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miR-483-5p promotes uterine leiomyoma progression by targeting TIMP2

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Abstract

Introduction and Objective. Uterine leiomyoma (UL) is a prevalent tumour of the female reproductive system which is poorly understood in terms of pathogenesis, and treatment options are limited. MicroRNAs (miRNAs) have been identified as being associated with tumorigenesis. The aim of the study is to elucide the function of miR-483-5p in promoting UL progression by targeting TIMP metallopeptidase inhibitor 2 (TIMP2) and its underlying molecular mechanisms.

Materials and Method. The study included 340 patients with UL. The expression level of miR-483-5p was measured using quantitative real-time PCR (qPCR). Following knockdown of miR-483-5p in uterine leiomyoma cells (UtLMCs), cell proliferation, migration, and apoptosis were assessed using CCK-8, Transwell, and flow cytometry assays. Subsequently, bioinformatics analysis and luciferase reporter gene detection identified the target of miR-483-5p.

Results. Compared to the adjacent myometrium, miR-483-5p was upregulated in UL tissues. Experiments revealed that miR-483-5p expression levels were increased in UtLMCs. It was determined that TIMP2 is the direct target of miR-483-5p. Furthermore, miR-483-5p significantly promoted the proliferation and migration of UtLMCs, and inhibited their apoptosis by targeting TIMP2.

Conclusion. Collectively, these findings reveal the molecular mechanism by which miR-483-5p negatively regulates TIMP2 to promote UL development, providing new insights for the targeted therapy of UL.

Key words

uterine leiomyoma, progression, TIMP2, miR-483-5p

INTRODUCTION

Uterine leiomyoma (UL) represents one of the most prevalent benign tumours occurring in the female reproductive system [1]. Its pathological characteristics manifest as abnormal proliferation of myometrial cells, involving complex interactions among hormones, growth factors, signalling pathways, and epigenetic regulation mechanisms [2–4]. Epidemiological investigations reveal that UL affects 20–50% of women during their reproductive years, while only approximately 30% of patients experience clinically significant symptoms that can profoundly impact quality of life, including pelvic pain, abdominal mass, menstrual disorders, dysmenorrhea, abnormal uterine bleeding, urinary frequency, incontinence, particularly infertility, and pregnancy loss [5–7]. Current research suggests multiple predisposing factors may contribute to UL development: family history, genetic variations, epigenetic modifications, early menarche, exposure to xenoestrogens, other diseases (hypertension, obesity, vitamin D deficiency), alcohol consumption, dietary habits, advanced maternal age, and nulliparity [8, 9]. However, the precise molecular mechanisms underlying UL pathogenesis remain largely elusive [10]. Contemporary clinical management strategies are primarily determined by symptom severity, tumour size, location, patient age, and fertility preservation considerations [1]. The two main therapeutic approaches include surgical intervention (myomectomy or hysterectomy) and pharmacological treatment [11, 12]. However, both methods present significant limitations – myomectomy carries a high recurrence rate within 5 years post-operation, and hysterectomy remains undesirable for women seeking uterine preservation [13]. In addition, medical therapies are restricted to a 6-month duration due to adverse effects, including bone density loss and diabetes risk [10]. These substantial limitations in both mechanistic understanding and therapeutic options underscore the critical need for exploring novel molecular pathways and developing targeted treatment strategies for UL.

In recent years, microRNAs (miRNAs) have gained widespread recognition in the regulation of gene expression. These single-stranded non-coding RNAs (~22–24 nucleotides in length), through sequence-specific binding to the 3'untranslated region (3'UTR) of the target gene, lead to its degradation or translational repression [14]. Through this mechanism, they are involved in a variety of biological processes, including cell proliferation, differentiation, apoptosis, and carcinogenesis [15]. Notably, emerging

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research highlights their pivotal role in elucidating molecular mechanisms of tumourigenesis [16]. Studies confirm that miRNAs exhibit dysregulated expression patterns in UL, suggesting their potential as novel diagnostic biomarkers and therapeutic targets [17, 18].

The primary focus of this study – miR-483-5p – has been demonstrated to be involved in the progression of multiple cancers, including lung cancer, clear cell renal cell carcinoma, and hepatocellular carcinoma [19–21]. A hallmark pathological feature of UL is the abnormal accumulation of extracellular matrix (ECM), resulting from metabolic imbalance [22]. Through the research on miR-483-5p in the field of tumours, it was initially suggested that it may also have certain potential value in UL. The dynamic equilibrium of ECM is primarily regulated by the coordinated activity of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) [23]; among these, TIMP2 serves as a crucial endogenous inhibitor of MMPs, playing a central role in maintaining ECM homeostasis. Notably, Gu et al. have demonstrated that miR-483-5p promotes malignant development of multiple myeloma by targetting TIMP2 [24].

