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Reciprocal enhancement of thrombosis by endothelial-to-mesenchymal transition induced by iliac vein compression

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ABSTRACT

Aims: Endothelial-to-mesenchymal transition (EndMT) is a pathophysiological change of vascular endothelium commonly seen in the cardiovascular system. Iliac vein compression syndrome (IVCS) is known to be often associated with intimal hyperplasia and thrombosis. However, whether EndMT exists in IVCS has not yet been reported. The purpose of this study was to investigate the relationship between EndMT and thrombosis in IVCS.
Main methods: Using IVCS models in pig and mouse, we detected intimal changes and thrombus in stenotic iliac vein by immunofluorescence staining. Primary human umbilical vein endothelial cells (HUVEC) were stimulated by transforming growth factor β1 (TGF-β1) and thrombin, and cell phenotypic transition and antithrombotic function of HUVEC were examined through q-PCR, western blot and ELISA. In the end, by immunofluorescence staining, we observed the effect of anticoagulant on interstitial changes of venous endothelial cells in IVCS models.
Key findings: We showed that iliac vein compression induced EndMT, of which its inhibition reduced thrombus formation. Further studies showed that HUVECs undergoing EndMT lost their anticoagulation and thrombolytic function. Interestingly, thrombin aggravated EndMT through TGF-β/Smad3 signaling. Moreover, compared with wild type (WT) mice, EndMT in stenotic iliac vein was reduced in WT mice fed with rivaroxaban or factor VII knockout mice, implying that anticoagulation alleviated EndMT in IVCS models.
Significance: Our findings indicate that EndMT and thrombosis reinforce reciprocally in IVCS, implying that targeting EndMT could be a potential strategy in prevention and treatment of thrombosis in IVCS.

1. Introduction

Iliac vein compression syndrome (IVCS) refers to the lower extremity and pelvic venous return dysfunction disease caused by left common iliac vein compression or the abnormal adhesion of structures within the cavity [1]. IVCS was shown to associate with thrombosis of the lower extremities, thereby causing a cluster of clinical symptoms [2]. Reduced velocity of blood at the distal area of vascular compression and activation of the coagulation system are the main mechanisms of thrombosis in IVCS [3]. And a large amount of thrombin is activated during vein thrombus formation.

Endothelial-to-mesenchymal transition (EndMT) defines a process whereby endothelial cells (ECs) lose their endothelial specification and acquire some mesenchymal cell features [4]. EndMT can be induced by many pathological conditions, such as inflammation, disturbed flow, oxidative stress, etc., during which ECs morphology and function change [5,6]. TGF-β1 is the primary stimulating factor of EndMT and inhibition TGF-β/Smad3 signaling can prevent EndMT [7,8]. EndMT can be seen in cardiac development as well as various diseases, such as cardiac fibrosis, kidney fibrosis, cerebral cavernous malformation, pulmonary hypertension and atherosclerosis [9–15]. However, the role of EndMT in thrombus formation has not yet been clarified.

In the present study, we found that iliac vein compression induced EndMT and inhibition of EndMT reduced the probability of thrombosis

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in IVCS animal models, as the antithrombotic function of endothelial cells undergoing EndMT is lost. In addition, we demonstrated that thrombin led to EndMT and inhibition of the coagulation system mitigates the transition.

2. Materials and methods

2.1. Animal models

All animal procedures were approved by the Institutional Animal Care and Use Committee of Soochow University. IVCS models were established in pig and mouse. The 8-to-10-weeks-old Bama pigs (30–35 kg) were used for iliac vein narrowing operation (n = 6). An anesthesia protocol was designed with premedication used by Shumianning II (Nanjing agricultural university China) 0.02 ml/kg, induction anesthesia given by propofol (Ehwa China) 5 mg/kg intravenously, and maintenance anesthesia with 3% pentobarbital sodium (Chemmart China). We used the retroperitoneal approach to expose the left common iliac aortic vein without entering the abdominal cavity. After measuring the diameter of the left common iliac vein, we ligated the iliac artery and vein to a glass rod with a diameter of 30% of the vein diameter and removed the glass rod after ligation. Angiography was performed to verify IVCS models 1 week postoperatively. Mice were anesthetized with 1% pentobarbital sodium (50 mg/kg). For the mouse IVCS model, we ligated the iliac artery, iliac vein, and a 5–0 polypropylene line together, and withdrew the 5–0 polypropylene line later. Stenosed vein segments were harvested 1 week (mouse) or 4 weeks (pig) after ligation. HemA mice with C57Bl/6 background homozygous for FVII knockout (exon 16) were purchased from Jackson lab [16]. Rivaroxaban was orally administered in drinking water (2 mg/l) for 7 days. Total of 40 mice were randomly divided into two groups and there was no difference in body weight between the two groups ($P > 0.05$). The experimental group was injected with oxymatrine intraperitoneally daily for 7 days (10 mg/kg), and the control group was injected with normal saline.

2.2. Immunostaining

Veins were fixed in 4% paraformaldehyde overnight and embedded in TissueTek O.C.T (4583, Sakura, United States). Frozen section (8 μm) was incubated with primary antibodies including platelet and endothelial cell adhesion molecule 1 (PECAM-1 CD31) (553370, BD Biosciences, United States), α-smooth muscle actin (α-SMA) (BM0002, Boster, China), von Willebrand factor (VWF) (6994, Abcam, USA), fibronectin (6328, Abcam, USA), and Cy3 donkey anti-goat IgG (HCL) (A0502, Beyotime, China). Fluorescence-labeled secondary antibodies (Alexa Fluor donkey anti-rabbit 488, Alexa Fluor rabbit anti-mouse 555, Alexa Fluor donkey-anti-mouse 647, Abcam, United States) and DAPI (C1002, Beyotime, China) were counterstained successively. Images were examined using a multicolor digital camera on an IX-81 laser confocal microscope (Olympus, Japan).

