Decreased CXCL5 expression associated with intrauterine adhesions in a rat model and human endometrial tissues

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Abstract

Background

Intrauterine adhesion (IUA) is a consequence of endometrium damage, leading to infertility, recurrent miscarriage, and various complications of pregnancy. IUA usually occurs after an infection or injury-related inflammation of the endometrium. CXCL5, a member of chemokines, is associated with the remodeling of connective tissues, but its roles in the formation of IUA remain uncertain. This study aims to investigate the expression and effect of CXCL5 in the development of IUA.

Results

CXCL5 protein level in the endometrium of IUA rat is significantly decreased compared with that in normal and sham-operated endometria of rats ($P < 0.001$). Furthermore, real-time PCR and western blotting assays also show that the CXCL5 expression in endometrial tissues from IUA patients is much lower than that in normal endometrium ($P < 0.01$), which is consistent with the results based on the rat model. CXCL5 significantly up-regulates MMP9 expression and slightly up-regulates p65 expression in human endometrial cells after CXCL5 overexpression.

Conclusions

These results show that CXCL5 plays an important role in the inhibition of IUA formation after injury by modulating MMP9 expression and therefore has a protective effect of CXCL5 in the adhesion formation of IUA. These findings provide valuable information for evaluating prognosis and guiding therapy of intrauterine adhesions.

Background

Intrauterine adhesion (IUA), also known as Asherman syndrome, is characterized by adhesions and fibrosis in endometrium within the uterus and/or cervix, and partial or complete obliteration of the uterine cavity [1, 2]. IUA occurs most commonly and frequently after trauma or infection, such as a simple operation on the uterus like a cesarean section, especially during the postpartum period with low estradiol levels [3]. The most common early symptoms of IUA patients are menstrual abnormalities, recurrent abdominal pain, and amenorrhea [4]. IUA often leads to infertility, recurrent miscarriage, and various complications of pregnancy, such as placenta implantation, placental abruption, preterm premature rupture of membranes, and developmental abnormalities in the fetus as a result of the loss of normal endometrium and insufficient the blood supply to the uterus [5–7]. In recent years, IUA has become one of the common causes of female infertility in China, and its incidence has been on the rise with uterine cavity surgery increasing [4, 8]. Thus, the prevention and treatment of IUA are of great value in maintaining women's health as well as meeting fertility requirements.

Very recently, hysteroscopy has been widely considered a gold standard to both diagnose and treat IUA. Meanwhile, various techniques for adhesiolysis and prevention of scar formation have also been applied in the clinic [9]. Indeed, the hysteroscopy with lysis of the scar tissue shows its huge advantage of restoring normal uterine function in many cases. However, such therapy is inapplicable in severe cases due to the likelihood of recurrence after surgical repair [10]. Reported statistics show that the postoperative endometrial recovery and clinical cure rate of patients with severe IUA are much worse than those with mild and middle IUA [11, 12]. These results indicate that the clinical prognosis of IUA patients is related to the degree, range, residual endometrial area before surgery, and endometrium growth. In order to further reveal the progression and relative molecular mechanisms underlying IUA, molecular biology studies are highly desired for the next-generation treatment and prevention of the postoperative re-adhesion of patients.

The basal layer is an essential structure in the human endometrium, located between the myometrium and the functional layer [6]. It contains various progenitors and stem cells that provide the regenerative capacity and participate in the remodeling of the endometrium after the loss of the functional layer in physiological menstrual cycles [9, 13]. Once the basal layer of the endometrium is severely damaged, endogenous endometrial stem cells are damaged, and thus endometrial regeneration ability is lost, usually leading to IUA [14, 15]. More importantly, a broad range of inflammatory chemokines, together with cytokines and cell adhesion molecules, are abnormally expressed during the development of IUA and participate in the regulation of secretion and the degradation of extracellular matrix components in the fibrosis of the endometrium [15, 16]. Therefore, the urgency for exploring the mechanism underlying the role of inflammatory chemokines in the process of adhesion formation has been raised for providing new therapeutic targets for IUA.

