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Prostatitis No.1 traditional chinese medicine significantly exhibited anti-inflammation role on prostatitis through miR-205-5p/YES1

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ABSTRACT

Prostatitis is one common male disease with a high prevalence. Traditional Chinese medicine (TCM) has been used as an alternative method for the treatment. However, the molecular mechanism of Prostatitis No.1 Traditional Chinese Medicine (P1TCM) on prostatitis is still unclear. For this purpose, the rat models were constructed and treated with PITCM of control, model, low (10 g/kg/d), medium (20 g/kg/d), and high (40 g/kg/d), as well as the transfections of medium dosage+NC mimic, and medium dosage+miR-205-5p mimic, medium dosage+NC mimic+pc-NC, medium dosage+miR-205-5p mimic+pc-NC, and medium dosage+miR-205-5p mimic+pc-v-YES-1 Yamaguchi sarcoma viral oncogene homolog 1 (*YES1*). Real-time quantitative PCR (qPCR) and western blotting analyses were carried out to evaluate the expression of miR-205-5p and *YES1*, respectively. The levels of interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α) were assessed by enzyme-linked immunosorbent assay (ELISA). The targeting role of miR-205-5p on *YES1* was predicted by StarBase and verified by a dual-luciferase reporter gene assay. Results showed that the optimal treatment of P1TCM relieved the damage of prostate tissue, decreased the immunity and inflammation factors, and reduced the expression level of miR-205-5p in prostate tissue and serum. miR-205-5p mimics significantly relieved tissue damage and reduced immunity and inflammatory functions. miR-205-5p targeted *YES1*. *YES1* was significantly upregulated in medium dosage treatment compared with Control, while downregulated compared with the Model. *YES1* was also upregulated in prostatitis patients. The pc-YES1 reversed the function of the miR-205-5p mimic. In conclusion, P1TCM significantly relieved the tissue damage and reduced prostate patients' inflammatory functions through miR-205-5p/YES1, which might be essential for clinical studies.

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Introduction

Prostatitis is one common male disease diagnosed with a prevalence of 1.8%–8.2% (1). However, it has been estimated that about 50% of men experience symptoms of prostatitis during their lifetime (2). Worse, prostatitis exhibited a high developing recurrence of 20%–50% (3). With epididymitis and orchitis, prostatitis accounts for 5%–12% of male infertility (4). The prostatitis contains chronic pelvic pain syndrome, asymptomatic inflammatory prostatitis, acute bacterial prostatitis, and chronic bacterial prostatitis, in which clinically non-bacterial prostatitis is more common than bacterial prostatitis. Bacterial, especially Gram-negative bacteria, is responsible for bacterial-type prostatitis (5, 6). The medical treatment for prostatitis contains anti-inflammatories, alpha-blockers, hormonal therapy, antimicrobials, and other medical therapies (7). However, ideal clinical efficacy can hardly be obtained, and these medicines exhibited side effects to some degree. Hence, a novel and effective treatment for prostatitis is required imminently.

Traditional Chinese medicine (TCM) has been widely used in clinical trials of prostatitis in recent years (8). According to TCM, the cause of prostatitis is mainly in the kidney. Yin deficiency and damp-heat, qi stagnation and blood stasis are the main pathogenic factors. By applying the unique diagnosis and treatment system of TCM to treat prostatitis, the clinical effect is remarkable (9). Recent studies have shown that the effective active ingredients in TCM can improve the blood supply of peripheral blood vessels and achieve the purpose of treatment (10). TCM has a unique advantage in the treatment of prostatitis by comprehensively regulating the body function through multi-level and multi-target mechanisms (11). Prostatitis No.1 traditional Chinese medicine (P1TCM) is an empirical formula invented by Professor Qingqi Zeng for the treatment of chronic prostatitis (12). P1TCM was mixed by SevenlobedYamRhizome, Dodder seed, Indian bread, Acorus tatarinowii Schott, Verbena officinalis (common vervain), Clematis chinensis Osbeck, angelica dahurica, and Yanhusuo W.T. Wang in a certain concentration. Animal experiments have shown that P1TCM is anti-inflammatory (12, 13), and clinical studies have also proved its safety and efficacy (14). Although P1TCM has been wi-

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dely used as an alternative and effective treatment of prostatitis in China, its molecular mechanism is still unclear.

