MiRNAs and Their Role in Venous Thromboembolic Complications

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Venous thromboembolic complications (VTCs), which include deep vein thrombosis (DVT) and pulmonary embolism (PE), have remained a pressing problem in modern clinical medicine for a long time. Despite the already wide arsenal of modern methods for diagnosing and treating this disease, VTCs rank third in the structure of causes of death among all cardiovascular diseases, behind myocardial infarction (MI) and ischemic stroke (IS). Numerous studies have confirmed the importance of understanding the molecular processes of VTCs for effective therapy and diagnosis. Significant progress has been made in VTC research, where the relative contribution of microRNAs (miRNAs) in the mechanism of thrombus formation and their consideration as therapeutic targets have been well studied.

miRNA venous thromboembolic complications cardiovascular disease hemostasis therapeutic targets biomarkers

1. Introduction

Venous thromboembolic complications (VTCs), which include deep vein thrombosis (DVT) and pulmonary embolism (PE), remain the most important problem in clinical medicine and affect the professional sphere of doctors of all specialties without exception. The importance of VTCs is due to their extremely high potential risk to the health and life of the patient. Improving the quality of prevention, diagnosis, prognosis, and treatment of VTCs can save the lives of thousands of people and ensure a noticeable reduction in financial pressure on the healthcare budget, thanks to the prevention of severe disabling diseases [1][2][3].

Currently, there are effective surgical and drug treatments for VTCs that can reduce disability in the population. However, the early diagnosis and prognosis of the disease is a key factor in the success of the treatment and is directly related to understanding the pathogenesis of VTCs, which leads to the possibility for forming risk groups for PE among patients with DVT [4]. Currently, the mechanisms of the incomplete resolution of venous thrombi in patients with DVP and patients who have suffered PE are completely unknown. It has been shown that the process of the fibrotic transformation of thrombotic masses is inextricably linked with the activities of inflammation and angiogenesis, as well as changes in the hemostasis system, in the regulation of which microRNAs (miRNAs) may take part [5][6]. It has been proven that miRNAs play a significant role in various biological processes, including the cell cycle, apoptosis, proliferation, and differentiation, regulating the expression of about 90% of all human genes.

In addition, their direct role in the pathogenesis of many human diseases, including the imbalance of the hemostatic system, such as thrombosis, has already been proven (**Table 1**) [7][8][9][10][11][12][13][14][15][16].

Table 1. The role of microRNAs (miRNAs) in thrombosis.

Thrombosis/Conditions Associated with Thrombosis	miRNA	Regulation	Study	Targets	Effect	References
AAF	miR-200b-3p	Up	In vitro	VEGF and Ang-II	Increases intimal hyperplasia to induce autogenous arteriovenous fistula thrombosis	[7]
Thrombosis	miR-1915-3p	Up	In vitro	RHOB	Enhances megakaryocyte (megakaryopoiesis) differentiation and platelet generation	[8]
COVID-19	miR-145 and miR-885	Down	Ex vivo (human serum) and in vitro	D-dimer	Increase endothelial cell apoptosis and display significantly impaired angiogenetic properties	[9]
Inflammation and arterial thrombosis	miR-146b-3p	Up	In vitro	P38MAPK/COX-2 pathway	TNF-α activates miR-146b-3p and then induces thrombosis	[10]
COVID-19	miR-16-5p	Down	In silico and bioinformatic analysis	EGFR, HSP90AA1, APP, TP53, PTEN, UBC, FN1, ELAVL1, and CALM1	Thrombosis-related predictive biomarker	[11]