To date, research on the molecular mechanisms of UL remains limited. In the current study, a highly evolutionarily conserved miRNA – miR-483-5p – was selected as the focus of this investigation. The study aimed to systematically elucidate the molecular mechanism of miR-483-5p and TIMP2 in the development of UL, providing a theoretical basis for developing miRNA-based precision therapeutic strategies.

MATERIALS AND METHOD

Research subjects. This study included 340 patients diagnosed with UL at the Zhongshan Torch Development Zone People's Hospital between January 2020 – June 2023. *Inclusion Criteria:* (1) diagnosed with UL via imaging examination and meeting diagnostic criteria; (2) no other endocrine disorders; (3) no other immune disorders; (4) no other haematological disorders or malignant tumors; (5) no severe hepatic or renal disease. Exclusion Criteria: (1) women in the gestational or lactation period; (2) use of hormonal medications within the past 3 months; (3) concurrent endometriosis; (4) severe psychiatric disorders.

Leiomyoma tissue and myometrium tissue were collected during hysterectomy or myomectomy procedures, along with clinical data such as age, BMI, menstrual irregularities, maximum tumour diameter, number of tumours, tumour location, and estradiol and progesterone levels measured during the follicular phase.

The study was approved by the Ethics Committee of this institution. The consent of all participants was obtained before the start of the experiment.

Cell culture and transfection. The uterine smooth muscle cells (UtSMCs; Shanghai Cell Bank, China) and human UL cells (UtLMCs; Sunncell, China) were maintained in DMEM (Gibco, USA, Cat# 11965092) supplemented with 10% FBS (Gibco, USA, Cat# A5256701). Cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2 until they reached the logarithmic growth phase. miR-483-5p mimics and inhibitors were then transfected into UL cells by Lipofectamine 2000 (Invitrogen, USA, Cat# 11668019) for overexpression or silencing of miR-483-5p. Additionally, negative controls (miR NC) were transfected into UL cells to investigate the functional role of miR-483-5p in UL. The transfection efficiency was evaluated based on the expression of miR-483-5p in the transfected cells.

Real-time quantitative PCR (RT-qPCR). The TRIzol (Thermo, USA, Cat. No. 15596018CN) method was chosen to extract total RNA from tissues and cells. The A_{260}/A_{280} ratio was measured using a NanoDrop spectrophotometer, and RNA samples with ratios between 1.8–2.1 were selected for cDNA synthesis via miRNA 1st Strand cDNA Synthesis Kit (Vazyme, China, Cat. No. MR101-01) and HiScript III RT SuperMix for qPCR (Vazyme, China, Cat. No. R323-01). Using *U6* (for miR-483-5p) and *GAPDH* (for mRNAs) as internal reference genes, qPCR amplification was carried out with miRNA Unimodal SYBR qPCR Master Mix (Vazyme, China, Cat. No. MQ102-01) and TB Green Premix Ex Taq (Takara, Japan, Cat. No. RR420A) in pre-configured reaction systems under programmed conditions. Primer sequences are provided in the Supplementary Materials (Tab. S1). qPCR reaction conditions: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, and 60 °C for 30 s. Finally, the $2^{-\Delta\Delta Ct}$ method was applied to analyze the data and compare the expression levels in the transfected cells.

Table S1. Primer sequences used in RT-qPCR

Name	Primer (5′–3′)
miR-483-5p	Forward: GCGAAGACGGGAGGAAAGA
	Reverse: AGTGCAGGGTCCGAGGTATT
TIMP2	Forward: GCTGCGAGTGCAAGATCAC
	Reverse: TGGTGCCCGTTGATGTTCTTC
U6	Forward: GCTTCGGCAGCACATATACTAAAAT
	Reverse: CGCTTCACGAATTTGCGTGTCAT
GAPDH	Forward: AGATCCCTCCAAAATCAAGTGG
	Reverse: GGCAGAGATGATGACCCTTTT

Cell proliferation assay. Cell suspensions from different treatment groups were seeded into 96-well plates and incubated for 24, 48, and 72 h, respectively. Following incubation, CCK-8 reagent (Yeasen, China, Cat. No. 40203ES60) was added to each well, and the plates were further incubated at 37 °C for 4 h. Absorbance was then measured at a wavelength of 450 nm.

Cell migration assay. Cells were seeded into the upper chamber of a Transwell insert (Thermo, USA, Cat. No. 140629), while complete medium was added to the lower chamber. The plate was then incubated for 24–48 h. After incubation, the upper chamber was gently rinsed with PBS to remove non-migratory cells. The medium in the lower chamber was carefully replaced with 4% paraformaldehyde (Beyotime, China, Cat. No. P0099-500ml) to fix the migrated cells for 15–20 min. Following fixation, the cells were washed with PBS and stained with 0.1% crystal violet (Solarbio, China, Cat. No. G1064) for 20–30 min. The stained cells on the membrane were visualized and counted under an inverted light microscope. The cell migration rate was calculated based on the counted cells.