MicroRNAs (miRNAs), about 17–25 nucleotides, target genes and play essential roles in regulating gene expression. miRNAs have been intensely studied for their immunoregulatory role and the regulation of the immune genes (15). Previous literatures have shown that miRNAs are implicated in the progression of prostatitis (16). In addition, Yan *et al*. (17) demonstrated that Jiedu Huoxue affects nonbacterial prostatitis via miRNAs regulation. MiR-205-5p is one biomarker reported to regulate prostate cancer pathogenesis (18, 19). Besides, it has been reported that miR-205-5p acts as tumor suppressor in many cancers, such as prostatic carcinoma and gastric cancer (20, 21). Therefore, whether related miR-205-5p and its targeted genes also regulate prostatitis treated with P1TCM is one meaningful subject to investigate.

YES, proto-oncogene 1 (*YES1*) is an important member of the Src family tyrosine kinases (SFKs) and a key regulator of tumor growth (22). The important role of *YES1* has also been reported to be involved in human cancer development, including prostate cancer (23). However, the function of *YES1* in prostatitis is obscure.

The present study investigated the role of P1TCM *in vivo* and clinical patients. Moreover, the regulation of miR-205-5p and its target gene on P1TCM-treated prostatitis was revealed. We might find a novel molecular therapeutic mechanism of P1TCM treating prostatitis through this study.

Materials and Methods

Preparation of recombinant lentivirus

Recombinant lentivirus LV-miR-205-5p and empty lentivirus were synthesized by Sangon Biotech (Shanghai, China). GV310 carrier was coded by enhanced green fluorescent protein (EGFP), digested by NheI and AgeI, and combined with miR-205-5p. Then, the GV310 was transfected into HEK 293T cells using a Lipofectamine 2000 kit (Thermo Fisher Scientific Inc.,). HEK 293T cells (Procell, Wuhan, China) were cultured in DMEM containing 10% FBS in an atmosphere of 5% CO_2 at 37°C. Two days after transfection, the LV-miR-205-5p was extracted from the mixture and assessed by enzyme-linked immunosorbent assay (ELISA). At the same time, the eGFP (LV-eGFP) carrier was used as Control.

Animals and treatments

Specific-pathogen-free (SPF) grade Sprague-Dawley (SD) rats at eight weeks old (male, weighing 200 ± 20 g) were obtained from Beijing Vital River Laboratory Animal Technology Co, Ltd. (Beijing, China), and housed at room temperature (25±0.5°C) with natural light, drank, and ate freely. All experimental animal operations must follow the experimental animal operation standards established by the Jiangsu Health Vocational College Animal Ethics Committee.

The rats were taken, intraperitoneally anesthetized, and the abdomen was depilated and disinfected. The skin and muscles were incised along the midline of the abdominal wall, the abdominal cavity was opened, and the prostate was exposed and injected with human *E. coli* (atcc25922, 2×10mcfu/l). Then, the abdominal muscles and skin were sutured.

A total of 200 SD rats were equally divided and injected with the following groups: Control, Model, low dosage (10 g/kg/d;), medium dosage (20 g/kg/d;), high dosage (40 g/kg/d;), medium dosage+NC mimic, and medium dosage+miR-205-5p mimic, medium dosage+NC mimic+pc-NC, medium dosage+miR-205-5p mimic+pc-NC, and medium dosage+miR-205-5p mimic+pc-v-YES-1 Yamaguchi sarcoma viral oncogene homolog 1 (*YES1*;). Two months after the injection, the rats were executed.

Calculation of the prostate index

After execution, the weight of rats and prostates was measured. The prostate index was calculated with (prostate weight/rat weight) ×10,000.

