Am J Cancer Res 2015;5(5):1706-1717
www.ajcr.us /ISSN:2156-6976/ajcr0004392

Original Article

Down regulation of MiR-93 contributes to endometriosis through targeting MMP3 and VEGFA

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Received December 3, 2014; Accepted April 12, 2015; Epub April 15, 2015; Published May 1, 2015

Abstract: *Objective:* This study aimed to explore the role of miRNAs in pathogenesis of endometriosis. *Methodology:* Endometrial samples from 57 females with endometriosis and 44 non-endometriotic controls were compared for the expression of a selected group of miRNAs. The regulatory function on downstream target was also explored. *Results:* The expression of miR-93 and miR106a was significantly reduced in endometriotic samples compared to that in non-endometriotic samples. High levels of MMP3 and VEGFA were detected in more than 50% ectopic endometrium tissues. A negative association was found between the expression of miR-93 and the protein levels of MMP3 (Pearson correlation, r=-0.39, P=0.0025) or VEGFA (Pearson correlation, r=-0.37, P=0.0047) in samples from endometriosis patients. Mechanistically, miR-93 targeted MMP3 and VEGFA by directly binding to the 3'UTR of *MMP3* and *VEGFA* mRNAs, and thereby inhibited the proliferation, migration and invasive capability of endometrial stromal cells (ESCs). *Conclusion:* The finding of this study suggests that deregulation of miR-93 contribute to endometriosis by up-regulation of MMP3 and VEGFA and thus provide potential therapeutic targets for the treatment of endometriosis.

Keywords: Endometriosis, microRNA, proliferation, migration, invasion

Introduction

Endometriosis is a polygenic gynecological disorder that is commonly diagnosed in women [1]. It is defined as the retrograde passage of endometrial tissue outside the uterine and its subsequent proliferation in the ectopic location. Endometriosis is associated with dysmenorrhea, dyspareunia, chronic pelvic pain, irregular uterine bleeding, and even ovarian cancer [2]. The development of new diagnostic or therapeutic methods for endometriosis is hampered due to the lack of knowledge regarding the etiology, pathogenesis and natural progression of endometriosis.

MicroRNAs (miRNAs) are short non-coding RNAs that repress gene expression by binding to the 3'UTR of mRNA directly and play crucial roles in various physiological processes. Abnormal expression of miRNAs has been found to be related to human diseases ranging from psychiatric disorders to malignant cancers [3-5]. It has been increasingly recognized that dysfunction of miRNAs is related to gynecological disorders, including abortion, polycystic ovary syndrome and premature ovarian failure [6-9]. Recently, it has been reported that disturbed expression of miRNAs is related to endometriosis. miRNAs play an important role in the pathogenesis of endometriosis. It has been found to be aberrantly expressed in endometriotic stromal cells. And altered plasma microRNAs can be used as novel biomarkers for diagnosis of endometriosis and endometriosis-associated ovarian cancer [10-12]. However, how miRNAs regulate endometriosis is unclear. More clinical data are needed to confirm the role of miRNAs in the pathogenesis of endometriosis meanwhile more functional studies are required to unveil the complex roles of dysregulated miRNAs. Therefore, the aim of this study was to explore the role of miRNAs in pathogenesis of endometriosis. To achieve this, in this study we examined the expression of the 11 selected miRNAs in the endometrial samples from patients with or without endometriosis. Furthermore, the regulatory function on downstream target was also explored.

Materials and methods

Clinical samples

All the protocol of this study was approved by Ren Ji hospital ethics committee. All patients have been provided with written informed consent prior to participation of this study.

Fifty seven Chinese-Han women with endometriosis were included in this study (mean age: 35.8, range: 22-47). All women underwent laparoscopic surgical examination of the abdominal cavity and completed excision of endometriotic tissue. None of the patients had received pre-operative hormonal therapy. Endometriosis was diagnosed either by clinical examination or by ultrasonography and confirmed by postoperative pathological examination of the surgically removed samples. Laparoscopic examination of the abdominal cavity excluded the presence of any other pelvic disorders that could potentially confound the data observed. The main symptoms for surgery in this group of patients were abdominal pain (71.9%) and sterility (28.1%).

Forty-four Chinese-Han asymptomatic women without endometriosis, who underwent surgery for laparoscopic tubal sterilization, were included in the control group (mean age: 36.5, range: 28-45). Absence of endometriosis was confirmed by surgical examination of the abdominal cavity.

The menstrual phase was identified according to the day of the reproductive cycle and histological analysis of the endometrium. At the time of surgery, 32 (56%) women with endometriosis were in the proliferative phase, and 25 (44%) were in the secretory phase of the menstrual cycle. And 23 (52%) controls were in the proliferative phase and 21 (48%) were in the secretory phase of the menstrual cycle. Women in the menstrual phase were excluded from the study. Each sample was divided and used for total RNA extraction, protein extraction, as well as cell isolation.

RNA extraction and quality determination

Total RNA was extracted from both the endometrial as well as endometriotic tissues and control endometrial tissues using the mirVana miRNA isolation kit (Ambion, Austin, TX, USA) according to the manufacturer's protocol. The RNA concentration and purity were determined by NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). Following the manufacturer's specifications, only samples with A260/A280 ratio of ~2.0 and A260/230 ratio in the range of 1.9-2.2 were considered for inclusion in this study.

Quantification of selected miRNAs by quantitative RT-PCR

Quantitative RT-PCR analysis was used to determine the relative level of 11 selected miRNAs (miR-93, -Let-7i, -183, -106b, -125a, -21, -202, -199a, -138, -16, -133b). The levels of miRNAs were detected by TaqMan miRNA RT-Real Time PCR. Single-stranded cDNA was synthesized by using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) and then amplified by TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) together with miRNA-specific TaqMan MGB probes (Applied Biosystems, Foster City, CA, USA). The U6 snRNA was used for normalization. Each sample in each group was measured in triplicate, and the experiment was repeated at least three times.

Western blotting

Protein extracts were boiled in SDS/β-mercaptoethanol sample buffer, and 30 μg samples were loaded into each lane of 10% polyacrylamide gels. The proteins were separated by electrophoresis, and the proteins in the gels were blotted onto PVDF membranes (Amersham Pharmacia Biotech, St. Albans, Herts, UK) by electrophoretic transfer. The membrane was incubated with mouse anti-VEGFA or anti-MMP3 monoclonal antibody (Abcam, Cambridge, MA, USA), and mouse anti-β-actin monoclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) at 37°C for 1 h. The specific protein-antibody complex was detected by using horseradish peroxidase conjugated rabbit anti-mouse IgG. Chemiluminescence reaction was carried out using the ECL kit (Pierce, Appleton, WI, USA) for detection. The β-actin signal was used as a loading control.