Apoptosis detection. Following centrifugation, all cells in the culture dish were collected and washed. According to the instructions of the FITC-Annexin V/PI detection kit (Abcam,

UK, Cat. No. ab14085), the cells were stained in groups and incubated in the dark for 15 min. Flow cytometry was then performed, and the results recorded for subsequent analysis.

Dual-luciferase reporter assay. The sequences of binding sites and mutation sites were inserted into the luciferase reporter plasmid pGL3-Basic (Novopro, China, Cat. No. V011544) to construct TIMP2-WT and TIMP2-MUT vectors, respectively. The plasmid pRL-TK served as an internal control. Both plasmids were co-transfected into UtLMCs. After 24 – 48 h of transfection, the cell lysis buffer was collected. The activities of both luciferases were measured sequentially by a dual-luciferase reporter assay kit (Vazyme, China, Cat. No. DL101-01), and the readings were recorded. The ratio of luciferase activity to Renilla luciferase activity was calculated, and by comparing the ratios between the experimental and control groups, the impact of miR-483-5p on gene expression was evaluated.

Prediction and validation of potential target genes. Four databases were selected, including TargetScan (release 8.0, https://www.targetscan.org/) [25], miRDB (version 6.0, https://mirdb.org/) [26], starBase (v2.0, http://starbase.sysu.edu.cn/) [27], and TarBase (v9.0, https://dianalab.e-ce.uth.gr/tarbasev9) [28], and collected target gene data from the selected databases. Data from different databases was integrated and cross-validated through the Weishengxin website. Finally, the expression levels of the predicted target genes were validated through RT-qPCR experiments.

Western blot. Total protein was extracted from UtLMCs with RIPA lysis buffer (Thermo, USA, Cat. No. 89900) containing protease inhibitors. Protein concentrations were measured with the BCA method, and equal quantities of protein were separated by 10% SDS-polyacrylamide gels prior to electrophoretic transfer to PVDF membranes. Following transfer, the membrane was blocked for 1.5 h at room temperature with 5% non-fat milk prepared in TBST. Subsequently, it was incubated with the following primary antibodies diluted in blocking buffer overnight at 4 °C: anti-TIMP2 (1:1000, Santa Cruz Biotechnology, USA, Cat. No. sc-21735) and anti-GAPDH (1:2000, Abcam, UK, Cat. No. ab263962). The membrane was exposed to HRP-labeled secondary antibody (1:5000, Thermo, USA) for 1 h. The antibodies used were anti-mouse IgG (Cat. No. C31430100) and anti-rabbit IgG (Cat. No. C31460100). Target proteins were detected using SuperPico ECL Chemiluminescence Kit (Vazyme, China, Cat. No. E422-01) and imaged with a chemiluminescence imaging system. Quantitative analysis of band intensity was performed using ImageJ software.

Statistical analyses. All experiments were performed with 3 independent biological replicates (each with 3 technical replicates) to ensure data accuracy and reproducibility. Statistical analyses were performed using SPSS 23.0 and GraphPad Prism 9.0 software. All data were validated for normality using the Shapiro-Wilk test. Pearson correlation analysis was performed to assess the association between the 2 variables. The results are expressed as mean ± SD. Differences between groups were assessed by Student's t-test (for 2 groups) or one-way ANOVA followed by Tukey's *post hoc* test for multiple comparisons, with a significance level of $P < 0.05$ considered statistically significant.

RESULTS

Clinical information of patients with UL. Table 1 shows the characteristics of the patients in the study in terms of their clinical features with leiomyoma The patients fell within the high-risk age range for leiomyoma (30–50 years). In the meantime, information was also collected about the levels of estradiol and progesterone in patients. No significant hormonal abnormalities were identified. Nearly half of the patients reported irregular menstruation, suggesting that leiomyomas may contribute to menstrual disturbances. Additionally, intramural leiomyomas were the most common type observed in the study.

Table 1. Clinical features of patients with uterine leiomyoma

Feature	Cases (n = 340)
Age (years)	40.96 ± 5.18
BMI (kg/m²)	23.83 ± 3.11
Irregular menstruation	
no	183 (53.8%)
yes	157 (46.2%)
Maximum diameter of myoma (cm)	5.70 ± 1.64
Number of myomas	
1	151 (44.4%)
>1	189 (55.6%)
Location	
Intramural myoma	229 (67.4%)
Subserosal myoma	67 (19.7%)
Submucosal myoma	44 (12.9%)
Estradiol (pmol/L)	148.90 ± 25.35
Progesterone (nmol/L)	1.77 ± 0.44

Expression level of miR-483-5p in leiomyoma tissue and cells. The miR-483-5p was detected in leiomyoma tissue and myometrium tissue collected from patients indicated that the expression level of miR-483-5p in leiomyoma tissue was significantly higher than that in myometrium tissue (Fig. 1A). As demonstrated in Figure 1B, a comparable outcome was observed, with the expression levels of miR-483-5p being considerably elevated in UtLMCs in comparison to UtSMCs.

