IL-19 induces MUC5AC overproduction in hepatolithiasis via stat3 pathway

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Abstract

Background

Intrahepatic biliary lithiasis is a benign biliary disease with complex pathological changes, high recurrence rate and difficult to cure. The pathological mechanism underlying hepatolithiasis development is closely related to chronic inflammation of intrahepatic bile duct and aberrant expression of mucin5ac (MUC5AC). The present study aimed to verify the up-regulation effect of IL-19 to MUC5AC in hepatolithiasis.

Results

He staining, PAS staining, immunohistochemistry and immunofluorescence were used to detect the expression of MUC5AC and IL-19 in bile duct tissue. The expressions of MUC5AC and STAT3 in bile duct tissue, epithelial cells and cell supernatant were detected by Western blot, PCR and ELISA. Chip experiment verified the binding of STAT3 to MUC5AC promoter sequence. The regulation effect of the stat3 signaling pathway was examined by stat3 siRNA transfection and using cryptotanshinone (CRY, a stat3 inhibitor). MUC5AC secretion level were significantly increased in mucosa of hepatolithiasis tissue compared to controls. After pretreatment with rhIL-19, the expression level of MUC5AC in HEiECs was significantly up-regulated. Knockdown of IL-20R or pretreatment with CRY attenuated the up-regulation effect of IL-19 to MUC5AC.

Conclusions

The present study suggest that the IL-19 can induce MUC5AC overproduction in hepatolithiasis via stat3 pathway. Intervention of IL-19 and its downstream molecules may provide support for the treatment and prevention of hepatolithiasis.

Background

Intrahepatic bile duct stones are rare in western countries, but very common in China (1). They have become a kind of benign bile duct disease which is difficult to cure because of their complex lesions and high recurrence rate (2, 3). The existing clinical treatment methods can only try to remove stones, but can not effectively prevent stone recurrence (3). The recurrence of stones makes patients have to accept multiple clinical treatments, which not only increases the risk and difficulty of treatment, but also increases the pain of patients (4). Therefore, it is of great clinical significance to study the mechanism of stone formation and explore effective methods to prevent stone formation and recurrence. During the development of hepatolithiasis, chronic inflammation of intrahepatic bile duct and excessive secretion of mucin are the key lesions (5). Mucins are identified as potent pronucleators of cholesterol stone formation through its binding with biliary lipids and subsequent acceleration of cholesterol crystal

nucleation in supersaturated bile (6). In addition, mucin interacts with calcium-binding proteins and induces the formation of calcium containing stones through calcium phosphate (7).Histologically, the glands in and around the bile duct wall of hepatolithiasis showed obvious proliferative pathological changes with inflammation and fibrosis (8). The proliferated bile duct epithelial tissue will produce a large number of mucin dominated by MUC5AC, which provides the skeleton and microenvironment for the formation of stones, which is an important mechanism of chronic proliferative cholangitis and stone formation (9).

A recent study elucidates that IL-24, which belong to the IL-20 subfamily, not only plays a vital role in anti-inflammatory and protective procedure in intestinal mucosa but also induces mucin deposition in the epithelial tissue during the development of inflammatory bowel disease (10).The IL-20 subfamily of cytokines is belong to the IL-10 family, which was composed of five relevant molecules, containing IL-19, IL-20, IL-22, IL-24, and IL-26 (11). IL-20 subfamily of cytokines, which secreted by immune cells have been proved the enhance effect in the processes of tissue repair (12, 13). Within the IL-20 subfamily, IL-19, IL-20, and IL-24 cytokines share the same IL- 20RA/IL-20RB and IL-22RA/IL-20RB receptor heterodimers to produce their physiological function (12, 13). However, the expression of these receptors is mainly restricted to the epithelial cells in most organ and the distribution of these receptors contain differences between tissues (13, 14). It is worth to investigate whether their receptors are expressed in bile duct epithelium and whether IL-19, which belongs to the same subfamily as IL-24, can also regulate the expression of mucin deposition.This study is purpose to investigate the expressions of mucin and MUC5AC in biliary tract mucosa and to determine the production of MUC5AC whether regulated by IL-19 in Intrahepatic.

Materials And Methods

*Cell culture.*Human intrahepatic biliary epithe- lial cells (HIBEpiCs; ScienCell Research Laboratories, Inc.) were routinely cultured in epithelial cell medium (containing 5ml epithelial cell growth factor, 10 ml fetal bovine serum, 5 ml penicillin/streptomycin solution; ScienCell Research Laboratories, Inc.) at 37 °C, 5% CO_2 and saturated humidity. The medium was changed regularly and subcultured once in about 2 days.

Cell pretreatment. To investigate the up-regulation effect of IL-19 to MUC5AC, HIBEpiCs were treated with different concentrations of recombinant human IL-19 (rhIL-19;100 , 300 ng/ml for experiment group and without rhIL-19 for control group) for 24 h. Cryptotanshinone (CRY; 10 μg/ml; cat. no.5624, Sigma-Aldrich; Merck KGaA), an stat3 inhibitor, was used in inhibitory experiments, (HIBEpiCs were incubated with 10 μg/ml CRY and 100ug/ml IL-19 for 24 h at Normal culture conditions.)

*Hematoxylin and eosin (H&E) staining.*The extracted bile duct tissue was fixed with 10% formalin and made into 4mm thick paraffin sections. Dewaxing with xylene, alcohol and distilled water. Put the slices into hematoxylin (cat. no.517-28-2 ; Sigma-Aldrich) aqueous solution for dyeing, and then put them into acid water and ammonia for color separation. Rinse the slices with distilled water for 1 hour. Then the

sections were dehydrated with 70% and 90% alcohol and stained with alcohol eosin (cat. no.548-24-3 ; Sigma-Aldrich) staining solution. The stained sections were dehydrated with pure alcohol, then made transparent with xylene, dripped with gum, covered with cover glass and sealed.pathological changes were observed under a light microscope and the bile duct wall thickness of each section were measured (DMM-300D; Shanghai Caikon Optical Instrument Co., Ltd., Shanghai, China).