Histological evaluations

The prostate tissue cell injury and inflammation degree were evaluated by hematoxylin and eosin (HE) staining. The tissues were removed and fixed with 10% paraformaldehyde solution, followed by routine dehydration and paraffin embedding to prepare 5 μm tissue sections. The sections were placed in an incubator and heated at 68°C for 1 h, and then placed in xylene for dewaxing for 30 min. The sections were then hydrated in 100, 95, 85 and 75% gradient alcohol. After washing, sections were stained with hematoxylin and eosin. The sections were then infiltrated with xylene, and observed under a light microscope. The prostatitis was identified into four degrees by two experienced pathologists in a blind fashion, including no, mild, moderate, and obvious inflammations.

Immunohistochemistry (IHC) staining was carried out to evaluate the protein expression levels of $CD3^+$ T cells and $CD19^+$ B cells under treatments using a cell isolation kit (Invitrogen) according to the instructions of the IHC protocol. Paraffin-embedded tissues were dewaxed, rehydrated and treated with 0.3% hydrogen peroxide by heat-induced technique for antigen extraction. Then slides were treated with primary antibodies at 4°C overnight, followed by covering with appropriate secondary antibodies for 30 minutes at room temperature. Slides were rinsed and treated with a DAB+ chromogen solution (Dako) for 5 minutes. A counter-stain of haematoxylin was applied. The cell numbers were calculated in five randomly selected sights. HE and IHC images were collected under a CX31 microscope (Tokyo, Japan).

Real-time Quantitative PCR (qPCR) analysis

The expression levels of miR-205-5p and *YES1* were evaluated in serum samples of normal (n=30) and prostatitis patients (n=30), in the prostatitis tissue of rats, and in transfections. Total RNA in serum and tissues was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). For the qPCR analysis of miR-205-5p, the one-step kit of miRNA Universal SYBR qPCR Master Mix (#MQ101-02; Vazyme Biotech Co. Ltd; Nanjing, China) was used. As for the qPCR analysis of *YES1*, HiScript II One Step qRT-PCR SYBR Green Kit (#221-01; Vazyme Biotech Co. Ltd) was employed. The qPCR reaction process and amplification were performed refer to the operation manual. Reaction conditions (40 cycles): 95°C, 5min; 95°C, 15s; 60°C, 34s. The $2^{-\Delta\Delta Ct}$ method was employed for calculating the relative expression level (24), using β-actin as the intern gene. The primers of miR-205-5p and *YES1* were synthesized by Genscript Co. Ltd (Nanjing, China)

Table 1. The sequence of specific primers for RT-qPCR.

Gene symbol	Specific primers
miR-205-5p	Forward: 5'-TCCTTCATCCACCGGAGTCTG-3' Reverse: 5'- CAGTGCGTGTCGTGGAGT-3'
YES1	Forward: 5'- CGGGGTACC ATGGGCTGCATTAAAAGTAAAG-3' Reverse: 5'- TGCTCTAGA TAAATTTTCTCCTGGCTGGTAC-3'
U6	Forward: 5'- CTCGCTTCGGCAGCACA-3' Reverse: 5'- AACGCTTCACGAATTTGCGT-3'
β-actin	Forward: 5'- GCCTTCATACATCAAGTT-3' Reverse: 5'- AATCTTCGCCTTAATACT-3'

and listed in Table 1.

Western blotting analysis

The protein expression of YES1 was evaluated in rat tissues and transfections. The proteins were extracted and qualified using RIPA lysis buffer (#R0278, Sigma, USA) and a BCA kit (Solarbio, China). Briefly, 80 μg of each protein was separated using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method. The protein on the gel was transferred onto the polyvinylidene fluoride (PVDF) membrane, which was washed by 25mL TBST for 5min, and blocked by 5% skimmed milk powder at 4℃ overnight. Then, the gels were cultured with primary antibodies against YES1 (1:1,000, #ab109265, Abcam, USA) and GAPDH (1:500, #ab37168, Abcam). These membranes were washed and incubated with Goat anti-Mouse/Rabbit IgG(H+L)-HRP (1:5,000; #AS014; ABclonal, Cambridge, MA, USA). Finally, the PVDF membrane was washed and placed into the electrogenerated chemiluminescence (ECL) solution (ECL808-25, Biomiga, San Diego, CA) for 1 min. The bands were observed under a Tanon 6600 Luminescence imaging workstation (Tanon, China). Using GAPDH as an internal reference, the YES1 band value was calculated by Image-Pro Plus 6.0 (Media Cybernetics, Inc.).