Cell culture

The endometrial stromal cells (ESCs) from participants without endometriosis were cultured as previously described [13]. Briefly, the minced eutopic endometrium was digested with collagenase type α (0.1%; Sigma, USA) for 30 min at 37°C and then filtrated through a 200 μmol/l wire sieve to remove debris. Following gentle

centrifugation, the supernatant was discarded, and the cells were resuspended in 1:1 formula of DMEM (Dulbecco's modified Eagle's medium)/F-12 (Gibco, USA). ESCs were separated from epithelial cells by passing over a 400 μmol/l wire sieve. The filtrated suspension was layered over Ficoll and centrifuged at 2000 rpm for 20 min to further remove leukocytes and erythrocytes. The middle layer of cells was collected and washed with D-Hanks. The ESCs were placed in a culture flask and allowed to adhere for 20 min. The adherent stromal cells were cultured as monolayer in flasks with DMEM/F-12 containing 10% fetal calf serum (FCS; Hyclone, Logan, UT, USA), 20 mmol/l HEPES, 100 IU/ml penicillin and 100 μg/ml streptomycin.

Dual luciferase assays

To generate 3'-UTR luciferase reporter, full length of 3'-UTR from MMP3 and a 533 bp segment of 3'UTR from VEGFA were cloned into the downstream of the firefly luciferase gene in pmirGLO (Promega, Madison, WI USA). MiR-93 mimic and miR-93 inhibitor were synthesized by GenePharma Co., Ltd (Shanghai, China). For luciferase reporter assays, HEK293T cells were seeded in 48-well plates. Luciferase reporter vectors were co-transfected with miR-93 mimic or miR-93 inhibitor by using lipofectamine 2000 (Invitrogen, Carlsbad, CA USA). Two days later, cells were harvested and assayed with the Dual-Luciferase Assay kit (Promega, Madison, WI USA). Each treatment was performed in triplicate in three independent experiments. The results were expressed as relative luciferase activity (Firefly LUC/Renilla LUC).

Cell proliferation assay

Cell proliferation was estimated by the MTT assay. ESCs were seeded in 96-well plates at low density (2×10^3) in DMEM-F12 culture and allowed to attach overnight. The cells were then transfected with miR-93 mimic or inhibitor, with scrambled sequence RNA as control. There were 3 wells in each group. Twenty microliters MTT (5 mg/ml) (Sigma, St. Louis, MO, USA) were added to each well 48 h after transfection, and the cells were incubated for further 4 h. The absorbance was recorded at A570 nm with a 96-well plate reader after the addition of DMSO.

The EdU proliferation assays were also used for detecting the effect of miR-93 on ESCs proliferation. ESCs were seeded in 12-well plates at low density (5×10^4) in DMEM culture. Cell proliferation was examined by EdU Cell Proliferation assay kit (Cell-Light Edu Apollo DNA in vitro Kit (Guangzhou RiboBio Co., Ltd, Guangzhou, China).

Cell migration and invasion assay

A typical Transwell assay (Costar, 6.5 mm diameter, 8 μm pore size) was used. A number of 3×10^4 cells in 200 μL serum-free medium were seeded to the top chamber, and 500 μL medium with high concentration of serum was added to the bottom. After 12 h, filters were then submerged in 4% PFA for 15 min and cells on the upper surface were removed by cotton swabs. The cells on the lower surface were stained with hematoxylin. Ten random fields were selected to determine the average number of cells per view field.

For cell invasion assays, the procedure was similar to the cell migration assay, except that transwell membranes were pre-coated with 24 μg/μl Matrigel (R&D Systems, USA), and the cells were incubated at 37°C for 8 hr in 5% CO_2 atmosphere. Cells adhering to the lower surface were determined in the same way as the cell migration assay.

Statistical analysis

All statistical analyses were performed using the SPSS v. 16.0 software (SPSS, Inc., Chicago, IL, USA). Student's t-test was employed to analyze the expression of miRNAs, the relationship between the expression of miR-93 and MMP3 or VEGFA protein level, and the luciferase activities and migrated or invaded cell numbers. The findings were considered to be significant at a P-value < 0.05.

Results

MiR-93 and miR-106b are down-regulated in endometriotic tissues

MiRNAs play crucial roles in various physiological activities. Microarray data suggests that the expression profile of miRNAs in endometriotic tissues is distinct from that in non-endometriotic tissues, indicating abnormal expression of miRNAs is related to endometriosis. To further examine the role of miRNAs in endometriosis, we focused on the 11 selected miRNAs which

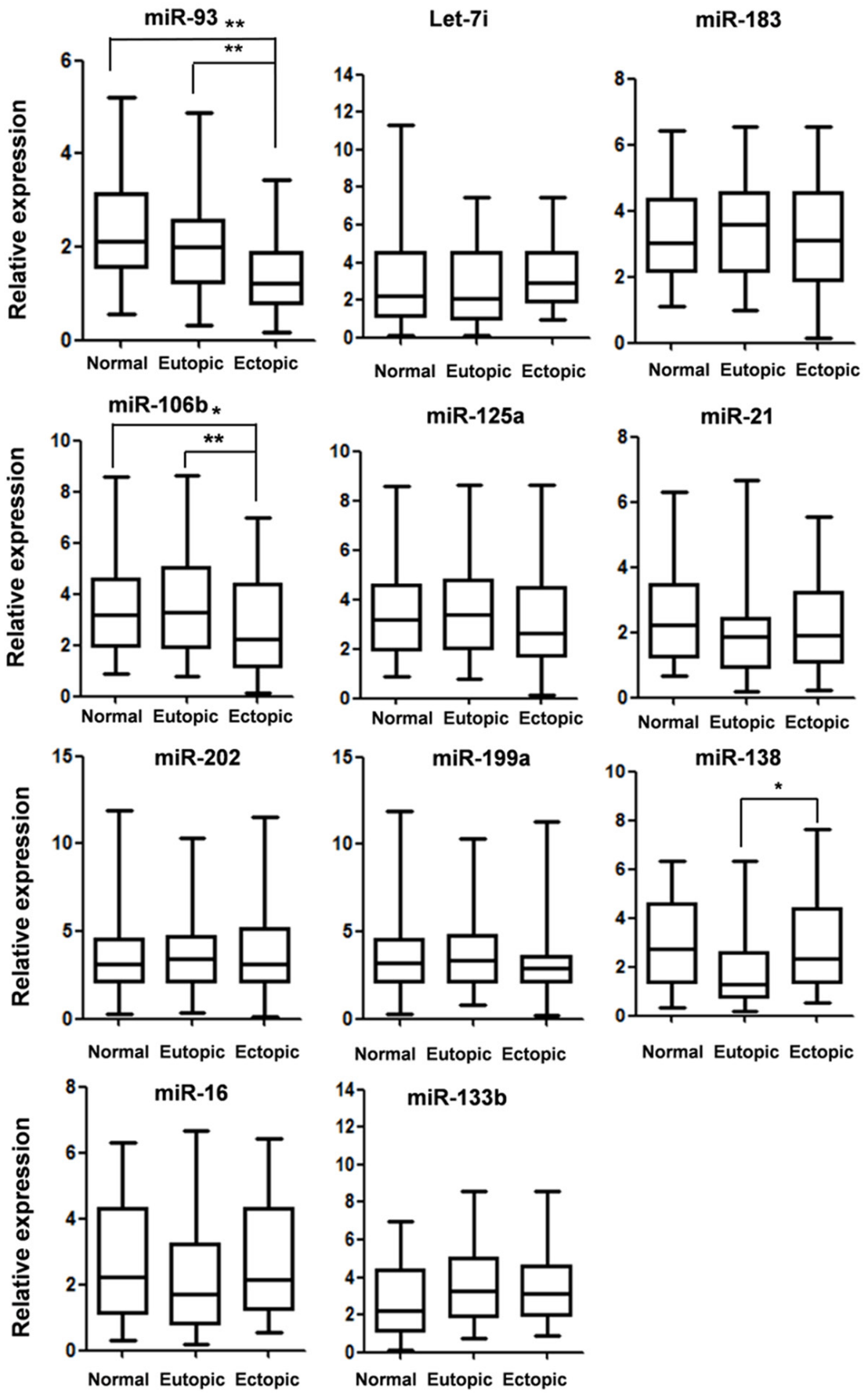
miR-93 **
Let-7i
miR-183
miR-106b *
miR-125a
miR-21
miR-202
miR-199a
miR-138
miR-16
miR-133b
Relative expression
Normal Eutopic Ectopic
**
*