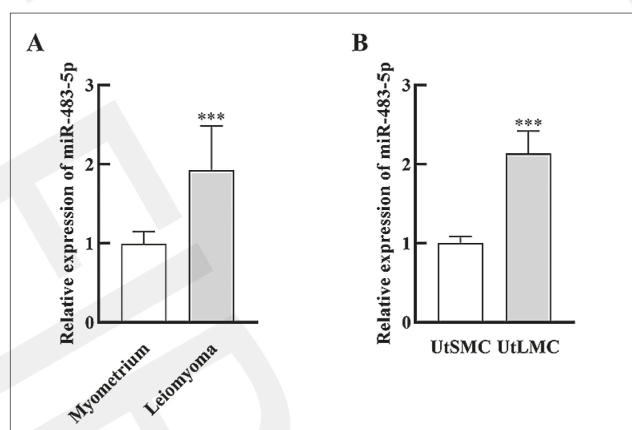

Figure 1. Expression levels of miR-483-5p in UL. (A) Comparative expression levels of miR-483-5p in myometrium tissue and leiomyoma tissue. (B) Expression levels of miR-483-5p in UtSMCs and UtLMCs.
***$P < 0.001$

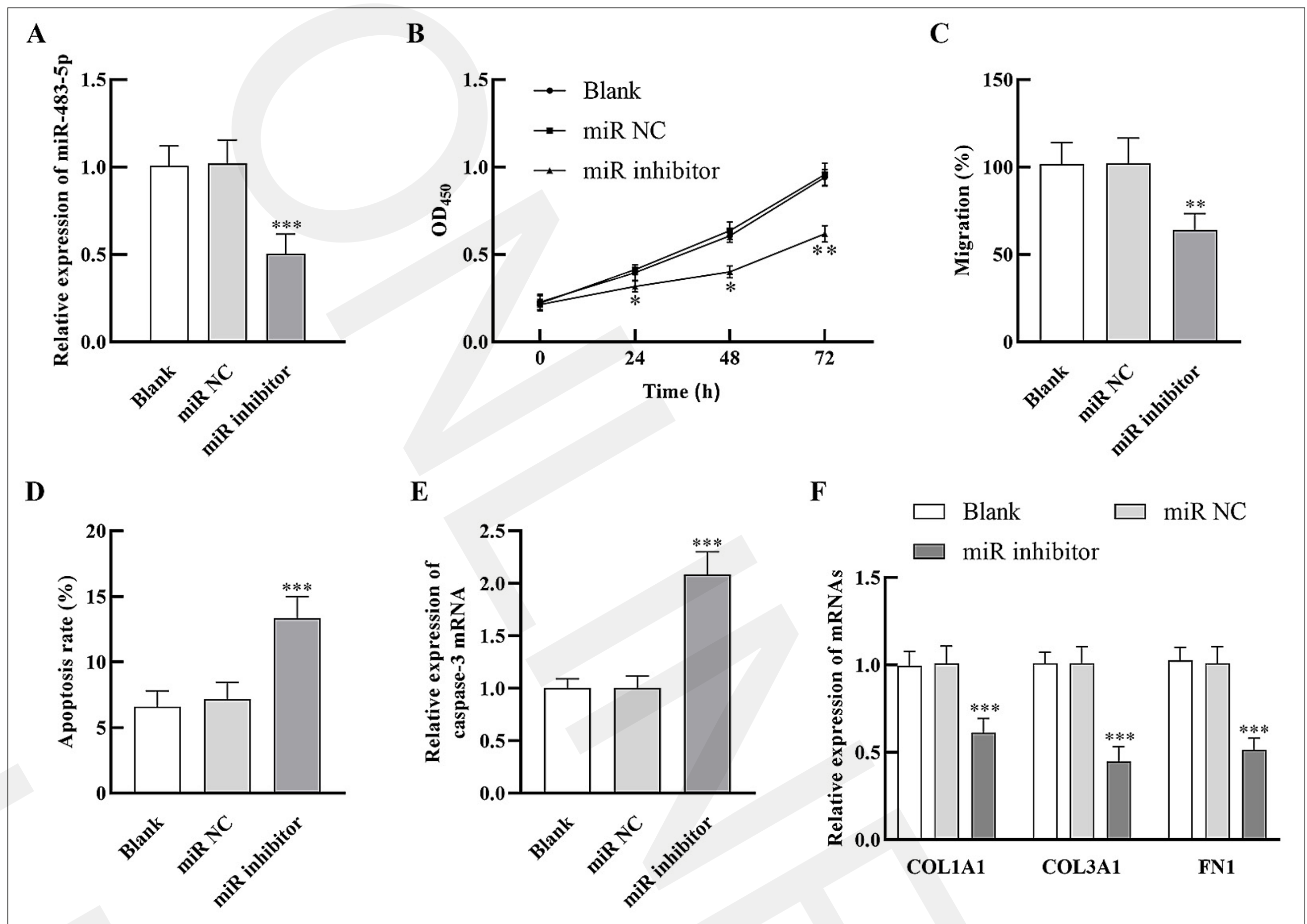

Figure 2. Functional analysis of miR inhibitor in transfected cells. (A) The miR inhibitor group exhibited significantly reduced expression of miR-483-5p relative to Blank and miR NC groups. (B) miR inhibitor group exhibited lower OD_{450} values than the Blank and miR NC groups. (C) miR inhibitor group demonstrated significantly reduced migration compared to the Blank and miR NC groups. (D and E) miR inhibitor group displayed markedly increased apoptosis rates (D) and caspase-3 expression (E) compared to Blank and miR NC groups. (F) Expression of COL1A1, COL3A1, and FN1 was significantly reduced in the miR inhibitor group, compared to the Blank and miR NC groups. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

It was concluded that miR-483-5p might be closely connected with the development of leiomyoma.

Regulatory effects of miR-483-5p on proliferation, migration, and apoptosis of leiomyoma cells. Based on the assessment of transfection efficiency, miR-483-5p expression was significantly downregulated in the miR-483-5p inhibitor group, confirming successful transfection and establishing a suitable basis for subsequent functional experiments (Fig. 2A). Cell proliferation was assessed using the CCK-8 assay. The OD_{450} values of Blank, miR NC, and miR inhibitor groups increased continuously with prolonged culture time, consistent with normal cell proliferation patterns. However, starting from 24 h, knockdown of miR-483-5p led to a substantial decrease in the OD_{450} value (Fig. 2B), suggesting that miR-483-5p plays a crucial role in promoting the proliferation of UtLMCs. A similar trend was observed in the migration assay, with