ELISA analysis

As biomarkers of inflammation factors, the levels of interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α) were detected in rat tissues using an ELISA analysis, using the corresponding kits purchased from Sigma-Aldrich (St. Louis, MO, USA). Briefly, the prostate tissues were washed, cut into pieces, and placed in 0.86% ice-cold saline. The tissues were homogenized, and centrifuged, and the supernatant was collected and stored at 4 ℃.

Dual-luciferase reporter gene assay

Firstly, the sponging and targeting role of miR-205-5p on *YES1* were predicted by StarBase. Secondly, the wild type (WT) and mutant (Mut) sequences of *YES1* were synthesized by GenePharma (Shanghai, China). YES1-WT and YES-Mut were constructed using MIR-REPORTTM vectors (Thermo Fisher Scientific). According to the manufacturer's manual, the vectors were transfected into HEK 293T cell lines using Lipofectamine 2000 kit (Thermo Fisher Scientific Inc.,). Finally, one Dual-Luciferase Reporter Assay System bought from Promega Corporation (Madison, WI, USA) was employed to detect the luciferase activity.

Statistical analysis

GraphPad Prism 8 (San Diego, CA, USA) was employed for the statistical analysis and chart drawing. Unpaired student's t-test, one-way ANOVA, and Tukey's multiple comparison analyses were performed depending on the groups. Data are exhibited as mean ± standard deviation (SD). $P<0.05$ was considered a significant difference.

Results

Selection of the target gene of miR-205-5p

The target genes of miR-205-5p were screened by the software ENCORI, Targetscan, and DIANA (Figure S1). A total of 12 common genes were selected, in which *YES1* was chosen for further study.

Selection of optimal P1TCM dosage on prostatitis

The *in vivo* experiment was conducted to select the optimal dosage of P1TCM (Figure 1A). Compared with the prostate index in the model group, the rats treated with P1TCM at dosages of low ($P<0.05$), medium ($P<0.01$), and high ($P<0.01$) exhibited significantly decreased prostate index (Figure 1B). We could observe from the HE staining that the prostate tissues were severely damaged in the Model group, which were remised with the treatments in low, medium, and high dosages of P1TCM (Figure 1C). As shown in Figure 1D, the $CD3^+$ T cells and $CD19^+$ B cells account for the majority of infiltrating inflammatory cells in rat models. Compared with the Model group, the number of $CD3^+$ T cell and $CD19^+$ B cell counts were significantly decreased with P1TCM treatments at low ($P<0.05$), medium ($P<0.01$), and high dosages ($P<0.01$). As for the inflammatory factors of IL-1β and TNF-α, their levels were significantly elevated in the Model group ($P<0.001$; Figure 1E). With the supplement of P1TCM, the level of IL-1β and TNF-α were significantly reduced with the dosages of low ($P<0.05$), medium ($P<0.01$), and high ($P<0.01$). The expression of miR-205-5p was significantly downregulated in the Model compared with the Control ($P<0.001$; Figure 1F). With the addition of P1TCM, the expression of miR-205-5p was significantly upregulated at low dosage ($P<0.001$), medium, and high dosage ($P<0.001$). Minor differences were observed at the medium and high dosages ($P>0.05$). We also evaluated the expression of miR-205-5p in the serum of clinical patients