*Periodic acid Schiff (PAS) staining.*The extracted bile duct tissue was fixed with 10% formalin and made into 4mm thick paraffin sections. After dewaxing the paraffin sections, rinse them with running water. After dropping oxidant, the slices were placed at room temperature for 8min and washed with running water. Rinse the slices at room temperature with water for 10min. After staining with hematoxylin, differentiate with acid differentiation solution and wash the sections with running water. Dehydrate the slices with ethanol, transparent with xylene, and then drip gum for sealing.The kit used in this experiment was as follow:cat. no.G1281; Beijing Solarbio Science & Technology Co., Ltd.) Mucin-like glycoprotein was observed purplish red under an light microscope (DMM-300D; Shanghai Caikon Optical Instrument Co., Ltd.) and percentage of positive mucin glyco area of each group have been quantified. The Image Pro-6 software was used in quantitative analysis.

Western blotting (WB). WB was used to detect the protein level of stat3. Protein extraction and sample preparation process same as described previously (3,6), with some modifications. Lysis buffer (Beyotime Institute of Biotechnology) was used in protein lysis process, Bradford protein assay kit (Bio-Rad Laboratories, Inc.) was used to detect protein concentration. The 1.5mg/ml protein sample was electrophoresed with 10% concentration separation gel and 5% concentration concentrated gel, and then transferred to polyvinylidene difluoride membranes.Prepare a sealing solution with a mass fraction of 5% with membrane washing buffer (TBS with 0.1%Tween-20;TBST) and nonfat dried milk, put the membrane into the sealing solution, and seal it at room temperature for 2 hours. Dilute the primary antibody against stat3 (1:1,000; cat. no. Ab68153; Abcam) and GADPH (1:3,000; cat. no. 60004-1-Ig; ProteinTech Group, Inc.) with primary antibody diluent. Cut the membrane according to the molecular weight of the target protein, put it into the prepared primary antibody, incubate membrane at room temperature for 1 h, and then put it into a 4 ℃ refrigerator shaking slowly overnight. After the primary antibody incubation, wash the membrane with TBST for 3 times, each time for 10 minutes. Dilute the HRP labeled secondary antibody with TBST, shake and incubate membrane in secondary antibody at room temperature for 2 h (horseradish peroxidase-conjugated anti-rabbit immunoglobulin G; 1:3,000; cat. nos. A23210 and WB0177; Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.), and then rinse it in TBST lotion for 3 times, 10 min each time. After adding the luminescent solution (EMD Millipore) on membrane, the gray scale of the band was scanned by gel Pro analyzer gel quantitative analysis software (version 6.3; Media Cybernetics, Inc.), and the expression of the target protein and internal reference protein were calculated.

Reverse transcription-quantitative PCR (RT-qPCR). Use RNA extraction kit (Invitrogen; Thermo Fisher Scientific, Inc.) to extract RNA from cells or tissue, and use disposable RNA free enzyme gun head in the extraction process. Then reverse transcriptional synthesis of cDNA was carried out using the Takara reverse transcription kit. Operate according to PrimeScript RT reagent kit (Takara Bio, Inc.), add

appropriate dyes, primers and cDNA templates, and then detect the amount of RNA in cells or tissue. RT-PCR primer sequence is as follows: MUC5AC (human) forward 5' -GGA ACT GTG GGGACA GCT CTT-3' and reverse, 5' -GTC ACA TTC CTC AGC GAG GTC-3'; GADPH (human) forward, 5'-ACAACTTTG GTATCGTGGAAGG-3' and reverse, 5'-GCCATCACGCCA CAGTTTC-3'; MUC5AC (rat) Forward: 5'-AGCACAGTTGCCTCAAGTCC-3' and Reverse: 5'-CTCGGCTACAGGTCCATCC-3'; GAPDH (rat) : Forward: 5'-ACA TGC CGC CTG GAG AAA CCT-3' and reverse : 5'- TCC ACC ACC CTG TTG CTG TAG-3 primers were used. Relative expression levels were normalization to the expression of GAPDH .

*Immunohistochemistry (IHC).*The bile duct tissue of patients underwent hepatectomy due to hepatolithiasis (experiment group) and hemangioma (control group) were obtained.The tissue collecting procedure was approved by the local ethics committee of the Affiliated Shengjing Hospital of China Medical University (approval no. 2017PS231K) and written information have been provided to participated patient. The basic information of relative patient have shown in table 1. The prepared 4mm paraffin sections were dewaxed with xylene and ethanol. Carry out antigen repairing and blocking endogenous peroxidase according to the instructions of the immunofluorescence kit (Beijing ZSGB-BIO Technology, Ltd.). The blocked sections were washed with PBS, and the primary MUC5AC (1:1,000; cat. no. ab3649; Abcam) or IL-19 antibody (1:1,000; cat. no. 154187; Abcam) was added , stored overnight at 4 ℃. The slices were washed with PBS for three times, then added with fluorescent secondary antibody (1:1,000; cat. no. BIR701-3; Beijing Borsi Technology Co., Ltd.) and incubated at room temperature for 1.5h. After cleaning the slices with PBS for three times, DAB (1:20; cat. no. AR1000; Wuhan Boster Biological Technology, Ltd.) was added and incubated at room temperature for 3min.Counter-stain the paraffin with hematoxylin at room temperature for 3 min and observe the staining results under the microscope light microscope (magnification, x200 and x400; Eclipse Ci-L; Nikon Corporation) after sealing with gum.According to the percentage of positive cell complete the quantitative analysis (0-25%, low; 25-50%, medium; >50%, high).

