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Platelet-enriched microRNAs as novel biomarkers in atherosclerotic and cardiovascular disease patients

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Review Article

Abstract

BACKGROUND: Cardiovascular disease (CVD) is a global health challenge. Various studies have shown that genetic and environmental factors play roles in the development and progression of CVD. Small non-coding RNAs, namely microRNAs (miRs), regulate gene expression and have key roles in essential cellular processes such as apoptosis, cell cycle, differentiation, and proliferation. Currently, clinical studies highlight the critical role of platelets and miRs in coronary thrombosis, atherosclerosis, and CVD.

METHODS: Using search engines such as PubMed and Scopus, articles studying platelet miRs and their effects on atherosclerosis and cardiovascular disease were reviewed.

RESULTS: This article presents a comprehensive analysis of the association of platelet-related miRs as prognostic, diagnostic, and therapeutic biomarkers with the pathogenesis of atherosclerosis and cardiovascular disease.

CONCLUSION: Taken together, data show that platelet-related miRs not only play important roles in the initial development of atherosclerosis and cardiovascular disease (CVD), but they are also considered prognostic and diagnostic biomarkers in CVD.

Keywords: Cardiovascular Diseases; Atherosclerosis; Platelet Function; Micrornas; Biomarkers

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Introduction

Cardiovascular diseases (CVDs) are the main cause of death worldwide[1]. CVDs represent a wide range of clinical manifestations, influenced by multiple factors such as genetic variability, and environmental and demographic factors, leading to the initiation, development, and progression of these disorders[2]. Currently, expanding clinical studies are focused on platelets, indicating their recognized role in the pathogenesis of coronary thrombosis and atherosclerosis due to their capacity to release regulatory molecules that affect various pathways. These pathways are key agents associated with the development of myocardial ischemia (MI) and, in turn, CVD[3]. Indeed, transcriptome machinery involving non-coding RNAs (miRs, lncRNA, mRNA) is necessary for the regulation of gene expression in platelets[4]. MicroRNAs are small non-coding RNAs consisting of 18-25 nucleotides, playing an essential role in cellular functions such as proliferation, cell cycle, apoptosis, differentiation, and hematopoiesis. Some studies have reported that miRs control about two-thirds of human genes through the degradation of messenger RNAs (mRNAs) or inhibition of

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translation[5]. In addition to their stable entity due to resistance to endogenous ribonuclease activity, miRs also display profound advantages for intercellular communication over short and long distances. For these reasons, microRNAs are currently studied as diagnostic and prognostic biomarkers and even for therapeutic applications[6]. Several research studies have shown that dysregulated platelet-derived miR expression contributes to cancer, cerebrovascular diseases, hypertension, Alzheimer's disease, and especially CVD[7]. Thus, the study of platelet miRs might deepen the understanding of the pathogenesis of atherosclerosis and a variety of diseases associated with heart injury. In this review, we summarize the association of currently recognized platelet microRNAs with cardiovascular disease pathogenesis. The potential of platelet-related miRNAs as diagnostic and prognostic biomarkers for atherosclerosis management in patients with CVD is also discussed.

Methods

In this review, search engines such as PubMed and Scopus were used (last accessed in January 2022) with the terms "Cardiovascular disease AND Platelet function OR Platelet miRNA" to review articles related to platelet-enriched microRNAs in atherosclerotic and cardiovascular disease patients and their roles in CVD.

Results

In this study, we first review CVD and platelet microRNAs, then evaluate the roles of miRs in CVD and other disorders known to be important factors causing cardiovascular disease. In the discussion section, we discuss the significant roles of miRs in cardiovascular disease and related conditions.

Role of platelet in the pathogenesis of cardiovascular disease

Today, several studies focus on platelets as regulators of vascular hemostasis in many organs, especially the heart, and their involvement in atherogenesis. Numerous pieces of evidence indicate that platelets are important factors in the initiation and development of atherosclerosis and thereby the incidence of CVD[8]. Clinical studies have shown that traditional CVD risk factors such as dyslipidemia, hypertension, diabetes, smoking, obesity, sedentary lifestyle, and insulin resistance due to high shear pressure and decreased blood flow lead to platelet hyperactivity. Hyperactive platelets interact with endothelial cells and monocytes via their CD62 (P-selectin), which in turn activates these cells. Activated platelets release various factors from their granules and microvesicles that affect the function of endothelial cells, leukocytes, and cardiomyocytes[9]. For example, CXCL1 released from activated platelets causes monocyte recruitment to atherosclerotic lesions[10]. CXCL4-derived platelets result in increased differentiation of macrophages, induce CXCL12 secretion from macrophages, and inhibit monocyte death, thereby playing an essential role in atherogenesis plaque formation[11,12]. CCL5 is released by monocytes acting on platelet activators, increasing angiogenesis and monocyte and T lymphocyte adhesion to the endothelium via ICAM-1 and VCAM-1[13]. CD40L-derived platelets induce inflammatory responses and stabilize platelet thrombin. PDGF, VEGF, and platelet cytokines promote the proliferation of vascular smooth muscle cells (VSMC) and the initiation and development of atherosclerosis[14]. Sphingosine-1-phosphate (S1P) released by platelets has either pro-inflammatory or anti-inflammatory effects through the activation of cardioprotective RISK and SAFE pathways[15,16]. This may be caused by the altered release of S1P. On the other hand, platelet-activating factor (PAF) has dual effects on cardiac functions; at high concentrations, it acts as a strong arrhythmogenic agent, whereas it has a cardioprotective role at very low concentrations[17]. Thus, platelets are main players not only in cardioprotection but also in the incidence of CVD. Interestingly, the dual roles of platelets provide more opportunities to study subjects regarding heart failure. Therefore, future studies may shed light on the precise role of blood platelets in CVD pathology and thereby detect useful prevention and treatment strategies in patients with CVD.