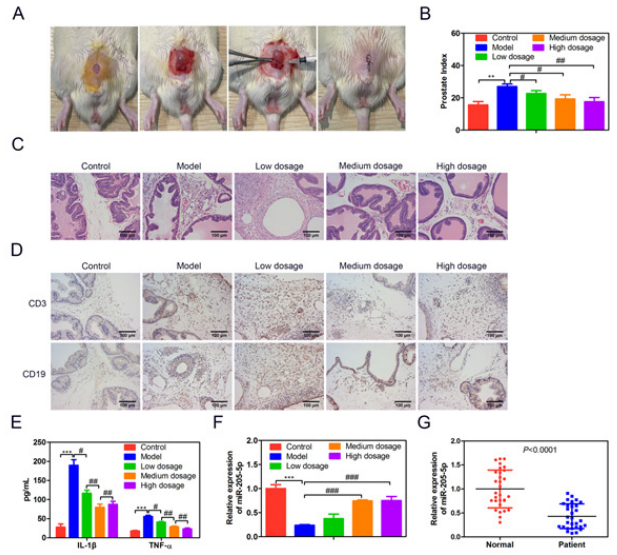

Figure 1. Selection of optimal P1TCM dosage on prostatitis. (A) The anesthetized rat was sterilized with iodophor in the lower abdomen and cut with a longitudinal median incision about 1 cm in length. (B) The prostate index was calculated with (prostate weight/rat weight) ×10000. (C) P1TCM alleviated rat prostatitis histological damage. * P<0.05 *vs* Model. (D) P1TCM decreased CD3$^+$ T cell and CD19$^+$ B cells in the prostatitis rat model. (E) The inflammatory factors of IL-1β and TNF-α were evaluated by ELISA assay. (F) qPCR analysis was performed to evaluate the expression level of miR-205-5p in rat models. (G) qPCR analysis was performed to evaluate the expression level of miR-205-5p in the serum of prostatitis patients. Data are shown as mean ± SD. ** P<0.01, *** P<0.001 *vs* Control. # P<0.05, ## P<0.01, ### P<0.001 *vs* Model.

by qPCR. It was significantly downregulated in prostatitis patients compared with normal patients (P<0.0001; Figure 1G). Combining the results above, we selected P1TCM at a medium dosage (20g/kg/d) as the optimal treatment for prostatitis. Moreover, the medium dosage can reduce the prostate index, relieve tissue damage, decrease inflammation factors, and reduce the expression level of miR-205-5p in prostate tissue and serum.

Overexpressed miR-205-5p significantly regulates tissue injury and inflammation in P1TCM-treated prostatitis

We assessed the role of P1TCM and miR-205-5p on prostatitis *in vivo*. The expression level of miR-205-5p was significantly upregulated in medium dosage+NC mimics and medium dosage+miR-205-5p mimic groups compared with Model (P<0.01; P<0.001; Figure 2A). As observed from HE staining, the combined treatment of medium dosage+miR-205-5p mimic exhibited a significantly decreased tissue injury compared with Model (P<0.01) and medium dosage+NC mimic (P<0.05; Figure 2B). The IHC experiment showed that the number of CD3$^+$ T cells and CD19$^+$ B cells were significantly reduced in the medium dosage+miR-205-5p mimic compared with Model (P<0.01) and medium dosage+NC mimic (P<0.05; Figure 2C). Compared with the Model and medium dosage+NC mimic, the inflammatory factor levels of IL-1β and TNF-α were significantly decreased in the medium dosage+miR-205-5p mimic (P<0.05; P<0.01; Figure 2D). Combined together, the combined treatment of P1TCM and miR-205-5p significantly reduced the tissue injury, reduced levels of inflammatory factors, upregulated the expression of miR-205-5p and downregulated the expression of *YES1*.

miR-205-5p targets YES1

Considering the opposed regulation trend of miR-205-5p and *YES1* in the above results, a further confirming experiment was performed. The bioinformatics analysis found that *YES1* has potential binding sites on miR-205-5p (Figure 3A). These binding sites between *YES1* and miR-