the inhibition of miR-483-5p resulting in a significant suppression of cell migration rates (Fig. 2C), further confirming its essential role in facilitating cell migration. Additionally, knockdown of miR-483-5p significantly increased the rate of cellular apoptosis (Fig. 2D) and the expression of caspase-3 (Fig. 2E), indicating that miR-483-5p inhibited apoptosis of UtLMCs. The downregulation of miR-483-5p also reduced the expression of key ECM components – COL1A1, COL3A1, and FN1 (Fig. 2F). In addition, it should be emphasized that no statistically significant differences were observed between the miR NC and the Blank group in any of the experiments.

The target gene TIMP2 of miR-483-5p. Three target genes were co-predicted by TargetScan, miRDB, starBase, and TarBase, representing the most noteworthy candidate genes in the research (Fig. 3A). Figure 3B illustrates the mRNA expression of these genes in UtSMCs and UtLMCs, respectively. The results revealed no significant differences in STK40 and ALCAM expression between the 2 cell types. However, TIMP2 gene expression showed a significant downregulation in UtLMCs compared to UtSMCs (Fig. 3B). The potential binding sites between miR-483-5p and TIMP2 were computationally predicted, followed by experimental validation of their interaction. It was found that there was some complementarity in the sequences, which might be the way that miR-483-5p regulates TIMP2 (Fig. 3C). Figure 3C shows the results of the dual-luciferase assay designed to evaluate the regulatory impacts of miR-483-5p on TIMP2. Analysis showed that the luciferase activity of TIMP2-WT in the miR mimic group was significantly reduced, indicating that elevated miR-483-5p levels could strongly suppress TIMP2-WT activity. This effect may be attributable to the specific binding of overexpressed miR-483-5p to TIMP2-WT 3'UTR, thereby inhibiting reporter gene activity and confirming the direct targeting of miR-483-5p to TIMP2.