micro RNA expression profiles changes related to atherosclerosis

Lipid disorders, hypertension, cigarette smoking, diabetes mellitus, and obesity are the main risk factors for atherosclerosis[18,19]. Recently, accumulating studies have proposed altered expression patterns of platelet miRs as important predictors and regulators of atherosclerosis[20]. Herein, therapeutic targeting of miRs to reduce atherosclerosis risk is of great

interest. Upregulation or downregulation of platelet-derived miRs in the predisposition condition of atherosclerosis presents the pathways involved in these diseases' pathogenesis. Therefore, a more in-depth understanding of therapeutic targeting of miRs may well translate to new prevention and management strategies for atherosclerosis as well as cardiovascular diseases. Here, we discuss the relationships between platelet-related miRNAs and atherosclerosis risk factors.

micro RNA changes upon hypertension

Hypertension, as one of the atherosclerotic risk factors, affects the expression and synthesis of platelet-derived miRNAs in CVD patients. For example, the expression of platelet miR-126, miR-223, and miR-22 is downregulated in hypertensive patients and may be considered indicators of future cardiovascular complications[21]. MiR-223, a hematopoietic-specific miRNA (found in platelets and myeloid cells) and non-hematopoietic cells (hepatocytes, cardiac myocytes, and endothelial cells), regulates the differentiation of megakaryocytes and erythrocytes. During macrophage polarization, miR-223 suppresses classic pro-inflammatory pathways and enhances alternative anti-inflammatory pathways by targeting Pknox1, Nfat5, and Rasa1, respectively. Hypoxia causes a marked downregulation of miR-223 in the murine heart with pulmonary hypertension and subsequently upregulates miR-223 targets (IGF-I and IGF-IR)[22]. Decreased levels of miR-223 and increased expression of IGF-IR were observed in patients with pulmonary hypertension. Interestingly, miR-223 overexpression following inhibition of IGF-IR led to the suppression of right-ventricular hypertrophy and improved young murine heart function under increased afterload or hypoxia. Thus, ischemia/reperfusion (I/R) in the murine heart was linked to miR-223 dysregulation[23]. In vivo, hypertensive rat model studies suggested that platelet miR-142-3p, by targeting BCLAF1, led to endothelial cell apoptosis and vascular remodeling under hypertension. The molecular mechanisms involved in this process remain unclear [24]. In animal studies, data showed that atherosclerosis induction under hypertensive conditions was associated with high expression of platelet miR-146a, miR-126, miR-223, miR-222, miR-214, and low expression of platelet miR-145, miR-10a, and miR-143. This study showed that microRNAs with different expressions in plasma and platelets under hypertension were related to hyperlipidemia[25]. Importantly, plasma miR-21 was upregulated in a cohort of hypertensive patients and related to systolic and diastolic blood pressure[26]. Fichtlscherer et al.[27] reported that the expression level of the miR-17 family in AMI, CAD, and renal disease with hypertension was decreased, suggesting a key role for the miR-17 family in the development of CVDs. Since hypertension has a critical role in the establishment and development of CVDs and a positive correlation with age and CVDs[28], understanding the precise molecular mechanisms involved in the initial development of CVDs and subsequently hypertension by platelet-related microRNAs might lead to the establishment of potential treatments for CVDs.

micro RNA changes upon obesity

Obesity, as a public health challenge, has reached pandemic levels and threatens human life. Approximately 4 million people die annually due to obesity or overweight complications[29,30]. Adipose tissue serves as both a lipid storage and an endocrine organ[31,32]. Adipose tissue releases adipocytokines related to CVDs, such as leptin, adipocyte fatty acid-binding protein, interleukin, lipocalin 2, and pigment epithelium-derived factor. Perivascular adipose tissue (PVAT) acts as an active endocrine organ, releasing various cytokines, adipokines, and growth factors that inhibit or stimulate CVD development. Some studies reported that PVAT dysfunction is linked to a decrease in the production of adiponectin, leptin resistance, TNF-α, IL-6, and the release of chemokines CCL5 and CCL2, which in turn cause oxidative stress and inflammation[33,34]. Leukocyte recruitment to the inflammatory site by releasing inflammatory molecules such as IL-17 or IFN-γ results in dysfunction of eNOS, VSMC, and endothelium fibrosis, subsequently causing vascular dysfunction. Studies have shown that PVAT dysfunction plays an important role in the pathogenesis of CVD risk factors, including obesity, diabetes mellitus, atherosclerosis, and hypertension[35,36]. There is numerous evidence that obesity causes platelet hyperactivity. Activated platelets release chemerin and adipokines involved in obesity, inflammation, and insulin resistance, causing increased proliferation, VSMC migration, eNOS inhibition, and activation

of NFKB signaling along with the expression of adhesion molecules, subsequently leading to vascular dysfunction and atherosclerosis development[37,38]. Moreover, growing evidence shows that platelets have microRNAs whose aberrant expression plays an essential role in obesity pathogenesis, thrombotic complications, and diabetes[39,40]. Downregulation of platelet miR-223 may be considered a possible mechanism for platelet activation by increased expression of the platelet ADP receptor P2Y12[41.] Importantly, miRs, through transcriptional and post-transcriptional factors, control the adipogenesis process during obesity. miR-143 and miR-375 increase the expression of PPARγ2 and C/EBP, leading to adipocyte differentiation in humans, mice, and the 3T3-L1 cell line, respectively[42,43]. Rayner et al.[44] indicated that miR-33 is a critical endogenous regulator of lipid metabolism and an essential player in atherosclerosis. In mice, deficiency of miR-33 is related to hyperlipidemia, obesity, and insulin resistance. In addition, some miRs in white adipose tissue are increased and decreased[45]. Altered expression of miRs related to obesity highlights the urgency for more studies to prevent and treat obesity effectively.

