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Circulating miRNAs in Peripheral Artery Disease: Mechanisms, Biomarkers, and Therapeutic Potential

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Abstract. Peripheral arterial disease (PAD) is a vascular disease that refers to lack or stenosed blood flow of arteries outside of the heart and brain circulation, mainly caused by atherosclerosis, and most cases present limb ischemia. Circulating microRNAs (miRNAs), which are small non-coding RNAs stably existing in blood, have attracted attention to the role of PAD pathogenesis. They regulate biological events such as endothelial injury, the growth and movement of vascular smooth muscles, inflammatory activity and neo-vascularization of vascular growth etc. This article summarizes the latest research progress on the role of circulating miRNAs in PAD and discusses its indication for the mechanism of disease and clinical application.

Keywords: microRNA, peripheral arterial disease, lower limb ischemia

1. Introduction

Peripheral artery disease (PAD) is driven by atherosclerosis and is defined by impaired blood flow from arterial narrowing or obstruction in the limbs, mainly in the lower limbs. More than 200 million people globally are affected by PAD and cause a considerable burden for global health [1]. Clinically, PAD might manifest as intermittent claudication, critical limb ischemia, and possibly limb amputation in advanced patients, and significantly increase the risk of cardiovascular and cerebrovascular disease [2]. The formation of PAD requires complex interactions of multiple pathological factors such as lipid accumulation, endothelial damage, chronic inflammatory responses, oxidized stress and thrombogenesis. Recently, in addition to existing risk factors, the epigenetic regulation has also been considered as an important factor in PAD formation.

MicroRNAs (miRNAs) are endogenous small non-coding RNA molecules (20 to 25 nt long) that regulate the expression of genes by binding to their target mRNAs at the level of transcription and translation. In PAD, miRNAs were found to be the potential pivotal regulator in different pathophysiological events, including endothelial dysfunction, inflammation, vascular smooth muscle cell proliferation and migration, and angiogenesis upon ischemic injury. Circulating miRNAs, which are detectable in various types of body fluids, are stable in nature, from diverse tissues and sources, and are readily available for detection and thus are potential non-invasive biomarkers.

Recent advances in sequencing technologies and molecular methods enabled us to characterize unique circulating miRNA signatures in PAD. These molecules have great potential in various clinical features, such as early diagnosis, risk stratification and prediction of clinical responses to

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treatments. Circulating miRNAs are also emerging as new potential targets for novel therapies. This Review summarizes current knowledge of the role of circulating miRNAs in PAD regarding their mechanistic effects, potential as diagnostic markers, therapeutic roles, and their significance for early detection and individualized therapies.

2. Biological characteristics of circulating miRNAs and their advantages as biomarkers

2.1. Origin, stability, and detection of circulating miRNAs

Circulating miRNAs (c-miRNAs) are ubiquitous in the body fluids, such as plasma, serum and other body fluids. The origin of c-miRNAs can be generally classified as active secretion and passive release. On one hand, miRNAs can be actively secreted from cells by extracellular vesicles (including exosomes and microvesicles), high-density lipoprotein complexes and associated miRNAs. On the other hand, miRNAs may be passively released into circulation during apoptosis and necrosis [3]. Compared with other RNA species, circulating miRNAs are very stable. For example, c-miRNAs are generally stable under various conditions, including repeated freeze–thaw process, changes of pH, and long-term storage, and this stability of c-miRNAs is directly related to their encapsulation in vesicles or linked by the protein complex [4, 5] Recent studies also further demonstrate that most plasma miRNAs are highly stable in time within an individual and only show very minor changes within weeks or months, which is considered highly repeatable [6]. Besides, research for pre-analytical variables shows that some factors (e.g. hemolysis) affect the measured level of miRNA, but circulating miRNAs are more stable than most usual RNA molecules [7].

Regarding detection technologies, the advancement of molecular biology and high-throughput technology greatly facilitate the study of circulating miRNAs. Usual approaches for analysis included RT-qPCR, microarray platforms and small RNA sequencing technologies, but new detection platforms developed based on nanomaterials and biosensor technologies in recent years are providing new information for rapid miRNA detection and potential clinical application [8].