Immunofluorescence (IF). IF experiment was used to compare the expression level of MUC5AC between experiment and control group. Climbed the pretreated HIBEpiCs (pretreated with 100 μg/ml of IL-19 for 24 h for experiment group) on slides and soaked with PBS for 3 times. Fixed the cells with 4% paraformaldehyde for 15min and washed with PBS three times. Permeated with 0.5% Triton X-100 at room temperature for 20 minutes and then washed with PBS. Sealed the slide with 1%BSA (cat. no A8020; Beijing Solarbio Science & Technology Co., Ltd.) at room temperature for 20 minutes and added MUC5AC primary antibody (1:100 dilution; cat. no. ab3649. Abcam), incubated overnight at 4 ℃ in dark environment. After washing with PBS, added fluorescent secondary antibody (goat anti-rat; cat. no. SA00009-1 and goat anti-rabbit; cat. no. FITC-10835; both 1:200; ProteinTech Group, Inc.) and incubate at room temperature in the dark environment for 1.5h. DAPI was added and incubated at room temperature in the dark environment for 5min. Observed the results under fluorescence microscope.

Enzyme-linked immunosorbent assay (ELISA). MUC5AC concentration of cell supernatants have been detected by ELISA.Concentrate the collected 2.5ml cell supernatant with ultrafiltration tube (EMD

Millipore) until the volume reached 250ul. Measure the concentration of MUC5AC in the concentrated cell supernatant according to the instructions of ELISA kit (cat. no. CSB-E10109h; Cusabio Technology, LLC).

Cell transfection. Short hairpin RNA (siRNA) plasmids (Shanghai GeneChem Co., Ltd) .Cultured cells to a density of 70-80%, then replaced the non-antibiotics medium. Added Lippo300 (Hanbio Biotechnology Co., Ltd.) and incubated at room temperature for 30min. Added STAT3 or IL-19siRNA and mixed well. After cultured for 48 hours, the transfection effect was verified by WB, PCR and other experiments.

The sequences of the siRNAs used are as follows:

Stat3 siRNA ,5’ - GGCUUUAGAGUUCACACAAUAGGAU-3’

IL-20R siRNA,5' -GAUGGCUUCCACCUGGUUATT- 3’

Chromatin immunoprecipitation (ChIP).ChIP assay Kit (Upstate Biotechnology, Billerica, MA, USA) used to performed chip experiment to verify the direct binding of stat3 to MUC5AC promoter sequence. HIBEpiCs cells were treated with IL-19 (100 ng/ml) for 24 h, and then were crosslinked with 1% formaldehyde for 10 min, and then following sonication. Diluted the soluble chromatin and immunoprecipitated it by anti-Stat3 antibody or IgG at 4 C for 12 h.Isolated DNA from the obtained chromatin-antibody complexes for PCR, using primers spanning the STAT3 binding elements in the promoter region of the MUC5AC gene.

Establishment of rat lithogene models. All animal studies were approved by the local ethics committee of the Affiliated Shengjing Hospital of China Medical University (approval no. 2017PS231K). Suitable size (male; 6 week old; body weight 220±15 g)of Sprague-Dawley rats (SD rat) were purchased from Beijing Dingguo Changsheng Biotechnology company.The rats were raised in 25 ℃ temperature, normal atmosphere and 12 h light/dark cycle environment, with free access to water and food. A total of 28 rats were randomly assigned to the experimental group and the control group. After general anesthesia, the rats were underwent laparotomy and exposed the common bile duct. Inserted the prepared polyethylene (PE) tube into the common bile duct of rats and fixed it. The end of the PE tube was fixed on the back of the rats through subcutaneous tunnel. For the rats in the experimental group, 100ul IL-19 (300ug/kg) was injected regularly through the PE tube, and for the rats in the control group was injected saline accordingly. Drawn blood sample of experimental animals and analyzed serum total bilirubin, ALT ,AST levels using a biochemical analyzer (cat. no. 98-11084-01; cat. no. 98-24010-US; cat. no. 98-24016-US; Catalyst One; IDEXX). Made bile smear to Verify the effectiveness of the modeling (Supplementary Figure1). After one week of modeling, the rats were sacrificed and the bile duct tissues were harvested for WB, PCR and IHC experiments.

*Statistical analysis.*All data were analyzed by SPSS 22.0 statistical software,and student t-test or one-way ANOVA model was used for analysis. $P<0.05$ "*" indicates significant. All data are presented as mean ± SD.

Result

The expressions of MUC5AC in bile duct tissue of hepatolithiasis increased

Chronic inflammation of intrahepatic bile duct and excessive mucin secretion are the pathological basis of the occurrence and development of intrahepatic bile duct stones. The differential levels of mucin-like glycoproteins in the bile duct tissues was analysed by PAS staining.The result demonstrated that mucin like glycoprotein was stained red purple, which was mainly expressed in the cytoplasm and cell membrane of epithelium and cavity of bile duct (Fig. 1). Compared with the control group, the positive PAS staining of bile duct epithelium and bile duct cavity in hepatolithes tissue were significantly increased. Presenting as the significantly increasing of positive stained mucin like glycoprotein in cytoplasm, bile duct epithelium membrane and bile duct cavity. H&E staining revealed that compared with control group the bile duct epithelium in hepatolithiasis group present obvious hyperplasia. The microtissue of the hyperplastic bile duct wall encapsulated the gland of the bile duct wall,which induced mucus stagnation and expansion of the adenoid cavity (Fig. 1). Compared with the control group, a longer diameter of the bile duct ,thicker bile duct wall large fibrous tissue and submucosal gland were found in hepatolithiasis group. The expression of MUC5AC was mainly concentrating on the apical side of the mucosa epithelia, indicate the metaplasia and hyperplasia of the bile duct epithelial cells in hepatolithiasis, resulting in MUC5AC overproduction.Immunohistochemical staining result showed quantitative of strong staining of MUC5AC proteins enhanced in the bile duct epithelium of patients with hepatolithiasisi (Fig. 1). Quantitative analysis indicate the MUC5AC expression level in hepatolithiasisi group extremely higher than control group.

The Expression Of Il-19 In Bile Duct Tissue Of Hepatolithiasis Increased

As a member of IL-20 subfamily, IL-19 cytokines share the common IL- 20RA/IL-20RB and IL-22RA/IL-20RB receptor with IL-20 and IL-24. The expression of these receptor mainly restricted in epithelial cell and existed difference between tissues. Immunofluorescence assay was used to detect the expression of IL-19 in bile duct epithelium of hepatolithiasis, and the relationship between IL-19 expression and MUC5AC expression (Fig. 2).The result suggested that both biomarker were mainly co-localized in epithelial cell,and compared with control group,the expression level of IL-19 and MUC5AC were obviously higher than hepatolithiasis tissue.This result proved that IL-19 and its receptor can be expressed in intrahepatic bile duct epithelium, and the expression level may be associated with the overexpression of MUC5AC.