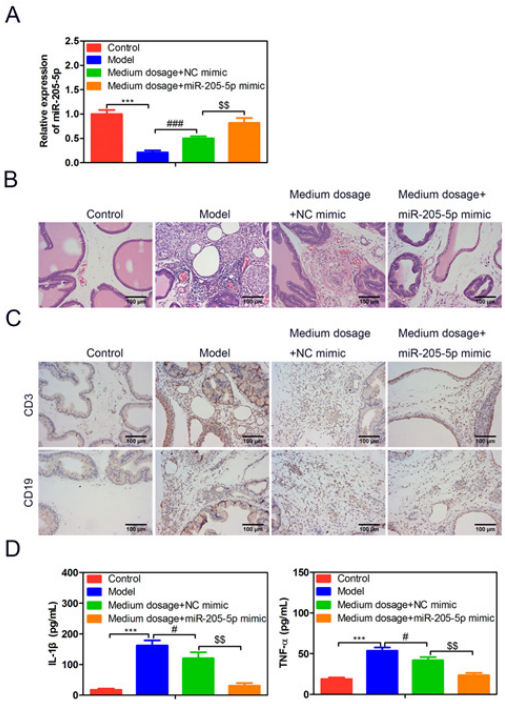

Figure 2. Overexpressed miR-205-5p significantly regulates tissue injury and inflammation in P1TCM-treated prostatitis. Overexpressed miR-205-5p was constructed, and the role of miR-205-5p on P1TCM-treated prostatitis was analyzed. (A) qPCR analysis was performed to evaluate the expression level of miR-205-5p in rat models. (B) HE staining revealed the tissue injury treated with P1TCM and overexpressed miR-205-5p. (C) IHC staining revealed the CD3$^+$ T cell and CD19$^+$ B cell numbers treated with P1TCM and overexpressed miR-205-5p. (D) The inflammatory factors of IL-1β and TNF-α were evaluated by ELISA assay. *** P<0.001 *vs* Control. # P<0.05, ## P<0.01 *vs* Model. $$ P<0.01 *vs* Medium dosage + NC mimic.

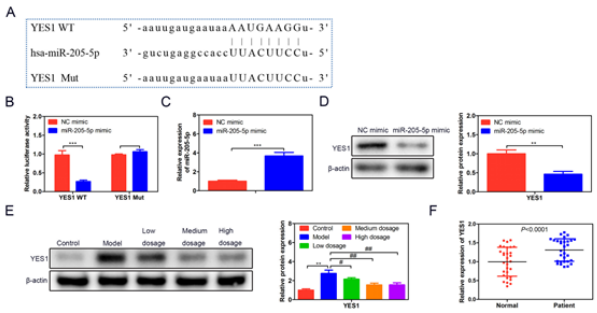

Figure 3. miR-205-5p targets YES1. (A) Starbase predicted the binding sites of miR-205-5p on YES1. (B) A dual-luciferase reporter assay evaluated these binding relationships between *YES1* and miR-205-5p. (C) The transfection of miR-205-5p was tested by qPCR. (D, E) The expression level of YES1 was assessed by western blotting in transfections and treatments. (F) The expression level of YES1 was assessed in the serum of the prostatitis patients. (A-D) ** P<0.01, *** P<0.001 *vs* NC mimic. (E-F) ** P<0.01 *vs* Control. # P<0.05, ## P<0.01 *vs* Model.

205-5p were then tested using a dual-luciferase reporter assay, in which overexpressed miR-205-5p significantly reduced the relative luciferase activity of YES1-WT, but not that of YES1-MUT (P<0.001; Figure 3B). To verify the luciferase assay, we evaluated the transfection efficacy by qPCR. The overexpressed expression of miR-205-5p in miR-205-5p mimic group revealed a successfully constructed transfection (P<0.001; Figure 3C). In contrast, overexpressed miR-205-5p significantly downregulated the expression of *YES1* compared with the NC mimic (P<0.01; Figure 3D). The expression level of miR-205-5p and *YES1* exhibited an opposite trend in the NC mimic and miR-205-5p mimic. Moreover, we assessed the expression level of *YES1* in different P1TCM treatments. The results showed significantly *YES1* was significantly upregulated in the Model compared with the Control (P<0.01; Figure 3E). With the addition of P1TCM, the expression level of *YES1* was gradually decreased, especially at the medium and high dosage (P<0.05; P<0.01; Figure 3E). Not only *in vivo* but the expression level of *YES1* was also assessed in clinical samples, which was significantly upregulated in prostatitis patients (P<0.0001; Figure 3F), opposing with that of miR-205-5p (Figure 1G). Taken together, we found a confirmed relationship between *YES1* and miR-205-5p. Moreover, they were negatively correlated *in vivo* and clinical samples.

