin, vimentin and CD34 (arrows) were all significantly decreased at the first third and fifth estrous cycles after the operation.

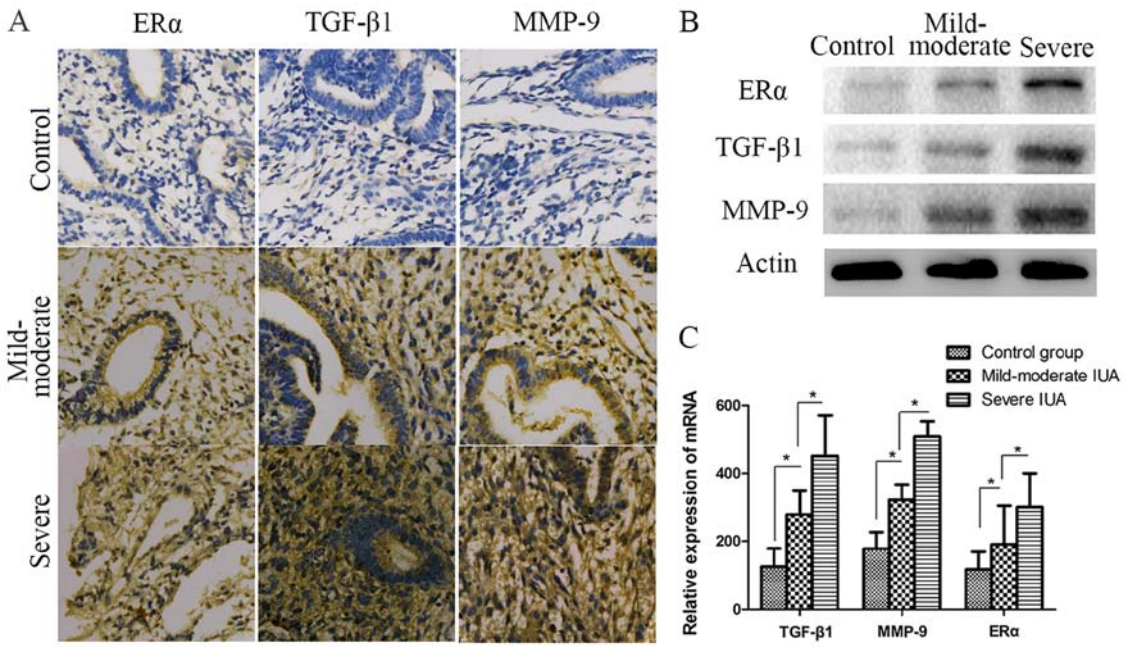

Figure 3. Expression levels of estrogen receptor α (ERα), transforming growth factor-β1 (TGF-β1) and matrix metalloproteinase-9 (MMP-9) in patients with intrauterine adhesions (IUAs). (A) The protein expression levels of ERα, TGF-β1 and MMP-9 in human endometrial tissues were detected by immunochemistry (magnification, x400). (A) Protein expression in endometrial tissues of the control group, mild-to-moderate IUAs and severe IUAs patients. (B) The protein expression levels of ERα, TGF-β1 and MMP-9 in human endometrial tissues were detected by western blotting. (C) The mRNA expression levels of ERα, TGF-β1 and MMP-9 in human endometrial tissues were detected by reverse transcription-quantitative polymerase chain reaction (*P<0.05).

after surgery at different time points, as previously mentioned (at postoperative days 1 and 3 and at the first, second, third, fourth and fifth estrous cycles), for protein and mRNA determination. Compared with the control group, ERα and TGF-β1 were significantly reduced on postoperative days 1 and 3, reaching their lowest level on postoperative day 3; subsequently, over time, the expression levels of ERα and TGF-β1 started to increase at the first postoperative estrous cycle, and then significantly exceeded the ones in the control group from the second postoperative estrous cycle onwards (P<0.05; Fig. 4).