2.2. Advantages over conventional biomarkers

Compared with the traditional protein-based biomarker, circulating miRNAs have several advantages, particularly with regard to their stability, sensitivity and dynamic responsiveness. With regard to stability, miRNAs are of short nucleotide lengths and have the protection of confinement in vesicle or being associated with RNA-binding protein complex, which render their resistance to RNase degradation very strong. Thus, circulating miRNAs can be stable in blood samples for a long time, and yield a reliable basis for clinical detection. When it comes to sensitivity and specificity, different diseases have unique miRNA expression features, corresponding with characteristic molecular fingerprints to improve the diagnostic accuracy. Moreover, changes of miRNA levels can be observed even at the early stage of disease development and, hence, are advantageous for early diagnosis.

It also occurs that circulating miRNAs are strongly dynamic responsive. Circulating miRNA expression can be altered according to the development of a disease, therapy applied or changes in environment, allowing for dynamic measurement of disease state. This characteristic showcases their great potential for the monitoring of disease and efficacy of therapy [9]. Another merit of this concept is that circulating miRNAs can be easily examined via minimally invasive or even noninvasive methods, because they are present in multiple biofluids, such as blood, urine and saliva. This increases patient cooperation and decreases potential adverse effects. Recently, with the advent

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of "liquid biopsy" concept, interest in the clinical application of circulating miRNAs has aroused greatly, which witnesses the great potential of circulating miRNAs as next-generation biomarkers [10].

3. Circulating miRNAs and core pathophysiological mechanisms of PAD

Onset and progression of PAD are due to intricate combinations of pathogenic elements, such as endothelial dysfunction, abnormal development of vascular smooth muscle cells (VSMC), chronic inflammation and thrombogenicity. Recent evidence suggests that circulating miRNAs, as important post-transcriptional regulators, regulate various pathological elements at multiple levels, and play an important role in the pathogenesis of PAD by reflecting modifications to relevant molecular levels.

3.1. Regulation of endothelial dysfunction and oxidative stress

Endothelial dysfunction is an early and crucial event of PAD and is mostly represented by decreased NO bioavailability and increased oxidative stress, which are modulated by circulating miRNAs by controlling several signalling pathways.

3.1.1. Key miRNAs targeting the eNOS signaling pathway

Endothelial nitric oxide synthase (eNOS) is responsible for maintaining vascular homeostasis through vasodilatory and anti-inflammatory effects. Diminished eNOS activity reflects endothelial dysfunction. Several miRNAs regulate NO production through PI3K/Akt/eNOS signalling pathway. Evidence suggests that miR-126 upregulates eNOS phosphorylation by PI3K/Akt pathway signalling, leading to increase NO production and improve endothelial function [11]. MiR-133a, miR-138 and miR-615-5p are involved in regulating eNOS expression and NO bioavailability to maintain vascular tone [12]. Several miRNAs also negatively regulate NO production and aggravate endothelial injury. Studies indicate a correlation between higher circulating level of miR-92a and lower NO bioavailability and pathological vascular dilatation [13]. All in all, these studies show that miRNAs contribute to endothelial dysfunction of PAD through regulation of the eNOS signalling pathway.

3.1.2. miRNAs associated with reactive oxygen species (ROS) generation

In PAD, oxidative stress comes from an imbalance indicated by the overproduction of reactive oxygen species (ROS) and impairment of antioxidant metabolism as a most important reason of disease progression. miRNAs are involved in both production and elimination of ROS and thus have bidirectional effects on oxidative stress. Abolhasani et al. [14] reported that miR-21 enhances accumulation of ROS by down-regulating gene expression of antioxidant system, which magnifies oxidative stress. Conversely, miR-210 may facilitate the adaptation of cells to oxidative injury. MiRNAs are involved in oxidative stress regulation via manipulating major antioxidant systems, such as Nrf2/Keap1 signalling pathway. Nrf2 is a transcription factor for maintenance of cellular redox system, which could be modulated by some miRNAs and finally affects the expression level of antioxidant enzymes [15]. Furthermore, a feedback regulation of ROS can change the expression of miRNAs, whereas miRNAs, at the same time, regulate ROS production, thus they form a feedback mechanism that dominates the generation of oxidative stress in PAD [16].

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3.2. Regulation of vascular smooth muscle cell phenotypic switching and proliferation

In PAD and atherosclerosis, vascular smooth muscle cells (VSMCs) undergo phenotypic transition from contractile to synthetic. miRNAs play a role in this process by regulating expression of several genes that confer properties of cell proliferation, migration and differentiation. Wang et al. [13] found that exosome-mediated transfer of miR-92a from endothelial cells to VSMCs drove phenotypic shift from contractile to synthetic states and enhanced cell proliferation, contributing to vascular remodelling.