micro RNA in lipid disorder

Several lines of evidence have established that hyperlipidemia is strongly associated with atherogenesis and CVDs[46]. Low-density lipoprotein (LDL) is an oxidized lipid that induces prothrombotic platelet activation via the CXCL12/CXCR4–CXCR7 axis and may play a critical role in CVD pathogenesis[47,48]. In addition, oxidized metabolites related to increased oxidized phospholipids, through CD36, tissue factor (TF), platelet-activating factor receptor (PAFR), and tissue factor pathway inhibitor (TFPI), result in endothelial dysfunction, inflammation, monocyte and macrophage differentiation, plaque formation, and atherogenesis[49,50]. Interestingly, the expression of CXCR4-CXCR7/CXCL12 on platelets is associated with CVD severity and may serve as a prognostic biomarker[51]. In hyperlipidemia, VSMCs over-express the scavenger receptor family (CXCL16, CD36)[52] and cholesterol receptors (LRP, LDL-R, VLDL-R), especially LRP-1, which facilitates LDL internalization and foam cell formation. Lipid-rich VSMCs with lower repair capacity, migration, and population count are the main characteristics of unstable plaques. Indeed, LDL concentration regulates the instability and susceptibility to rupture of atherosclerotic plaques in advanced stages[53,54]. Importantly, the lipid-rich core of atherosclerotic plaques is more than six times more thrombogenic due to the induced release of TF from foam cells and macrophages. Rupture-prone plaques consist of a thin fibrous cap, a tight lipid core, and are hypocellular with lower collagen. Plaque stability depends on the balance of collagen synthesis by VSMCs and collagen degradation by collagenases, gelatinases, and matrix metalloproteinases (MMPs)[55,56].

Current studies focus on the association of microRNAs and lipid disorders. miRNAs, as regulators of gene expression involved in lipid metabolism, play key roles in differentiation and lipid storage by adipocytes. For example, the expression of the JAZF1 gene, involved in insulin resistance, lipid metabolism, and gluconeogenesis, is downregulated in prediabetic patients, thereby causing atherosclerosis development in these patients[57,58]. Surprisingly, early life nutrition leads to increased miR-483 expression, which is related to insulin resistance, lipotoxicity, and endothelial apoptosis, thereby impairing integrin and endothelial regeneration and highlighting metabolic disease risk. Furthermore, this miR is linked to diabetes mellitus and CVDs[59]. miR-33a and miR-33b target ABCA1, the regulator of cholesterol metabolism, resulting in decreased VLDL and increased HDL in plasma. Thus, miR-33 inhibition, along with the efflux of macrophage cholesterol, regulates plaque formation[60,61]. Several studies have reported that inflammatory cytokines play an essential role in this balance since platelet miRs, which constitute the majority of circulating miRs, can alter the expression of inflammatory cytokines and thereby play a leading role in determining the fate of atherosclerotic plaques[62,63]. Besides platelet miRs, plasma miRs such as miR-378a-5p, targeting MAPK1, promote adipogenesis in 3T3-L cells[64]. Overexpression of miR-130, miR-27a, and miR-27b inhibits PPARγ, the master regulator of adipogenesis, halting adipocyte differentiation and decreasing the adipogenesis process[65,66]. Furthermore, lipid tissue acts on endogenous tissue, resulting in the release of various materials into the blood, altering gene expression profiles and the formation of different diseases, especially CVDs.

Diabetes mellitus-associated micro RNA changes

Mounting evidence has shown that diabetes mellitus significantly increases the likelihood of developing CVDs[67]. Since hyperactive platelets contribute to diabetes, current attention is focused on the association of hyperactive platelets with different diseases such as CVDs. Interestingly, platelet miRs compose large amounts of circulating miRs in plasma [68,69]. Several studies have investigated the expression of platelet-related miRs in diabetes mellitus. For example, Legrand et al.[70] revealed that during hyperglycemia, hyperactive platelets released PDGFRβ and downregulated miR-223, resulting in proliferation, blocked dedifferentiation of VSMCs, and restenosis (intimal hyperplasia) in response to stent agents[71]. High activation of calpain and proteolytic cleavage of Dicer may be responsible for the significant downregulation of platelet-related miRs (miR-142, miR-223, miR-126, miR-155) in patients with diabetes and diabetic mice[72]. Downregulation of platelet miR-223, miR-140, miR-126, and miR-26b contributes to increased expression of P2Y12 and P-selectin, participating in platelet hyperactivation[73]. Zampetaki et al.[74] observed that low expression of platelet-derived miR-197 is related to abnormal platelet function in diabetes patients[75]. Hyperglycemia is responsible for low expression of miR-223 and miR-146a, promoting platelet activation and, in turn, initiating and developing cardiovascular complications in diabetes patients[76]. Since plasminogen activator inhibitor-1 (PAI-1) is a target of miR-30c, increased expression of PAI-1 contributes to increased arterial occlusion and atherosclerosis development in murine models[77]. miR-126 is not only associated with platelet activation and cardiovascular events via regulation of thrombin generation but also plays a critical role in wound repair, angiogenesis, and the protection of vascular integrity[39,78,79]. Downregulation of miR-126 inhibits vascular protection effects and thrombogenicity by Notch1 suppression and platelet TF, respectively. Taken together, low expression of miR-126 may help accelerate CVD development[80]. Wound repair in diabetic patients is a clinical challenge responsible for reducing life quality, prolonged hospitalization, and even lower limb amputations[81]. In a diabetic cohort, the expression of miR-191 was investigated directly in peripheral blood and wound fluid, and it was reported that during proinflammatory conditions, overexpression of platelet or endothelial cell-related miR-191, targeting zonula occludens-1 of dermal cells, attenuates dermal cell adherence and therefore delays tissue repair. Additionally, a marked association has been reported among circulating miR-191 and MIF, PDGF-bb, and IL-1b[82].