2.3. ELISA

Human serum TGF-β1 level was measured by the ELISA Kit (EL-0162c, Elabscience). Briefly, 40 μl of human serum was added to 360 μl of dilution buffer, and incubated with biotinylated detection antibody and HRP conjugate. After incubated with substrate reagent for 15 min, the color intensity was measured at 450 nm. Quantikine Human Thrombomodulin (TM) Kit (DTHBD0 R&D Systems) was used to measure TM in the supernatant of HUVEC cultures according to the manufacturer's instructions.

2.4. HUVEC culture

Primary HUVECs (PCS-100-010, ATCC, USA) were maintained in vascular cell basal medium (PCS-100-030, ATCC, USA) containing ascorbic acid (PCS-999-006, ATCC, USA), FBS (PCS-999-010, ATCC, USA), rhEGF (PCS-999-018, ATCC, USA), heparin sulfate (PCS-999-011, ATCC, USA), L-glutamine (PCS-999-017, ATCC, USA), rhVEGF (PCS-999-024, ATCC, USA), rhFGF-b (PCS-999-020, ATCC, USA), rhIGF-1 (PCS-999-021, ATCC, USA), hydrocortisone (PCS-999-014, ATCC, USA). Cells were stimulated with thrombin (T9326, Sigma Aldrich, USA) and 2 mM $CaCl_2$ or TGFβ1 (100-21, PeproTech, USA) 48 h after starving for 24 h.

2.5. RNA isolation and QPCR

Total RNAs from HUVECs were extracted using the RNA simple Total RNA kit (Tiangen, DP419, China). RNA was quantified using a NanoDrop 2000 spectrometer (Thermo Scientific, USA). Reverse transcriptions were performed by using the 5xAll-In-One RT Master Mix (abm, G490, Canada). Subsequently, quantitative real-time PCR (qPCR) was performed using the SYBR Green FastMix Reaction Mixes kit (Roche, 4887352001, Switzerland) in a real-time-PCR System (LightCycler 480, Roche, Switzerland). RNA expression was analyzed using $2^{-\triangle\triangle CT}$ methods. Primer sequences are given in Supplementary materials 1: Table S1.

2.6. Western blot analysis

Cells were lysed with RIPA Lysis Buffer (1% Triton X-100, 1% deoxycholate, 0.1% SDS, 10 mM Tris and 150 mM NaCl) with protease and phosphatase inhibitor cocktail (Santa Cruz Biotechnology Inc., Heidelberg, Germany). 30 μg of total protein from each sample was transferred to NC membrane and then incubated with primary antibody overnight at 4 °C, following by fluorescent secondary antibodies (goat anti-rabbit IRDye 800CW, goat anti-mouse IRDye 800CW, LI-COR Odyssey, USA) for 1 h at room temperature. Primary antibodies include mouse anti-human α-SMA (1:500 BM0002, Boster, China), rabbit anti human CD31 (1:200 134168, Abcam, USA), rabbit anti human VE-cadherin (1:1000 2500, CST, USA), rabbit anti human Smad3 (1:200, BM3919, Boster, China), rabbit anti human p-Smad3 (1:200 BM4033, Boster, China), rabbit anti human VWF (1 μg/ml 6994, Abcam, USA), rabbit anti human fibroblast specific protein-1 (FSP-1) (1:500 41532, Abcam USA). Chemiluminescence measurements were performed using the Odyssey infrared imaging system (LI-COR Biosciences, USA). Densitometric analysis was done using the Image J software (NIH) to quantify protein expression levels.

2.7. Statistical analysis

Statistical analysis was performed using Prism 7.0 (GraphPad) and SPSS 21.0 (SPSS, Chicago, IL, USA). Student's *t*-test, and the χ^2 test were employed to compare continuous and categorical data respectively. Data are presented as mean ± SEM (standard error of the mean). Two-tailed P values < 0.05 were considered as statistically significant.

3. Results

3.1. Iliac vein compression induced EndMT and inhibition of EndMT reduced the probability of thrombosis in IVCS models

Vascular ECs are single layer cells that cover the vascular lumen's interior. Dysfunction of ECs can lead to intimal hyperplasia and thrombosis [17]. To investigate changes of venous intima in IVCS, we established IVCS animal models in pig and mouse (Fig. 1a). By immunofluorescence staining, we found that the stenosed iliac vein segments had a higher level of the mesenchymal cell markers, α-SMA and, fibronectin expression ($P < 0.005$) and reduced expression of endothelial-specific markers, CD31 and, VWF ($P < 0.005$). Moreover, co-