Il-19 Promoted The Expressions Of Muc5ac In Hibepics

In order to further confirm whether IL-19 can promote the secretion of MUC5AC in intrahepatic bile duct epithelium, we intervened with different concentrations of IL-19 on intrahepatic bile duct epithelial cells.

RT-PCR results showed that the expression of MUC5AC mRNA increased with the increase of the concentration of IL-19 (Fig. 3A), which confirmed that IL-19 could improve the transcription level of MUC5AC. ELISA experiments confirmed that with the increase of IL-19 concentration, the content of MUC5AC in cell supernatant increased significantly (Fig. 3B), which confirmed that IL-19 could promote the secretion of MUC5AC in intrahepatic bile duct cells. The cell fluorescence experiment confirmed that after the intervention of IL-19, the intracellular MUC5AC content of intrahepatic bile duct cells in the experimental group was significantly higher than that in the control group (Fig. 3C). These result proved that IL-19 can increase the expression of MUC5AC at the transcriptional and translation levels. The results of ELISA and PCR showed that the promoting effect of IL-19 on the transcriptional expression of MUC5AC decreased significantly after intrahepatic bile duct epithelium cell transfected with IL-20r siRNA (Fig. 3D,E).

Il-19 Induced Muc5ac Overproduction Via Stat3 Pathway In Vitro And Vivo

Next, we studied which pathway IL-19 intervenes to regulate MUC5AC secretion.Previous study presented that IL-19,which similar to IL-6,could bind with its receptor and activates stat3.WB results showed that the expression of stat3 in intrahepatic bile duct epithelial cells treated with different concentrations of IL-19 was significantly higher than that in the control group (Fig. 4A), and increased with the increase of intervention concentration. The expression of stat3 in intrahepatic bile duct cells decreased significantly after IL-20r siRNA transfection (Fig. 4B), which confirmed that IL-19 intervention could activate and increase the expression of stat3 in intrahepatic bile duct cells. The results of ELISA and PCR showed that CRY intervention and stat3 siRNA transfection could reduce the promoting effect of IL-19 on the expression of MUC5AC in intrahepatic bile duct epithelial cells (Fig. 4C,D). This further confirmed that IL-19 promoted the expression of MUC5AC by activating stat3.In further experiments, we predicted the binding sites between stat3 and MUC5AC promoter sequence through the database (jaspar.genereg.net), and confirmed that stat3 can bind to MUC5AC promoter sequence through chip experiment (Fig. 4E). This result indicate that stat3's regulating effect on MUC5AC is completed by directly combining with the promoter sequence of MUC5AC.

To further verified the regulatory effect of IL-19 on MUC5AC, an intrahepatic bile duct stone model of SD rats was established (Supplementary Fig. 1). The results of PCR showed that the content of MUC5AC mRNA in bile duct epithelium of model group increased significantly (Fig. 5A). WB results showed that the expression of stat3 protein in bile duct epithelium in the model group was significantly higher than that in the control group (Fig. 5B). The results of immunohistochemistry showed that the content of IL-19 in bile duct epithelium in the model group was significantly higher than that in the control group (Fig. 5C). Quantitative analysis indicate the IL-19 expression level in model group extremely higher than control group. The results of correlation analysis showed that the expression of IL-19 and stat3 and the expression of stat3 and MUC5AC were positively correlated (Fig. 5D,E). These results suggest that IL-19

can promote the expression and secretion of MUC5AC by regulating stat3 during the formation of rat intrahepatic bile duct stone model.

Discussion

Intrahepatic bile duct stones are common diseases that seriously threaten the health of Chinese people (1). Although there are many clinical schemes for the treatment of intrahepatic bile duct stones, there are still some problems, such as the repeated recurrence of intrahepatic bile duct stones can not be completely cured (2, 3). Therefore, it is of great significance to clarify the etiology of intrahepatic bile duct stones and avoid recurrence for the treatment of intrahepatic bile duct stones. Histologically, the glands in the bile duct wall and around the bile duct in the liver of hepatolithiasis showed obvious proliferative pathological changes with inflammation and fibrosis (8). Hyperplastic glands and bile duct epithelium produce a large amount of mucin, which provides a suitable microenvironment for the formation of stones (15). Starting the process of stone formation and growth with calcium salt and lipid as the core is an important mechanism of chronic hyperplastic cholangitis and stone formation (16). At present, 20 mucin genes have been identified, of which MUC5AC is the main mucin secreted by bile duct (6, 17). It is highly expressed in patients with primary hepatolithiasis and is an important nuclear factor, Which acts as a barrier for the digestive epithelium to maintain normal physical activity in the hard luminal environment (5,7,). Previous studies have demonstrated that overexpression of MUC5AC in both the superficial epithelium and the deep folds are likely associated with the formation of several types of gallstones (6,8,). At present, there are many studies related to the increased secretion of MUC5AC causing intrahepatic bile duct stones (9, 16, 20). Previous studies of our research group have also confirmed that PKC – NOx – ROS and micro130b-sp1 signal pathway can regulate the overexpression of MUC5AC in the pathogenesis of intrahepatic bile duct stones (21, 22). However, there is no study that can fully explain the underlying molecular mechanism of MUC5AC overexpression. A recent study elucidated IL-24, ont only plays a essential role in physiological function like anti-inflammatory and protective procedure but also induces pathological process like mucin deposition in the epithelial tissue during the development of inflammatory bowel disease (10). IL-24 is member of IL-20 subfamily, which is part of the IL-10 family and is composed of five relevant cytokines, containing IL-19, IL-20, IL-22, IL-24, and IL-26. IL-20 subfamily of cytokines, which secreted by immune cells have been proved the enhance effect in the processes of tissue repair (10, 11, 13). Within the IL-20 subfamily, IL-19, IL-20, and IL-24 cytokines share the same IL-20RA/IL-20RB and IL-22RA/IL-20RB receptor heterodimers to produce their physiological function (11–13). However, the expression of these receptors is mainly restricted to the epithelial cells in most organ and the distribution of these receptors contain differences between tissues (11–13).Therefore, we suppose whether IL-19 participates in MUC5AC secretion procedure in the epithelial cells of biliary tissue. In this study, we found that IL-19 was expressed in the bile duct epithelium of patients with intrahepatic bile duct stones, and the content was significantly higher than that of the control group. After the intervention of IL-19, the secretion of MUC5AC in intrahepatic bile duct epithelial cells increased significantly. It is confirmed that IL-19 can regulate the expression of MUC5AC in intrahepatic bile duct epithelium.Previous study have revealed several signaling pathway participating in bile duct chronic