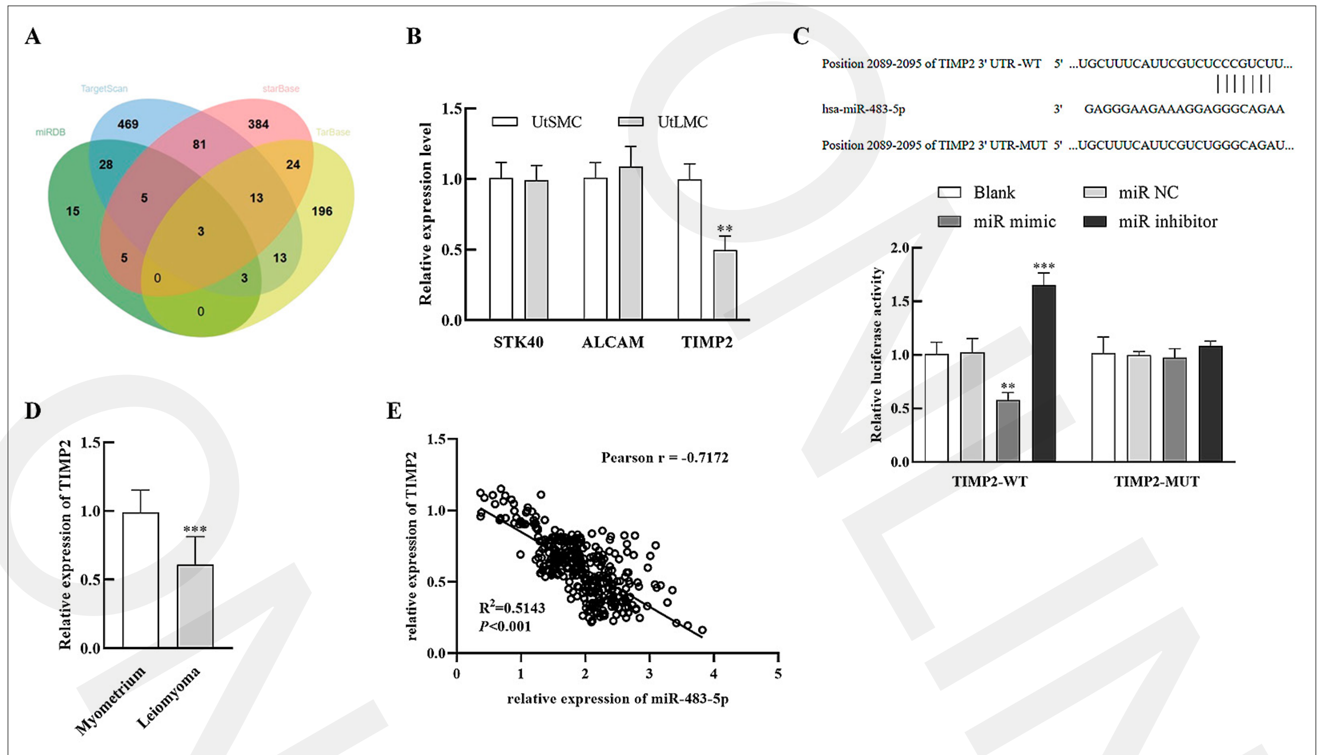

Figure 3. Exploration of the correlation between the predicted target gene TIMP2 and miR-483-5p. (A) The overlap of genes related to miR-483-5p was predicted from 4 databases. (B) Expression of the 3 overlapping genes in UtSMCs and UtLMCs only demonstrated that TIMP2 was significantly downregulated in UtLMCs. (C) Partial complementary pairings for the sequence alignment of miR-483-5p and TIMP2. Transfection with miR inhibitor significantly upregulated TIMP2-WT luciferase activity, whereas transfection with miR mimic significantly downregulated it. (D) Expression level of TIMP2 in leiomyoma was significantly lower than that in the adjacent myometrium. (E) A significant correlation was observed between miR-483-5p and TIMP2 (n = 340).
$^{**}P < 0.01$, $^{***}P < 0.001$

On the contrary, after inhibiting miR-483-5p, the luciferase activity significantly increased. For the TIMP2-MUT group, neither the overexpression nor the inhibition of miR-483-5p markedly affected the luciferase activity (Fig. 3C). This indicates that the regulatory effect depends on specific binding sites.

In addition, TIMP2 expression was significantly lower in leiomyoma than in myometrial tissue (Fig. 3D) and was significantly correlated with miR-483-5p (r = -0.7172; $P < 0.001$) (Fig. 3E), suggesting a close relationship between them.

TIMP2 mediates the functional mechanism of miR-483-5p. According to the results shown in Figure 4A, miR-483-5p inhibited the expression of TIMP2 at both mRNA and protein levels. This further indicated that miR-483-5p was able to target and negatively regulate TIMP2. Subsequent experiments further demonstrated that miR-483-5p promoted cell proliferation and migration (Fig. 4B-C) while suppressing apoptosis (Fig. 4D-E) by negatively regulating TIMP2. In addition, downregulation of TIMP2 also alleviated the suppression of COL1A1, COL3A1, and FN1 expression by miR-483-5p knockdown (Fig. 4F). These findings collectively indicate that TIMP2 serves as a key downstream effector molecule of miR-483-5p.

DISCUSSION

The pathogenesis of UL, a frequently occurring benign tumour in women at reproductive age, remains poorly understood, which has significantly hindered the development of effective therapeutic strategies [29]. miRNAs are emerging as highly promising diagnostic biomarkers and therapeutic targets across a range of tumour types [30]. As an evolutionarily conserved miRNA, miR-483-5p has been suspected to be involved in the development of multiple human diseases, such as metabolic disorders and various malignancies [31, 32]. Among them, miR-483-5p is recognized for its significant impact on tumour progression by mediating cell proliferation, metastasis, and apoptosis in clear cell renal cell carcinoma and hepatocellular carcinoma [20, 21]. However, its role in UL has not been previously investigated.

