LncRNA WTAPP1 Promotes Migration and Angiogenesis of Endothelial Progenitor Cells via MMP1 Through MicroRNA 3120 and Akt/PI3K/Autophagy Pathways

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**ABSTRACT**

Efficient recruitment and angiogenesis of endothelial progenitor cells (EPCs) are critical during a thrombus event. However, the details of EPC recruitment and the regulation of angiogenesis have not been fully determined. The aim of this study was to determine the role of the long noncoding (lnc)RNA Wilms tumor 1 associated protein pseudogene 1 (WTAPP1) in regulation of the migration and angiogenesis of EPCs. EPCs were isolated from human peripheral blood and characterized by flow cytometry, after which lentivirus-mediated lncRNA WTAPP1 overexpression and knockdown were performed. Scratch assay, Transwell assay, and in vitro and in vivo tube formation assays were performed to measure cell migration, invasion, and angiogenic abilities, respectively. Moreover, a microarray screen, bioinformatic prediction, and quantitative PCR and Western blot of miRNAs interacting with lncRNA WTAPP1 were conducted. Western blot was carried out to elucidate the relationship among WTAPP1, miR-3120-5P, and MMP-1 in the autophagy pathway. WTAPP1 positively regulated migration, invasion, and in vitro and in vivo tube formation in EPCs by increasing MMP-1 expression and activating PI3K/Akt/mTOR signaling. Furthermore, WTAPP1 contains a putative miR-3120-5P binding site. Suppression of WTAPP1 by miR-3120-5P decreased the level of MMP-1. In addition, we demonstrated that suppression of the autophagy pathway is involved in the effects of WTAPP1 on EPC migration and angiogenesis. The lncRNA WTAPP1, a molecular decoy for miR-3120-5P, regulates MMP-1 expression via the PI3K/Akt and autophagy pathways, thereby mediating cell migration and angiogenesis in EPCs. Acting as a potential therapeutic target, the lncRNA WTAPP1 may play an important role in the pathogenesis of DVT. Stem Cells 2018; 36:1863–1874

**SIGNIFICANCE STATEMENT**

The aim of this study was to determine the role of the long non-coding (lnc)RNA WTAPP1 in the regulation of migration and angiogenesis of endothelial progenitor cells. It was found that WTAPP1 positively regulated migration, invasion, and in vitro and in vivo tube formation in EPCs by increasing MMP-1 expression and activating PI3K/Akt/mTOR signaling. Furthermore, lncRNA WTAPP1, as a molecular decoy for miR-3120-5p, regulates MMP-1 expression via autophagy pathways. Acting as a potential therapeutic target, lncRNA WTAPP1 may play an important role in angiogenesis.

**INTRODUCTION**

Deep vein thrombosis (DVT), with an incidence of 0.1%–0.27% per year, is a peripheral vascular disease commonly encountered in clinical practice [1, 2]. Treatment for DVT may involve anticoagulation therapy, inferior vena cava filter placement, aggressive clot removal, and secondary prevention and prophylactic modalities [3]. Despite adequate therapy, 20%–50% of patients will develop post-thrombosis syndrome within 2 years, whereas others will experience long-term complications, such as postphlebitic syndrome (40%) and chronic thromboembolic pulmonary hypertension (4%) [4, 5]. The limited and inefficient treatment methods currently available in clinical practice necessitate a greater understanding of the molecular and cellular mechanisms that are involved in the pathogenesis of DVT.
In the setting of DVT, endothelial progenitor cells (EPCs), which are a type of bone marrow-derived circulating progenitor cell in the endothelial lineage, have been reported to have the abilities to home in on and migrate to disease sites to facilitate thrombus recanalization and resolution by neovascularization, increase new blood vessel formation at the injured site, and secrete a variety of vasoactive and angiogenic factors to improve angiogenesis [6–8]. Furthermore, EPCs have the capacity to protect differentiated endothelial cells from apoptosis, thereby preserving their angiogenic potential under conditions of oxidative stress [9]. In our previous study, we demonstrated that the transplantation of normal and gene-modified bone marrow-derived EPCs altered the vein microenvironment and facilitated thrombus resolution in a rat model of vein thrombosis [10–12]. However, the number of EPCs in peripheral blood is limited. Moreover, the capacity for angiogenesis is influenced by adverse conditions in the microenvironment. Both of these factors can impair the effects of thrombus resolution by EPCs [13–15]. Therefore, in a clinical context, it is critically important to promote the effective recruitment of EPCs to the thrombus and enhance angiogenesis.