Platelet miRs in atherogenesis

Platelets are key players in atherosclerosis development. Platelet activation occurs through interaction with dysfunctional endothelial cells, increased proinflammatory and prothrombotic mediators, and reactive oxygen species (ROS) generation, along with hypertension, hyperlipidemia, diabetes, smoking, and hypercholesterolemia[83]. Hyperactivated platelets adhere to endothelial cells and von Willebrand factor (VWF) via GP Ibα, GP IIb/IIIa, and P-selectin, resulting in firm adhesion mediated between platelets and endothelial cells by integrin binding[84]. Activated platelets release several mediators, including proinflammatory chemokines and cytokines, promoting cell adherence, leukocyte recruitment, proliferation, coagulation, proteolysis, and inflammatory processes, thereby promoting atherosclerosis lesions[85]. Collectively, these events stimulate and migrate VSMCs to the vascular intima and fibroblast proliferation, subsequently increasing collagen synthesis and the emergence of atherosclerosis lesions in the arterial intima[86]. Indeed, crosstalk between platelets and surrounding cells such as endothelial cells, VSMCs, and leukocytes, especially monocytes, via cytokines, plays a pivotal role in atherosclerosis development[20]. Platelet miRs play a crucial role in all steps of atherogenesis. For example, platelet-related miR-223 induces apoptosis in endothelial cells by targeting IGF-1R following endothelial dysfunction[87]. Moreover, platelet miR-22 targets ICAM-1 by blocking NFKB and MAPK pathways, resulting in halted adherence, monocyte migration, and foam cell transition to plaque lesions[88,89]. Interestingly, miR-223 accelerates atherosclerotic development in ApoE−/− mice on a western diet[90]. Additionally, Shan et al.[91] found that downregulation of miR-223 markedly increased neointimal lesion formation in ApoE−/− mice. These data indicate the importance of miR-223 in preventing atherosclerosis development. Surprisingly, upregulation of miR-223 induces proliferation, migration, and apoptosis of

VSMCs, thereby inhibiting atherogenesis[91]. Another platelet-derived miR, miR-126, promotes endothelial cell proliferation and repair following hyperlipidemic stress and suppresses atherosclerosis by inhibiting Notch1 and PI3K[92,93]. Additionally, miR-126 amplifies the proangiogenic function of transcription factors VEGF and FGF by suppressing SPRED-1, an internal inhibitor of angiogenesis signaling, and activating MAPK pathway signaling[79]. Notably, platelet-related miRs are involved in atherosclerosis development (Figure 1). In summary, miR-126, through angiogenesis amplification, provides protection against atherogenesis. Microarray results indicated that increased platelet miR-21-5p is strongly associated with cardiac enzymes such as creatine kinase and troponin. The direct role of miR-21 in platelet function remains unclear but may target KEGG and PI3K/Akt pathways related to platelet activation[94,95].

Biological functions of platelet miRs

MicroRNAs responsible for regulating gene expression may alter physiological to pathological processes[96]. Notably, platelet-related miRs compose the main plasma miRs[68,69]). Changes in miR expression and their target genes have been shown in different diseases, including CVDs97). In mouse models, platelet miR-142-3p plays a critical role in the organization of tubulin and actin in megakaryocytes. miR-142 results in impaired megakaryocyte maturation, thrombocytopenia, and disrupted activation of integrin α2bβ3, the leading aggregation receptor

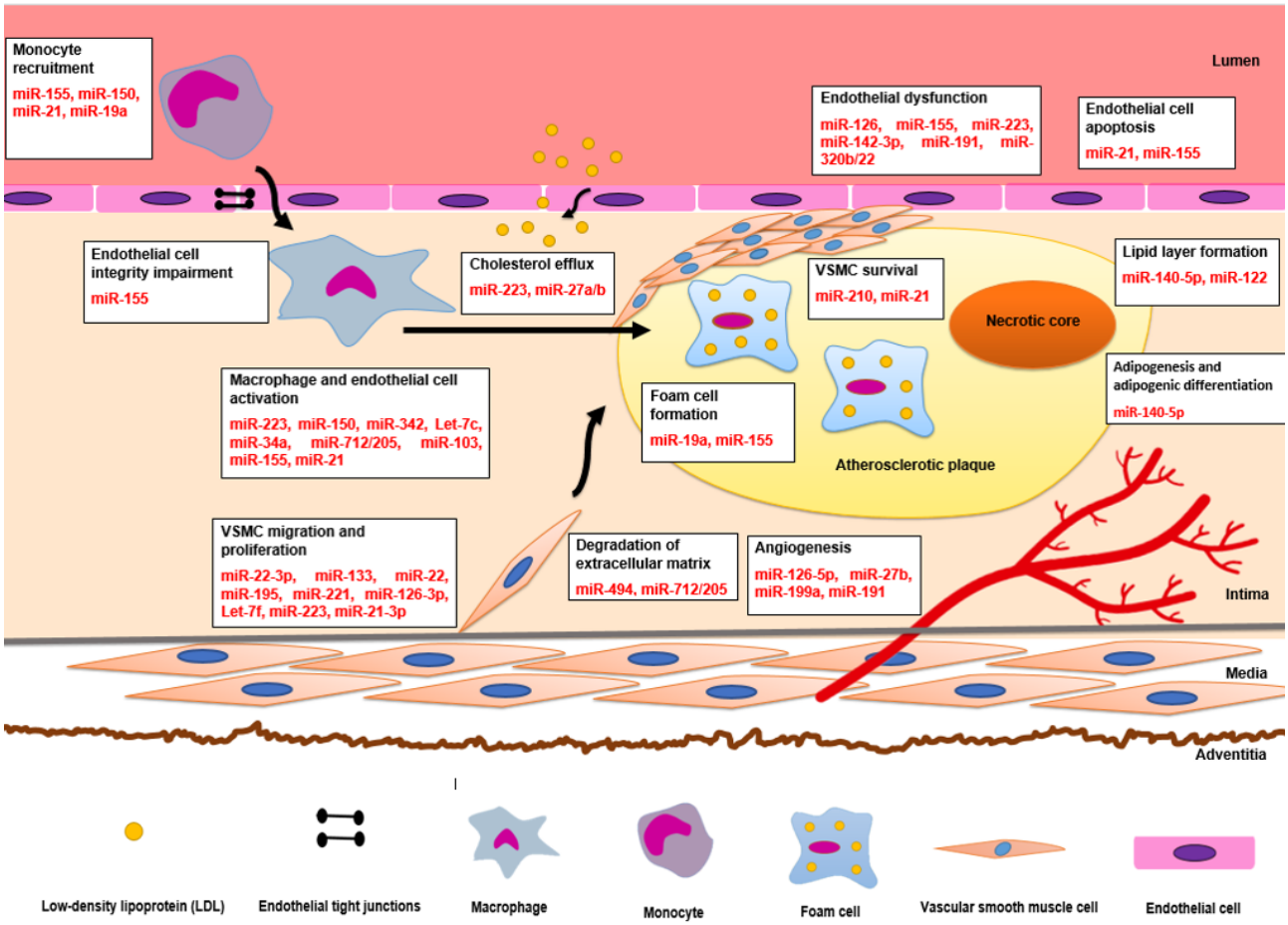

Figure 1. Role of platelet miRs in atherosclerosis development. Susceptible condition in the initiation and progress of the atherosclerosis process such as hypertension, diabetes mellitus, smoking, hyperlipidemia, and obesity induces inflammation leading to endothelial dysfunction, and platelet activation in interaction with dysfunctional endothelial causing to release of inflammatory chemokines and miRs. Cross-talk between platelet and surrounding cells involved in atherosclerosis lesions including monocyte, VSMC, macrophage, and endothelial result in monocyte recruitment, migration, and proliferation VSMC, transition monocyte to macrophage, macrophage to foam cell, and angiogenesis in the intima.