The expression of MMP-9 during the early phase was significantly increased from day 1 after surgery, reaching its

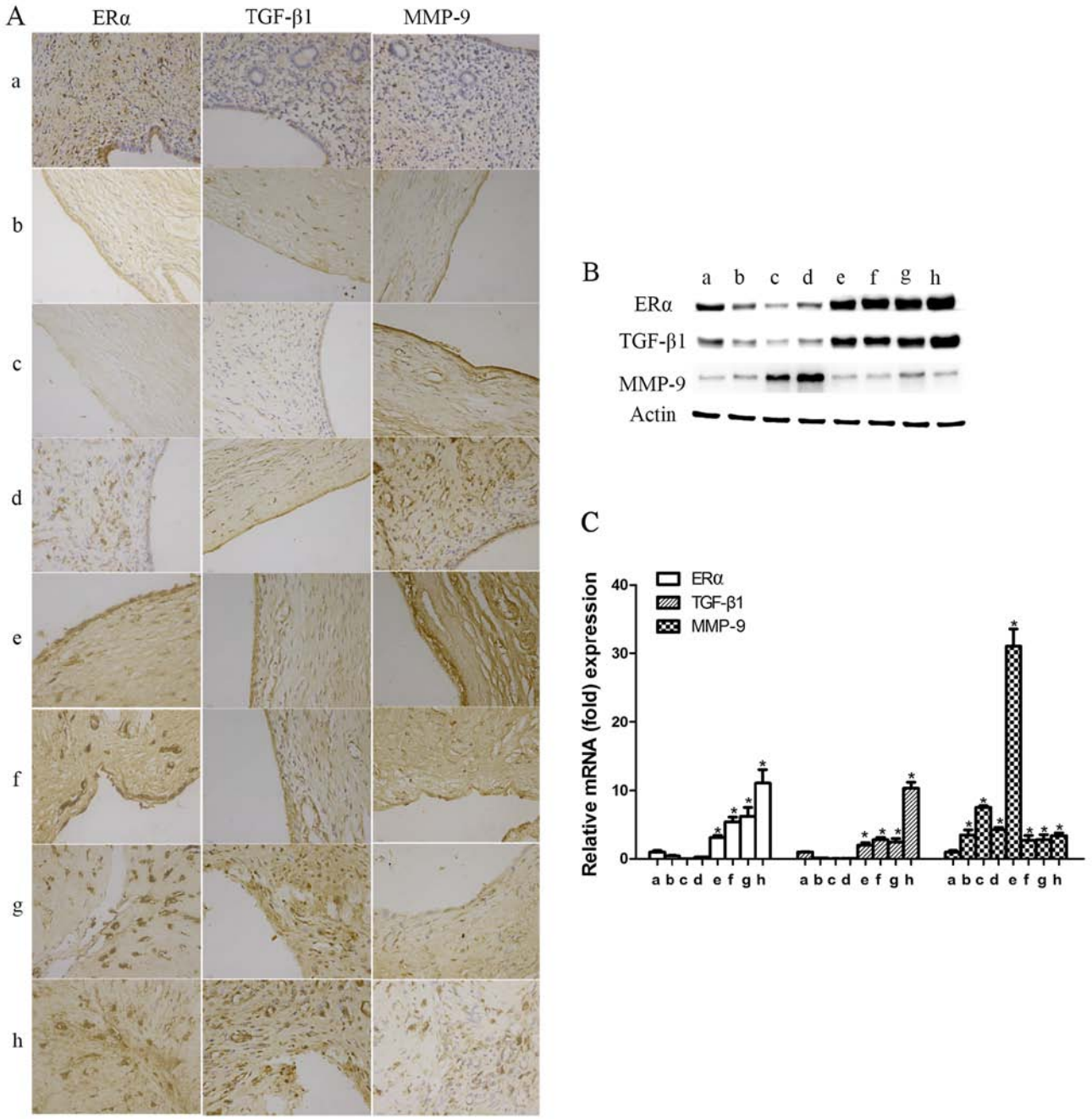

Figure 4. Expression levels of estrogen receptor α (ERα), transforming growth factor-β1 (TGF-β1), matrix metalloproteinase-9 (MMP-9) in rats with intrauterine adhesions (IUAs). (A) The expression levels of ERα, TGF-β1 and MMP-9 in rat endometrial tissues were detected by immunochemistry (magnification, 400). (B) The relative protein expression was detected by western blotting, and the results were in accordance with those of immunochemistry. (C) The mRNA expression levels of ERα, TGF-β1 and MMP-9 in rat endometrial tissues were detected by reverse transcription-quantitative polymerase chain reaction. a, ERα, TGF-β1 and MMP-9 in the endometrium of the control group; b→h, ERα, TGF-β1 and MMP-9 in the endometrium of the experimental groups on postoperative days 1 and 3, and in the first, second, third, fourth and fifth estrous cycles (*P<0.05).

highest level at the second postoperative estrous cycle (P<0.05; Fig. 4). Similar to humans, MMP-9 expression was found to be significantly upregulated at the fifth estrous cycle (P<0.05; Fig. 4). These findings suggest that TGF-β1, MMP-9 and ERα were involved in IUA development and progression, but the underlying mechanism has not been reported in detail.

To determine whether the SDF-1/CXCR-4 axis affects the pathogenesis of IUAs, the expression of SDF-1 and CXCR-4 was measured in the endometrium of patients and rats with IUAs. In the human experiments, the expression of SDF-1 and CXCR-4 at the protein and mRNA level, whether in the mild-to-moderate or the severe IUA group, did not differ significantly compared with the control group (P>0.05; Fig. 5). In the rat experiments, with progressing time after surgery, SDF-1 expression exhibited an increasing tendency in the early phase, reaching its highest level at the second postoperative estrus phase (P<0.05), after which time it again decreased (Fig. 6); as regards CXCR-4, there was no significant upregulation observed at any of the detection time points (P>0.05; Fig. 6).