Long noncoding RNAs (lncRNAs), with lengths of >200 nucleotides, are a recently discovered novel class of genes with regulatory function that lack protein-coding ability. Many studies have shown that lncRNAs exert critical functions in a wide range of cellular processes, including X-chromosome inactivation, splicing, imprinting, epigenetic control, and the regulation of gene transcription [16–18]. Furthermore, lncRNAs exhibit dysregulated expression in various human diseases, such as cancer [19], cardiovascular disease [20], and neurodegenerative disease [21]. According to the most recent evidence [22,23], lncRNAs regulate adult stem cell function and may serve as novel therapeutic targets in vascular disease. However, their roles in EPC function and the pathogenesis of DVT remain largely unknown.

In our previous study, we found that several lncRNAs were differentially expressed in EPCs derived from the peripheral blood of DVT patients compared with the levels in healthy controls [24]. Among these lncRNAs, Wilms tumor 1 associated protein pseudogene 1 (WTAPP1) was found to be significantly downregulated and thus might be involved in angiogenesis in thrombi. Here, we aimed to investigate the function of WTAPP1 in human EPCs and examine its mechanisms of action. Our results showed that WTAPP1 acts as a molecular decoy for miR-3120-5p and affects EPC recruitment by regulating MMP-1 expression, which plays an important role in the context, it is critically important to promote the effective recruitment of EPCs to the thrombus and enhance angiogenesis.

Materials and Methods

Ethical Approval of the Study Protocol

All research involving human participants was approved by the institutional review board of Soochow University, Suzhou, China. All participants gave written informed consent. The study protocol was approved by the Institutional Animal Care and Use Committee of Soochow University. The study was conducted following international guidelines for animal experimentation.

Isolation and Characterization of EPCs

EPCs were isolated and characterized as described previously [25]. Peripheral blood mononuclear cells (PBMCs) were isolated from healthy volunteers with Ficoll-Isopaque Plus (Histopaque-1077; Sigma, MO, USA) by gradient centrifugation. The PBMCs were seeded onto type 1 collagen-coated six-well cell culture plates, cultured in endothelial basal medium-2 (EBM-2) MV BulletKit (Lonza, MD, USA), and maintained at 37°C in a 5% CO2 incubator. The medium was changed after 4 days.

EPCs were identified by confocal microscopy and flow cytometry. The cells were incubated with FITC-UEA-1 (Sigma Deisenhofen, Germany) and Dil-Ac-LDL as described previously [25,26]. Double-positive staining for Dil-AcLDL and FITC-UEA-1 was considered to represent EPCs. The phenotypes of EPCs were analyzed with regard to their surface expression of CD31, CD34, CD45, CD133, and CD309 (all antibodies were purchased from Becton-Dickinson, NJ, USA). The EPCs from passages 3 and 4 were used.

Cell Treatment

Recombinant lentiviral particles expressing lncRNA WTAPP1, WTAPP1 short interfering (si)RNA, miR-3120-5P mimic and inhibitor, Atg-5 siRNA, and vehicle controls were obtained from GenePharm Co. Ltd. (Shanghai, China). EPCs were grown to approximately 40% confluence and infected with lentiviral particles in complete medium for 48 hours. To increase the infection efficiency, cells were cotreated with the cationic polymer polybrene (8 μg/ml in water). Neither the lentivirus nor polybrene affected cell viability. The siRNA had no off-target effects, and the siRNA or overexpression vector had no effect on EPC adherence, shape, or viability at the indicated multiplicity of infection and treatment duration. In the autophagy pathway experiments, EPCs that were treated with WTAPP1 lncRNA or WTAPP1 siRNA lentivirus were also incubated with 10 nM rapamycin (Beeyotime Biotechnology, Nantong, China).