CXC motif chemokine ligand 5 (CXCL5), also known as epithelial neutrophil-activating peptide-78 (ENA-78), is a member of the CXC subfamily of chemokines and first cloned in 1994 by Chang [17]. After binding with its receptor CXCR2, CXCL5 enables the recruitment of neutrophils followed by the promotion of angiogenesis and the remodeling of connective tissues [18]. Recently, Konduru, A.S. reported that the knockdown of endogenous p65 suppresses CXCL5 gene transcription *in vivo* [19], indicating that the CXCL5 expression level is regulated by the NF- κ B pathway. In contrast, the silence of CXCL5 expression inhibited the NF- κ B pathway in cells also has been demonstrated [20]. Moreover, the mRNA and protein levels of p65 significantly increase and associate with the degree of adhesion in IUA patients [21, 22], suggesting CXCL5 might be involved in the development of IUA mediated by the NF- κ B pathway. It is also noted that MMP9, a class of the matrix metalloproteinase, plays an essential role in the degradation of the extracellular matrix and the fibrous tissue of IUA patients. The low expression of MMP9 in IUA patients results in excessive deposition of extracellular matrix as well as the normal endometrium is replaced by fibrous connective tissue, ultimately leading to endometrium fibrosis and adhesion formation of the endometrium [23]. Although the CXCL5/CXCR2 axis is essential for the cell adhesion and migration followed by matrix remodeling by activating PI3K/AKT-induced up-regulation of MMP9 in bladder cancer [24], the role of CXCL5 in the process of the adhesion formation and fibrosis proliferation in IUA remains unclear.

In this study, we investigate the expression of CXCL5 and its potential role in IUA. CXCL5 has a decreased expression both in IUA animal models and patient endometria. Such low expression of CXCL5 may induce MMP9 decreased expression and lead to the formation of intrauterine adhesion. These results show that CXCL5 plays a key role in inhibiting IUA formation and thus is a promising therapeutic target for the prevention and treatment of IUA.

Results

Morphological changes of endometrium in IUA animal models

Previous studies have shown a correlation between the chemokines with the degradation of the extracellular matrix and fibrosis [25, 26]. In order to investigate the potential role of CXCL5 in IUA pathogenesis, we first established a rat model of endometrium adhesion with phenol mucilage. All rat uteruses were resected after 14 days of operation, and the gross morphology displayed that contracture occurred in rats' uterus of the IUA group rather than that in normal and sham-operated groups (Fig. 1A-C). Microscopy images in Fig. 1D-F show that the endometrial cavity surface is covered by regularly arranged columnar epithelium with abundant endometrial glands both in control and sham-operated groups. Compared with the normal and sham-operated groups in Fig. 1, the uterine endometrial glands of IUA rat models are remarkably reduced with tissue hyperplasia, and large amounts of macrophages and plasma cells are replaced by collagen fibers, indicating that inflammation is involved in IUA pathogenesis.

Degree of endometrial fibrosis in the IUA rat model

To evaluate the degree of endometrial fibrosis, we performed Masson staining and observed a lot of stromal blue collagen fibers in the IUA model group, which were tightly arranged in the endometrium (Fig. 2). Besides, the histological examination of IUA uterus shows the presence of endometrium adhesion and fibrosis. HE and Masson staining results confirm the IUA rat model was successfully established.

CXCL5 down-regulation associated with IUA in rat models

Previous studies [20, 21, 23, 24] imply that the abnormal expression of CXCL5 might play an important role in the occurrence of IUA according to existing publications. To this end, we examined the CXCL5 expression in the endometrium of the IUA rats, sham-operated, and normal group by immunohistochemistry. The uteruses of normal and sham-operated groups show intense positive CXCL5 staining, which is mainly expressed in the cytoplasm of glandular epithelial and stromal cells (Fig. 3). The staining quantification indicates that CXCL5 expression level in the IUA group is significantly lower than those in both the sham-operated group and normal group, while there is no obvious difference between normal and sham-operated groups (Fig. 3J). All these results indicate that CXCL5 is involved in the suppression of the endometrium adhesion formation during the genesis and development of IUA.