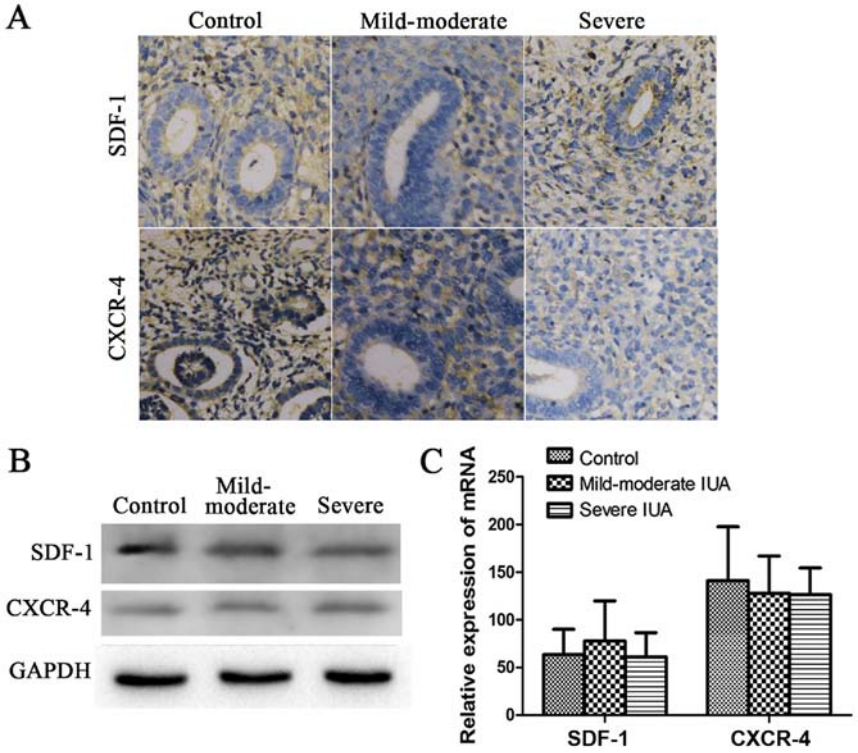

Figure 5. Expression levels of stromal derived factor-1 (SDF-1)/chemokine (C-X-C motif) receptor 4 (CXCR-4) axis in patients with intrauterine adhesions (IUAs). (A) The protein expression levels of SDF-1 and CXCR-4 in the human endometrial tissues were detected by immunochemistry (magnification, x400). (B) The protein expression levels of SDF-1 and CXCR-4 in the human endometrial tissues were detected by western blotting. (C) The mRNA expression levels of SDF-1 and CXCR-4 in the human endometrial tissues were detected by reverse transcription-quantitative polymerase chain reaction (P>0.05). GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

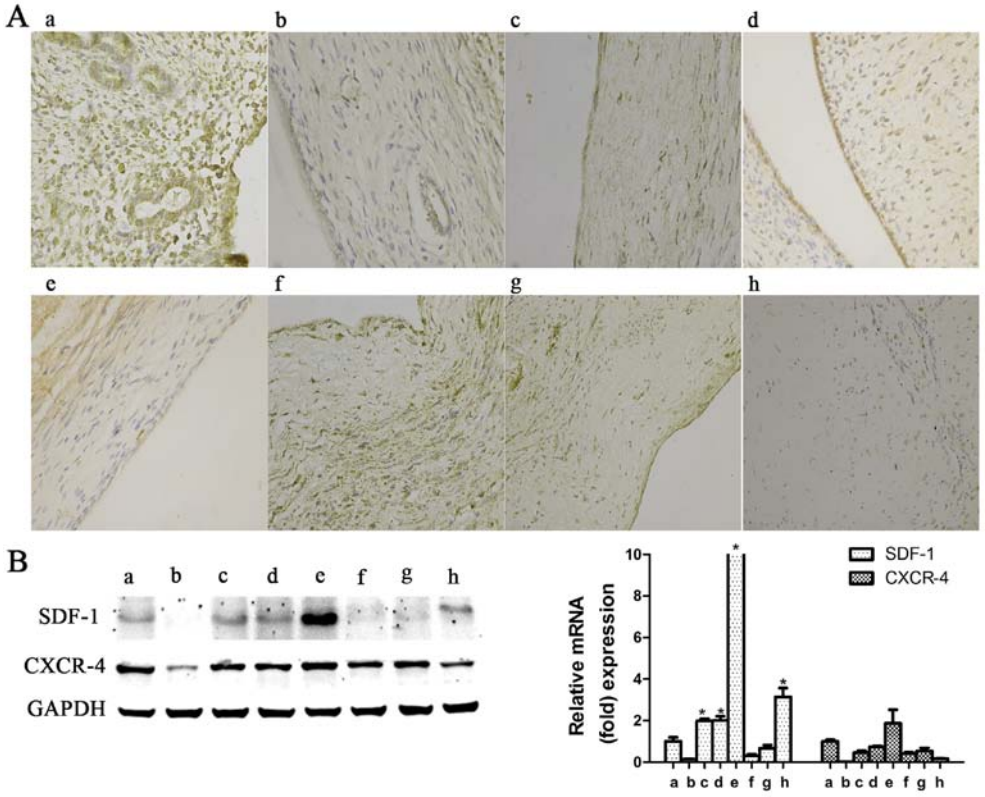

Figure 6. Expression levels of stromal derived factor-1 (SDF-1)/chemokine (C-X-C motif) receptor 4 (CXCR-4) axis in rats with intrauterine adhesions (IUAs). (A) The expression level of CXCR-4 in rat endometrium with IUAs detected by immunochemistry (magnification, x400). (B) SDF-1 and CXCR-4 were detected by western blotting in rat endometrium. The mRNA expression levels of SDF-1 and CXCR-4 in rat endometrial tissues were detected by reverse transcription-quantitative polymerase chain reaction. a, Control group endometrium; b→h, endometrium in experimental groups on days 1 and 3, and in the first, second, third, fourth and fifth estrous cycles after the operation, respectively (*P<0.05). GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Discussion