Wound Healing Migration Assay

Wound healing migration assay was performed with EPCs following published methods [27]. Briefly, EPCs (4 × 10^6 cells) that were infected with WTAPP1 lncRNA or WTAPP1 siRNA lentivirus were seeded in a six-well plate and grown to nearly 100% confluence; then, a linear scratch was made with a 200-μl pipette tip. After being washed with PBS twice, the cells were incubated with serum-free EBM-2 medium for specific times. Images were taken at 0 and 24 hours at ×40 magnification, and the wound size was measured in three wells per group.

Matrigel Cell Invasion Assay

A Transwell chamber was used (8 μm, 24-well plate) for the invasion assay. The insert membranes were coated with diluted Matrigel. EPCs (1 × 10^5) that were infected with WTAPP1 lncRNA, WTAPP1 siRNA, or miR-3120-5P mimic or inhibitor lentivirus were added to the upper chamber and cultured for 24 hours. Then, the insert membranes were cut and stained with crystal violet (Beeyotime Technology, China), and the number of invading cells was counted under an inverted microscope and photographed. All experiments were performed at least three times independently.
In Vitro Tube Formation Assay
An in vitro Matrigel tube formation assay was performed to determine the angiogenic activity of EPCs as described previously [25]. Briefly, EPCs (5 × 10⁴ cells) were infected with WTAPP1 lncRNA, WTAPP1 siRNA, or miR-3120-5P mimic or inhibitor lentivirus and then seeded in Matrigel-coated 48-well plates, after which the tubular structures of EPCs in the Matrigel were examined under a microscope after 12 hours of incubation. The total number of tubes that formed, which represents the degree of angiogenesis in vitro, was scanned and quantified in three random fields (×100 magnification) per well.

In Vivo Angiogenesis
An in vivo tube formation assay was performed as described previously [28, 29]. Male athymic nude (nu/nu) mice, aged 6 weeks, were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. EPCs (5 × 10⁵ cells) that were infected with WTAPP1 lncRNA or WTAPP1 siRNA lentivirus were resuspended in 200 μl of Matrigel (BD) on ice and implanted into the flank of the nude mice (n = 6 for each treatment) by subcutaneous injection using a 25-gauge needle. After 7 days, the Matrigel implants were removed, fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned. The histological sections were stained with H&E to examine the presence of luminal structures. Microvessels were defined as luminal structures that contained erythrocytes; four randomly selected fields in each section were counted under ×400 magnification. Images were analyzed under an Olympus CKX41 microscope with OlympusMicro software.

Microarray Analysis and Computational Analysis
miRNA expression profiling was performed using miRNA microarray analysis and miRNA array probes (LC Sciences), in accordance with previously described methods [26]. Triplicates of
each sample were analyzed. The significance of differences between EPCs with Inc-WTAPP1 knockdown and normal controls for a given detectable miRNA signal was calculated. Differentially detected signals were identified through fold change filtering (fold change ≥2.0 or ≤0.5) and paired t test (p < .05).

**Luciferase Assay.** For luciferase reporter experiments, the 3′-UTR segments of MMP1 predicted to interact with miR-3120-5P were amplified by PCR and inserted into the GV251 vector immediately downstream from the stop codon of luciferase (Promega). For the measurement of luciferase activity, HEK293 T cells were cotransfected in 24-well plates with 100 ng of luciferase plasmid and 50 ng of Renilla plasmid (Ambion) as a control, as well as with 400 ng of miR-3120-5p mimics or negative control miRNA. Forty-eight hours later, the activities of luciferase and Renilla plasmid were assessed via the Dual Luciferase Reporter 1000 Assay System (Promega, WI, USA).

**Real-Time Reverse-Transcription Polymerase Chain Reaction**

After the EPCs had been treated as described above, total cellular RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Real-time reverse-transcription polymerase chain reaction was carried out using SYBR Green Q-PCR Mix (Thermo Scientific, MBI, USA) on a Roche LightCycler 480 (Roche, Switzerland). The expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 snRNA was assessed simultaneously in all samples as internal controls. Relative gene expression was calculated using the 2−ΔΔCT method [30]. The oligonucleotide primers for WTAPP1 lncRNA, MMP1, GAPDH, miRNA-3120-5P, and U6 snRNA are listed in Supporting Information Table S1.