The study observed that miR-483-5p was markedly upregulated in leiomyoma tissue compared with adjacent myometrium tissue, as well as in UtLMCs relative to UtSMCs. As previously reported, exosomes derived from MM-MSCs exert their effects on multiple myeloma cells by increasing the level of miR483-5p, thereby facilitating the malignant development of multiple myeloma [24]. These findings therefore strongly suggest that miR-483-5p may have a significant impact on UL pathogenesis. The function and mechanism of miR-483-5p were also analyzed, and demonstrated that miR-483-5p motivates cell proliferation and migration. These results are consistent with previous reports indicating its role in promoting tumour growth in various cancers [24, 33, 34]. Moreover, miR-21 was widely recognized as being able to inhibit the function of tumour suppressors, promote cell proliferation, and ultimately promote tumour formation [35, 36]. It has been found to be upregulated in UL [37]. Additionally, Huang et al. discovered that the increased levels of miR-29 can inhibit UL cell growth

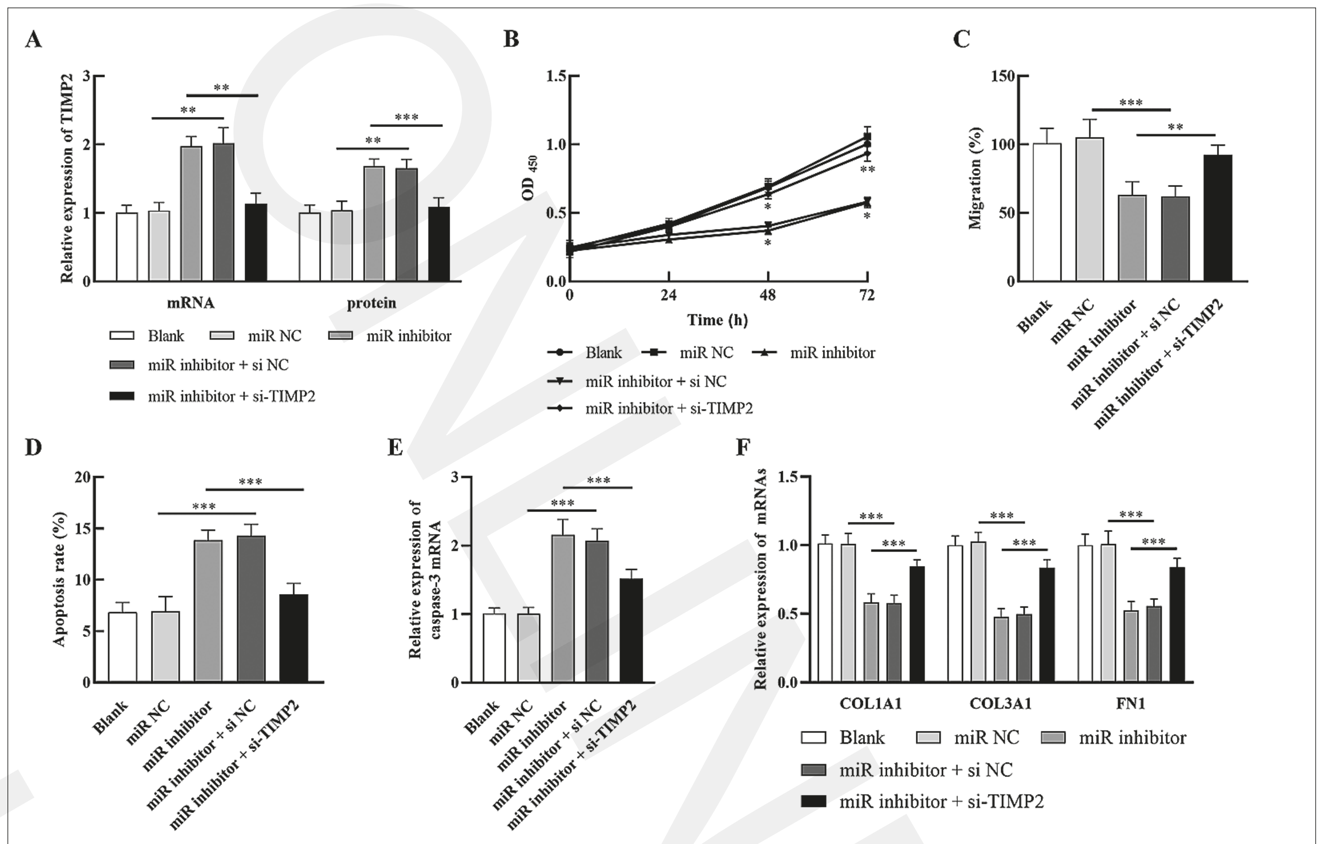

Figure 4. The mechanism analysis of miR-483-5p targeting TIMP2 to promote UL progression. (A) At both mRNA and protein levels, TIMP2 expression was significantly higher in the miR inhibitor group compared to the blank group, while the miR inhibitor +si-TIMP2 group showed significantly lower expression than the miR inhibitor group. (B) The value of OD_{450} in the miR inhibitor group was significantly reduced in comparison with the blank group, while the miR inhibitor + si-TIMP2 group was significantly higher than the miR inhibitor group at 48–72 h. (C) Migration rate of miR inhibitor + si-TIMP2 was significantly higher than that of the miR inhibitor group. (D and E) Apoptosis rate (D) and caspase-3 expression (E) in the miR inhibitor + si-TIMP2 group were significantly lower than those in the miR inhibitor group. (F) miR inhibitor + si-TIMP2 group exhibited significantly higher expression of COL1A1, COL3A1 and FN1, compared to the miR inhibitor group.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