In the present study, significant upregulation of TGF-β1, MMP-9 and ERα expression was detected in patients and rats with IUAs, despite a minor fluctuation of the MMP-9 level in rats. In addition, we found that SDF-1 and CXCR-4 did not differ significantly in the endometrium of the patients, whereas in the endometrium of the rats, SDF-1 expression was significantly increased during the early postoperative phase and then sharply declined; however, CXCR-4 expression in rat endometrium did not differ significantly after surgery. These results suggest that the formation and development of IUAs is mainly associated with excessive fibrosis and insufficient restoration of the endometrium induced by various cytokines and growth factors.

At high-power magnification, it was observed that, along with the development and progression of IUAs, fibrotic tissues gradually covered or replaced the normal endometrium and promoted the formation of IUAs (Figs. 1 and 2). This outcome suggests that fibrosis plays a key role in IUAs, which was also demonstrated by previous studies (3,4). TGF-β1, as a pivotal mediator and indicator of fibrogenesis, has been found to be implicated in the pathogenesis of numerous fibrotic diseases, such as cardiac fibrotic and hypertrophic remodeling, hepatic fibrosis and chronic kidney diseases (23,24). An increased TGF-β1 level is often present in tissues exhibiting an uncontrolled fibrotic response. In the present study, animal and human subjects were used to investigate the expression of TGF-β1 in endometrium with IUAs, and it was observed that, compared with the control group, the mRNA and protein levels of TGF-β1 were significantly upregulated in the experimental groups, and the degree of endometrial fibrosis was consistent with the expression level of TGF-β1 during the formation of IUAs (Figs. 3 and 4). Based on these results, it may be argued that TGF-β1 contributes to fibrosis development and progression of IUAs, which was also supported by Salma *et al* (4) and other scholars (25,26). Moreover, as a downstream target gene of TGF-β1, MMP-9 has been previously considered to be an anti-fibrotic factor due to its ability to degrade and remodel the ECM (7,27). It was previously reported that MMP-9 expression in IUAs was inversely correlated with endometrial fibrosis (26). However, in the present study, we found that MMP-9 was involved in the development and progression of IUAs with its pro-fibrotic function, particularly at the early phase. This result is consistent with previous findings indicating that MMP-9 inhibitors could effectively alleviate chronic kidney fibrotic diseases, particularly at the early stages (28). Therefore, it is hypothesized that, in addition to their contribution to the generation of myofibroblasts through EMT and involvement in the pro-fibrotic role of interstitial macrophages, MMPs are also dysregulated and involved in every aspect of inflammation and tissue repair (29).

ERα is a well-known nuclear transcription factor, proven to promote the proliferation or metabolism of endometrial epithelial cells after combining with estrogen, and increasing the synthesis of intracellular DNA and protein (30). Estrogen is usually included in the clinical therapy of IUAs to promote the proliferation and repair of the endometrium. A successful case of prolonged estrogen supplementation prior to conventional controlled ovarian hyperstimulation in a woman who had experienced repeated implantation failure due to an unresponsive thin endometrium was presented by Shen *et al* (31). In addition, Cai *et al* (32) also demonstrated that the administration of estrogen exerts a preventive effect on the development of endometrial fibrosis in rats and rabbits. The TGF-β1 signaling pathway may be interposed by functional coadjutant interactions between Smad and other types of transcriptional factors, kinase receptors and nuclear receptors. Inhibition of ERα-dependent TGF-β1/Smad signaling may involve the regulation of renal fibroblast activation with its potential preventive mechanism (33).