Reduced expression of CXCL5 in endometria of IUA patients

In order to further confirm the low expression of CXCL5 in IUA patients, we then examined the expression level of CXCL5 in the endometria of 6 IUA patients and 6 normal patients, respectively. The mRNA expression level of CXCL5 in the endometrial samples of IUA patients (Fig. 4A) is significantly down-regulated ($P < 0.01$) compared with the normal endometria (Ctrl). Similarly, CXCL5 protein expression in the endometria of IUA patients also is remarkably lower than that in the normal endometria (Fig. 4B). Figure 4C shows that the statistically significant difference of CXCL5 level ($P < 0.01$) during the two groups of patients. These results indicate that a reduced expression level of CXCL5 exists in endometrial tissues both of IUA rats and patients, and give strong evidence that CXCL5 plays a protective role in inhibiting genesis of IUA.

CXCL5 positively regulates MMP9 and p65 expression in endometrial cells

It has been reported that low expression of MMP9 exists in IUA endometrial tissues, and its protein level can be regulated by CXCL5 through PI3K/AKT signaling [23]. Recently, Xue *et al.* demonstrated that the p65 expression is increased in IUA, and CXCL5 and p65 positively promote the protein expression of each other [22]. To further confirm the correlation between the expression of CXCL5, MMP9, and p65 in IUA endometrial tissues, we performed western blotting to detect the protein level of CXCL5, MMP9, and p65 after transfecting overexpression plasmid of CXCL5 in Ishikawa cells. A significant increase in MMP9 expression and a slight increase in p65 expression were observed with the increase of CXCL5. Representative results are shown in Fig. 4D, and the relative expression of proteins was calculated in Fig. 4E, indicating that the overexpressed CXCL5 up-regulates both MMP9 and p65 protein levels in human endometrial cells. It is important to point out that the low expression of CXCL5 in the endometrium of both IUA rats and patients in Figs. 3 and 4, and previous studies have shown that MMP9 is poorly expressed while p65 is highly expressed in IUA samples [22, 27]. These facts confirm that the low expression of CXCL5 inhibits the adhesion formation in IUA by decreasing the protein level of MMP9 rather than p65.

Discussion

IUA is the most common syndrome affecting fertility and pregnancy outcomes and usually occurs after an infection or injury-related inflammation [3]. The present clinical treatment of IUA is mainly restoring the shape of the uterine cavity, but the efficacy is unsatisfactory in the repair of endometrium function [9], driving the enthusiasm for research on the molecular mechanism of IUA pathogenesis. Here, we first demonstrate a reduced CXCL5 expression in the endometrium of both IUA rats and patients and show that CXCL5 plays key roles in protecting endometrium and preventing fibrosis and adhesion formation.

Collecting a large number of human endometrium samples is known to be difficult in obtaining ethical approval from the Ethics Committee, together with the experimental time and cost. Compared with clinical samples, the IUA rat model is more widely used for the immunohistochemistry analysis of endometrium in IUA [6, 28], because it can closely mimic the development and pathological characteristics of IUA patients and its experimental conditions can be easy to control. To investigate the molecular mechanism of IUA genesis, we established an IUA rat model to mimic the condition in human IUA by using the phenol mucilage method. Different from many other model animals, A rat has two distinct cervixes and uterine bodies, which is an important advantage for studying IUA. The pathological characteristics of rats more closely mimic the human disease, and the cost in feeding rats is very low, compared with other larger animals such as rabbits. On the other hand, the size of the rat is still large enough for the convenience of performing surgical procedures compared with other smaller animals such as mice. Therefore, rats are commonly used for establishing an ideal model for uterine diseases, particularly for IUA.