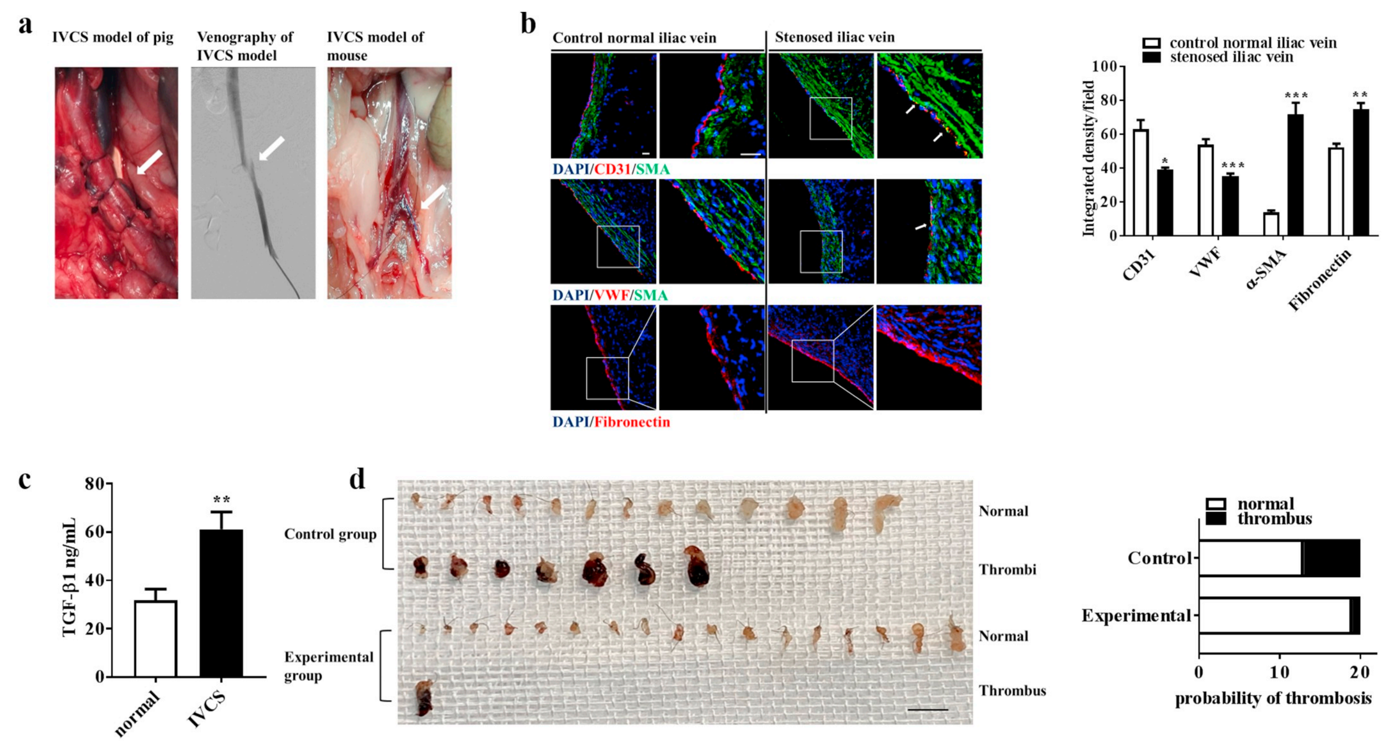

Fig. 1. Iliac vein compression induced EndMT and inhibition of EndMT reduced the probability of thrombosis in IVCS models. a IVCS animal models in pig and mouse. The arrows indicate stenosed sections. b Representative immunofluorescence of pig iliac vein sections stained for CD31 (red), VWF (red), α-SMA (green), fibronectin (red), and DAPI (blue). Arrows indicate ECs expressing both CD31 and α-SMA or VWF and α-SMA in stenosed iliac vein. Scale Bar = 20 μm. c TGF-β1 concentration in IVCS patients compared with normal individuals control. Data are mean ± SEM (n = 10 per group). $^{**}P < 0.005$. d Probability of thrombosis in IVCS in WT mouse (control group) compared with WT mouse injected with oxymatrine (experimental group) ($P < 0.05$). Scale Bar = 5 mm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

expression of CD31 and VWF with α-SMA, suggesting ECs in their intermediate stages of EndMT, can be seen in the endothelium of the stenosed iliac vein but not in the normal iliac vein (Fig. 1b and Supplementary materials 2: Fig. S1). All these results indicated that EndMT occurred in compressed iliac venous ECs. To investigate whether EndMT exists in IVCS patients, We used ELISA to measure TGF-β1 concentration in human blood. Results showed that TGF-β1 was significantly elevated in IVCS patients compared with normal individuals ($P < 0.005$ n = 10 per group) (Fig. 1c and Supplementary materials 1: Table S2).

IVCS is often combined with iliofemoral vein thrombosis [18]. To investigate the impact of EndMT on thrombus formation, mice were intraperitoneally injected with oxymatrine, a specific inhibitor of the TGF-β/Smad3 signaling pathway, daily after ligation of the iliac vein, while the control group was injected with normal saline. The distal iliac vein below the ligated site was collected 7 days after the initial operation. In the control group, 35% of iliac veins generated thrombi, while in the oxymatrine injected group, 5% of iliac veins were found to form thrombi ($P < 0.05$, n = 20 per group), (Fig. 1d). These results indicate that EndMT promotes venous thrombus formation in IVCS.