by suppressing the STAT3 signalling pathway [38]. Based on this, it supports the hypothesis that miR-483-5p may contribute to UL development through similar mechanisms. Notably, the current study also revealed that miR-483-5p exerts anti-apoptotic effects in UtLMCs, mirroring its reported function in lung cancer cells, where it regulates apoptosis through targeting RBM5 [39]. The imbalance between cell proliferation and apoptosis is the core driving force for the progression of UL. miR-199a-5p played an anti-tumour role in UL by inhibiting cell proliferation and promoting cell apoptosis [40]. Therefore, it can be inferred that miR-483-5p may participate in the progression of UL by fostering cell growth and inhibiting programmed cell death.

It has been previously reported that miR-483-5p targets TIMP2 and participates in fibrosis signal transduction in hepatocellular carcinoma [21]. The present study has shown that there is a negative correlation between the expression of miR-483-5p and TIMP2 in UL tissues. Mechanistically, the experiment identified TIMP2 as a direct downstream target of miR-483-5p in UL cells. TIMP2 is a well-characterized matrix metalloproteinase (MMP) inhibitor and acts in a seemingly contradictory dual role in ECM remodelling: it is not only a key inhibitor of MMP-2 but also an essential co-factor in its activation process [41]. Furthermore, it was found that TIMP2 possesses growth-regulating activity independent of MMP inhibition, making it a multifunctional signalling molecule [42]. #

Frequent downregulation has been demonstrated to be not only related to the development and advancement of cardiovascular diseases, but also expressed at a lower level in various malignant tumours, and its deficiency can promote tumor progression [43, 44]. Earlier research has reported that miR-483-5p facilitates tumour progression in multiple myeloma through direct targeting of TIMP2 [24]. In UL cells, miR-483-5p negatively regulates TIMP2 expression. Knockdown of TIMP2 attenuates the phenotypic changes resulting from miR-483-5p inhibition, supporting the hypothesis that TIMP2 mediation is a key mechanism through which miR-483-5p facilitates UL development.

The development of UL is driven by dysregulation of ECM metabolism [45], manifested by a marked overproduction of ECM components, including collagens (predominantly types I and III), fibronectin, laminin, and hyaluronic acid, by UL cells. The subsequent accumulation and remodelling of the ECM are critical for establishing the distinct physical architecture of these benign tumours [23]. In the current study, inhibition of miR-483-5p resulted in reduced expression of key ECM components COL1A1, COL3A1, and FN1 in UL cells, suggesting that miR-483-5p may be involved in ECM metabolic dysregulation in UL. Interestingly, the study found that downregulating TIMP2 alleviated the suppression of ECM component expression caused by miR-483-5p inhibition. This reveals the potential role of the miR-483-5p/TIMP2 axis in the imbalance of ECM metabolism

in UL: miR-483-5p directly targets and suppresses TIMP2 expression, thereby disrupting the normal regulation of ECM metabolism by TIMP2. This disruption may promote the abnormal accumulation of ECM components, ultimately driving the fibrotic process in UL. This mechanism not only explains the protective role of TIMP2 in UL development, but also uncovers the potential function of miR-483-5p as a pro-fibrotic factor.

Limitations of the study. Although the study provides evidence supporting the potential function of the miR-483-5p/TIMP2 axis in UL progression, several limitations should be acknowledged. First, the relatively small sample size, with all samples collected from a single hospital, may impose certain restrictions on the generalizability of the findings and introduce potential selection bias. Second, in the analysis of the clinical and pathological characteristics of patients, although BMI is regarded as a risk factor for UL [46], no significant correlation was observed in the obtained results. Moreover, genetic and racial factors related to disease onset [10] were not included in the analysis scope of this study. Third, as the study primarily relied on *in vitro* experiments, the lack of validation through *in vivo* animal models represents a critical limitation that should be addressed in future research.

Subsequent studies plan to expand sample sizes through multicentre collaboration and the introduction of animal models to validate the functional role of the miR-483-5p/TIMP2 axis. This approach will enable a more comprehensive exploration of the risk factors and molecular mechanisms underlying the development of UL.

CONCLUSION

In summary, miR-483-5p is significantly upregulated in UL tissues and is closely associated with UL progression. Mechanistically, miR-483-5p enhances the progression of UL by directly targeting and negatively regulating TIMP2.

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