Phenol mucilage is widely used for establishing the inflammation-induced adhesion animal model in IUA [21, 29, 30] and other various medical studies, such as peritoneal adhesion [31], cervicitis [32], pelvic inflammatory [33], *et al.* for its high effectivity and reliability. Phenol mucilage contains 3 toxic ingredients, including liquefied phenol, Arabic gum, and glycerin. Liquefied phenol is a chemical burn agent, which is highly corrosive and can destroy local tissues and cause an inflammatory reaction. Arabic gum and glycerin are thickeners, which can reduce the damage to adjacent tissues due to the diffusion of phenol. This chemical method is more controllable and easier to operate than the mechanical injury method, and therefore is suitable to be used as a relatively steady and reproducible inflammation-induced animal model of IUA. Although inflammation at the site of endometrial injury is widely considered as the leading cause of IUA [1–3], other factors such as mechanical damage may also lead to IUA and even have been used to make IUA model in animals [28]. To this end, it is necessary to establish a sham-operated group to exclude mechanical damage, including the effects of surgical procedures and injection process on the IUA model rats and thus to confirm that the pathological characteristics in IUA group are only caused by a given concentration of toxic agents. In order to simulate the endometrial injury of IUA rats caused by the operation and injection, we established a sham-operated group by the treatment with PBS, which is the non-toxic agent [28, 30]. This IUA model is not only beneficial for the understanding of IUA phenotype, molecular mechanisms of adhesion formation, but is also useful for choosing the effective therapeutic strategies for IUA prevention and treatment. As mentioned above, the stricture appearance of the uterus at the IUA model rats was observed, endometrium becoming thin and atrophic, the number of glands reduced, and collagen fibers proliferated significantly (Figs. 1 and 2). These changes are consistent with the pathological manifestation of human IUA, confirming that our model is a qualified IUA rat model.

The immunohistochemistry results of rats show that the protein level of CXCL5 in the IUA group is significantly lower than those in the normal and sham-operated groups (Fig. 3). Additional measurements of mRNA level and protein level of CXCL5 in humans also confirm the low expression of CXCL5 in the endometria of IUA patients (Fig. 4A-B). The above results suggest that the expression of CXCL5 is

associated with the maintenance of normal intimal functions and should play an important role in inhibiting IUA formation.

Previous studies show that CXCL5 is involved in PI3K/AKT signal pathway to regulate the expression of matrix metalloproteinases (MMPs), which play critical roles in fibrogenesis and fibrosis [24]. After binding to its receptor CXCR2, CXCL5 induces the elevated phosphorylation of AKT and activates PI3K/AKT signaling pathway. MMP9, a group of hydrolases, can degrade type IV and V collagens and is essential for the breakdown of extracellular matrix in normal physiological and disease processes [34]. In addition, Hu *et al.* demonstrated the low MMP9 expression in endometrial tissues of IUA and indicated the lack of MMP9 expression is closely related to the IUA development [27]. Combined with the above important fact, the significantly reduced expression of CXCL5 in both IUA animal models and patient endometria found in this study indicates that the low CXCL5 expression leads to the reduced expression of MMP9. This conclusion, together with our second finding that the enhanced CXCL5 expression in endometrial cells leads to the increased expression of MMP9, reveals an evidently positive correlation between the expressions of CXCL5 and MMP9. Based on the above analyses, we suggest a molecular mechanism of CXCL5 in IUA as follows: the low expression of CXCL5 induces to a decreased expression of MMP9 through the PI3K/AKT signaling pathway, leading to the accumulation of extracellular matrix and proliferation of collagen fibers on the damaged endometrium; then normal endometrium growth is inhibited; finally, intrauterine adhesion is formed.