**Western Blot Analysis**

EPCs (1 × 10⁶ cells) that had been treated with lentivirus as described above were lysed in RIPA buffer and subjected to high-speed centrifugation and quantification by the bicinchoninic acid
Cellular proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. After being blocked with 5% non-fat milk in Tris-buffered saline–Tween 20, the membranes were incubated with primary antibodies against PI3K, phospho-PI3K, Akt, phospho-Akt, mTOR, phospho-mTOR, LC3, ATG5, and p62. Then, the appropriate horseradish peroxidase-conjugated secondary antibodies were applied. β-Actin (Sigma) was used as a loading control. The protein bands were detected with SuperSignal West PicoChemiluminescent Substrate (Pierce, Rockford, IL, USA) on X-ray films (Kodak, Tokyo, Japan).

Statistical Analyses
All statistical analyses were performed using SPSS v21 (SPSS, Chicago, IL, USA). Data are presented as mean ± SD. Student’s t test or one-way ANOVA was used to examine the differences between two groups or for multiple-group comparisons. Correlation analyses of the expression levels of WTAPP1, MMP-1, and miR-3120-5p were performed using Pearson’s correlation. p < .05 was considered to be statistically significant.

RESULTS
Characterization of EPCs
To examine the role of IncRNAs in EPCs, we obtained human peripheral blood, isolated EPCs by density gradient centrifugation, and characterized them by flow cytometry and confocal microscopy. These cells were positive for CD309 and CD31, stained weakly for CD34, and were negative for CD45 and CD133 (Fig. 1A). Moreover, a functional assay revealed that these cells had the ability to take up DiI-ac-LDL and were bound by FITC-UEA-1 on their membrane (Fig. 1B). These findings confirmed our successful isolation of EPCs for further use in subsequent experiments.

WTAPP1 Positively Regulates Cell Migration, Invasion, and Tube Formation in EPCs
To determine the role of WTAPP1 in EPCs, we performed gene knockdown and overexpression by lentiviral infection and examined cell function. WTAPP1 knockdown and overexpression, respectively, increased and decreased MMP-1 in EPCs. (A): Lentivirus-mediated IncRNA WTAPP1 knockdown and overexpression were performed in EPCs, followed by an evaluation of MMP-1 by real-time quantitative PCR, association analyses between MMP-1 and IncRNA WTAPP1 levels, and Western blot of MMP-1. (A): IncRNA WTAPP1 knockdown and overexpression, respectively, increased and decreased MMP-1 in EPCs. (B): Correlation analyses indicated that there was a positive linear association between MMP-1 and IncRNA WTAPP1, with an r² value of .472. (C): Western blot revealed that IncRNA WTAPP1 knockdown and overexpression, respectively, increased and decreased the levels of MMP-1 in EPCs. (D): Relative quantification of protein levels of MMP-1, *, p < .05 and ***, p < .001 for between-group comparisons. Abbreviations: IncRNA, long noncoding; siRNA, short interfering RNA; WTAPP1, Wilms tumor 1 associated protein pseudogene 1.
formation in EPCs. These results suggest that WTAPP1 positively regulates EPC migration and angiogenesis.

**WTAPP1 Positively Regulates the Expression of MMP-1 in EPCs**

To determine the mechanism of WTAPP1-mediated effects in EPCs, we examined the expression of MMP-1 in EPCs during WTAPP1 knockdown and overexpression. WTAPP1 positively regulated the expression of MMP-1 (Fig. 3A). Correlation analysis, which was performed based on the results of RT-PCR, confirmed the significant association between WTAPP1 and MMP-1 expression ($r^2 = .472, p < .05$; Fig. 3B). Furthermore, the protein level of MMP-1 was consistent with its mRNA content (Fig. 3C, 3D). These results indicate that WTAPP1 exerts its effects in EPCs by altering MMP-1 expression.