3.2. EndMT attenuated the anti-thrombotic function of ECs

The endothelium acts as a vascular barrier, and its integrity is a key factor in avoiding the activation of endogenous and exogenous coagulation systems [19,20]. At the same time, ECs can exert anticoagulation and thrombolytic functions by secreting TM, NO and tissue plasminogen activator [21,22]. To induce EndMT, we added TGF-β1 to HUVECs. As Smad3 phosphorylation (p-Smad3) was reported to be an indicator of TGF-β1 activity [7], we examined p-Smad3 in HUVECs stimulated by TGF-β1. Results showed that TGF-β1 induced Smad3 phosphorylation with the peak at 10 ng/ml and this dose was used throughout the study (Supplementary materials 2 Fig. S2). HUVECs became elongated and irregular, with enhanced α-SMA expression, and decreased expression of CD31 and VWF when stimulated by TGF-β1, suggesting EndMT of HUVECs was induced ($P < 0.05$), (Fig. 2a, b). To investigate the effect of mesenchymal transition on the antithrombotic function of endothelial cells, we measured the expression of VE-cadherin, TM and eNOS by q-PCR, TM level in cell culture supernatant (STM) by ELISA, and NO production through nitric oxide assay kit- in HUVECs. Results showed that the m-RNA expression of TM, eNOS, and NO was reduced (Fig. 2c) and the concentration of NO and TM in cell culture supernatant decreased in HUVECs undergoing EndMT ($P < 0.05$) (Fig. 2d and Additional file1 Tables S3 and 4). The expression of VE-cadherin, an indicator of adhesion between ECs, decreased, suggesting that the tight junction between ECs had loosened in HUVEC undergoing EndMT ($P < 0.05$) (Fig. 2e).

3.3. Thrombogenesis aggravate EndMT

Endothelial cells undergo EndMT by shear stress and inflammation [5]. Although we have shown that EndMT promotes thrombus formation in IVCS, whether thrombus formation reciprocally enhances EndMT is unknown. As shown in Fig. 3a, immunofluorescence staining showed an increased α-SMA and reduced CD31 expression in stenosed iliac vein with thrombus formation, implying that EndMT occurred more obviously at stenosed vein with thrombus formation. Therefore, we asked whether thrombus formation aggravates EndMT. Since locally activated thrombin concentration increases in thrombogenesis, we used thrombin to stimulate HUVECs. As expected p-Smad3 increased in