inflammation and mucus overproduction. However, neither is available on the perfectly adequate explanation of the underlying mechanism of mucus regulation.Previous study about most frequently reported pathways include STAT6, ERK1/2, TNFα and NF–κB (23–25). Stat family, which first discovered in 1988 is responsible for regulating the transcription of multiple key genes involved in cell proliferation, diferentiation, apoptosis, metastasis, infammation, immunity, cell survival, and angiogenesis (26).Previous research have elucidated that IL-19 binding to its receptor and led to the activation of stat3 which similar to other members of the IL-10 family of cytokines (27–30).Therefore, we wondered whether the promoting effect of IL-19 on MUC5AC was achieved through the activation of Stat3.Our results confirmed that the expression of STAT3 in intrahepatic bile duct epithelial cells increased significantly after IL-19 intervention.When intrahepatic bile duct epithelial cells were pretreated with IL-19 and CRY,which have been demonstrated to inhibited the STAT3 pathway, the gene and protein expressions of MUC5AC were suppressed.In addition, chip experiment confirmed that stat3 can bind to the promoter sequence of MUC5AC and participate in the expression regulation of MUC5AC.

In conclusion,our study indicates that IL-19 could promoting MUC5AC secretion via stat3 pathway.This is the first study demonstrating the regulatory effect of IL-19 to MUC5AC in biliary epithelium.Suggesting IL-19 play a crucial role in mucus production in hepatolithus ,Interventions targeting on IL-19 and its downstream molecule could provide value for the treatment and prevention of hepatolithus.

Declarations

Ethics approval and consent to participate

The study is reported in accordance with ARRIVE guidelines. All animal experiments were approved by the local ethics committee of the Shengjing Hospital of China Medical University (approval no. 2017PS231K). All animal experiments were ethically performed according to with the guidelines of the Experimental Animal Welfare Ethics Committee of the Affiliated Shengjing Hospital of China Medical University.

Human informed consent statements

Experiments involving human participants were conducted according to the declaration of Helsinki and approved by the the local ethics committee of the Shengjing Hospital of China Medical University (approval no. 2017PS927K) in accordance with its guidelines for the protection of human subjects. Informed consent was obtained from all subjects and/or their legal guardian in this study.

Patient consent for publication

Not applicable.

Availability of data and materials

All datasets generated for this study are included in the manuscript/Supplementary Files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SW and YW were in charge of correspondence.SW and XW designed the experiment. XW performed the experiments. XW wrote the manuscript. SW and YW revised the manuscript. All the authors read and approved the fifinal manuscript.

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Table 1

Table I. The basic information of clinical specimens

	Control	Hepatoliths
Total number	8	12
Age(years),mean±SD	51.3±16.4	58.9±14.3
Male(n,%)	3,37.5%	5,41.7%
Female(n,%)	5,62.5%	7,58.3%