In view of those findings, we examined endometrial tissues of humans and rats with IUAs and observed that the expression of ERα in the experimental groups was significantly higher compared with that in the control group, and that ERα expression in severe IUA endometrium was significantly higher compared with that in mild-to-moderate IUA endometrium (Figs. 3 and 4). Another notable finding is that attracted the expression tendency of ERα corresponded to the expression of TGF-β1 in human and rat IUAs. These results suggest that the formation of IUAs is possibly associated with abnormal upregulation of ERα, which is consistent with the findings in cardiac failure (34). In the present study, the active upregulation of ERα may have been derived from lack of estrogen in the endometrium with IUAs. The activation of endometrial excessive fibrosis unconventionally stimulated by TGF-β1 in IUAs induced ERα upregulation. ERα in endometrium with IUAs is prevented from interacting with estrogen, resulting in a relative lack of estrogen at the endometrium rather than a shortage of estrogen in the circulation, with further abnormal upregulation of ERα in the IUA endometrium. Estrogen superabundance is detrimental to endocrine organ function and even aggravates fibrosis to some degree. Therefore, for IUAs patients, individualizing the dose of estrogen is advocated in current clinical practice.

BMSCs, a category of CXCR-4-expressing bone-derived multifunctional stem cells that are capable of differentiating into lineages of cells, have been investigated as a therapy for a number of diseases. Zhao *et al* (12,13) and Alawadhi *et al* (35) have also demonstrated that ectogenic BMSCs administered systemically or locally have the potential to selectively migrate to and repair the injury, but the underlying mechanism has not been reported in detail. It is well known that chemokine SDF-1 and its special receptor CXCR-4 play a crucial role in BMSC homing (14,15,36). Our previous cell study demonstrated that ERα may promote BMSC proliferation and migration via SDF-1/CXCR-4 (17). In the present study, the expressions of SDF-1 and CXCR-4 exhibited no obvious change in patient endometrium (Fig. 5). SDF-1 is a dynamically altered chemokine secreted by damaged tissues. Samples of endometrium with IUAs, particularly in patients with fertility requirements, cannot be frequently collected for investigation. In the present study, animal groups with different sampling time points were established to monitor variations in SDF-1 and CXCR-4 after surgery in rats. There was no significant upregulation of CXCR-4 expression in the endometrium with IUAs, while SDF-1 exhibited an increasing tendency at the early phase (Fig. 6). In view of this finding, it was hypothesized that SDF-1 may be one of inflammatory mediators of short-term endometrial damage, but not a BMSC-specific chemokine. In addition, it may also be hypothesized that autologous CXCR-4-expressing BMSCs are incapable of homing and differentiation to endometrial cells for a short time following endometrial damage;

however, the recovery effect of SDF-1/CXCR-4 axis-mediated BMSC homing for IUAs in the long-term requires further investigation.

In conclusion, the present study investigated the expression of fibrotic factors, ERα and SDF-1/CXCR-4 axis in IUAs. It is recommended that the dose of estrogen should be individualized in the treatment of IUAs. In addition, it may be hypothesized that the formation and development of IUAs are closely associated with an abnormal endometrial fibrosis microenvironment (niche) involved with TGF-β1 and MMP-9, and inadequent normal endometrial regeneration involved with various growth factors and cytokines. Thus, specific interventions are crucial for suppressing excessive fibrosis and providing a protective effect by promoting endometrial restoration during the early stages of endometrial injury. However, the specific underlying mechanisms require further elucidation, and whether SDF-1/CXCR-4 axis-mediated BMSC homing and differentiation is required for endometrial restoration requires verification in future studies.

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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

ZQ and WX conducted the experiments and the data analysis and wrote the article. HJ and YR revised the article and provided a critical review of concepts. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Ethics Committee of the First Affiliated Hospital of Chongqing Medical University approved the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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