CXCL5 can up-regulate the p65 expression and activates the NF- κ B signaling pathway, while p65 can also bind the promoter of the CXCL5 gene and induces its transcription in cancer cells [19, 35]. Very recently, evidence accumulated shows that the NF- κ B pathway is associated with disorders of fibrogenesis and up-regulated in IUA genesis [21]. However, it remains unclear whether CXCL5 regulates the p65 expression in the NF- κ B signaling pathway in IUA. Importantly, Xue *et al.* found the overexpression of p65 in IUA [22]. To further investigate the correlation between p65 and the low-expressed CXCL5 in IUA found in this study, we detected the p65 expression level in CXCL5-overexpressed endometrial cells. Surprisingly, we observed a slight increase in p65 protein level with the increase of CXCL5 expression in endometrial cells, indicating that the p65 expression is not merely regulated by CXCL5 in IUA, and its increased expression in IUA can be attributed to the regulation by other more important factors. Thus, we speculate that the NF- κ B signaling pathway is not the principle molecular mechanism by which CXCL5 inhibits the formation of adhesion in IUA.

Conclusions

CXCL5 shows a significantly reduced expression in endometrial tissue of IUA both in rats and humans, and CXCL5 positively regulates MMP9 expression, indicating that CXCL5 plays a protecting role in the adhesion formation and fibrogenesis of IUA through modulating MMP9 and therefore is a potential biomarker. Based on these findings, we proposed a molecular mechanism of CXCL5 in IUA. Our results provide important information for evaluating and target the therapy of intrauterine adhesions.

Methods

Patients and tissue samples

Intrauterine adhesion and control tissues were collected from The Second Affiliated Hospital of Anhui Medical University between October 2017 and July 2018. 6 endometrial tissues were collected from intrauterine adhesion patients diagnosed by hysteroscopy, and 6 normal endometrium tissues from secondary infertility control individuals were collected as controls. There is no significant difference in age, weight, or parity between the intrauterine adhesion and control group. The study was approved by the Ethics Committee of the Anhui Medical University (No. 20160034) and complied with the Declaration of Helsinki. All patients of this study were aware and signed an informed consent agreement.

Creation of a rat model for intrauterine adhesions

Adult female Sprague-Dawley (SD) rats aged 8 weeks (weight: ~220g) were obtained from the Experimental Animal Center of Anhui Medical University and used to create the IUA model by phenol mucilage as the description [21, 32]. All rats were raised in the laboratory for 1 week of adaption. Vaginal smear was used to evaluate the phases of the estrous cycle, and all animal surgeries were performed during their diestrus stage of the cycle. 40 rats were randomly grouped into three groups: IUA group (20 rats for phenol mucilage treatment), sham-operated group (10 rats with vehicle treatment), and normal group (10 rats with no treatment). Firstly, 5% pentobarbital sodium was intraperitoneally injected for the anesthetization of rats. Then, intrauterine injections of 0.04ml phenol mucilage (Liquefied phenol 25% v/v, Arabian gum 5% w/v, Glycerin 20% v/v) were administered on adult female rats to cause endometrial injury in IUA group. The sham-operated group received an equal volume of PBS. The rats were housed with free access to food and water in an environment-controlled room at 22°C with a 12h light and dark cycle. Two weeks after the surgery, all rats were euthanized using a CO₂ flow at a rate of 10 L/min for 7 min, and endometrium tissue samples were collected for further analyses. This study was performed in strict accordance with the recommendation from the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All animal experiments were performed at the Anhui Medical University and approved by the Ethics Committee on Animal Research of the Anhui Medical University (No. LLSC20180106).

H&E staining and Masson staining

Endometrium tissues underwent standard paraffin embedding and were cut into 5-μm thick serial sections [36]. Hematoxylin & eosin (H&E) staining and Masson staining were performed as previously described. For HE staining, the sections were incubated with hematoxylin solution for 5 min and then stained with eosin solution for 3 min. For Masson staining, after deparaffinization and rehydration, the

sections were stained with hematoxylin for 5-10 min, and Masson staining mixture for 5 min. Then the sections were immersed in Phosphomolybdic Acid Solution for 1 min and Aniline Blue Solution for 5 min and then examined and photographed by microscopy.