Figure 1. *miR-93 and miR-106 were down-regulated in ectopic endometrium.* The expression levels of 11 selected miRNAs in ectopic and eutopic endometrium samples from endometriosis patients, as well as normal endometrium samples from general population were detected using stem-loop qRT-PCR. The results were analyzed using student t-test, and $P < 0.05$ was considered statistically significant. *$P < 0.05$, **$P < 0.01$.

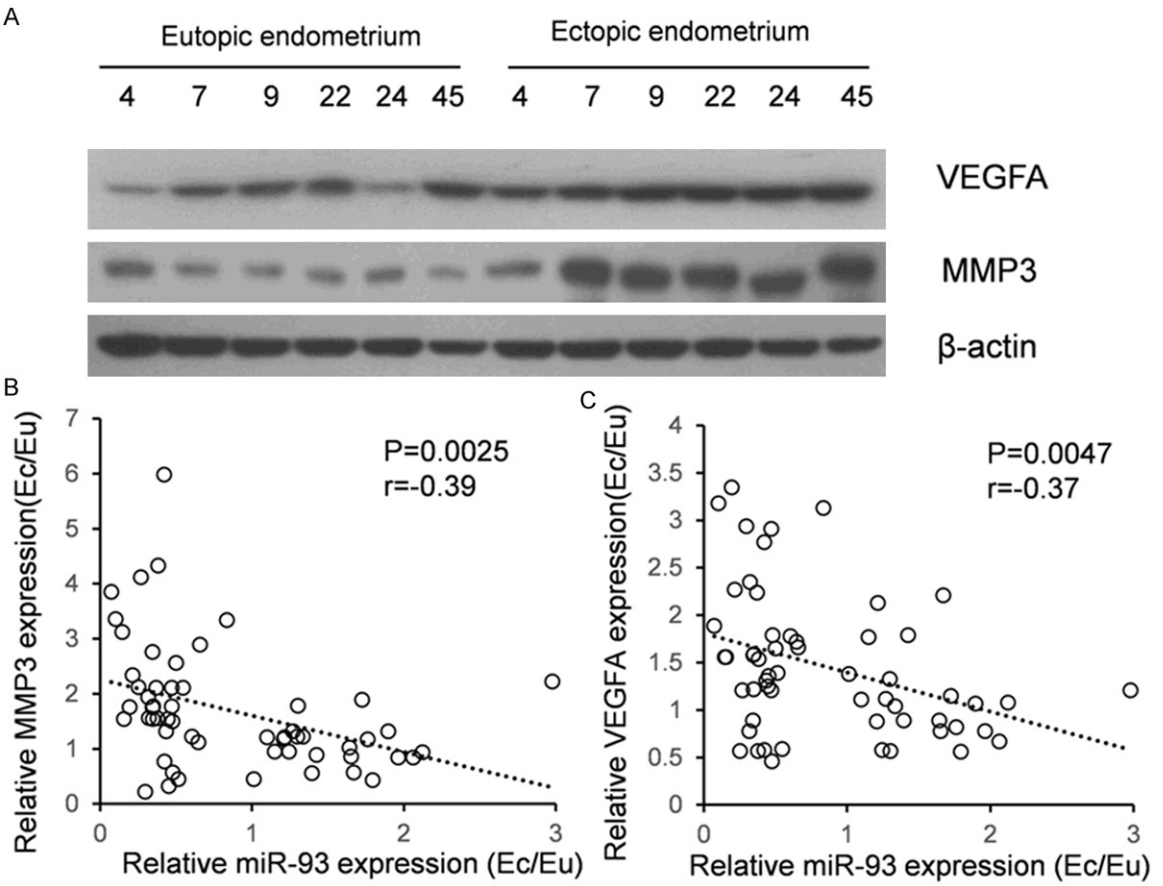

Figure 2. *The correlation between miR-93 expression and protein levels of MMP3 and VEGFA in endometriosis patients.* A. The expressions of MMP3 and VEGFA in ectopic and eutopic endometrium samples were detected by western blot. To evaluate the relative expressions of MMP3 and VEGFA, the band intensities of western blot were semi-quantitatively calculated by Quantity One software. B. An inverse correlation was defined between miR-93 and MMP3 levels in 57 clinical samples of endometriosis patients (Pearson correlation, r=-0.39, P=0.0025). C. An inverse correlation was identified between miR-93 and VEGFA levels in 57 clinical samples of endometriosis patients (Pearson correlation, r=-0.37, P=0.0047).

have been reported differentially expressed in endometriotic tissues and examined their expression in endometrium from Chinese-Han patients with or without endometriosis. As shown in **Figure 1**, the expression levels of miR-93 or miR-106a were significantly reduced in endometriotic samples compared with the normal endometrium. Because miRNAs are well-recognized as suppressors for gene expression by targeting the 3'UTR of mRNAs, we searched for the targets of miR-93 or miR-106a by using online bioinformatics tools: targetscan (http://www.targetscan.org/) and miRanda (http://www.microrna.org). To our surprise, VEGFA and MMP3, which play important roles in the development of endometriosis, were the potential targets of miR-93 and miR-106b.