Thrombosis/Conditions Associated with Thrombosis	miRNA	Regulation	Study	Targets	Effect	References
Multiple myeloma	miR-532	Down	Ex vivo (human serum) and in vitro	TF, EPCR, ERK 1/2, MAPK, and NF-κB	Increases multiple myeloma-induced endothelial cell thrombosis	[12]
Photochemical-induced carotid thrombosis	miR-223	Down	In vivo	IGF-1R	Attenuates thrombosis	[13]
Primary antiphospholipid syndrome	miR-483-3p miR-326	Up Down	In vitro	PI3K/AKT and NOTCH	Contribute to primary antiphospholipid syndrome pathogenesis by producing endothelial cell proliferation, monocyte activation, and adhesion/procoagulant factors	[14]
Atherosclerotic plaque rupture with thrombosis	miR-466h-5p	Up	In vivo	Bcl2	Promotes atherosclerotic plaque rupture and thrombosis	[15]
Catheter-related thrombosis	miR-92a-3p	Down	In vivo	MAPK/NF-κB	Inhibits oxidative stress injury and prevents catheter-related thrombosis formation	[16]

Vascular
mitogen-
A1, Heat
ase and
tensin homolog; UBC, Ubiquitin C; FN1, Fibronectin 1; ELAVL1, ELAV like protein 1; CALM1, Calmodulin 1; TF, Tissue factor; EPCR, Endothelial protein C receptor; ERK 1/2, Extracellular signal-regulated kinase; MAPK, p38 mitogen activated protein kinase; NF-κB, Nuclear factor-κB; TNF-α, Tumor necrosis factor alpha; IGF-1R, insulin-like growth factor 1; PI3K, Phosphoinositide 3-kinases; NOTCH, Neurogenic locus notch homolog protein 1; Bcl2, B-cell lymphoma 2.

The relevance of the problem of PE is due to the difficulties of timely diagnosis due to the polymorphism of clinical syndromes. To detect PE, various research methods are used—both laboratory and instrumental. The degree of their information content varies [17]. Among the most important, from the point of view of PE verification, are methods, such as the determination of the D-dimer content, electrocardiography, ultrasound (echocardiography), and radiation methods (chest radiography, lung ventilation perfusion scan (VQ Scan), computed tomography (CT) of the chest, and angiopulmonography) [18]. The determination of the D-dimer content, ultrasonography, and venography are most often used in the diagnosis of DVT, after surgery [19]. Ultrasonography has certain limitations. In contrast, venography is invasive and increases the risk of complications, including blood clot rupture and allergic reactions, which also limit its use [19]. One solution to this problem is to find accurate, effective, and non-invasive methods for diagnosing VTCs. And in this regard, circulating miRNAs are already being studied as potential biomarkers in VTCs. Circulating miRNAs can be secreted from cells into human biological fluids as a part of extracellular vesicles (EVs) (microvesicles and exosomes) or can be a part of RNA-protein complexes, such as miRNA/Argonaute-2 (Ago2) and miRNA/nucleophosmin 1 (NPM1) [6][20]. Such miRNAs are resistant to nucleases, are specific to certain pathological conditions, and are easy to detect using modern laboratory methods, which makes them potential biomarkers for patients with VTCs [6]. The discovery of aberrant miRNA expression in VTCs suggests the possibility of creating new diagnostic and prognostic tools. The analysis of miRNA target genes could lead to new horizons in understanding the pathogenesis of VTCs and, thereby, new treatments (**Figure 1**).