Immunohistochemistry and scoring

Immunohistochemistry was performed according to the manufacturer's instructions (Beijing Zhongshan Golden Bridge). Fully processed with 3% H₂O₂ for 15 min and washed by PBS (phosphate-buffered saline) buffer, then the sections were blocked by 10% goat serum for 30 min at room temperature. Antibody against CXCL5 (ab198505, Abcam Company) was incubated with the sections overnight at 4°C. After incubated with biotinylated secondary antibody and biotinylated horseradish peroxidase complex for 15 min, the sections were visualized by diaminobenzidine (DAB).

The number of CXCL5 positive cells was counted in three randomly chosen fields using a semi-quantitative method by multiplying the multiplication of the number of positively-expressed cells and their positive intensity [21]. The proportion of positive cells classified into 5 grades (0-4): 0%, 25%, 50%, 75% and 100%. The intensity of the stain was scored from 0 to 3 points: 0-no staining, 1-weak, 2-moderate, 3-strong. The score was calculated by multiplying the proportion and intensity score (0-12). The samples were observed under a light microscope (40×, 100×, 200×, 400× magnification).

RNA isolation and real-time PCR analysis

Total RNA from the endometrium tissues was isolated, as previously described [37]. RNA was extracted using RNAiso Plus reagent (TaKaRa Biotechnology). RNA samples (2μg) were further reverse-transcribed with the PrimeScriptTMP RT Master Mix (TaKaRa Biotechnology) according to the manufacturer's instructions. The PCR amplification was performed by using SYBR® Premix Ex TaqTMP II (TaKaRa Biotechnology). CXCL5 primer sequence is: 5'-CTCAAGCTGCTCCTTTCTCG-3', 5'-GCGATCATTTTGGGGTTAAT-3'. GAPDH primer sequence is: 5'-GAAGGTGAAGGTCGGACTC-3', 5'-GAAGATGGTGATGGGATTTC-3'. All PCR reactions were run in a multiplex real-time PCR machine (StepOne, Applied Biosystems, USA). GAPDH quantification served as an internal control for normalization. Relative differences in mRNA levels over control values were calculated using the Δ Ct method according to the manufacturer's protocol. The results of PCR reactions were independently repeated at least twice.

Cell culture and transfection

Ishikawa cells, a human endometrial epithelial cell line, were obtained from the American Type Culture Collection (ATCC). Cells were cultured in RPMI-1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and antibiotics under the condition of 37°C and 5% CO₂. CXCL5 expression vector (2µg, 1µg) and control plasmid pcDNA3.1 were transfected to cells using jetPRIME transfection reagent (Polyplus) according to the description of the protocol. After incubation with the transfection mix for 24 h, then the cell growth medium was replaced, and the cells were harvested from the dishes following another 24 h incubation. Finally, the cells were assayed by Western blotting.

Western Blotting

Cells and tissue samples were lysed with a RIPA buffer containing a protease inhibitor cocktail (B14001, Bimake). Protein concentrations were measured by bicinchoninic acid assay (BCA). Equal amounts of protein mixtures (30µg) were run on SDS-PAGE; the proteins were transferred to PVDF membrane (Millipore) and incubated in PBST (Tween-20) with 5% non-fat milk for 1 h at room temperature. The blot was incubated with primary antibodies including anti-CXCL5 (1:100, no. ab198505, Abcam Company), anti-MMP9 (1:1000, no. 3852, Cell Signaling Technology), anti-p65 (1:1000, no. 8242, Cell Signaling Technology) and anti-β-actin (1:20000, no. KC-5A08, Shanghai Kangcheng) overnight at 4°C. Secondary antibodies that were conjugated to horseradish peroxidase were incubated with the blot for 1 h at room temperature. The proteins that were revealed by Western blotting were visualized by using the enhanced chemiluminescence reagents (ECL, Servicebio). The densities of bands were analyzed by ImageJ software and normalized to GAPDH.

Statistical Analysis

All the data are expressed as mean ± standard deviation (SD) and analyzed by GraphPad Prism software version 6.0 (San Diego, CA, USA). Comparisons between groups were studied by Student's *t*-test. For analysis of immunohistochemistry staining, a two-way ANOVA test was used. *P* < 0.05 was considered statistically significant.