MMP3 and VEGFA are down-regulated in ectopic endometrium and inversely correlated with miR-93 level

We further examined the protein levels of MMP3 and VEGFA in the samples from endometriosis patients and normal controls by western blot. The expression of MMP3 was up-regulated in 36 ectopic tissue samples (63.1%), and

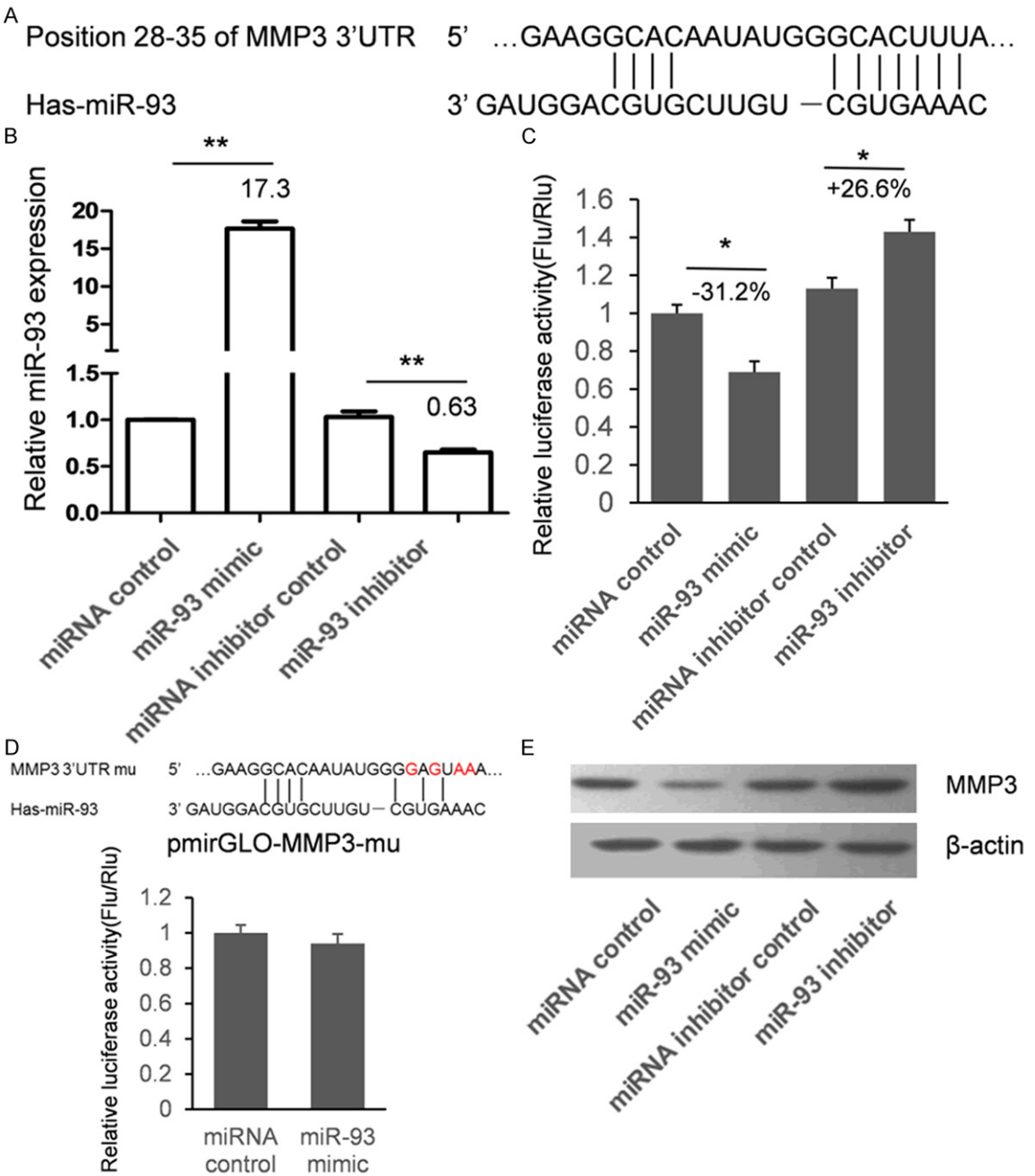

Figure 3. *The expression of MMP3 is suppressed by miR-93.* (A) Schematic diagram for the predicted interaction between miR-93 and MMP3 mRNA. HEK293T cells were co-transfected with pmir-GLO-MMP3 and miR-93 mimic or miR-93 inhibitor for dual-luciferase assay. The miR-93 expression (B) and luciferase activities (C) were detected 48 hours after transfection. (D) Reporter plasmid containing mutant MMP3 3'UTR were applied to determine the binding region of miR-93. HEK293T cells were co-transfected with pmir-GLO-MMP3-mu and miR93 mimic or miRNA control. The luciferase activities were detected 48 hours after transfection. The results were analyzed by student t-test and $P < 0.05$ was considered as statistically significant. *$P < 0.05$, **$P < 0.01$. (E) ESCs were transfected with miR-93 mimic or inhibitor with scramble single strand or double strand RNA as control. MMP3 protein levels were detected by western blot after 48 hours.

VEGFA was overexpressed in 30 ectopic tissue samples (52.4%) (**Figure 2A** and data not shown).

To evaluate the relative expression of MMP3 and VEGFA, the band intensity was determined by Quantity One software with β-actin as load-