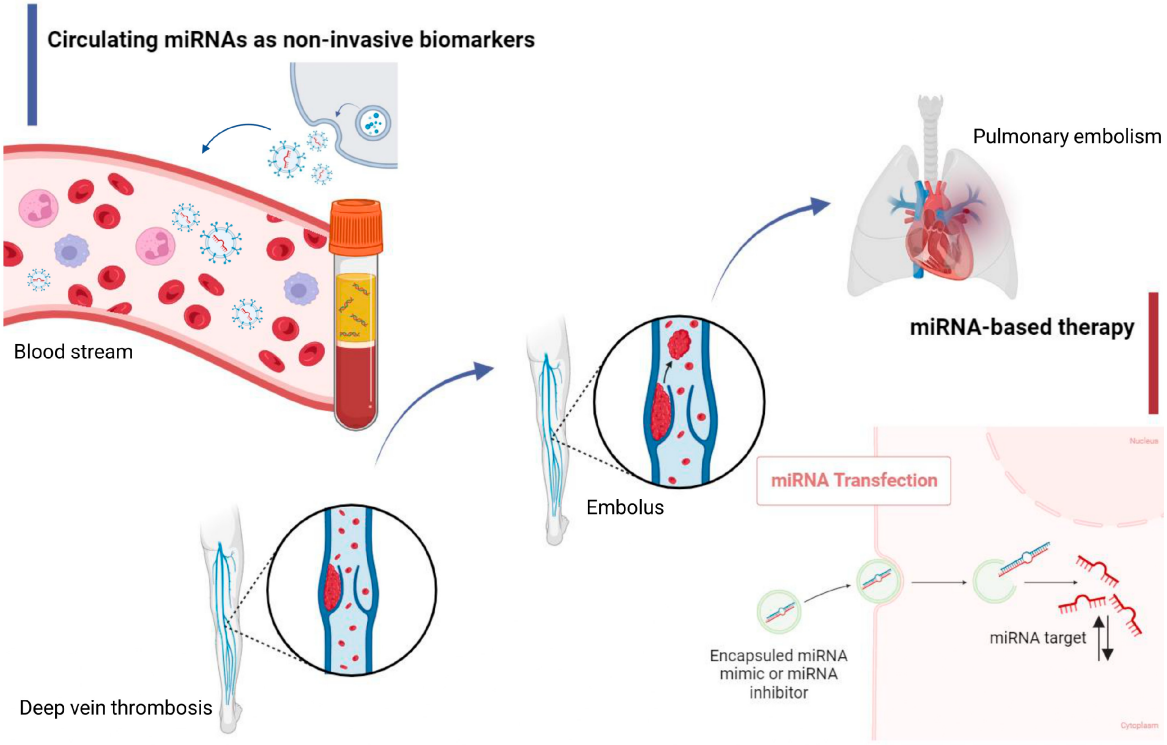

Figure 1. Schematic illustration of the capabilities of microRNAs (miRNAs) in venous thromboembolic complications (VTCs). There are two directions for studying miRNAs in deep vein thrombosis (DVT) and pulmonary embolism (PE): (1) the transfection of a miRNA mimic or inhibitor to change the expression of a target miRNA with the possibility of therapeutic interventions and (2) the consideration of circulating miRNAs as non-invasive biomarkers for the purpose of diagnosing DVT and the possibility of prognosticating PE.

2. MiRNAs and Hemostasis

According to the literature, miRNAs control the expression of several key factors of hemostasis (platelet biogenesis and function, coagulation factors, anticoagulant mechanisms, and fibrinolysis), indicating that the dysregulation of these miRNAs can lead to hemostatic imbalance and, accordingly, increased thrombosis or bleeding (**Figure 2**, **Figure 3** and **Figure 4**) [21][22][23][24][25][26].