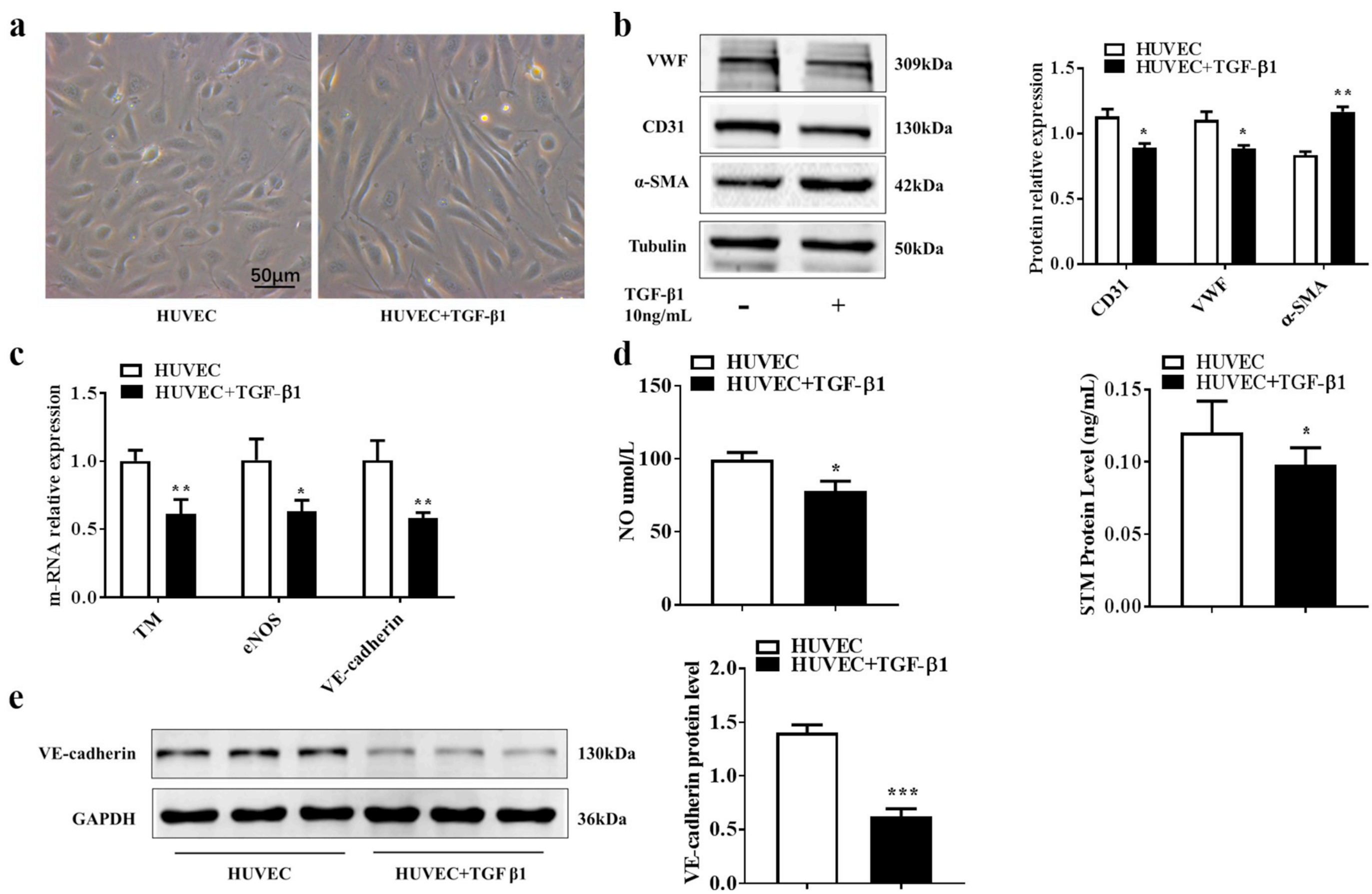

Fig. 2. EndMT attenuated the anti-thrombotic function of ECs. (a) Cellular morphology of HUVEC stimulated by TGF-β1 compared with normal HUVEC. Scale Bar = 50 μm. (b) The protein levels of VWF, CD31, and α-SMA in HUVEC stimulated by TGF-β1. The quantification of protein levels is shown in the right panel. Data are mean ± SEM. Results are representative of 3 independent experiments. $^*P < 0.05$; $^{**}P < 0.005$. (c) MRNA expression of TM, eNOS and VE-cadherin was analyzed by qPCR normalized to GAPDH. Fold changes are shown. Data are mean ± SEM. Results are representative of 3 independent experiments. $^*P < 0.05$; $^{**}P < 0.005$. (d) Concentration of NO (left) through nitrate reductase method and the STM antigen (right) by ELISA kit in cell culture supernatant. Data are mean ± SEM. (n = 10 per group). $^*P < 0.05$ vs control group. (e) Representative western analysis of the protein levels of VE-cadherin in HUVEC. The quantification of protein levels is shown in right panel. Data are mean ± SEM. $^{***}P < 0.001$.

HUVEC stimulated by thrombin, with an obvious transition at 10 U/ml up to 48 h, so we used this dose in the following experiments (Fig. 3b). Results showed that when added with thrombin, mRNA and protein level of CD31, VWF in HUVECs decreased, while the expression of α-SMA, FSP-1 increased. However, when treated with oxymatrine, these changes were not observed ($P < 0.05$), (Fig. 3c, d). These results suggest that activated thrombin triggered EndMT through TGF-β/Smad3 signaling.

3.4. Anticoagulation alleviated EndMT in IVCS

Slowing of blood flow after venous stenosis can activate the coagulation system [18]. To investigate the effects of anti-coagulation on EndMT, we performed the IVCS model using FVII$^{-/}$ and WT mice fed with rivaroxaban. Although expression of CD31, and VWF in stenosed left iliac vein was increased in both FVII$^{-/-}$ and WT mice that had been fed with rivaroxaban, expression of α-SMA, and FSP-1 were decreased, indicating that EndMT was alleviated when the coagulation system was inhibited (Fig. 4a). We collected clinical coagulation parameters of normal individuals, patients with simple saphenous vein varices and normal iliac vein confirmed by angiography and IVCS patients without thrombosis. Among those coagulation parameters, D-Dimer, an indicator of activation of coagulation and fibrinolytic systems, was significantly higher in the IVCS group than in the normal individual control group and simple saphenous vein varices patient group, indicating that the coagulation system was activated in IVCS even in the absence of thrombus formation($P < 0.001$), (Fig. 4b). Further analysis showed that D-Dimer was the independent factor of IVCS patients ($P < 0.05$), (Additional file1 Table S5). In view of our experimental results, we believe that anticoagulant therapy is beneficial to alleviate the interstitial changes of venous ECs in IVCS.

4. Discussion

In this study, we explored the role of EndMT, a form of EC transition, in thrombosis. We found that EndMT occurred when the iliac vein was compressed and inhibition of EndMT, through blocking TGF-β/Smad3 signaling, reduced the probability of thrombosis in IVCS models. Thrombin exacerbated EndMT via TGF-β/Smad3 signaling, while anticoagulation mitigated the interstitial change in stenosed venous ECs. The proposed mechanisms were illustrated in Fig. 5.

IVCS is a common cause of acute and chronic venous thromboembolism [18,23]. We created IVCS animal models by surgically restricting iliac vein luminal diameter in both pig and mouse to