in platelets[98]. Pathogenic thrombosis results from excessive integrin activation by vWF and fibrinogen, with overexpression of platelet miR-142-3p in ACS patients compared to healthy controls[96]. Moreover, miR-142-3p induces reduced IL-10 and TGF-β secretion, downregulates Rac1 and Rac1-GTPase expression, decreases T-reg induction, and increases hemorrhage risk[99,100]. Interestingly, platelet-derived miRs impair circadian rhythm, playing a pivotal role in ischemia and myocardial infarction (MI) in CVD patients[101]. Highly expressed platelet miR-107 targets the CLOCK gene, a circadian fluctuation-related gene, responsible for thrombocytopenia along with simultaneous hyperaggregability in ACS patients. Additionally, platelet-related miR-320b/22 regulates the expression of proinflammatory mediators ICAM-1 in endothelial cells of ST-segment elevation myocardial infarction (STEMI) patients. Low miR-320b/22 expression is associated with endothelial cell dysfunction and inflammation, the cornerstone of atherosclerosis and CVDs[102].

Some platelet miRNAs are linked to the platelet aggregation process. For example, miR-34b-3p targets thromboxane A synthase 1 (TBXAS1), and miR-19b-1-5p targets PDE5, NOS3, and GUCY1A3, regulating the NO-cGMP signaling pathway[103,104]. Platelet miR-96 targets VAMP8/endobrevin, involved in platelet degranulation[105]. miR-15b-5p, via regulation of direct expression of PRKCQ, MYLK, SRGN, FYN, and FCER1G related to the GPVI signaling pathway, plays a prominent role in collagen-induced activation[106]. In other words, platelet miRs not only participate in hemostasis and platelet aggregation but are also involved in biological processes such as fibrosis (miR-21, miR-199a/b, miR-30c)[107,108] and angiogenesis (miR-27b, miR-199a)[109,110]. miR-21-5p targets Wiskott-Aldrich syndrome protein, thereby attenuating the release of TGF-β1. miR-21-null mice had higher megakaryocyte and lower leukocyte and platelet numbers[111]. Platelet miRs, via microparticles (MPs), transfer to neighboring cells, affecting gene expression and leading to altered cell function. For instance, the impact of MPs-related miR-4306 is mediated by targeting VEGFA and ERK1/2/NF-κB signaling pathways, resulting in migration inhibition and lower macrophage presence in the hearts of mice with MI[112]. miR-223 is linked to cardiac dysfunction (CD36, SLC8A1, MEF2C), cardiomyocyte proliferation (TGFBR3, SLC8A1), heart hypertrophy (HDAC4, LIF, IL6ST), and vascular remodeling (MEF2C, FBXW7, LIF)[108]. Moreover, the functional impact of platelet miR-223 is mediated by P2Y12-regulated platelet response to the antiplatelet drug clopidogrel[41]. Platelet-derived miR-126 regulates angiogenesis and vascular integrity by suppressing inhibitors of Delta-like 1 homolog (Dlk1) and Notch1 ligand, protecting against atherosclerosis. Additionally, by inhibiting endothelial cell apoptosis in deep vein thrombosis (DVT), it suppresses PI3K/Akt signaling. Three targets of platelet miR-126, including P2Y12, disintegrin, and MMP9, participate in the inhibition of platelet adherence[92]. Platelet miRs associated with CVD are listed in Table 1.

Platelet miRs as diagnostic biomarkers

In 1993, the discovery of microRNA by Lee et al.[113] provided new insights into the regulation of gene expression, the pathogenesis of different diseases, and the potential diagnostic value of these miRs. Today, miRNAs, due to their easy accessibility, high stability in body fluids, and their expression changes related to disease and health conditions, could be considered logical biomarkers in various diseases, especially CVDs[114] (Figure 2). Platelet miR-223, through targeting PDGFRβ, promotes VSMC differentiation and leads to coronary pathology in Kawasaki disease (KD), suggesting that detection of miR-223 may identify patients with high-risk coronary arteries[115]. Other studies reported that downregulation of platelet miR-223, associated with hyperactive platelets, may be helpful in identifying high on-treatment platelet reactivity (HTPR), non-responsiveness to clopidogrel, and susceptibility to atherosclerosis[116]. However, in a large study of patients with acute chest pain and suspected MI, miR-223 was not able to distinguish MI patients from other causes of angina[117]. A study investigating miR-19a-3p and let-7f expression in acute ischemia (IS) shows the diagnostic value for IS with AUC 0.755 (miR-19a-3p) and 0.874 (let-7f)[118]. Diehl et al.[119] found that overexpression of miR-19 is related to cardiac hypertrophy and angiogenesis. miR-21-5p, with the highest expression in platelets, may serve as a modern diagnostic biomarker in MI, ischemia, and pulmonary emboli[20]. miR-21-5p is significantly associated with cardiac troponin, creatine kinase, systolic and diastolic pressure, and platelet activation through PI3K/Akt signaling, thereby showing a