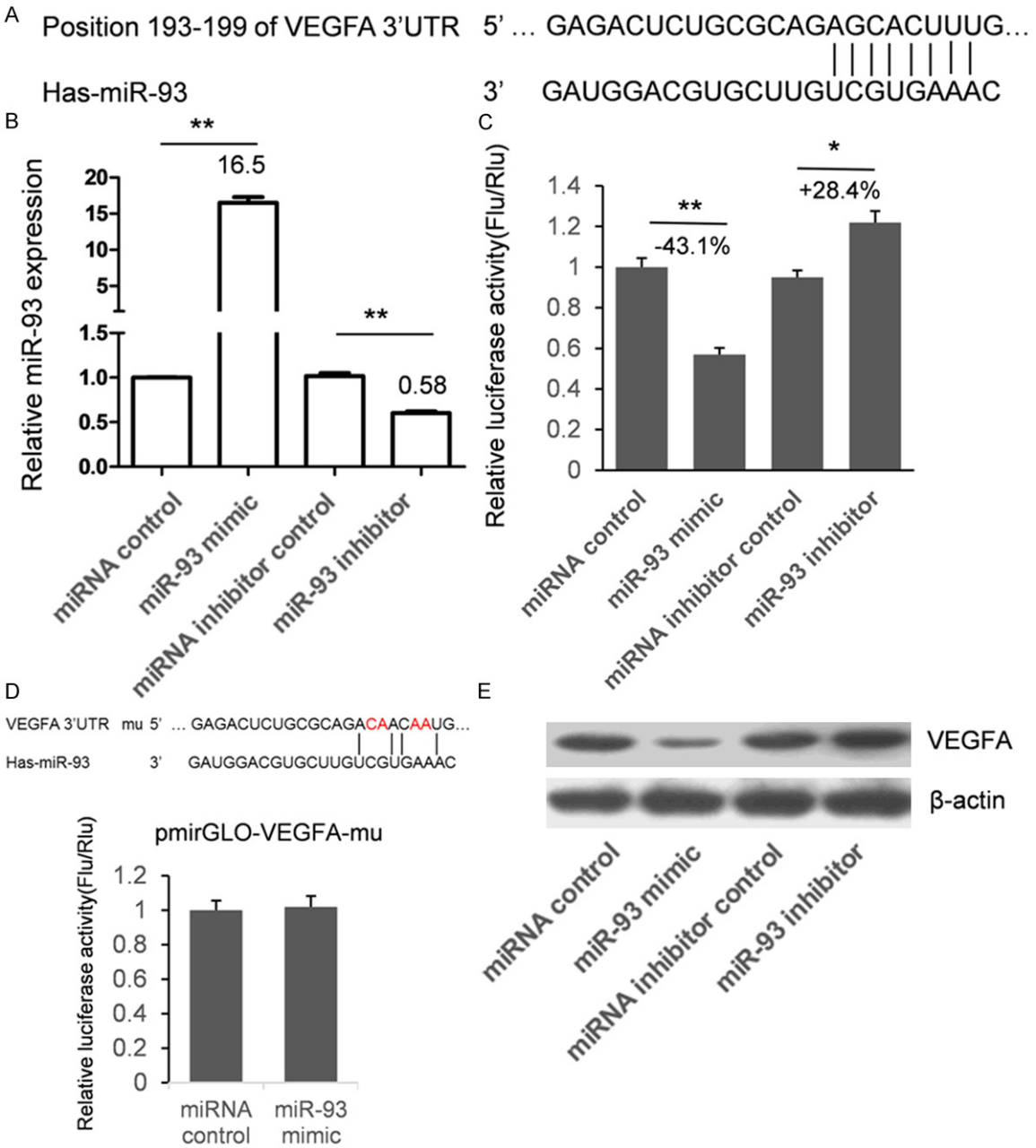

Figure 4. *The expression of VEGFA is suppressed by miR-93.* (A) Schematic diagram for the predicted interaction between miR-93 and VEGFA mRNA. HEK293T cells were co-transfected with pmir-GLO-VEGFA and miR-93 mimic or miR-93 inhibitor for dual-luciferase assay. The miR-93 expression (B) and luciferase activities (C) were detected 48 hours after transfection. (D) Reporter plasmid containing mutant VEGFA 3'UTR were used to determine the binding region of miR-93. HEK293T cells were co-transfected with pmir-GLO-VEGFA-mu and miR93 mimic or miRNA control. The luciferase activities were detected 48 hours after transfection. The results were analyzed by student t-test and $P < 0.05$ was considered as statistically significant. *$P < 0.05$, **$P < 0.01$. (E) ESCs were transfected with miR-93 mimic or inhibitor with scramble single strand or double strand RNA as control. VEGFA protein levels were detected by western blot.

ing control. Interestingly, we found an inverse correlation between the expression levels of miR-93 and the protein levels of MMP3 or VEGFA in 57 clinical samples of endometriosis. Low levels of miR-93 were associated with high levels of MMP3 (Pearson correlation, r-0.39; $P < 0.01$; **Figure 2B**) and VEGFA (Pearson correlation, r-0.37; $P < 0.01$; **Figure 2C**) protein, indicating a potential role of miR-93 in regulating the expression of MMP3 and VEGFA.