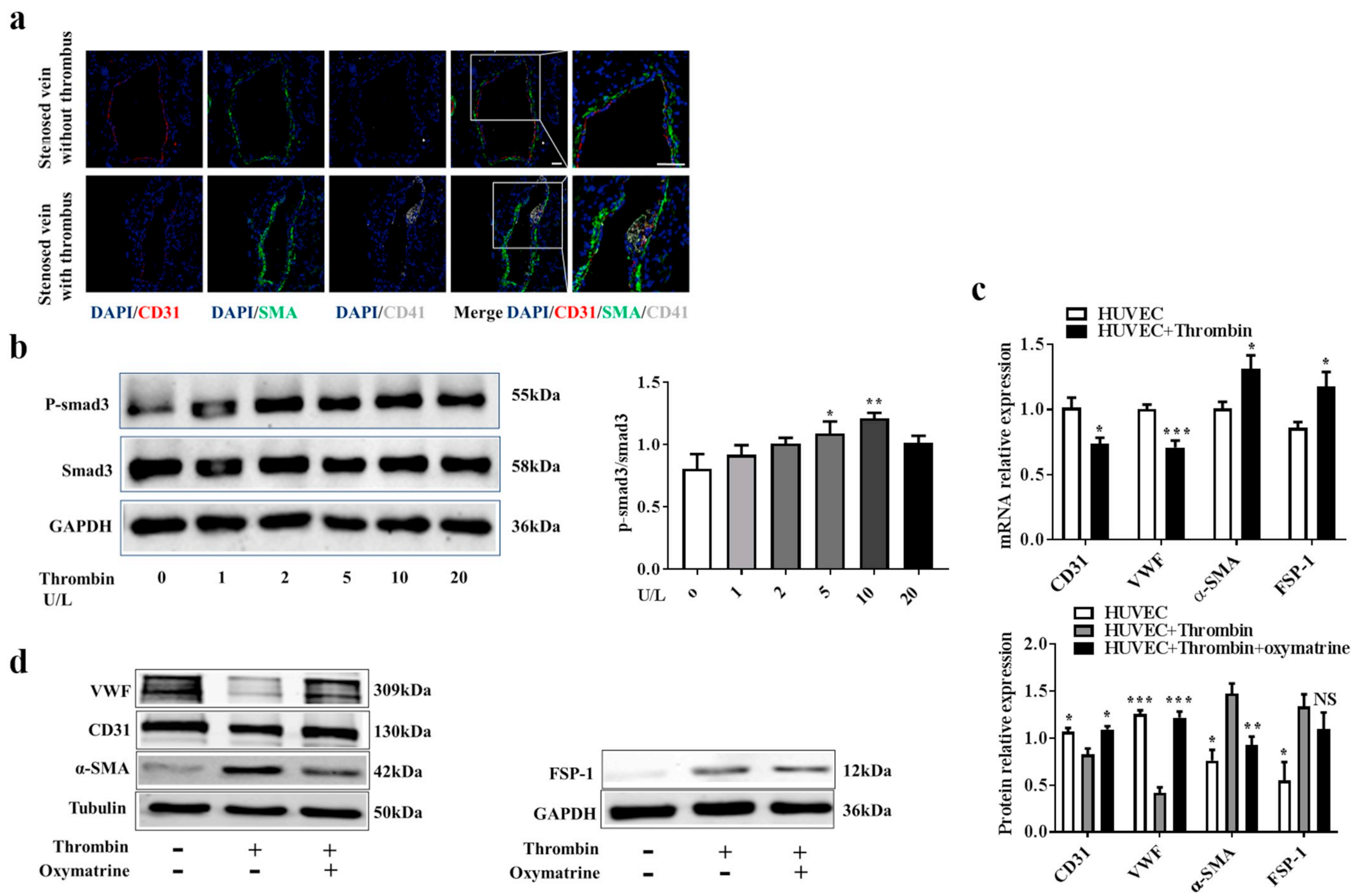

Fig. 3. Thrombogenesis promotes EndMT. (a) Representative immunofluorescence indicated a gain of more mesenchymal, α-SMA (green), and less endothelial markers, CD31 (red), in stenosed iliac vein with thrombus compared with those without thrombus. CD41 (white). Scale Bar = 50 μm. (b) Western analysis of HUVEC treated with thrombin at indicated doses for 48 h. Quantification of p-Smad3/smad3 is shown on right. Data are mean ± SEM. Results are representative of 3 independent experiments. *$P < 0.05$; **$P < 0.005$. (c) HUVEC treated with 10 U/ml thrombin mRNA was harvested for q-PCR. Quantification of mRNA levels of CD31, VWF, α-SMA or FSP-1 was normalized to GAPDH. Fold changes are Shown. Data are mean ± SEM. Results are representative of 3 independent experiments. *$P < 0.05$; ***$P < 0.001$. (d) Representative western blotting of the protein levels of CD31, VWF, α-SMA, and FSP-1 in HUVECs after treatment with thrombin or thrombin with oxymatrine co-incubation. The quantification of protein levels is shown in the right panel. Data are mean ± SEM. Results are representative of 3 independent experiments. *$P < 0.05$; **$P < 0.005$, ***$P < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

understand better the pathologic change of stenosis vein in IVCS. Angiography proved that iliac vein stenosis was successfully achieved. In most cases, direct vascular constriction was used to establish stenosis models [24,25]. Since the iliac vein is compressed by the iliac artery in IVCS, the continuous pulsation of the artery is an important factor in the stimulation of venous intima hyperplasia. Therefore, we bound the artery and vein together to establish the IVCS model. At the same time, we adopted the extraperitoneal surgical approach to reduce inflammatory reaction and trauma. Through IVCS models, we found that iliac vein compression induces EndMT, as some ECs lost endothelial special characters and acquired mesenchymal phenotype in stenosed veins. Further, ELISA was used to detect the concentration of TGF-β1 in serum from IVCS patients and normal individuals control, and results show increasing expression of TGF-β1 in IVCS patients, which suggested that there exists EndMT in IVCS patients. Venous compression results in reduced velocity of blood and activation of coagulation system leading to chronic inflammation and EC dysfunction [5]. ECs sense changes of hemodynamic and plasma factors through receptors on cell surface, and transmit signal through Smad family into the nucleus, which activates nuclear transcription factors, such as slug, snail, twist, zeb1, and zeb2 to initiate EndMT [26,27]. Previous studies on EndMT focused on the arterial intima, such as cerebral hemangioma, atherosclerosis [12,28–31]. We detected this phenomenon on stenosed venous ECs, providing a new field for the research of EndMT.

ECs regulate intimal barrier function, which controls the efflux of plasma proteins and infiltration of blood cells into the subendothelium. The destruction of endothelium integrity causes exposure of the subendothelial matrix leading to activation of the endogenous coagulation system and platelets [32,33]. In addition, ECs can secrete anticoagulant and thrombolytic factors to inhibit thrombosis during thrombosis, and keep vascular patency [32]. We stimulated HUVEC with TGF-β1 to induce EndMT and found that tight junction between ECs had loosened, and secretion of vasodilators and thrombolytic factors, such as NO and TM, decreased. All of these results indicated that the antithrombotic function of ECs undergoing EndMT is weakened and venous with EndMT are prone to thrombosis. Furthermore, Using IVCS models, we confirmed that inhibition of EndMT reduced the probability of thrombosis. IVCS is the most important factor affecting deep venous thrombosis of the lower extremity. Venous intimal hyperplasia and abnormal adhesion structure are the main pathological changes [33].