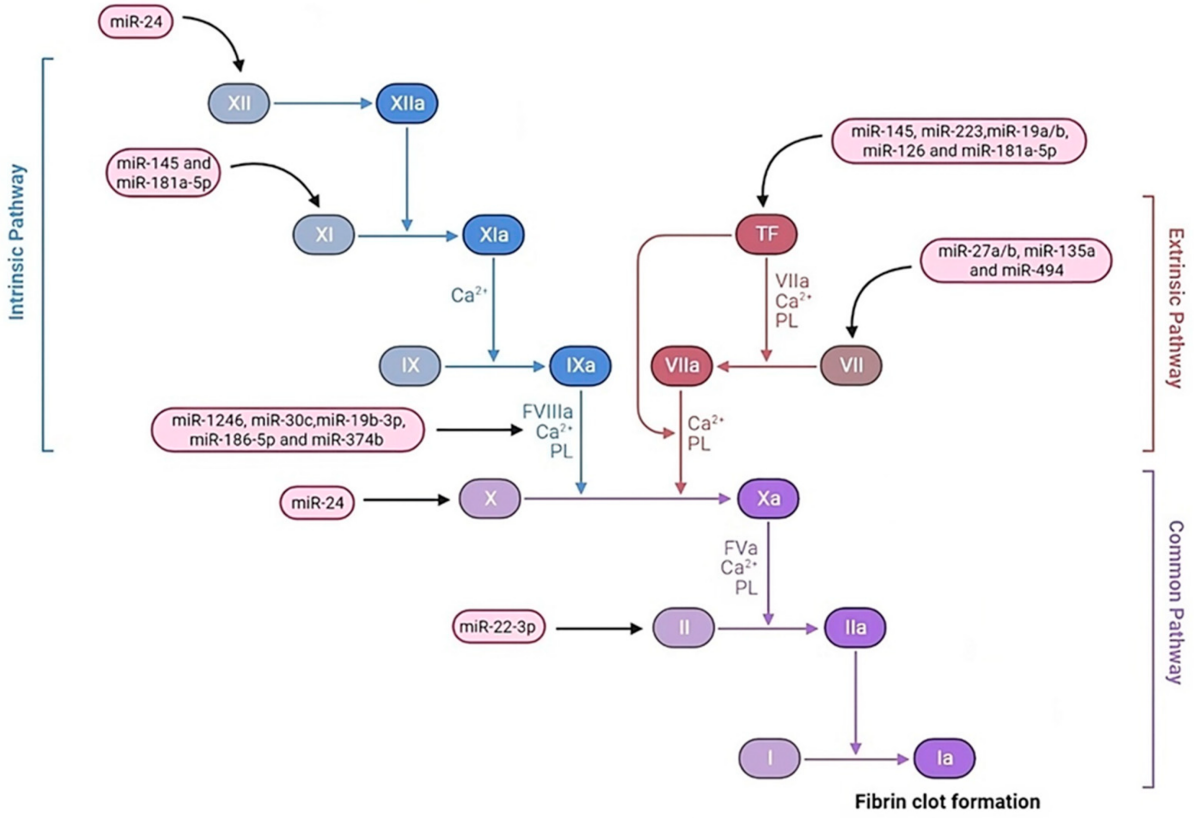

Figure 2. Schematic illustration of microRNA (miRNA) regulation of blood coagulation and fibrinolysis pathways. Several miRNAs are presented that are reported to regulate key factors of hemostasis. Note: TF, Tissue factor; PL, Platelets.