Figures

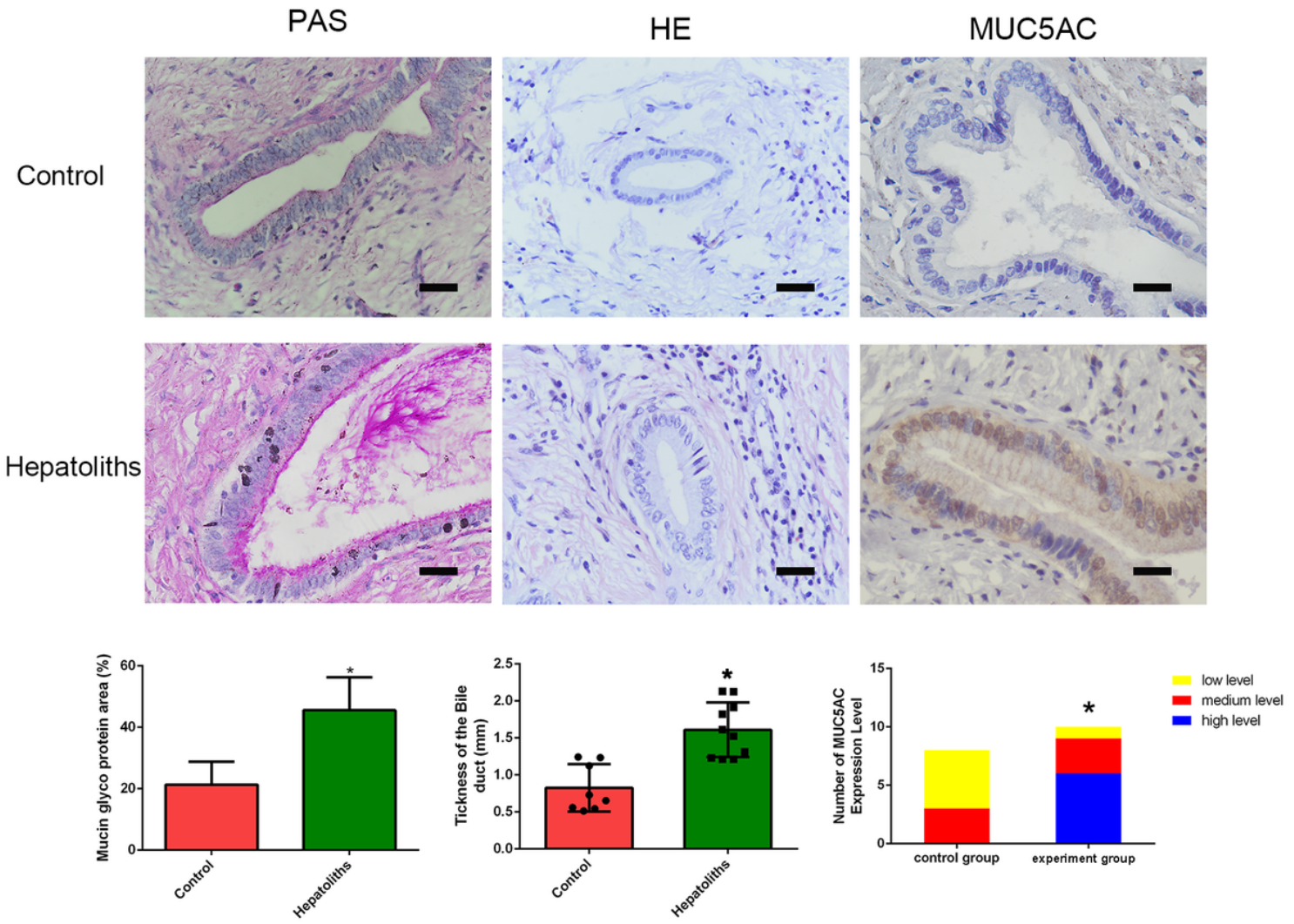

Figure 1

Pathological observation of bile duct tissues in hepatolithiasis patient and controls. Pas staining showing the pathology of bile duct tissues and the percentage of positive mucin glyco area of each group have been quantified. HE staining showing the pathology of bile duct tissues and the bile duct wall thickness of each group have been measured. IHC staining the MUC5AC expression state in hepatolithiasis patient (experiment group) and controls.Differences among the groups were analyzed by T-test. *P <0.05 compared with the control group. Scale bars: 50 μm.

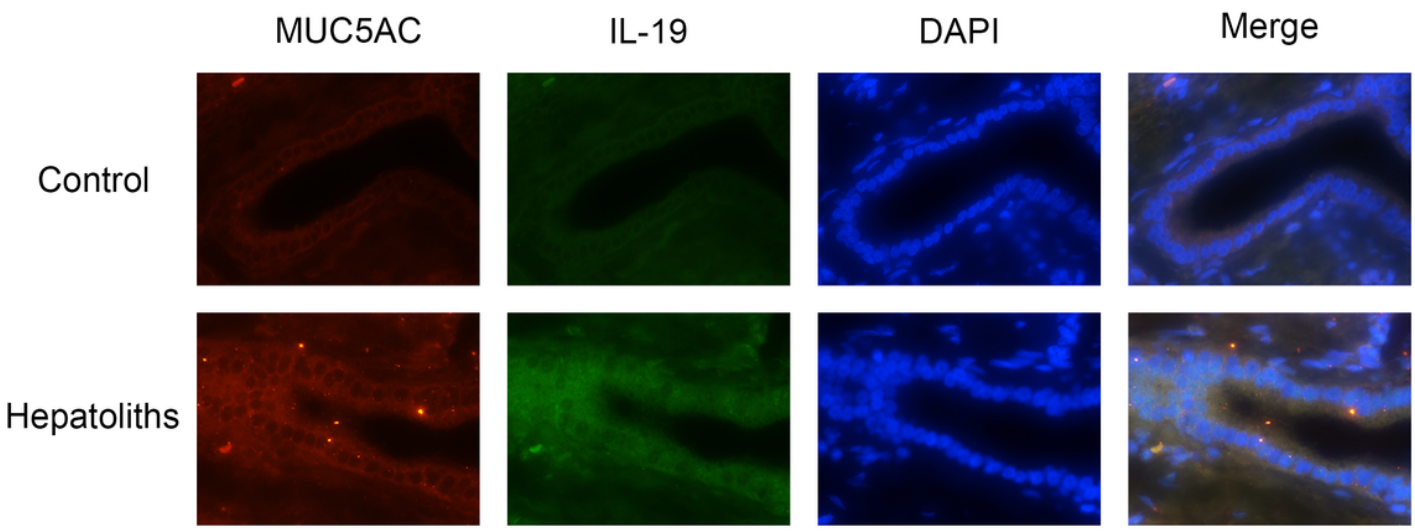

Figure 2

The co-expression of IL-19 (green) and MUC5AC (red) in the bile duct tissues from the hepatolithiasis and controls . Nuclei are stained with DAPI (blue). Scale bars: 50μm.