Table 1. Studies investigating platelet miRs in cardiovascular disease patients

Study	Platelet miRs	Diseases	Findings	References
Zietzer	miR-222-3p miR-223-3p miR-126-3p	AF	Platelet activation by LPS causes high expression of miR-223-3p and miR-222-3p and subsequent-derived used immigration endothelial cells AF related to increased EV-derived platelet	(138)
Wang	miR-223	AIS	Low expression of miR-223 in ACS patients responsible for increased risk of HTPR	(139)
Szelenberger	miR-223-3p miR-126-3p	ACS	Overexpression of miR-223-3p in ACS but significant difference in miR-126-3p expression was not seen	(140)
Eyileten	miR-19a-3p miR-186-5p let7-f	AIS	Patients with HPR remarkably increased EV derived platelet (CD 62, CD 45) in comparison to HPR normal on the first day of acute stroke miR-19a-3p AUC =0.755, P =0.004 Let7f AUC=0.874, P=0.0001 Platelet EV AUC =0.776 P=0.001 Leukocyte AUC =0.715 P=0.008 Patients with moderate stroke have significant expression of miR-19a-3p in comparison with minor stroke patients on the first day of ischemic stroke AUC=0.867 P=0.001	(114)
Szelenberger	miR-142-3p miR-107 miR-338-3p miR-223-3p miR-21-5p miR-130b-3p miR-301a-3p miR-221-3p	ACS	Increased expression of eight Ma combination patients associated with reactivity and function of platelet The combination of miR-142-3p and AST results in the discrimination of ACS patients from healthy control by 82% sensitivity and 88% specificity	(92)
Stojkovic	miR-21 miR-126 miR-191 miR-24 miR-27b miR-28 miR-150 miR-197 miR-223 miR-320a	ACS	miR-21 and miR-126 induced MPA formation in ACS patients with DAPT High expression of miR-21 and miR126 in ACS	(124)
Singh	miR-19b-and 1-5p	ACS	Downregulation of miR-19b-1-5p related to high risk of MACCE, and platelet aggregation during ASA administration miR-19-b-1-5p indicated ASA resistance and MACCE predictor in ACS patient	(141)
Mukaihara	miR-126-5p miR-126-3p	CABG, PAD	Low expression of miR-126-3p due to endothelial dysfunction may cause increased IP-VEGFa A miR-126-3p overexpression occur instantly after CABG and decreased to a preoperative level Serum level of miR-126 decreased in PAD patients comprised to patients without PAD	(142)
Liu	miR-223-3p	CAD	GAS5 polymorphism may affect clopidogrel response in CAD patients with poor metabolizer CYP2C19 GAS5 mediated expression of P2Y12 and clopidogrel response via miR-223-3p	(143)

Continued Table 1

Study	Platelet miRs	Diseases	Findings	References
Lin	miR-142-3p miR-24-3p miR-411-3p miR- 103a- 2- 5p miR- 548av- 3p miR- 3184- 3p miR- 17- 5p miR- 339- 5p miR- 454- 3p miR- 106b- 5p miR-32- 5p miR- 10401- 3p miR-140- 5p miR- 30e- 5p miR- 330- 5p miR-138- 5p miR- 181a- 5p let- 7i- 3p miR- 324- 5p miR- 660- 5p miR- 3074- 5p miR-30d- 5p miR- 186- 5p miR- 25- 3p miR- 107 miR- 378e let- 7d- 3p miR- 6819- 3p let- 7e- 5p miR- 490- 5p miR- 6721- 5p miR-6729- 3p miR- 505- 5p miR- 150- 5p miR- 11401 miR- 6749- 3p miR-100- 5p miR- 483- 3p miR- 4647 miR- 4676- 5p miR-3181 miR- 4446- 3p miR-6772- 5p miR- 6850-5p miR- 6732- 5p miR- 6837- 5p miR-27b- 5p miR- 23b- 5p miR- 1236- 3p miR-2110 miR- 485- 5p miR- 485- 3p miR- 654- 5p miR- 1180- 3p miR-10a- 3p miR- 378f let- 7c- 5p let- 7b- 5p miR- 574- 5p miR- 342- 5p miR- 320a- 3p miR-3064- 5p miR- 320c miR- 190b- 5p miR-1260b miR- 7- 5p	CAD	Upregulation of miR-142-3p and miR-24-3p whereas downregulation of miR-411-3p in clopidogrel resistance may predict clopidogrel resistance in CAD patients	(144)

Continued Table 1

Study	Platelet miRs	Diseases	Findings	References
Li	miR-223 miR-126	STEMI	miR-126 and be miR-223 have a key role in DAPT resistance and may potential biomarkers in STEMI Low expression of miR-126 and miR-223 related to DAPT resistance	(128)
Yang	miR-4306	CAD	miR-4306 expression was decreased in CAD patients, suggesting poor prognostic factor in these patients miR-4306 inhibits migration of HMDMs in vitro and thereby reduced macrophage count in heart of MI mice	(108)
Marketou	miR-223 miR-126 miR-22	Essential hypertension	Low expression of miR-126 and miR-22 in hypertensive patients, suggested a strong predictor for CVD Negative association to SBP (for miR-22 r=-0.43, p<0.001; for miR-223 r=-0.47, p<0.001)	(21)
Ding	miR-204-5p	ACS	Overexpression miR-204-5p in ACS patients with HPR after DAPT administration (AUC=0.667) Expression of miR-204-5p related to Gensini score	(145)
Chen	miR-365-3p miR-96-5p miR-495-3p miR-107 miR-223-3p miR-15a-5p miR-339-3p	CAD	miR-339-3p and miR-365-3p with 74. and 3%, 90% sensitivity and 71. and 4%, 93.3% specificity, respectively for HTPR detection after 24 hours following drug administration SYNTAX score positively related to miR-223-3p and miR-365-3p in 24 hours (p ≤ 0.006)	(146)
Kanuri	(n=321 miRs)	CAD	NGS shows shown that 70 miRs have a significantly different expression, 37 miRs with overexpose, recession, and 33 miRs along with low expression	(147)
Li	miR-21 miR-126 miR-150 miR-223 miR-1	STEMI	Low expression of miR-126 and miR-21 whereas overexpression of miR-150 and miR-223 in STEMI patients MiR-126 strongly related to cTnI (r=-0.556, p=0.011) miR-1 in STEMI was no significant statistical difference	(148)
Goren	miR-150	Chronic systolic heart failure	Downregulation of miR-150 in systolic heart failure	(149)

Several studied have investigated platelet miRs in different kind of CVDs involving AF, ACS, AIS, CABG, PAD, CAD, STEMI, Essential hypertension, chronic systolic heart failure in order to deep understanding of etiology and pathphysiology of initiation and development of CVD and in turn, find novel and practical diagnostic, prognostic and treatment biomarkers for patients with CVD.