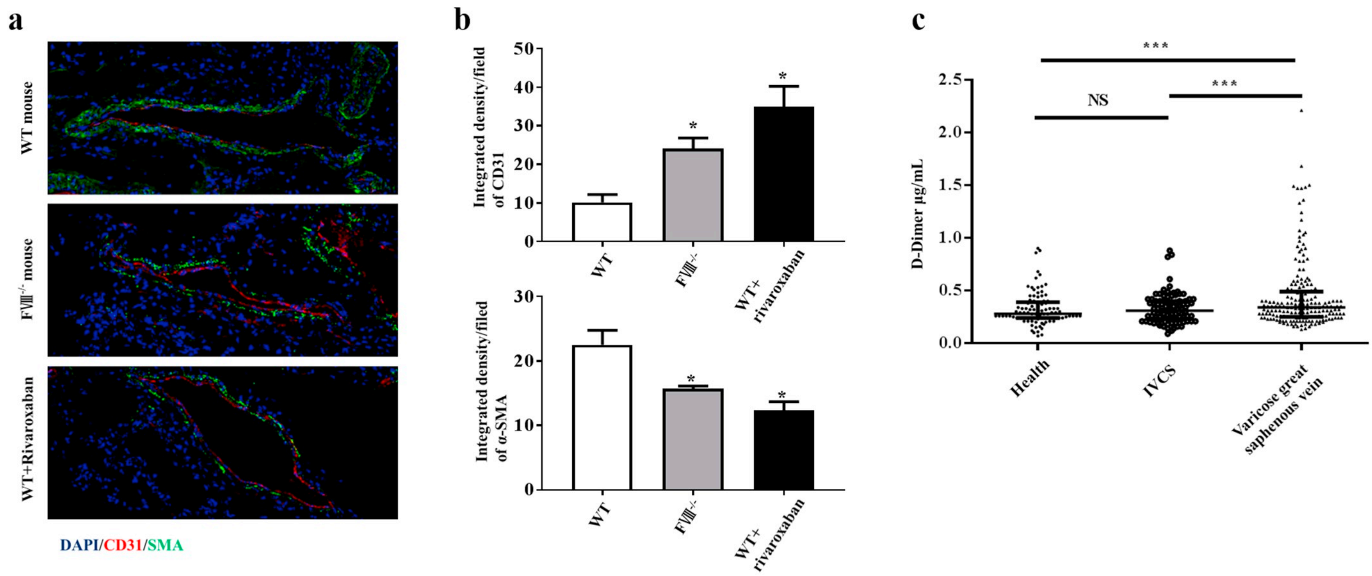

Fig. 4. Anticoagulation alleviates EndMT in IVCS. (a) Characterization of the endothelial phenotype by immunofluorescence in stenosed iliac venous from control WT mice with $FVII^{-/-}$ mice or WT mice fed with rivaroxaban. CD31 (red), α-SMA (green), and DAPI (blue). Quantification of fluorescence intensity is shown in the right panel. Scale Bar = 50 μm. Data are mean ± SEM. $*P < 0.05$. (b) The concentration of d-dimer in normal control, simple saphenous vein varices and IVCS without thrombus. Data are median with interquartile range. $***P < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Anticoagulation, catheter thrombolysis and, endovascular therapy with stenting remain the main treatment strategy for IVCS with thrombosis. However, each of them has its complications, such as bleeding, stent thrombosis, etc. [34,35]. Reducing the probability of thrombosis by inhibiting EndMT provides a new idea for IVCS treatment.

During thrombosis, activated thrombin, platelets and various factors accumulate on the surface of ECs, which alters the function of ECs while forming thrombus [36]. The local concentration of activated thrombin was significantly increased during thrombosis, and thrombin could regulate endothelial barrier permeability [37]. Thus, we used thrombin to act on HUVEC and found that thrombin can promote EndMT through the TGF-β/Smad3 pathway, indicating that EndMT were aggravated by thrombin during thrombosis. These results showed that EndMT and thrombosis have a close interaction. Under pathological conditions, such as disturb flow or hypoxia, interstitial changes occurred in ECs, and ECs function was weaken, leading to thrombosis. Locally activated thrombin after thrombus aggravated EndMT, and thrombolytic function of ECs was lost. Thereby, the thrombus expanded. EndMT and thrombosis synergistically promote limb swelling, pain and venous valves dysfunction.

IVCS is often combined with venous thrombosis, however, long-time anti-coagulation therapy is controversial in IVCS patients especially to those without thrombus. Clinical data suggest a potential activation of the coagulation system in IVCS patients. In addition, using mouse models of IVCS, we found that anticoagulation therapy through FVII knockout or taking rivaroxaban can alleviate EndMT. These results provided a theoretical basis for long-time anticoagulant therapy in IVCS patients.

This study does not discuss who is in a dominant position in the reciprocal enhancement of EndMT and thrombosis. Future studies are warranted to address the sequence of EndMT and thrombosis at the startup and to provide more mechanistic insights on how thrombosis and EndMT regulate each other.