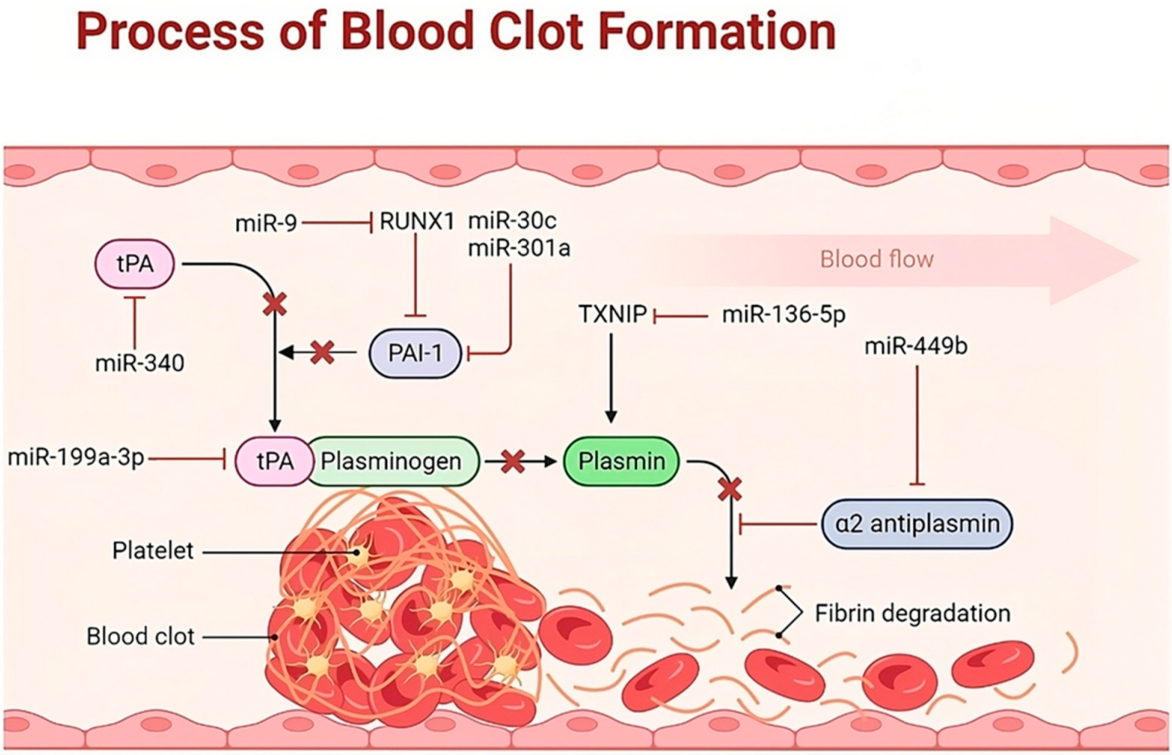

Figure 3. The illustration of a panel of regulatory miRNAs potentially involved in the process of blood clot formation. The normal coagulation pathway is a balance between the procoagulant pathway responsible for thrombus formation and the mechanisms that inhibit it beyond the site of injury. This delicate balance can be disrupted whenever the procoagulant activity of coagulation factors increases or the activity of natural inhibitors decreases under the regulatory action of microRNAs (miRNAs). Note: tPA, Tissue plasminogen activator; PAI-1, Plasminogen activator inhibitor-1; RUNX1, Runt-related transcription factor 1; TXNIP, Thioredoxin interacting protein.