AF: Atrial fibrillation, LPS: lipopolysaccharide, EV: extracellular vesicle, AIS: Acute ischemic stroke, ACS: acute coronary syndrome, HTPR: high on-treatment platelet reactivity, HPR: high platelet reactivity, AUC: area under the curve, AST: aspartate transaminase, MPA: Monocyte–Platelet Aggregate, MACCE: major adverse cardiac and cerebrovascular events, CABG: Coronary artery bypass grafting, PAD: peripheral artery disease, IP-VEGFA: Intra-platelet vascular endothelial growth factor-A, CAD: Coronary artery disease, GAS5: growth arrest-specific 5, STEMI: ST-elevation myocardial infarction, DAPT: Dual antiplatelet therapy, HMDMs: human monocyte-derived macrophages, MI: myocardial infarction, SBP: systolic blood pressure, NGS: next-generation sequencing, cTnI: cardiac troponin I

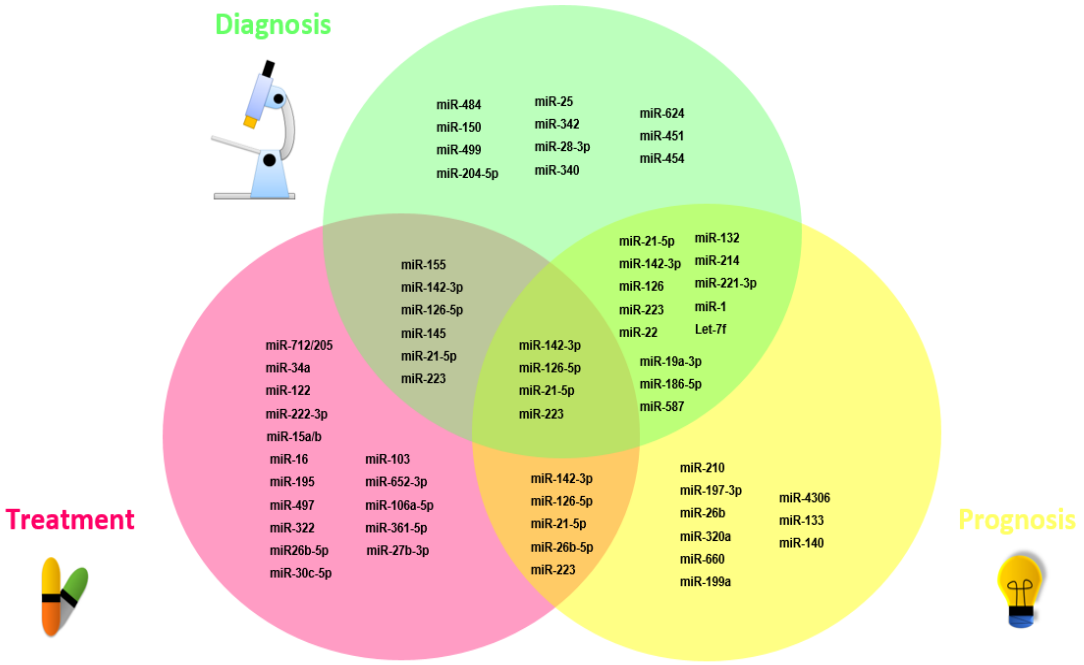

Figure 2. Platelet miRs as diagnostic, prognostic, and therapeutic biomarkers for cardiovascular diseases. Venn diagram of platelet miRs as diagnostic, prognostic, and treatment biomarkers in CVD. Overlapping fields represent common platelet-related miRs in diagnostic, prognostic, and treatment biomarkers. In the center of the diagram, four miRs (142-3b, 126-5p, 21-5p, 223) have been considered as common diagnostic, prognostic, and treatment biomarkers in CVD.

strong relation to susceptibility to MI[120]. Our study indicated that platelet miR-484 discriminated between ACS patients and healthy controls (sensitivity 69.5%, specificity 84.6%, and AUC 0.786). In addition, platelet-related miR-484 had potential power in diagnosing ACS subgroups compared to controls (NSTEMI vs. control AUC 0.910, UA vs. control AUC 0.978). These miRs could also diagnose UA and NSTEMI patients with negative troponin, a main clinical challenge, with 100% sensitivity and 83% specificity, suggesting potential diagnostic biomarkers in ACS and its subgroups[121]. Low expression of miR-155 may act as atheroprotective and anti-inflammatory in atherosclerosis development, plaque rupture, and inflammatory response in CAD and CHD patients. However, a study showed overexpression of miR-155 at 0.5 and 1 hour after plaque rupture[122]. These conflicting results could be due to different measurement techniques for miRs (PCR, microarray), endogenous control genes, sample preparation methods, sample sources (plasma/serum and whole blood), lack of standardization, various anticoagulants (EDTA vs. sodium citrate), and small sample sizes[123]. Despite extensive studies on miRs and their prospective diagnostic power in CVDs, no miRs as diagnostic biomarkers have been validated. Therefore, our knowledge of microRNAs is in an early stage of development, and the potential diagnostic power of miRs in different diseases warrants widespread investigation in the future.