In addition, miR-143/145 and miR-21 regulate the phenotypic state of VSMCs by influencing the proliferation and migration of VSMCs via transcription factor and other signalling pathways. Changes in the concentration of circulating miRNAs reflect the extent of vascular remodelling and therefore may be a useful biomarker for monitoring PAD.

3.3. Regulation of inflammation and immune responses

Chronic inflammation is one of the major drivers of the progression of PAD. MiRNAs control inflammation by regulating immune cell function and cytokines expression.

3.3.1. miRNAs regulating macrophage polarization and cytokine production

In atherosclerosis, macrophages play an important role in plaque formation. microRNAs modulate macrophage polarity to the pro-inflammatory M1 or anti-inflammatory M2 phenotype. It has been shown that miR-181b and miR-223 could suppress inflammatory responses by inhibiting the NLRP3 inflammasome and suppressing plaque formation [12]. In addition, miRNAs also affect inflammatory responses by targeting related signalling pathways, such as NF-κB, which in turn influences the synthesis of inflammatory factors such as TNF-α and IL-6. Changes in circulating miRNA levels, for instance, can thus represent a measurement of inflammation, making miRNAs important in PAD-associated inflammation.

3.3.2. miRNAs associated with neutrophil extracellular trap (NET) formation

Activated neutrophils discharge DNA-based networks, called neutrophil extracellular traps (NETs), which contain histones and granule proteins. NETs play a role in thrombosis regulation and inflammation. Study work in this field is just beginning, but accumulating findings indicate that miRNAs can modulate vascular inflammation by controlling neutrophil activation and NET formation [17].

3.4. Role in ischemia-induced angiogenesis and collateral vessel formation

Reduced perfusion in the limbs in PAD causes angiogenesis and collateral vessel development as an attempt at compensation to restore blood perfusion. miRNAs, such as miR-126 and miR-210, play a role in the growth and migration of endothelial cells through modulating the VEGF- and HIF-1α-associated signalling pathways [18]. Among them, miR-210 is widespread in response to hypoxic conditions, with its expression substantially upregulated under hypoxic conditions, assisting cellular adaptation to hypoxia.

In addition, miRNAs modulate endothelial cells interaction with surrounding cells, enabling further development of collateral vessels. Altered levels of circulating miRNAs might reflect

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angiogenic potential, and therefore their potential is a useful marker of collateral development and predictability of clinical outcomes in PAD.

4. Circulating miRNAs as potential therapeutic targets in PAD

As the function of miRNAs in PAD has evolved and matured, miRNAs have slowly turned from biomarkers to promising therapeutic candidates. MiRNAs modulate multiple signalling pathways and take part in angiogenesis, inflammation, phenotype switch of vascular cells, presenting great potential for precision therapy of PAD. However, multiple hurdles still restrict these findings from being clinically applied.

4.1. miRNA-based therapeutic strategies

Current therapies involving targeting miRNAs can mainly be categorized into two types: giving the restoration of downregulated protective miRNAs by miRNA mimics and giving inhibition of upregulated pathogenic miRNAs by the inhibition effects of antisense oligonucleotides, such as antagomirs or anti-miRs. The idea of these therapies is to reverse the disease-related gene network disorder by modulating the expression level of miRNA, to balance the dysregulated gene network. For example, previous studies found that enhancement of endothelial repair and angiogenesis can be achieved by miR-126 mimics [19], while suppression of miR-92a by antagomirs can enhance endothelial function and promote blood vessel regeneration [20]. In recent years, chemical modifications of miRNA-based therapeutics have significantly advanced their stability and their ability to deliver in vivo, providing important technical support for the clinical applications of the new drugs [21].

Nevertheless, miRNAs also show a 'one-to-many' effect, meaning one miRNA controls several genes. This advantage of miRNAs makes them more powerful effectors, but also exposes their efforts to possible off-target effects and unintended outcomes. The wide effects of miRNA modulation need to be taken into account carefully when employing miRNA therapeutics.