The effect of P1TCM on prostatitis was regulated by the miR-205-5p/YES1 axis through regulating the inflammatory cells and inflammation factors

After confirming the targeting role between *YES1* and miR-205-5p, we further studied their roles in prostatitis. The expression level of miR-205-5p and *YES1* exhibited an opposed regulation trend in the treatments of medium dosage+NC mimic+pc-NC, medium dosage+miR-205-5p mimic+pc-NC, and medium dosage+ miR-205-5p mimic+pc-YES1 (P<0.05; P<0.01; P<0.001; Figure 4A). No significant differences were observed in the expression level of miR-205-5p in medium dosage+miR-205-5p mimic+pc-NC and medium dosage+ miR-205-5p mimic+pc-YES1 groups (P>0.05). We can observe from the HE staining that the tissues were severely damaged in the medium dosage+ miR-205-5p mimic+pc-YES1 group compared with the medium dosage+miR-205-5p mimic+pc-NC (P<0.05; Figure 4B), revealing that overexpressed YES1 significantly reversed the role of miR-205-5p mimic on tissue cell injury. A similar expressed trend was assessed in the IHC experiment at CD3 and CD19 cells (Figure 4C). pc-YES1 significantly reversed the function of miR-205-5p. The levels of IL-1β and TNF-α were significantly increased in the medium dosage+ miR-205-5p mimic+pc-YES1 group when compared with medium dosage+miR-205-5p mimic+pc-NC (P<0.05; P<0.01; Figure 4D). In summary, we conclude that miR-205-5p combined with the treatment of P1TCM significantly relieved the tissue injury, reduced IHC expression, and decreased the levels of inflammatory factors. Moreover, the function of miR-205-5p was reversed by its target gene of *YES1*.

Discussion

Prostatitis is one common disease puzzling men worldwide (25). P1TCM is one treatment for chronic prostatitis used in several hospitals in China. Moreover, miRNAs were reported to be involved in disease regulation in various aspects (26). In the present study, we first selected the optimal concentration of P1TCM on prostatitis. Then, we investigated the role of miR-205-5p and its target gene in regulating P1TCM-treated prostatitis. This study drew the following conclusions: (1) Medium concentration (20g/kg/d) of P1TCM on rats for 28 days was selected as the optimal dosage. (2) The medium P1TCM could significantly reduce the tissue injury and inflammation of prostatitis rats. (3) *YES1* was confirmed to be one target gene of miR-205-5p. (4) The effects of P1TCM on prostatitis were regulated by the miR-205-5p/YES1 axis.

To the best of our knowledge, prostatitis is a complex pathological process involving inflammation (27). In the present study, the prostatitis rat model was successfully constructed, and different concentration treatment of P1TCM was employed on rats. We found that the 20g/kg/d of P1TCM can significantly reduce the tissue injury and inflammation of prostatitis rats, demonstrating that P1TCM protects against prostatitis through anti-inflammatory effects. The infiltrating inflammatory cells of CD 3^+ T cells and $CD19^+$ B cells, as well as the inflammatory