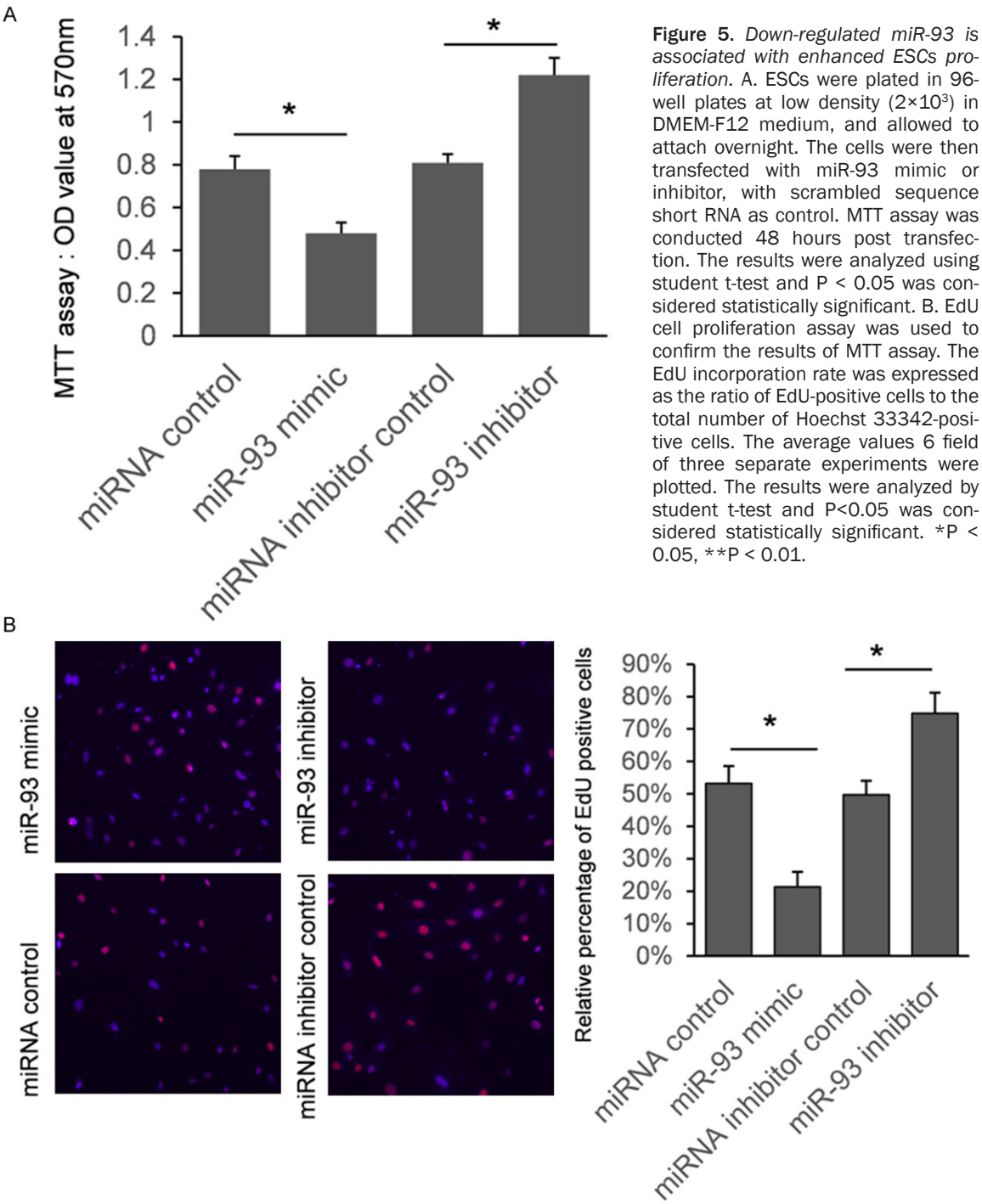

Figure 5. *Down-regulated miR-93 is associated with enhanced ESCs proliferation.* A. ESCs were plated in 96-well plates at low density (2×10^3) in DMEM-F12 medium, and allowed to attach overnight. The cells were then transfected with miR-93 mimic or inhibitor, with scrambled sequence short RNA as control. MTT assay was conducted 48 hours post transfection. The results were analyzed using student t-test and $P < 0.05$ was considered statistically significant. B. EdU cell proliferation assay was used to confirm the results of MTT assay. The EdU incorporation rate was expressed as the ratio of EdU-positive cells to the total number of Hoechst 33342-positive cells. The average values 6 field of three separate experiments were plotted. The results were analyzed by student t-test and $P<0.05$ was considered statistically significant. *$P < 0.05$, **$P < 0.01$.

MiR-93 represses MMP3 and VEGFA expression by targeting 3'UTR directly

To examine whether miR-93 directly targeted MMP3 or VEGFA mRNA, we generated pmirGLO-MMP3 and pmirGLO-VEGFA constructs in which full length of 329 bp 3'-UTR of MMP3 mRNA or a 533 bp segment of 3'UTR from VEGFA mRNA were cloned downstream of the firefly luciferase coding region in pmirGLO vector respectively. We next transfected pmirGLO-MMP3 or pmirGLO-VEGFA together with miR-93 mimic or inhibitor into HEK293T cells followed by luciferase reporter assay. As shown in **Figures 3B** and **4B**, the expression of miR-93 was significantly up-regulated in cells transfect-

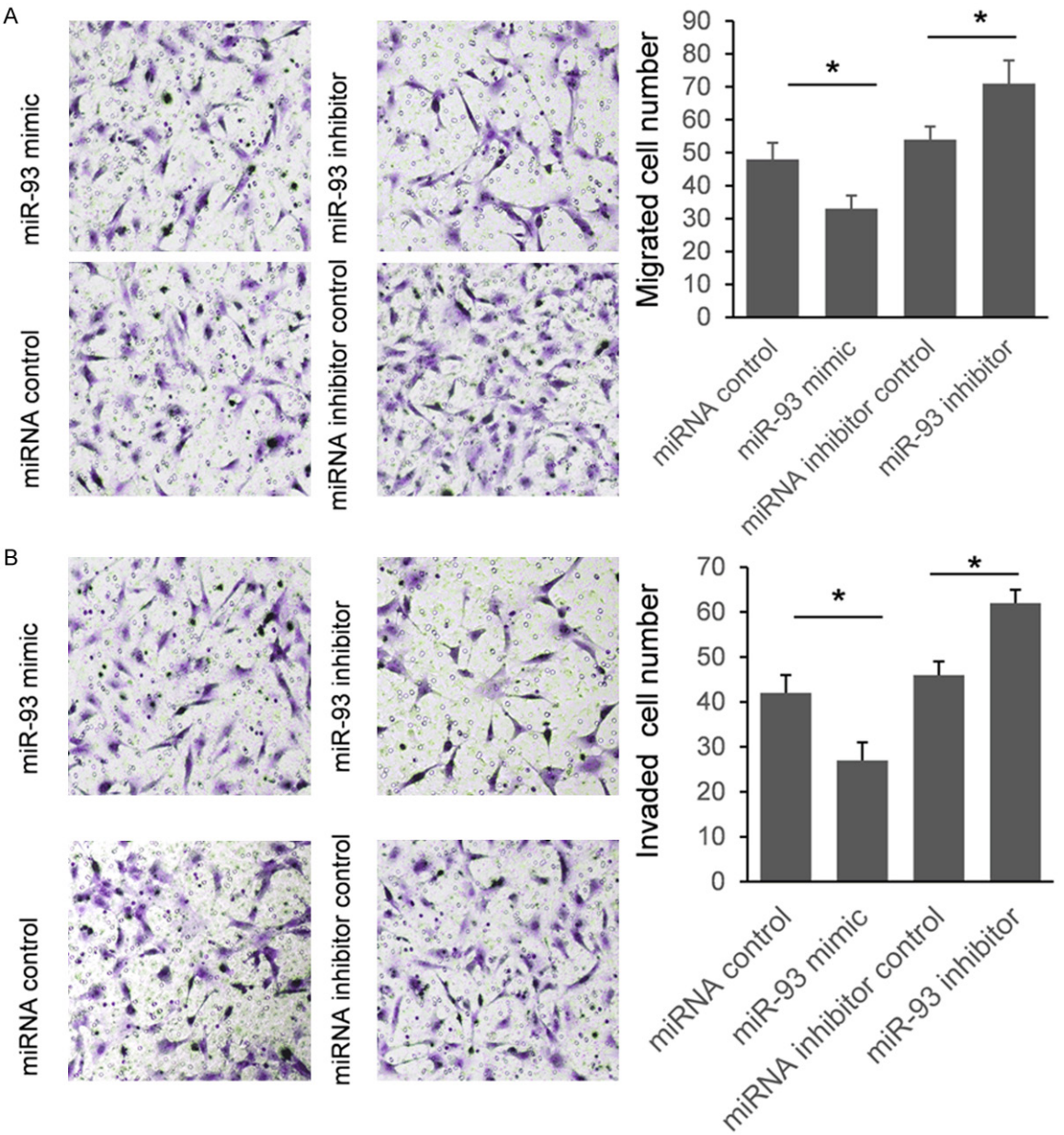

Figure 6. *Down regulated miR-93 is related to enhanced esophageal cancer cells migration and invasion.* ESCs were transfected with the miR-93 mimic or inhibitor with scramble RNA as control. The transfected cells were harvested and subjected to the following assays 48 hours after transfection. A. For migration assays, the transfected cells (3×10^4 cells/ml) were seeded in the top of an 8.0 mm-pore membrane chamber. Following a 12 hours incubation period, cells that passed through the membrane to attach to the bottom of membrane were fixed and stained with hematoxylin. Cells were scraped and removed from the top of chamber. Membranes were mounted on cover slides, and cells were counted. Cell migration was quantified by counting the amount of cells passing through the pores from five different fields per sample at 400× selected in a random manner. B. Cell invasion assays were carried out using matrigel pre-coated transwell chamber. 1×10^5 were seeded and incubated for 8 hours. All experiments were performed in duplicate. The results were analyzed using student t-test and $P < 0.05$ was considered statistically significant. *$P < 0.05$, **$P < 0.01$.