4.2. Targeting specific miRNAs in PAD animal models

In recent years, intervention studies in animal models of PAD and limb ischemia towards specific miRNAs have yielded positive results. Of these, miR-92a is one of the most studied targets, as it negatively regulates angiogenesis in endothelial cells. Blocking miR-92a results in amelioration of endothelial function, induction of angiogenesis in the ischemic tissue and increased perfusion to the limb. More importantly, intercellular crosstalk between ECs and VSMCs is regulated by miR-92a, it drives phenotypic conversion of VSMCs from contractile to synthetic state and promotes vascular remodelling [13]. Ye et al. [22] reported that the contractile phenotypic state of VSMCs is maintained by miR-143/145 cluster. A decrease in this cluster causes vascular remodelling and development of atherosclerosis. Restoration of their expression inhibits abnormal VSMCs proliferation and prevents vascular remodelling.

In addition, several miRNA mimics have been shown to augment angiogenesis in preclinical studies of PAD, which highlight their therapeutic potential. This experimental evidence is particularly important for miRNA-targeted therapies[23].

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4.3. Challenges in clinical translation

Although promising therapeutic prospects, the clinical application of miRNA-based therapies in PAD faces several challenges. First, effective delivery systems are a bottleneck. miRNAs are unstable in vivo and poorly able to deliver to target tissues specifically [21]. Second, off-target effects are an issue of safety. Due to the rich ability of miRNAs to target numerous genes at once, one might have undesirable biological effects. Some miRNA therapy has been interrupted in the clinical trial stage owing to its toxicity, hence good safety assessment before application is essential [23]. Third, individual variation will affect therapeutic effects. The heterogeneity of individuals, heterogeneity of disease stage, heterogeneity of individual metabolism in PAD patients leads to the difference in the miRNA expression pattern and function, which in turn influence the therapeutic effect. Besides, lack of replicability of studies due to inconsistency of the detection methods and data processing.

5. Conclusion

As a type of emerging biomarkers, circulating miRNAs are closely associated with PAD and are involved in PAD progression. They affect a series of pathologic events, including endothelial injury, oxidative imbalance, VSMC phenotype change, inflammation and new vessel formation, which deeply understand the molecular mechanism of PAD. In addition, their unique expression pattern indicates their potential use for early PAD diagnosis and prediction. In addition, miRNA-based therapeutic methods and technology have demonstrated favorable effects in preliminary animal studies, indicating their great potential for precision treatment as novel methods of PAD. Some issues should be overcome before a clinical application of miRNA can be achieved. This involves lack of standardized methods of detection, difficulty of delivery methods and concerns about safety and off-target effects. Future studies will need to focus on large and multicentre studies to confirm the clinical utility of circulating miRNAs, while also advancing detection technologies and optimizing therapeutic strategies. Progress in these areas is necessary to enable the clinical use of miRNA-based approaches and enhance outcomes in PAD treatment.

References

[1] Song P, Rudan D, Zhu Y, et al. Global, regional, and national prevalence and risk factors for peripheral artery disease in 2015: an updated systematic review and analysis. Lancet Glob Health. 2019; 7(8): e1020-e1030. doi: 10.1016/S2214-109X(19)30255-4

[2] Poredoš P, Šabovič M, Božič Mijovski M, et al. Inflammatory and Prothrombotic Biomarkers, DNA Polymorphisms, MicroRNAs and Personalized Medicine for Patients with Peripheral Arterial Disease. Int J Mol Sci. 2022; 23(19): 12054. doi: 10.3390/ijms231912054

[3] Saenz-Pipaon G, Martinez-Aguilar E, Orbe J, et al. The Role of Circulating Biomarkers in Peripheral Arterial Disease. Int J Mol Sci. 2021; 22(7): 3601. doi: 10.3390/ijms22073601

[4] Karaca Dogan B, Salman Yilmaz S, Izgi GN, Ozen M. Circulating non-coding RNAs as a tool for liquid biopsy in solid tumors. Epigenomics. 2025; 17(5): 335-358. doi: 10.1080/17501911.2025.2467021

[5] Sanz-Rubio D, Martin-Burriel I, Gil A, et al. Stability of Circulating Exosomal miRNAs in Healthy Subjects. Sci Rep. 2018; 8(1): 10306. doi: 10.1038/s41598-018-28748-5

[6] Sandau US, Wiedrick JT, McFarland TJ, et al. Analysis of the longitudinal stability of human plasma miRNAs and implications for disease biomarkers. Sci Rep. 2024; 14(1): 2148. doi: 10.1038/s41598-024-52681-5

[7] Katayama ES, Hue JJ, Loftus AW, et al. Stability of microRNAs in serum and plasma reveal promise as a circulating biomarker. Noncoding RNA Res. 2025; 15: 132-141. doi: 10.1016/j.ncrna.2025.08.001