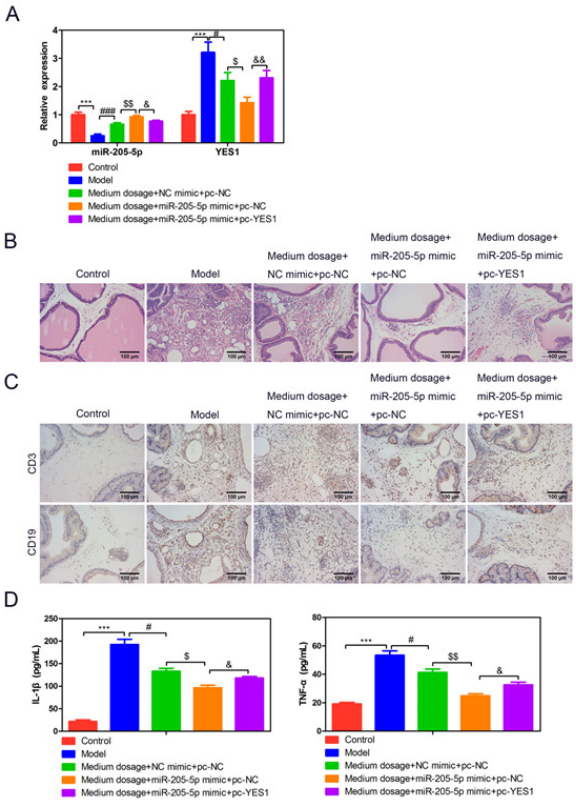

Figure 4. The miR-205-5p/YES1 axis regulated the effect of P1TCM on prostatitis through regulating the inflammatory cells and inflammation factors. (A) Overexpressed miR-205-5p and YES1 were constructed, and the role of miR-205-5p/YES1 on P1TCM-treated prostatitis was analyzed. (A) qPCR analysis was performed to evaluate the expression level of miR-205-5p and YES1 in rat models. (B) HE staining revealed the tissue injury treated with P1TCM and overexpressed miR-205-5p and YES1. (C) IHC staining revealed the $CD3^+$ T cell and $CD19^+$ B cell numbers treated with P1TCM and overexpressed miR-205-5p and YES1. (D) The inflammatory factors of IL-1β and TNF-α were evaluated by ELISA assay. *** P<0.001 *vs* Control. # P<0.05, ### P<0.001 *vs* Model. $ P<0.05, $$ P<0.01 *vs* Medium dosage + NC mimic + pc-NC. & P<0.05, && P<0.01 *vs* Medium dosage + miR-205-5p mimic + pc-NC.

factors of IL-1β and TNF-α, were significantly influenced. The anti-inflammation effect of P1TCM identified in our present study was consistent with that in other studies (12, 13). Therefore, we further investigated the role of miR-205-5p/YES1 on prostatitis.

miR-205-5p has been widely studied as one molecule that plays an essential role in inflammation (28, 29). For example, miR-205-5p is involved in ischemic/reperfusion (I/R) injury through suppressing inflammation (30). Zhang *et al*. (28) demonstrated that knockdown miR-205-5p alleviates the inflammatory response in allergic rhinitis. MiR-205-5p is also studied in prostate-related diseases. For example, Li *et al*. (31) demonstrated that miR-205-5p inhibited cell migration and invasion in prostatic carcinoma by targeting zinc finger E-box binding homeobox 1 (ZEB1). miR-205-5p is also one promising biomarker of prostate and bladder cancer (19). miR-205-5p is involved in prostate cancer pathogenesis by inhibiting cancer cell aggressiveness (18). However, none of the studies have reported the role of miR-205-5p on prostatitis, not to mention their role in regulating P1TCM-treated prostatitis. Our present study revealed the targeting role of miR-205-5p on prostatitis for the first time, which was significantly downregulated in prostatitis patients and has been upregulated under P1TCM treatment. Overexpressed miR-205-5p significantly reduced tissue injury and decreased the inflammatory cells and the levels of inflammatory factors.

As well-known, miRNAs function posttranscriptionally by usually base-pairing to the mRNA 3'-untranslated regions to repress protein synthesis (32). Consistently, miR-205-5p exerts its effect on the progression of disease via targeting its mRNAs (33). Here, we explored the miR-205-5p/YES1 axis on regulating graded P1TCM-treated prostatitis. *YES1* is reported to be a targetable oncogene in cancers (34). YES1 has been reported to be a therapeutic target and biomarker in cancers (35). Besides, YES1 functions as an oncogene in prostate cancer (36). Its role in regulating prostatitis has never been revealed. We found that the expression of *YES1* was significantly downregulated under graded treated P1TCM. *YES1* exhibited counteract role of miR-205-5p in regulating P1TCM-treated prostatitis. Taken together, we concluded that the miR-205-5p/YES1 axis regulated the effect of P1TCM on prostatitis through regulating the inflammatory cells and inflammation factors.

There are several limitations in this study. First, the samples in our study were relatively small. Second, the main functional component of P1TCM on prostatitis was not explored in detail. In addition, whether P1TCM regulated prostatitis through certain signaling pathways was obscure. Therefore, our study needs to perform more experiments to perfect our study.

Our present study revealed the role of miR-205-5p/YES1 on P1TCM-treated prostatitis for the first time. We concluded that the miR-205-5p/YES1 axis regulated the role of P1TCM on prostatitis through the anti-inflammation effect.

Conflict of interest

All authors confirm that there are no conflicts of interest in this study.

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