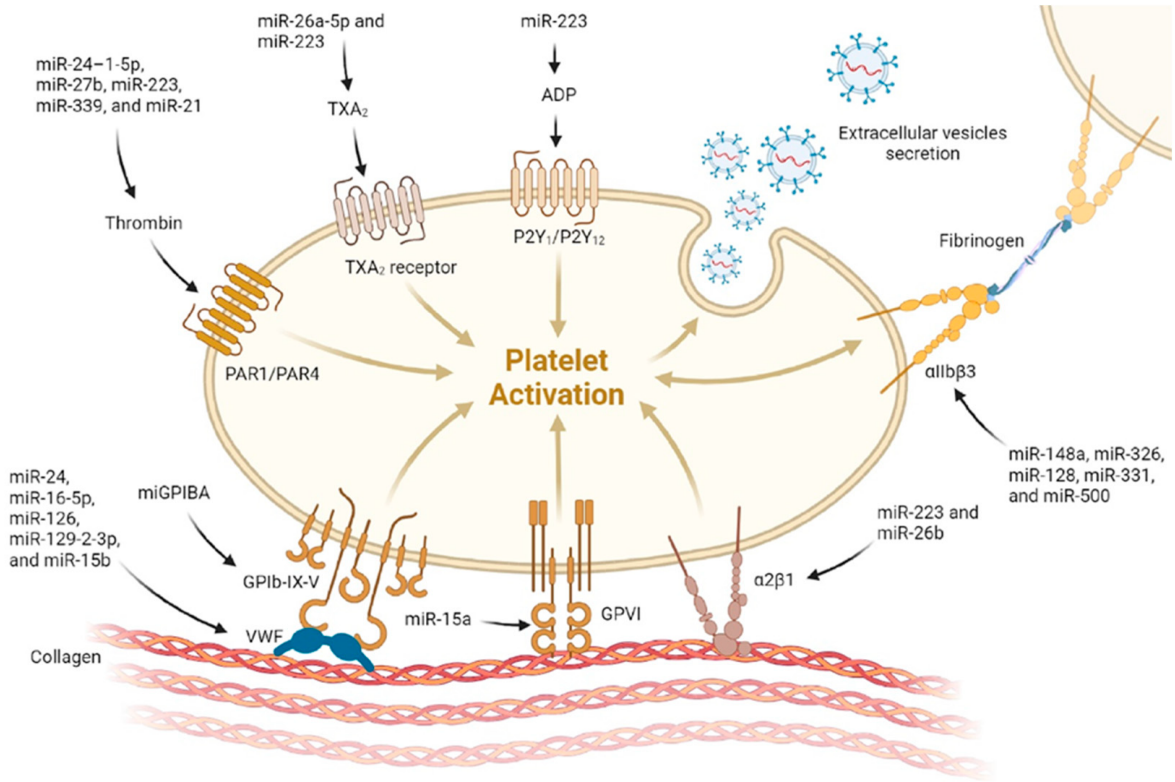

Figure 4. MicroRNA (miRNA) regulation of platelet activity. Abundant evidence demonstrates a critical role for miRNAs in platelet biology and platelet production and activation. Moreover, from the literature, activated platelets release extracellular vesicles (EVs) (exosomes and microvesicles), which contain a wide range of proteins and nucleic acids, including miRNAs, such as miR-223, miR-25-3p, miR-339, miR-21, and miR-328.

Tissue factor (TF) is a critical factor in the initiation of coagulation, and its overexpression has been extensively studied in patients with cancer complicated by coagulopathy [27]. While the regulatory role of miRNAs was actively being studied for TF, some miRNAs were found to directly inhibit TF expression and were active in various tumor cell lines. For instance, in breast cancer (BC) cell lines, miR-19 directly inhibits TF expression, and miR-19a inhibits TF expression in colon cancer (CC) cells [28][29]. In addition, Li et al. found that the activation of miR-223 resulted in decreased TF expression and the inhibition of tumor necrosis factor-α (TNF-α) expression in the aorta tissue of C57BL/6J mice and cultured endothelial cells (ECs) (EA.hy926 cells and human umbilical vein endothelial cells (HUVECs)) [30]. This study demonstrates the miR-223-mediated suppression of TF expression, suggesting a novel molecular mechanism for controlling the coagulation cascade and suggesting a clue against thrombogenesis during the process of atherosclerotic plaque rupture. The relationships between miR-93, miR-106, and TF were observed in leiomyoma cells, where the suppression of miR-93 and miR-106 expression levels and the associated increase in TF expression promoted inflammatory and metabolic processes in tumor cells [31]. In human microvascular ECs, miR-19a and miR-126 have been shown to modulate endothelial thrombogenicity through the direct inhibition of TF expression and activity. In addition, changes in the expression levels of miR-19a and miR-126 were induced by the inflammatory stimulus, TNF-α.

Teruel et al. demonstrated significantly lower expression levels of miR-19b and miR-20a in monocytes from patients with antiphospholipid syndrome (APS) and systemic lupus erythematosus (SLE) compared with controls, and miR-19b and miR-20a expressions were inversely correlated with TF expression in the corresponding monocytes [32]. Thus, these results showed the role of miR-19b and miR-20a in hypercoagulability in conditions observed in patients with APS and SLE. Also, miRNA has been identified as being associated with plasminogen activator inhibitor-1 (PAI-1), a key modulator of the fibrinolytic pathway. Recently, increased PAI-1 expression and thrombus formation have been associated with decreased miR-30c expression in patients with type 2 diabetes mellitus (DM) [33]. Fibrin formation may be under the control of miR-409-3p, which was identified in HuH-7 cells through miRNA library screening and decreased steady-state levels of all the fibrinogen genes (fibrinogen alpha (FGA), fibrinogen beta (FGB), and fibrinogen gamma (FGG)) [34]. It was found that when miR-409-3p is activated, fibrinogen formation in HuH-7 cells decreases. These data suggest that thrombus growth and persistence may be subject to miRNA-mediated regulation of fibrinolysis through PAI-1 and fibrinogen synthesis.