**Involvement of PI3K/Akt/mTOR Signaling in WTAPP1-Mediated Effects in EPCs**

According to previous reports, the PI3K/Akt/mTOR signaling pathway is involved in regulation of the migration and angiogenic function of cells [31, 32]. To further determine the effects of WTAPP1 on intracellular pathways, we examined PI3K/Akt/mTOR signaling. Our results indicated the downregulation and upregulation of PI3K, Akt, and mTOR phosphorylation in EPCs with IncRNA WTAPP1 knockdown and overexpression, respectively (Fig. 4A–4D). These results confirm the involvement of PI3K/Akt/mTOR signaling in the WTAPP1-mediated effects on EPCs.

**LncRNA WTAPP1 Functions as an miR-3120-5p Decoy in EPCs**

Since there is no direct interaction between the lncRNA WTAPP1 and MMP-1, we further analyzed the associated regulatory mechanism by introducing an miRNA, based on the finding that lncRNAs act as decoys to sequester miRNAs and prevent them from binding to their targets, thus modulating many functional mRNA targets through translation. By microarray analysis (Fig. 5A) and quantitative PCR (Fig. 5B), we selected miR-3120-5p as a candidate miRNA for the above purpose. Moreover, using a bioinformatic tool (web-server InCeDB; http://gyanxet-beta.com/InceDb/), we predicted lncRNA-miRNA interactions between lncRNA WTAPP1 and miR-3120-5p (Fig. 5C). By correlation analysis, we confirmed the interaction between IncRNA WTAPP1 and miR-3120-5p (Fig. 5D).

To further explore the potential molecular mechanism underlying the effect of miR-3120-5p, a luciferase reporter assay was performed. Based on the TargetScan databases, MMP1 was predicted to have a putative miR-3120-5p binding site within its 3’-UTR (Fig. 5C). To confirm that MMP1 were regulated by miR-3120-5p, the 3’-UTR segments were cloned into a reporter plasmid.
downstream from luciferase, and reporter assays were then performed. It was found that miR-3120-5p mimics robustly reduced MMP1 reporter gene expression; the luciferase activities were only 0.7, significantly lower than those in the control (Fig. 5E).

These findings suggested that miR-3120-5p suppressed MMP1 by direct binding to its 3'-UTR segment. To confirm the ability of miR-3120-5p to inhibit MMP1 expression, we transfected EPCs with miR-3120-5p mimics and inhibitor. EPCs transfected with miR-3120-5p mimics exhibited significantly decreased MMP1 protein level, while EPCs transfected with miR-3120-5p inhibitor exhibited an increased MMP1 protein level (Fig. 5F, 5G).

In addition, we analyzed the protein level of MMP-1 during lncRNA WTAPP1 overexpression or knockdown (Fig. 5F, 5G). LncRNA WTAPP1 knockdown and overexpression abolished these effects.

Figure 5. LncRNA WTAPP1 functions as an miR-3120-5p decoy in human peripheral blood-derived endothelial progenitor cells (EPCs). (A): Heatmap analysis of dysregulated miRNAs in normal and lncRNA WTAPP1 knockdown EPCs. A total of 17 significantly dysregulated miRNAs were found and differentially expressed in normal and lncRNA WTAPP1 knockdown EPCs. miR-3120-5p was selected for further analysis. (B): Real-time quantitative PCR confirmed the upregulation of miR-3120-5p in lncRNA WTAPP1 knockdown EPCs, which was consistent with the microarray results. *** p < .001 for between-group comparison. (C): The predicted positions of miR-3120-5p binding sites in the MMP-1 and lncRNA WTAPP1 transcripts. (D): Correlation analyses indicated a negative linear association between the expression of LncRNA WTAPP1 and miR-3120-5p, with an r2 value of .548. (E): Luciferase reporter assays were performed on HEK293 T cells. Each bar represents mean ± SEM (n = 3, ***, p < .001). (F): Western blot analysis of MMP-1 in EPCs transfected with miR-3120-5p mimic or inhibitor and lncRNA WTAPP1 knockdown and -overexpression lentivirus. miR-3120-5p inhibition and overexpression, respectively, increased and decreased the expression of MMP-1, whereas LncRNA WTAPP1 knockdown and overexpression abolished these effects. (G): Relative quantification of Western blot results in (F). * p < .05 and ** p < .01 compared with vehicle control. #, p < .01 and §, p < .05 compared to EPCs transfected with miR-3120-5p mimic or inhibitor. Abbreviations: lncRNA, long noncoding; siRNA, short interfering RNA; WTAPP1, Wilms tumor 1 associated protein pseudogene 1.
effects of miR-3120-5p inhibition and overexpression on MMP-1 expression. These results suggest that lncRNA WTAPP1 functions as an miR-3120-5p decoy in EPCs.