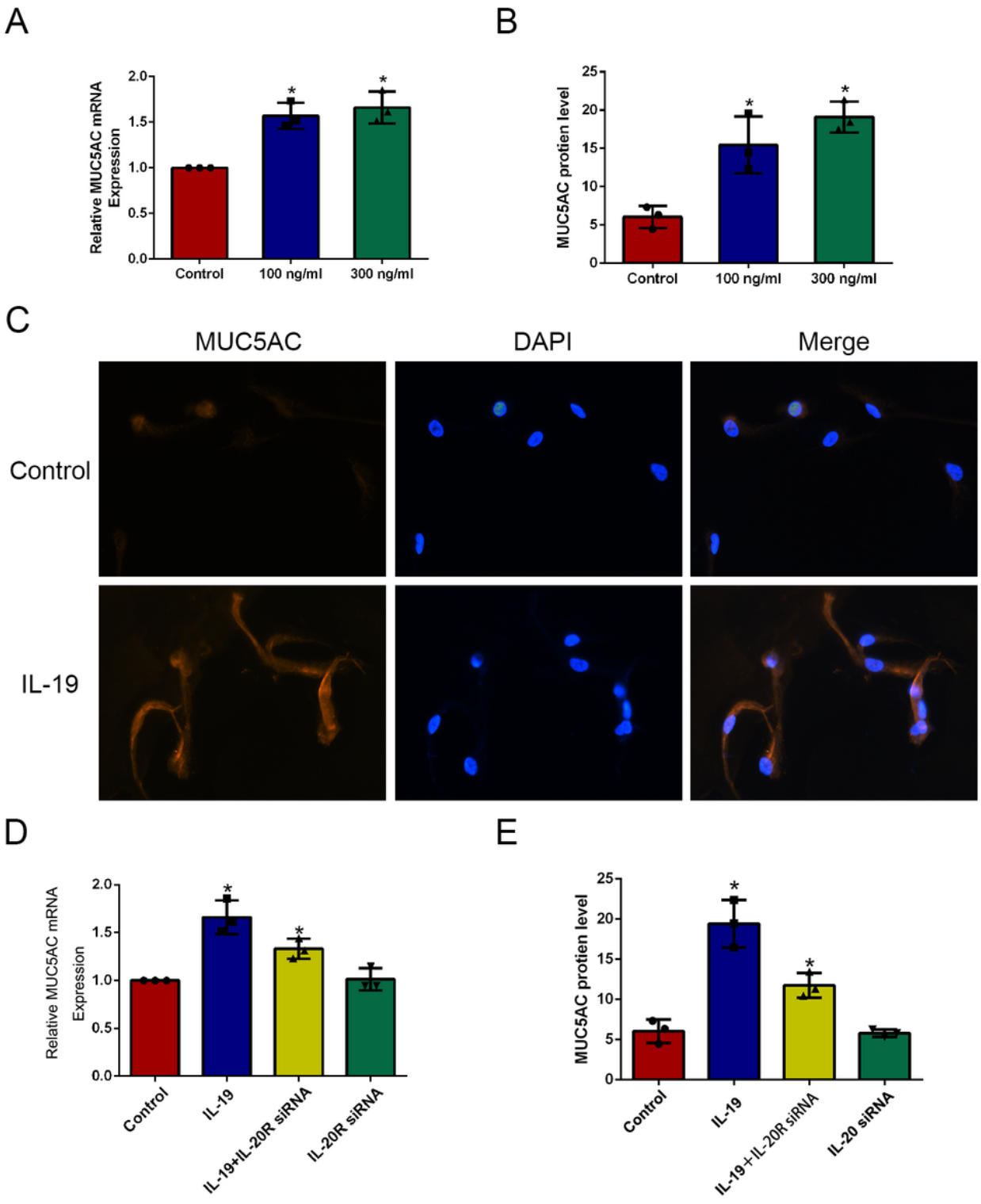

Figure 3

IL-19 up-regulate muc5ac expression in intrahepatic bile duct epithelium.(A).PCR detection of MUC5AC mRNA level in intrahepatic bile duct epithelium cell in experiment group (IL-19 intervence) and control group.(B).ELISA result of detecting MUC5AC in intrahepatic bile duct epithelium supernatant in experiment group and control group.(C)Immunofluorescence result of MUC5AC(red) expression level in experiment group (IL-19 intervence) and control group,nuclei are stained with DAPI (blue).(D)PCR

detection of MUC5AC mRNA level in each group.(E)ELISA result of detecting MUC5AC in each group.Statistical signifificance was calculated according to the one-way ANOVA analysis. *P < 0.05 compared with the control group.Scale bars: 20μm.