3. MiRNA and DVT

Currently, the molecular mechanisms of DVT pathogenesis are not fully understood, which significantly limits the creation of new therapeutic, diagnostic, and prognostic tools. It is known that the processes of thrombus lysis and the restoration of the venous wall are associated with proinflammatory cytokines, chemokines, and leukocytes. Additionally, the definition of Virchow's triad considers three major factors that contribute to the formation of blood clots, including changes in blood flow, endothelial damage, and a hypercoagulable state. However, the molecular mechanism of DVT is not fully understood. Recent studies have suggested that miRNAs are involved in the formation and development of DVT. In their study, Zhang et al. observed increased interleukin 6 (IL-6) expression in the peripheral blood mononuclear cells (PBMCs) of patients with DVT and investigated the expression profile of miR-338-5p in patients with DVT, in the HUVEC cell line, and in DVT animal models, using microarrays [35]. The authors provided evidence that miR-338-5p plays an important role as a suppressor of IL-6 by showing that decreased miR-338-5p expression increased IL-6 expression and promoted DVT formation. Tang et al. designed a study for the purpose of verifying the effects of miR-495 targeting interleukin 1 receptor type 1 (IL1R1) on lower extremity DVT through the toll-like receptor 4 (TLR4) signaling pathway in vitro and in vivo. As revealed throughout their study, the overexpression of miR-495 promoted the proliferation and inhibition of the apoptosis of femoral vein ECs through the direct regulation of the 3′ untranslated region (3′-UTR) of messenger RNA (mRNA) IL1R1 expression via the TLR4-signaling pathway [36].

Knowledge accumulated in recent years suggests that DVT is closely associated with the fibrotic remodeling of vein walls, which consists of a switch in the vascular smooth muscle cell (VSMC) phenotype from contractile to synthetic, with VSMC proliferation, collagen deposition, and damage to the extracellular matrix (ECM) by matrix metalloproteinases (MMPs). More recently, the involvement of MMPs together with the tissue inhibitors of metalloproteinases (TIMPs) in the mechanism of DVT has been recognized as significant and is actively being discussed [37][38]. Francis et al. reported that high plasma levels of MMPs, including MMP-1, -2, -3, -7, -8, and -9, are observed in patients with acute DVT [39]. Ai et al. observed a decrease in the expression of miR-411, while the

expressions of hypoxia-inducible factor-1α (HIF-1α), Collagen I, as well as MMP-2 were increased in the vein walls and corresponding VSMCs obtained from rats with DVT [40]. HIF-1α is known to play important roles in cell proliferation, migration, and differentiation; vasoconstriction or vasodilation; ECM degradation; and angiogenesis [41][42]. In addition, HIF-1α regulates many genes, and mediates vein wall fibrosis remodeling, implying HIF-1α might be a potential target for the treatment of vein wall fibrosis in DVT [43]. Eventually, the increased expression levels of miR-411 inhibited HIF-1α expression, leading to the downregulation of MMP-2 in VSMCs and, consequently, alleviated vein wall fibrosis in vitro.

Endothelial nitric oxide (NO) regulates several physiological processes, such as platelet adhesion and aggregation, which may influence susceptibility or resistance to thrombosis [44]. There is evidence that the expression level of endothelial nitric oxide synthase (NOS3), which plays an important role in NO synthesis, was significantly reduced in MVEC cell lines by the overexpression of miR-195 and miR-582 [45]. The inhibition of the expression of miR-195 and miR-582 in ECs could promote an increase in NOS3 expression, indicating that NOS3 mRNA was a target for miR-195 and miR-582. In other words, the aberrant expression of miR-195 and miR-582 can cause endothelial dysfunction by regulating vascular homeostasis and can ultimately lead to thrombus formation in both arteries and veins.