Platelet miRs as prognostic biomarkers

Currently, due to the high morbidity and mortality of CVDs, new studies focus on platelet miRs not only serving as diagnostic biomarkers but also as prognostic tools for the prevention of cardiovascular diseases and reducing potential risk before ischemia occurs[118]. Several studies have investigated the expression profiles of platelet-related miRs in CVD patients to identify the role of miRs in the pathophysiologic process of CVDs[124]. Szelenberger et al.[96] found that overexpression of platelet miR-21-5p and miR-142-3p may be considered prognostic biomarkers in pulmonary embolism, stroke, and ACS patients, respectively. Obesity, smoking, and diabetes are the main risk factors for CVD development, and altering miRNA expression results in disease development[18,19]. Interestingly, upregulation of circulating miR-126 in patients with diabetes, MI, and atrial fibrillation is related to

increased platelet reactivity, development of insulin resistance, atherosclerosis risk, and predictive value for future atherothrombotic events[74,125-128]. In a study analyzing the expression of platelet miR-223, miR-126, and miR-22 in patients with hypertension, it was reported that miR-22 and miR-223 were significantly downregulated in these patients and negatively associated with systolic blood pressure. Moreover, ROC curves show that these platelet-derived miRs are strong prognostic biomarkers for CVD[21]. One of the largest cohorts on the prognostic value of miRNAs in over 1000 CAD patients suggested that platelet miR-210, miR-132, and miR-140 might be prognostic biomarkers of cardiovascular death[129]. In addition, miR-197 and miR-223 could control predictors of cardiovascular death[130]. In a case–control study, STEMI patients showed the superiority of miR-26b, miR-320a, and miR-660 over cardiac troponin in risk stratification of CVD patients and the prediction of future events[131]. Also, some platelet miRs could predict treatment response to antiplatelet therapy. In a study examining miR-126 and miR-223 expression in STEMI patients with and without DAPT resistance, decreased miR-223 and increased P2Y12 expression resulted in hyperactivity of platelets and clopidogrel resistance. Therefore, miR-223 and miR-126 have potential prognostic value in patient survival and treatment resistance[132]. In contrast, the results of some miRNA studies were contradictory. For example, low expression of miR-223 is considered a poor prognostic factor in MI[74], while another study found that overexpression of miR-223 consistently indicated high mortality risk in CVD[130]. However, further studies will provide novel insights into better identification of disease pathogenesis, especially CVD, and help develop effective prevention and treatment strategies for CVDs in the near future.

Therapeutic potential of platelet-related miRs in atherosclerosis

Platelet miRs participate in essential biological processes, including the regulation of lipid metabolism, vascular homeostasis, angiogenesis, cell proliferation, inflammation, platelet activation, and pathological processes in cardiovascular disease development[133]. Various studies have shown that dysregulation of platelet miRs, following altered gene expression involving cardiovascular homeostasis regulation, causes atherosclerosis development and subsequently CVDs[20]. Thus, in recent years, studies have focused on platelet-derived miRs as potential treatment tools. miR-155 modulates inflammatory signal transduction in atherosclerosis pathogenesis, involving VSMCs, dendritic cells (DCs), macrophages, and endothelial cell leukocyte differentiation. Therefore, miR-155 might be applied as a novel therapeutic target in atherosclerosis treatment[134]. Result analysis of qPCR and microarray indicated upregulated platelet miR-142-3p in hypertension patients. miR-142-3p, by targeting Bcl-2-associated transcription factor (BCLAF)1, renders endothelial cell dysfunction and accelerates hypertension development, suggesting a new treatment target in high blood pressure[24]. Anti-atherogenic and anti-apoptotic effects of miR-126-5p are induced by Notch1, DLK1, E-sel, and VCAM-1. Additionally, miR-126-5p mediates reduced atherosclerosis lesions[93].

Currently, depending on altered miR expression in different diseases, anti-miRs and miR mimics oligonucleotides could be utilized as attractive therapeutic markers in clinical trials. For example, overexpression of miR-145 is associated with the stability of atherosclerosis plaque via reduction of plaque size, increase in collagen components and VSMC numbers, and decrease in macrophage count in brachiocephalic arteries and aortic sinuses. Thus, miR-145 mimics might be a potential treatment strategy for atherosclerosis[135]. However, some studies have used anti-miRs for the suppression of pro-atherogenic miRNAs as anti-atherogenic therapies. In the atherosclerotic mouse model, miR-712/205, along with proatherogenic and proinflammatory effects by activation of metalloproteases and disintegrin, results in atherosclerosis. Collectively, anti-miR-712 with the VCAM1-targeting peptide could inhibit mRNA-miR interaction without influencing gene expression and be applied as a novel treatment method[136]. A study showed that anti-miR-34a and anti-miR-122 cause arrest of metabolic syndrome and atherosclerosis deterioration[137,138]. Plasma miR-222-3p and miR-21-5p are involved in the physiological and pathological processes of CVDs related to high venous thromboembolism (VTE) and MI, suggesting therapeutic targets for CVD[139]. A recent study reported that the miR-15 family (miR-322, miR-497, miR-195, miR-16, miR-15a/b), miR-26b-5p, miR-30c-5p, miR-106a-5p, miR-652-3p, miR-103, miR-361-5p, and miR-27b-3p

are strongly associated with heart failure and cardiac hypertrophy[140]. Despite substantial evidence about platelet miRs, there are several limitations in the study of miRs, such as small sample size, unknown source of miRs, lack of a standard for the evaluation of miR expression, contamination during sample preparation, and the use of different gene controls in several studies[123,141]. In summary, limitations in miR studies may result in conflicting results.

Conclusion

Several pieces of evidence have suggested that platelet-related miRs not only play an important role in the initial development of atherosclerosis and subsequent cardiovascular disease (CVD) but are also considered prognostic, diagnostic, and treatment biomarkers in CVD. Currently, numerous studies focus on platelet miRs to understand CVD pathogenesis. Inconsistent experimental results arise from limitations in miR studies. Analysis of miRs in different biological fluids (PRP, PPP), ambiguous sources of released miRs, lack of standardization of tests, different gene controls, genetic variety, and drug interactions are reported as responsible for the study restrictions of miRs. Future studies focusing on overcoming these limitations will lead to better insights into platelet-derived miRs as prognostic, diagnostic, and treatment biomarkers and the subsequent pathogenesis of atherosclerosis and CVD

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Conflict of interests

The authors declare no conflict of interest.

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Author's Contributions

Parisa Masoudikabir, Fatemeh Sigarchian and Mohamad Reza Shirazi: review and analyzed all related papers and drafted manuscript. Mohamad Esmail Ghydari consulted the and edited the manuscript. Mohsen Hamidpour conceived of the study, designed and coordinated it, and finalized the manuscript. All authors approval for publication

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