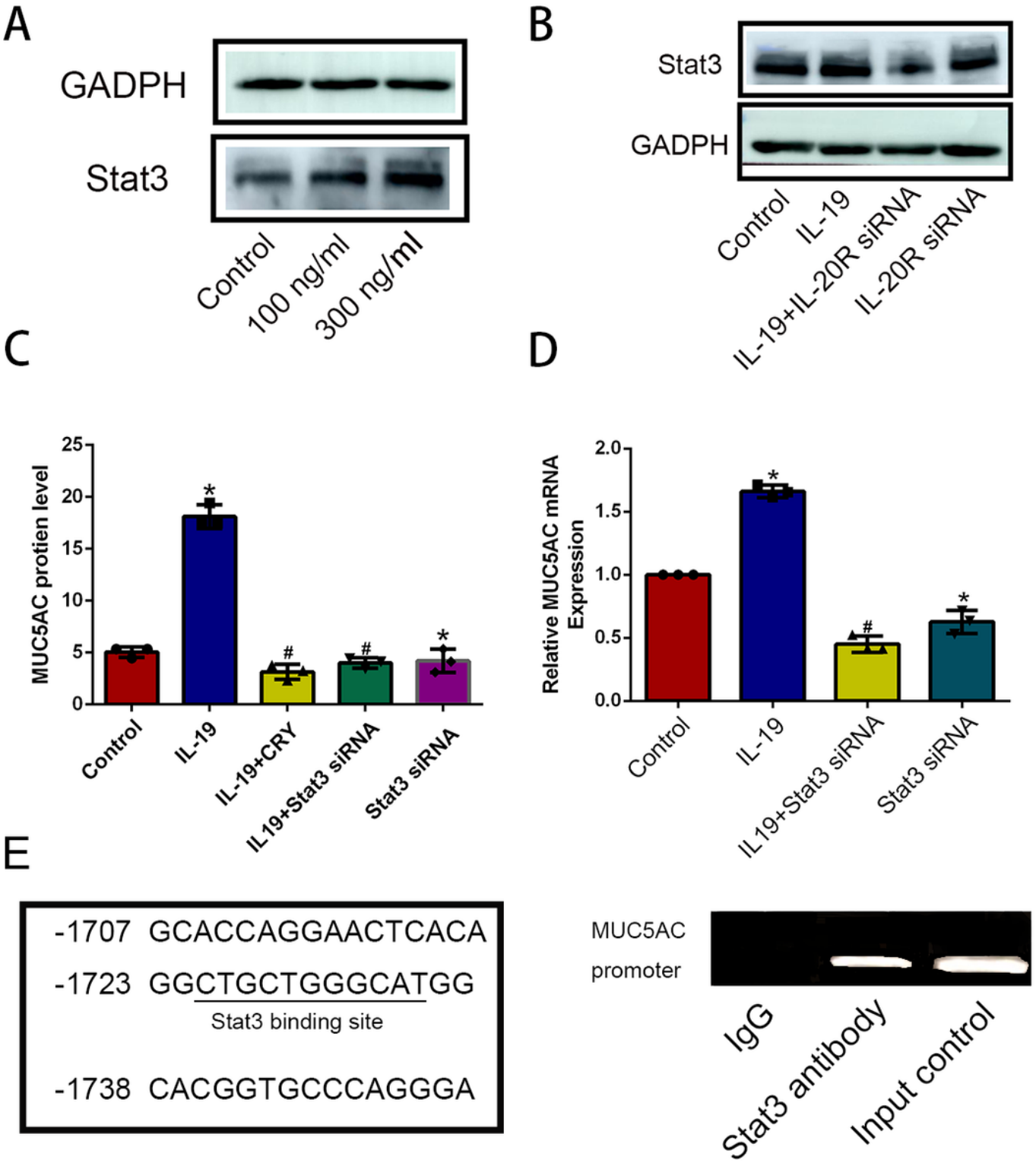

Figure 4

IL-19 up-regulate muc5ac expression via stat3 pathway.(A,B).western blot was used to detect the expression level of stat3 protein level in each group.(C)ELISA result of detecting MUC5AC protein level in each group.(D).PCR detection of MUC5AC mRNA level in each group.(E)The possible binding sites of stat3 in MUC5AC promoter sequence were predicted by database (jaspar.genereg.net) and verified by chip experiment.Statistical signifificance was calculated according to the Student t-test. *P < 0.05 compared with the control group.#P < 0.05 compared with the rhIL-19-treated group.

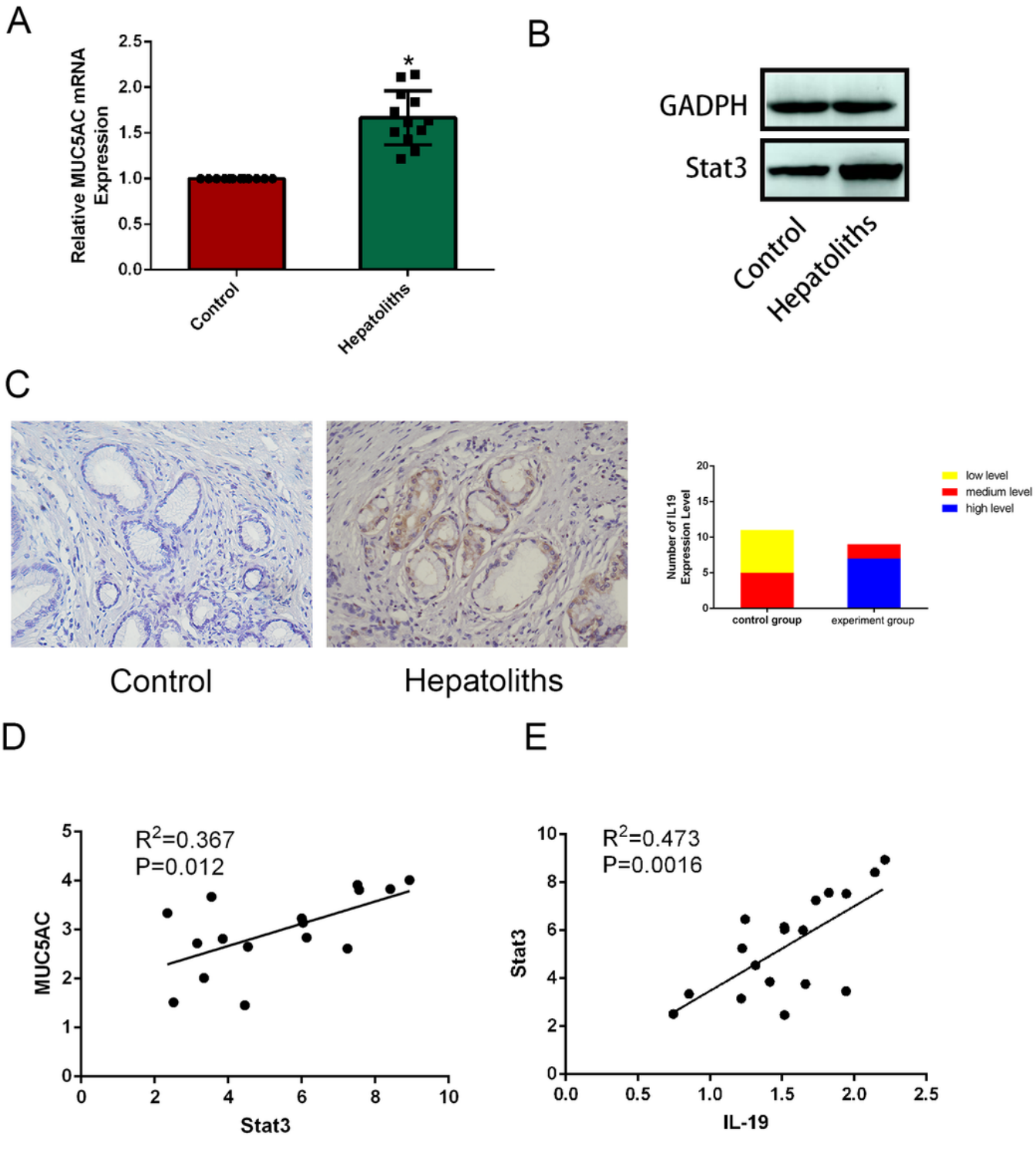

Figure 5

Verification of up-regulation effect of IL-19 and stat3 to MUC5AC following lithogene model establishment.(A).PCR detection of MUC5AC mRNA level in bile duct tissues in rats.(B).western blot was used to detect the expression level of stat3 protein level in bile duct tissues in rats.(C).IHC staining the IL-19 expression state in bile duct tissues in rats.(D.E),Correlation analysis of IL-19 between stat3 and stat3 between MUC5AC in bile duct tissues in rats.

Supplementary Files

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