ed with miR-93, up to 17.3 ($P < 0.01$) and 16.5 fold ($P < 0.01$) respectively; whereas miR-93 levels were significantly down regulated to 63% ($P < 0.01$) or 58% ($P < 0.01$) relative to corresponding control. Meanwhile, the luciferase activity was significantly suppressed by miR-93, about 31.2% ($P < 0.05$) for pmir-GLO-MMP3 (**Figure 3C**) and 43.1% ($P < 0.01$) for pmirGLO-

VEGFA (**Figure 4C**), respectively. In contrast, the luciferase activity was significantly up-regulated by the miR-93 antagonist compared with the miRNA inhibitor control, about 26.6% ($P < 0.05$) for pmirGLO-MMP3 (**Figure 3C**) and 28.4% ($P < 0.05$) for pmirGLO-VEGFA (**Figure 4C**), respectively. These results indicate that miR-93 targets the 3'-UTR of MMP3 and VEGFA mRNAs, leading to down-regulation of MMP3 and VEGFA protein.

We further determined the binding region of miR-93 on the 3'UTR of MMP3 or VEGFA mRNAs. The putative binding regions of miR-93 in the 3'-UTR of MMP3 or VEGFA mRNAs were mutated with 4 non-related nucleotides (designated as pmirGLO-MMP3-mu or pmirGLO-VEGFA-mu) followed by luciferase reporter assay. As shown in **Figures 3D** and **4D**, the luciferase activities were not significantly reduced in cells transfected with miR-93 mimic compared with miRNA-control ($P > 0.05$). These data indicate that miR-93 suppresses the expression of MMP3 and VEGFA through binding to seed sequence at the 3'-UTR of MMP3 and VEGFA mRNA.

To further examine whether the expression of endogenous MMP3 and VEGFA was suppressed by miR-93, endometrial stromal cells (ESCs) were transfected with miR-93 mimics or inhibitor and the protein levels of MMP3 and VEGFA were detected by Western blot 48 hours after transfection. Compared with the corresponding controls, the levels of MMP3 and VEGFA protein were heavily suppressed by miR-93 mimics and slightly up-regulated by miR-93 inhibitor in ESCs (**Figures 3E** and **4E**). These results indicated that miR-93 repressed the expression of endogenous MMP3 and VEGFA in ESCs by directly targeting 3'UTR.

MiR-93 suppresses ESCs proliferation, migration and invasion

MMP3 and VEGFA critically regulate cell growth and migration. We thus examined whether miR-93 regulated the proliferation or migration of ESCs. As shown in **Figure 5A**, the proliferation ability of ESCs was significantly reduced by miR-93 mimic, about 38.5% ($P < 0.05$) compared to the controls. And the cell proliferation was significantly up-regulated by miR-93 inhibitor, about 50.6% ($P < 0.05$) in MTT assays. In addition, similar results were obtained by EdU cell proliferation assay (**Figure 5B**). The results showed that the number of proliferating cells was reduced by 51.2% ($P < 0.05$) when miR-93 was up-regulated, and increased by 46.2% ($P < 0.05$) when miR-93 inhibitor was added, respectively. These data suggests that miR-93 controls the proliferation of ESCs.

To further explore the effect of miR-93 on cell migratory and invasive behavior, typical transwell assay was performed. As shown in **Figure 6A**, the number of migrated ESCs was significantly reduced by 31.2% when miR-93 mimics was transfected, and it was increased by 30.5% when miR-93 inhibitor was transfected. Meanwhile, the number of invaded ESCs was reduce by 35.8% ($P < 0.05$) when miR-93 was up-regulated. In contrast, the numbers of invasive cells was comparable between the miR-93 inhibitor and control groups (**Figure 6B**). Together, these data suggest that miR-93 critically regulates the proliferation and migration by inhibiting the expression of MMP3 and VEGFA.

Discussion

Endometriosis is a commonly diagnosed disease in women, the pathogenesis of which is unclear. Recently, several studies have examined the expression of miRNAs of eutopic and ectopic endometrium from women with endometriosis [11, 14-16], in which several miRNAs have simultaneously been identified as dysfunction miRNAs in endometriosis. In this study, we chose 11 miRNAs according to the reported data, and detected their expression pattern in Chinese-Han endometriosis population. We found that the expression of miR-93 was significantly down-regulated in human ectopic endometrium samples compared with the normal endometrium samples ($P=0.006$). Meanwhile, high levels of MMP3 and VEGFA were detected in more than 50% ectopic endometrium samples, indicating that a negative association exists between the expression of miR-93 and the expression of MMP3 (Pearson correlation, $r=-0.39$, $P=0.0025$) or VEGFA (Pearson correlation, $r=-0.37$, $P=0.0047$) in endometriotic tissues. Furthermore, miR-93 targeted the 3'UTR of MMP3 and VEGFA, thus inhibited the expression of MMP3 and VEGFA. These findings suggest that the down-regulation of miR-93 correlates with the pathogenesis of endometriosis which is probably due to reduced repression of the expression of MMP3 and VEGFA protein. To

our knowledge, we provided the first evidence about the correlation between miR-93 and MMP3 or VEGFA in endometriotic clinical samples.

Matrix metalloproteinase (MMP) family proteins are involved in the breakdown of extracellular matrix during tissue remodeling in normal physiological as well as disease processes by lysing the basal membrane collagen and inducing further synthesis of MMPs [17, 18]. It is now widely accepted that the overexpression of MMPs especially MMP3 may accelerate the process of invasion and tissue remodeling, which is hypothesized to contribute to the pathogenesis of endometriosis. VEGFA, a 35-45 kD heparin-binding glycoprotein, stimulates the mitogenesis of endothelial cells and its overexpression is often related to tumorigenesis. There are reports indicating that overexpressed VEGFA exists in the eutopic endometrium samples and in the serum or peritoneal fluid of endometriosis patients [19, 20]. In the present study, we demonstrated that down-regulated miR-93 resulted in excessive proliferation, migration and invasion of ESCs. Considering that miR-93 directly targets MMP3 and VEGFA, it is conceivable that miR-93 inhibits endometriosis. In addition, it is also predicted that miR-93 targets integrin-β8 and TGFβR2 [21, 22]. Further investigations are needed to examine whether other targets of miR-93 regulate endometriosis.