[8] Vafadar A, Alavimanesh S, Babadi S, Hosseinpour V, Ehtiati S, Savardashtaki A. MicroRNA biosensors for cardiovascular disease detection. Clin Chim Acta. 2026; 578: 120525. doi: 10.1016/j.cca.2025.120525

[9] Wu Y, Li Q, Zhang R, Dai X, Chen W, Xing D. Circulating microRNAs: Biomarkers of disease. Clin Chim Acta. 2021; 516: 46-54. doi: 10.1016/j.cca.2021.01.008
[10] Felekkis K, Papaneophytou C. The Circulating Biomarkers League: Combining miRNAs with Cell-Free DNAs and Proteins. Int J Mol Sci. 2024; 25(6): 3403. doi: 10.3390/ijms25063403
[11] Song W, Liang Q, Cai M, Tian Z. HIF-1α-induced up-regulation of microRNA-126 contributes to the effectiveness of exercise training on myocardial angiogenesis in myocardial infarction rats. J Cell Mol Med. 2020; 24(22): 12970-12979. doi: 10.1111/jcmm.15892
[12] Silva VR, Azar A, Goncalves ER, et al. MicroRNA-Mediated Regulation of Vascular Endothelium: From Pro-Inflammation to Atherosclerosis. Int J Mol Sci. 2025; 26(13): 5919. doi: 10.3390/ijms26135919
[13] Wang C, Wu H, Xing Y, et al. Endothelial-derived extracellular microRNA-92a promotes arterial stiffness by regulating phenotype changes of vascular smooth muscle cells. Sci Rep. 2022; 12(1): 344. doi: 10.1038/s41598-021-04341-1
[14] Abolhasani S, Ahmadi Y, Fattahi D, Rostami Y, Chollou KM. microRNA-Mediated Regulation of Oxidative Stress in Cardiovascular Diseases. J Clin Lab Anal. 2025; 39(9): e70017. doi: 10.1002/jcla.70017
[15] Saha S. Role of microRNA in Oxidative Stress. Stresses. 2024; 4(2): 269-281. doi: 10.3390/stresses4020016
[16] Mansour RM, Hemdan M, Eldein KMA, et al. Crosstalk between miRNAs and oxidative stress: implications for cancer pathogenesis and therapy. Funct Integr Genomics. 2025; 25(1): 259. doi: 10.1007/s10142-025-01737-4
[17] Chen L, Hu L, Li Q, Ma J, Li H. Exosome-encapsulated miR-505 from ox-LDL-treated vascular endothelial cells aggravates atherosclerosis by inducing NET formation. Acta Biochim Biophys Sin (Shanghai). 2019; 51(12): 1233-1241. doi: 10.1093/abbs/gmz123
[18] Naderi R, Mohaddes G, Mohammadi M, et al. The Effect of Garlic and Voluntary Exercise on Cardiac Angiogenesis in Diabetes: The Role of MiR-126 and MiR-210. Arq Bras Cardiol. 2019; 112(2): 154-162. doi: 10.5935/abc.20190002
[19] Gao S, Gao H, Dai L, et al. miR-126 regulates angiogenesis in myocardial ischemia by targeting HIF-1α. Exp Cell Res. 2021; 409(2): 112925. doi: 10.1016/j.yexcr.2021.112925
[20] Gou L, Zhao L, Song W, et al. Inhibition of miR-92a Suppresses Oxidative Stress and Improves Endothelial Function by Upregulating Heme Oxygenase-1 in db/db Mice. Antioxid Redox Signal. 2018; 28(5): 358-370. doi: 10.1089/ars.2017.7005
[21] Tian H, Cheng L, Liang Y, et al. MicroRNA therapeutic delivery strategies: A review. Journal of Drug Delivery Science and Technology. 2024; 93: 105430. doi: 10.1016/j.jddst.2024.105430
[22] Ye Z, Zhu S, Li G, et al. Early matrix softening contributes to vascular smooth muscle cell phenotype switching and aortic dissection through down-regulation of microRNA-143/145. J Mol Cell Cardiol. 2024; 192: 1-12. doi: 10.1016/j.yjmcc.2024.05.002
[23] Brillante S, Volpe M, Indrieri A. Advances in MicroRNA Therapeutics: From Preclinical to Clinical Studies. Hum Gene Ther. 2024; 35(17-18): 628-648. doi: 10.1089/hum.2024.113