4. MiRNA and PE

PE is a critical and potentially life-threatening medical condition characterized by the formation of blood clots, typically in the deep veins of the legs, which can dislodge and travel to the pulmonary arteries, causing obstruction and compromising cardiopulmonary function. Pulmonary hypertension, often resulting from recurrent PE, can lead to progressive respiratory dysfunction and long-term disability. Considering these serious consequences, there is a pressing need for improved innovative therapeutic strategies for PE [46]. Recent advancements in medical research have uncovered a burgeoning interest in the role of miRNAs in the context of PE pathogenesis. Zhu et al. identified miRNA profiles in both in vitro and in vivo models of acute PE [47]. The analysis identified specific miRNAs affected by hypoxia/reoxygenation (H/R) or acute PE induction. Notably, some miRNAs were upregulated (miR-34a-5p, miR-324-5p, and miR-331-3p), while others were downregulated (miR-429, miR-491-5p, and miR-449a). Treatment with urokinase-type plasminogen activator (uPA) effectively restored these miRNA levels to those observed in healthy controls, both in laboratory cell models and in live mice. Additionally, the study involved predicting the target genes of these miRNAs and exploring their potential functions. The identified targets were linked to critical biological processes, including cell growth, proliferation, and inflammation. To further validate their findings, the researchers conducted experiments in which they artificially increased the levels of miR-449a, using a mimic. This manipulation completely reversed the protective effect of uPA in the in vitro H/R model. In acute PE, miR-34a-3p was identified as one of the pivotal miRNAs that directly regulate the expression of dual-specificity phosphatase-1 (DUSP1) [48]. Upregulated miR-34a-3p was found to effectively inhibit the proliferation of pulmonary artery smooth muscle cells (PASMCs), a hallmark of acute PE progression. This suggests that miR-34a-3p may offer potential therapeutic avenues for managing acute PE by targeting pulmonary vascular proliferation.

Chronic thromboembolic pulmonary hypertension (CTEPH) is a rare form of pathology that develops because of the chronic obstruction of large/medium branches of the pulmonary arteries and secondary changes in the microvasculature of the lungs. CTEPH is a late complication of acute PE, with an incidence of 0.1–9.1% during the first two years after the episode [49]. The complex pathogenesis of CTEPH remains not fully understood to date. In an experimental study on a line of PASMCs obtained from the surgical specimens of patients with CTEPH, Wang et al. demonstrated the inhibitory effect of let-7d on cell proliferation by increasing the expression level of the p21 cell-cycle inhibitor mRNA [50]. In other words, the authors concluded that the relationship between the reduced let-7d expression and excessive proliferation of PASMCs in material from patients with CTEPH plays a role in the pathogenesis of the disease. Chen et al., in a study on 190 patients with CTEPH and a control group of patients without CTEPH, confirmed the previously proven association of the development of the disease with the polymorphism of the fibrinogen alpha gene (FGA) and demonstrated the direct role of miR-759 in the regulation of FGA expression through interactions with the polymorphic site (deletion/insertion (Del/Ins)) polymorphism of 28 base pairs (bp) in the 3′-UTRs of mRNAs [51].

Although the comprehension of the role of miRNAs in PE is still in its nascent stages, these findings underscore the potential of miRNAs to be instrumental in unraveling the intricate mechanisms of PE and potentially devising novel treatment strategies. However, it is crucial to emphasize that further research, particularly in larger cohorts and comprehensive confirmatory experiments, is imperative before clinical applications can be fully realized.

5. Circulating miRNAs vs. Traditional Biomarkers

Numerous studies have attempted to find accurate and early diagnostic laboratory biomarkers for PE and DVT. However, to date, plasma D-dimer is the only well-established and clinically applicable biomarker for the identification of PE and DVT. The D-dimer test reflects the level of fibrin degradation. A negative D-dimer result is relative evidence to exclude PE and DVT, but a positive result has low specificity and low diagnostic and prognostic value for VTCs [52]. D-dimer concentrations may also be elevated in non-thrombotic disorders, including disseminated intravascular coagulation (DIC), infection, and stroke [53][54]. In recent years, one of the most promising new biomarkers is soluble P-selectin, which has higher specificity than D-dimer in the non-invasive diagnosis of PE and DVT [55]. However, D-dimer and P-selectin alone or in combination do not have high sensitivity or specificity in diagnosing thrombus formation.

Circulating miRNAs, as biomarkers of PE and DVT, have been less studied, although circulating miRNAs may be more sensitive and accurate in the diagnosis and prognosis of VTCs. Recently, several studies have been carried out showing that circulating microRNAs are preferable as biomarkers because they are highly stable in human biological fluids, such as blood, and can be detected in the early stages of disease development, while protein structures, such as D-dimer, are found in blood only when damage has already occurred [56][57]. In addition, miRNAs play a role in almost all cellular functions, including hemostatic balance [21][22][23][24][25][26].

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