In conclusion, the present study identified that low miR-93 is related to elevated expression of MMP3 and VEGFA, which contribute to the pathogenesis of endometriosis. These findings provided new potential therapeutic target for treatment of endometriosis.

Disclosure of conflict of interest

None to disclose.

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References

[1] Nyholt DR, Low SK, Anderson CA, Painter JN, Uno S, Morris AP, MacGregor S, Gordon SD, Henders AK, Martin NG, Attia J, Holliday EG, McEvoy M, Scott RJ, Kennedy SH, Treloar SA, Missmer SA, Adachi S, Tanaka K, Nakamura Y, Zondervan KT, Zembutsu H and Montgomery GW. Genome-wide association meta-analysis identifies new endometriosis risk loci. Nat Genet 2012; 44: 1355-1359.

[2] Eskenazi B and Warner ML. Epidemiology of endometriosis. Obstet Gynecol Clin North Am 1997; 24: 235-258.

[3] Maes OC, Chertkow HM, Wang E and Schipper HM. MicroRNA: Implications for Alzheimer Disease and other Human CNS Disorders. Curr Genomics 2009; 10: 154-168.

[4] Xu J, Li Y, Wang F, Wang X, Cheng B, Ye F, Xie X, Zhou C and Lu W. Suppressed miR-424 expression via upregulation of target gene Chk1 contributes to the progression of cervical cancer. Oncogene 2013; 32: 976-987.

[5] Farazi TA, Hoell JI, Morozov P and Tuschl T. MicroRNAs in human cancer. Adv Exp Med Biol 2013; 774: 1-20.

[6] Roth LW, McCallie B, Alvero R, Schoolcraft WB, Minjarez D and Katz-Jaffe MG. Altered microRNA and gene expression in the follicular fluid of women with polycystic ovary syndrome. J Assist Reprod Genet 2014; 31: 355-362.

[7] Rah H, Jeon YJ, Shim SH, Cha SH, Choi DH, Kwon H, Kim JH, Shin JE and Kim NK. Association of miR-146aC > G, miR-196a2T > C, and miR-499A > G polymorphisms with risk of premature ovarian failure in Korean women. Reprod Sci 2013; 20: 60-68.

[8] Hu Y, Liu CM, Qi L, He TZ, Shi-Guo L, Hao CJ, Cui Y, Zhang N, Xia HF and Ma X. Two common SNPs in pri-miR-125a alter the mature miRNA expression and associate with recurrent pregnancy loss in a Han-Chinese population. RNA Biol 2011; 8: 861-872.

[9] Dong F, Zhang Y, Xia F, Yang Y, Xiong S, Jin L and Zhang J. Genome-wide miRNA profiling of villus and decidua of recurrent spontaneous abortion patients. Reproduction 2014; 148: 33-41.

[10] Suryawanshi S, Vlad AM, Lin HM, Mantia-Smaldone G, Laskey R, Lee M, Lin Y, Donnellan N, Klein-Patel M, Lee T, Mansuria S, Elishaev E, Budiu R, Edwards RP and Huang X. Plasma microRNAs as novel biomarkers for endometriosis and endometriosis-associated ovarian cancer. Clin Cancer Res 2013; 19: 1213-1224.

[11] Braza-Boils A, Mari-Alexandre J, Gilabert J, Sanchez-Izquierdo D, Espana F, Estelles A and Gilabert-Estelles J. MicroRNA expression profile in endometriosis: its relation to angiogenesis and fibrinolytic factors. Hum Reprod 2014; 29: 978-988.

[12] Lin SC, Wang CC, Wu MH, Yang SH, Li YH and Tsai SJ. Hypoxia-induced microRNA-20a expression increases ERK phosphorylation and angiogenic gene expression in endometriotic

stromal cells. J Clin Endocrinol Metab 2012; 97: E1515-1523.
[13] Shi YL, Luo XZ, Zhu XY, Hua KQ, Zhu Y and Li DJ. Effects of combined 17beta-estradiol with TCDD on secretion of chemokine IL-8 and expression of its receptor CXCR1 in endometriotic focus-associated cells in co-culture. Hum Reprod 2006; 21: 870-879.
[14] Pan Q, Luo X, Toloubeydokhti T and Chegini N. The expression profile of micro-RNA in endometrium and endometriosis and the influence of ovarian steroids on their expression. Mol Hum Reprod 2007; 13: 797-806.
[15] Burney RO, Hamilton AE, Aghajanova L, Vo KC, Nezhat CN, Lessey BA and Giudice LC. MicroRNA expression profiling of eutopic secretory endometrium in women with versus without endometriosis. Mol Hum Reprod 2009; 15: 625-631.
[16] Shi XY, Gu L, Chen J, Guo XR and Shi YL. Downregulation of miR-183 inhibits apoptosis and enhances the invasive potential of endometrial stromal cells in endometriosis. Int J Mol Med 2014; 33: 59-67.
[17] Brinckerhoff CE, Rutter JL and Benbow U. Interstitial collagenases as markers of tumor progression. Clin Cancer Res 2000; 6: 4823-4830.
[18] Van Themsche C, Potworowski EF and St-Pierre Y. Stromelysin-1 (MMP-3) is inducible in T lymphoma cells and accelerates the growth of lymphoid tumors in vivo. Biochem Biophys Res Commun 2004; 315: 884-891.
[19] Wang H, Gorpudolo N, Li Y, Feng D, Wang Z and Zhang Y. Elevated vascular endothelia growth factor-A in the serum and peritoneal fluid of patients with endometriosis. J Huazhong Univ Sci Technolog Med Sci 2009; 29: 637-641.
[20] Machado DE, Abrao MS, Berardo PT, Takiya CM and Nasciutti LE. Vascular density and distribution of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 (Flk-1) are significantly higher in patients with deeply infiltrating endometriosis affecting the rectum. Fertil Steril 2008; 90: 148-155.
[21] Lyu X, Fang W, Cai L, Zheng H, Ye Y, Zhang L, Li J, Peng H, Cho WC, Wang E, Marincola FM, Yao K, Cai H, Li J and Li X. TGFbetaR2 is a major target of miR-93 in nasopharyngeal carcinoma aggressiveness. Mol Cancer 2014; 13: 51.
[22] Fang L, Deng Z, Shatseva T, Yang J, Peng C, Du WW, Yee AJ, Ang LC, He C, Shan SW and Yang BB. MicroRNA miR-93 promotes tumor growth and angiogenesis by targeting integrin-beta8. Oncogene 2011; 30: 806-821.