Abbreviations

CXCL5, CXC motif chemokine ligand 5; CXCR2, CXC motif chemokine receptor 2; DAB, diaminobenzidine; ENA-78, epithelial neutrophil-activating peptide-78; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HE, Hematoxylin and eosin; IUA, Intrauterine adhesions; MMP9, matrix metalloproteinase 9; MMPs, matrix metalloproteinases; NF-κB, nuclear factor kappa-B; PBS, Phosphate Buffered Saline; PBST, Phosphate Buffered Saline Tween 20; PVDF, Polyvinylidene Fluoride.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Anhui Medical University, China (No. 20160034). Written informed consent was obtained from all participants. Animal experiments were reviewed and approved by the Ethics Committee on Animal Research of the Anhui Medical University (No. LLSC20180106).

Consent for publication

Not applicable.

Availability of data and materials

All materials and data used in the study are available and can be provided as necessary by contacting the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SS proposed and supervised the project, designed the study and analyzed data. ZF performed experiments under the supervision of SS and BW. YH and CS helped with sample collection and western blotting. LZ, GZ helped with analysis and interpretation of data. SS, ZF and YG wrote the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Abnormal morphology in IUA rats. (A-C) Representative images of the isolated uteruses from three groups of rats. Black arrowhead points to the intrauterine adhesion. (D-L) 40 rats were randomly separated into three different groups: normal group (10 rats with no treatment), sham-operated group (10 rats with vehicle treatment), IUA group (20 rats with phenol mucilage treatment). One side of the uterus in each rat was treated with phenol mucilage or vehicle treatment. The uteruses from three groups were collected for HE staining 14 days after treatment. Representative photomicrographs of uteruses sections obtained from three group rats at 40× (D-F, scale bars: 400µm), 100× (G-I, scale bars: 200µm) and 400× (J-L, scale bars: 50µm) original magnification. In IUA rats, the uterine cavity was smaller than the other two groups.

Figure 2

Masson staining for uteruses in three group rats. Selected representative images of uteruses in each group reveals the interstitial fibrosis changes. Blue staining in IUA rats demonstrated severer adhesion compared to normal and sham-operated rats. Normal group, n=10, Sham-operated group, n=10, IUA group, n=20. (A-C) Magnification: 40×, scale bar, 400µm. (D-F) Magnification: 100×, scale bar, 200µm. (G-I) Magnification: 400×, scale bar, 50µm.

Figure 3

The expression of CXCL5 in IUA rats detected by IHC. (A-I) The representative localization of CXCL5 (brown stained) was observed in three different rat groups under light microscopy. Normal group, n=10, Sham-operated group, n=10, IUA group, n=20. (A-C) Magnification is 40×, and the scale bar is 400μm. (D-F) Magnification is 200×, and the scale bar is 100μm. (G-I) Magnification is 400×, and the scale bar is 50μm. (J) Summarization of proportion and intensity of positive staining. CXCL5 has lower staining in the IUA group compared with that in normal and sham-operated groups. Statistical significance of three groups was assessed, ***P<0.001.

Figure 4

Down-expression of CXCL5 in IUA endometrium and the correlation between CXCL5, MMP9, and p65. (A) The expression of CXCL5 in 6 cases of normal endometria (Ctrl), 6 cases of IUA were measured by real-time PCR. Levels of all mRNA were normalized to that of GAPDH mRNA. (B) The protein levels of CXCL5 in endometrial tissues of two groups were detected by Western blotting. (C) A quantitative analysis of CXCL5 expression in IUA and normal endometrium tissues normalized to β-actin expression. (D) Ishikawa cells were transfected with CXCL5 (2μg, 1μg) or vector, and the expression of CXCL5, MMP9, and p65 was measured by western blotting. β-actin was used as an internal control. (E) Quantitative analysis of relative protein expression is shown. Statistical significance of differences between the two groups was assessed t-test. *P<0.05, **P<0.01.

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