**Involvement of the Autophagy Pathway in the LncRNA WTAPP1-Mediated Effects in EPCs**

The autophagy pathway is involved in the migration and angiogenic behavior of EPCs. To determine the role of the autophagy pathway in the effects of the lncRNA WTAPP1, we examined the autophagy pathway components LC3, Atg5, and P62. LncRNA WTAPP1 knockdown and overexpression, respectively, increased and decreased LC3 and Atg5, whereas it decreased and increased P62 in lncRNA WTAPP1-knockdown and -overexpressing EPCs. Rapamycin and Atg5 siRNA, respectively, increased and decreased the expression of autophagy pathway components in lncRNA WTAPP1-overexpressing and -knockdown EPCs. (A): Relative quantification of MMP-1 in (A). (B): Relative quantification of LC3 in (A). (C): Relative quantification of Atg5 in (A). (D): Relative quantification of P62 in (A). *, p < .05; **, p < .01; and ***, p < .001 for between-group comparisons. Abbreviations: siRNA, short interfering RNA; WTAPP1, Wilms tumor 1 associated protein pseudogene 1.

We also observed that the autophagy activator rapamycin and the inhibitor Atg5 siRNA increased and decreased, respectively, the expression of autophagy pathway components in EPCs during lncRNA WTAPP1 overexpression and knockdown (Fig. 6A–6E). These results confirm the involvement of the autophagy pathway in the lncRNA WTAPP1-mediated effects on EPCs.

**miR-3120-5P Negatively Regulates Cell Migration and Tube Formation in EPCs**

Based on the interaction between miR-3120-5P and MMP-1, we examined the effects of miR-3120-5P on cell migration and angiogenesis in EPCs. miR-3120-5P overexpression and knockdown, respectively, decreased and enhanced cell invasion (Fig. 7A) and
tube formation (Fig. 7B). These results confirm the inhibitory function of miR-3120 on EPC migration and angiogenesis. Based on these results, we proposed that lncRNA WTAPP1, as a molecular decoy for miR-3120-5p, regulates MMP-1 expression via the PI3K/Akt and autophagy pathways, thereby mediating cell migration and angiogenesis in EPCs (Fig. 7C).

**DISCUSSION**

Venous thrombus resolution and recanalization, together forming a complex and spontaneous process in humans, require synergy between various cell types, the main steps of which are infiltration of inflammatory cells into the thrombus, tissue remodeling, and angiogenesis [33]. Accumulating evidence has suggested that EPCs are recruited to the site of the thrombus and involved in the resolution and recanalization [34, 35]. However, the detailed mechanisms of EPC recruitment and angiogenesis remain unknown.

As a class of newly discovered genes, lncRNAs, which have gene regulatory functions but lack protein-coding ability, have been suggested to play a critical role in regulating physiological functions [36]. Emerging evidence suggests that lncRNAs function in vascular biology and contribute to vascular diseases, including atherosclerosis, hypertension, stroke, and peripheral artery diseases [37, 38]. However, few studies focusing on the regulatory role of lncRNAs in EPC recruitment and angiogenesis have been reported. Our previous study involving microarray analysis showed that some differentially expressed lncRNAs were found in EPCs from patients with DVT. Among these, WTAPP1, located at chr11q22.2, with the seqname of ENST00000544704 and the gene Symbol RP11-725k16.4, was predicted to be a lncRNA with pro-angiogenic effects [24]. In this study, we investigated the role of the lncRNA WTAPP1 in EPCs. Our results demonstrated that it positively regulates migration, invasion, and in vitro and in vivo tube formation ability in EPCs by increasing MMP-1 expression and activating the PI3K/Akt/mTOR signaling pathway. In addition, we showed that WTAPP1 contains a putative miR-3120-5P binding site and confirmed their interaction by correlation analysis. Suppression of WTAPP1 by miR-3120-5P also decreased the level of MMP-1.
Furthermore, we identified that suppression of the autophagy pathway was involved in the effects of WTAPP1 on EPC migration and angiogenesis. MMP1, an interstitial collagenase that is synthesized and secreted by endothelial cells and inflammatory cells, plays an important role in the migration of cells in thrombi [39]. Ivy et al. [40] also found that MMP1 is necessary for the migration of human bone marrow-derived mesenchymal stem cells. It may promote cell invasion and migration via protease-activated receptor 1 [41] and by activating MMP2 and MMP9 [42, 43]. Furthermore, MMP1 itself is a necessary proteinase for angiogenesis [44, 45], especially in the initial stages of this process. MMP1 can induce the growth of human umbilical vein endothelial cells and subsequent sprouting angiogenesis both in vivo [46] and in vitro [47]. These findings are consistent with our results.