5. Conclusions

In summary, the data presented in this work suggest a vascular endothelium changes-EndMT in IVCS. Due to EndMT, the tight junction between endothelial cells becomes incomplete, and the dysfunction of the EC leads to a decrease in the secretion of anticoagulant and thrombolytic factors. We confirmed that inhibition of EndMT can reduce the probability of thrombosis. Reciprocal enhancement of thrombosis by EndMT, suggest that inhibition EndMT could be a new strategy in the prevention and treatments for thrombosis in IVCS.

Abbreviations

EndMT	endothelial-to-mesenchymal transition
IVCS	iliac vein compression syndrome
HUVEC	human umbilical vein endothelial cells
TGF-β1	transforming growth factor β1
ECs	endothelial cells
WT	wild type
$FVII^{-/}$	factor VII knockout
CD31	platelet and endothelial cell adhesion molecule 1
α-SMA	α-smooth muscle actin
VWF	von Willebrand factor
FSP-1	fibroblast specific protein-1
TM	thrombomodulin
STM	TM in culture supernatant
Fig	figure

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Authors' contributions

XL and HL designed the study. XD and TY supervised the study. LH conducted the experiments. WW analyzed the data, interpreted the data and composed the manuscript. LS, WL, LX collect source data.

All authors read and approved the final manuscript.

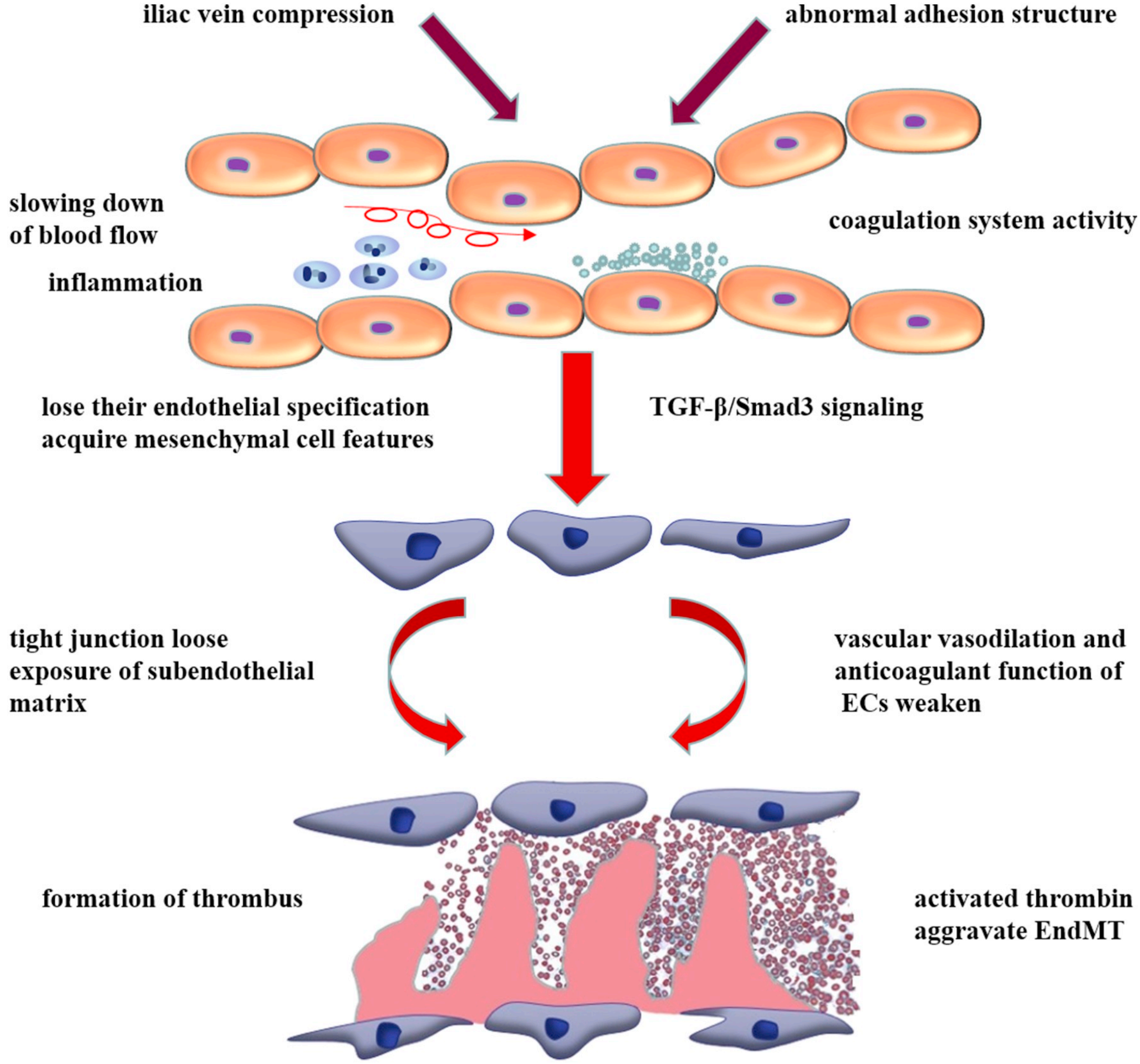

Fig. 5. Schematic mechanisms of the relationship between EndMT and thrombosis in IVCS. Orange cells represent normal endothelial cells. Light blue cells represent inflammatory cells. Dark blue cells represent endothelial cells underwent EndMT. Blue granules represent coagulation factors. Red granules represent stasis erythrocytes. The red ridge represents the platelet girder. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

All authors agree to the publication of this manuscript.

Ethics approval

This program was approved by the Ethics Committee of Soochow University (ECSU-201800069) and obtains informed consent of the people for using they clinic data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.116659.

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