Increasing evidence has revealed that several lncRNAs act as competing endogenous RNAs (ceRNAs) via their miRNA response elements for specific miRNA targets and could result in the failure of a single or multiple miRNAs to bind to their targets [48, 49]. Several lncRNAs, including PTENP1 [50], H19 [51], and CCAT1 [52], have been suggested to function as ceRNAs in regulating biological activities. In the present study, miRNA array analysis was performed in EPCs that had been transfected with WTAPP1 siRNA. Compared with normal EPCs, there were three upregulated miRNAs. The results of qRT-PCR confirmed these findings, demonstrating that miRNA-3120-SP was upregulated in the lncRNA WTAPP1-downregulated EPCs; a negative association was found by Pearson’s correlation analysis. There were many coupled gene sequences in miRNA-3120-SP with lncRNA WTAPP1 and MMP1 mRNA. We found that miRNA3120-5P bridges the regulation of MMP1 by lncRNA WTAPP1. Moreover, in a previous study, miRNA3120-5P was shown to target heat shock cognate protein 70 [53], which is essential for Akt signaling in endothelial function [54]. In the present study, miRNA-3120-5P could target MMP1 and inhibit Akt, subsequently inhibiting angiogenesis, revealing an opposite role to that of lncRNA WTAPP1.

As shown in a previous study, PI3K/Akt/mTOR signaling plays an important role in EPC migration and angiogenesis [55]. This pathway has also been shown to facilitate MMP1-mediated cell proliferation and migration [56] and negatively regulate autophagy [57, 58], which has protective effects in humans by degrading cytoplasmic contents using its own lysosomal machinery in cells [59, 60]. Recent studies have also revealed a crucial function of lncRNAs in the modulation of cell autophagy in vitro and in vivo, which could be involved in the development and progression of multiple diseases, including vascular diseases [61, 62].

Here, we found that suppression of the autophagy pathway was involved in the effects of WTAPP1 on EPC migration and angiogenesis. Moreover, according to a recent study, lncRNAs also influence drug sensitivity by modulating autophagy. Overexpression of the lncRNA Risa, which regulates insulin sensitivity and autophagy in hepatocytes and myotubes, could significantly decrease autophagy, thus relieving insulin resistance. Atg7 or Atg5 knockdown by siRNA inhibits the effects of Risa on insulin resistance in vivo [63]. These findings suggest that modulation of the lncRNA–autophagy axis is a potential approach for controlling diseases. Thus, further investigation of lncRNAs involved in the regulation of autophagy may identify novel strategies that can enhance the benefits of pharmacotherapy in the treatment of DVT.

In conclusion, lncRNA WTAPP1, as a molecular decoy for miRNA-3120-5p, regulates MMP-1 expression via the PI3K/Akt and autophagy pathways, thereby mediating cell migration and angiogenesis in EPCs, which play an important role in the pathogenesis of DVT.

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AUTHOR CONTRIBUTIONS

W.D., D.M., and L.L.: laboratory work, data analysis and interpretation, manuscript writing, and final approval of the manuscript; L.X., Z.L., and M.Z.: laboratory work, data analysis and interpretation, manuscript writing, and final approval of the manuscript; W.B.: conception/design and fund raising, and final approval of the manuscript; X.Q.: conception/design, data analysis, and interpretation, manuscript writing, and final approval of the manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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