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LITERATURE REVIEW

MicroRNAs in Atherosclerosis: Key Players, Diagnostic Potential, and Therapeutic Opportunities

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Abstract: Atherosclerosis is a significant contributor to cardiovascular diseases, often progressing silently over many years before clinical symptoms manifest. The multifaceted pathogenesis of atherosclerosis, involving processes such as endothelial dysfunction and inflammation, presents challenges in early diagnosis and effective treatment. MicroRNAs (miRNAs), a class of non-coding RNA molecules with post-transcriptional gene regulation abilities, have emerged as potential key players in atherosclerosis pathophysiology. However, there is a research gap in fully understanding the distinct roles and therapeutic potential of specific miRNAs in atherosclerosis management. This review aims to investigate and synthesize the current knowledge on prominent miRNAs associated with atherosclerosis initiation and progression, highlighting the need for precise diagnostic biomarkers and innovative therapeutic strategies. The objectives of this study are to elucidate the roles of key miRNAs in atherosclerosis development, identify diagnostic biomarker candidates, and explore potential therapeutic interventions targeting miRNAs to mitigate atherosclerosis-related complications efficiently.

Keywords: Atherosclerosis, Mirna, Epigenetics, Cardiovascular Disease, Artery

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Introduction

Atherosclerosis is one of the primary contributors to cardiovascular disease, significantly impacting health and leading to increased rates of illness and death, progresses through distinct phases of initiation, plaque advancement, and damage, involving complex cellular interactions within the arterial walls [1]. Early development of atherosclerosis often occurs asymptomatically over years, underscoring the need for preemptive diagnostic and therapeutic strategies to mitigate adverse cardiovascular outcomes [2]. The complex development of atherosclerosis is affected by both genetic and epigenetic factors, with new research underscoring the regulatory function of microRNAs (miRNAs) in influencing crucial pathways related to the formation and progression of atherosclerotic plaques [3, 4]. MiRNAs are small non-coding RNA molecules that play a significant role in regulating post-transcriptional gene expression, drawing attention for their potential effects on the pathophysiology of atherosclerosis. Nonetheless, gaining a thorough understanding of the specific contributions of individual

miRNAs, along with their diagnostic and therapeutic potential, is vital to exploring new strategies for managing the disease. This review's uniqueness stems from its integration of existing knowledge regarding specific miRNAs associated with atherosclerosis and their importance as possible diagnostic markers and therapeutic targets. By filling this knowledge gap, the review seeks to shed light on the unique molecular mechanisms driving the progression of atherosclerosis and explores the potential of miRNA-based therapies to enhance clinical outcomes.

Methods

This review utilized a systematic search strategy across major scientific databases to identify relevant studies focusing on miRNAs associated with atherosclerosis pathogenesis. Selection criteria included articles published within a specified timeframe, with a main emphasis on exploring the functions of miRNAs in the progression of atherosclerosis. Data extraction involved summarizing key findings related to the functions of specific miRNAs, their molecular targets, and implications for atherosclerosis progression. The methodology of this review was designed to deliver an extensive summary of existing research on the role of miRNAs in atherosclerosis and explored potential avenues for future research and therapeutic interventions in the field.

MiR-181b

Extensive research findings consistently highlight the significant role of miR-181b in suppressing endothelial inflammatory responses. This microRNA functions as a powerful inhibitor by specifically targeting the transmission of NF-kB pathway is involved in both acute and chronic vascular conditions. Notably, miR-181b acts exclusively in endothelial cells and does not impact leukocytes. The mechanism revolves around the targeting of importin-α3, an essential protein that aids in the movement of NF-kB into the cytoplasm and nucleus [6]. In leukocytes, importin-α5 serves as the dominant isoform for mediating the nuclear import of NF-kB, and miR-181b does not affect its function. Consequently, the NF-kB signaling pathway in leukocytes remains unaffected. With systemic intravenous administration, miR-181b decreased the activation of NF-kB in endothelial cells [7]. Due to this, leukocyte recruitment is suppressed and atherosclerotic lesions are formed in ApoE−/-mice prone to atherosclerosis. Furthermore, it is noteworthy that the anti-atherosclerotic properties of miR-181b are independent of any alterations in the lipid profile. This finding aligns with studies conducted on knockout mice with endothelial-specific NF-kB (IKKy) and transgenic mice expressing dominant-negative IkBa, which exhibited protection against the formation of atherosclerotic lesions [8]. In vitro studies suggest that various pro-inflammatory stimuli, including TNF-α and LPS, lead to a decrease in miR-181b levels in endothelial cells. In vivo studies revealed that after only four weeks on a high-cholesterol diet, ApoE−/- mice demonstrated a significant decline in miR-181b expression by approximately 53% in the vascular endothelium and approximately 40% in mouse plasma [9]. This indicates that altered levels of miR-181b expression might increase the likelihood of vascular wall inflammation in individuals. Additionally, reduced miR-181b expression was observed in the blood plasma of people diagnosed with Coronary Artery Disease (CAD) through angiography. Therapeutically targeting miR-181b specifically in the endothelial cells to inhibit NF-kB activity holds promise in maintaining optimal protection against infectious pathogens, compared to targeting myeloid cells [10]. Notably, the delivery of miR-181b has demonstrated inhibitory effects on endothelial inflammation and has demonstrated protective effects against sepsis in mice. These results highlight the significant role of miR-181b as a regulator of NF-kB signaling in the vascular endothelium in response to different stimuli. Furthermore, these results present new prospects for "substitution" therapy aimed at combating inflammation [11].

MiR-146a

miR-146a is an additional microRNA that is responsive to cytokines and contributes to the protection of blood vessel walls against atherosclerosis. In Endothelial Cells (ECs), cytokines such as TNF-α and IL-1β trigger the expression of miR-146a and miR-146b, albeit after a delay that aligns with the reduction of inflammatory gene activity. Increased miR-146a levels in ECs demonstrate a suppressive effect on the cytokine response [12], indicating that it may function in a negative feedback loop to curb the spread of inflammation signals within ECs. Furthermore, miR-146a expression rises in atherosclerotic plaques found in both humans and mice. By directly targeting HuR, an RNA-binding protein that downregulates endothelial nitric oxide synthase (eNOS), miR-146a inhibits the NF-kB and MAPK signaling pathways [13]. It also targets TRAF6 and IRAK1, two adapter proteins, to reduce the activation of EC adhesion molecules. Unlike miR-181b, miR-146a modulates NF-kB signaling in both ECs and macrophages. Overexpression of ApoE in ApoE−/− macrophages increases miR-146a levels, resulting in a decreased progression of atherosclerotic lesions both locally in macrophages and systemically when miR-146a is administered [14]. Future research is required to clarify the specific cell types and mechanisms through which miR-146a

exerts these beneficial effects in experimental atherosclerosis. Additionally, due to its anti-inflammatory properties in regulating various immune cells, miR-146a likely plays a broader role in mitigating inflammatory responses. Importantly, miR-146 serves as another crucial cytokine-sensitive microRNA that may help diminish EC inflammation through negative feedback mechanisms [15].

MiR-92

miR-92a, part of the miR-17~92 cluster, is predominantly expressed in endothelial cells, and its levels fluctuate in response to shear stress, as observed in both experimental and biological contexts. In vitro studies demonstrate that exposure to pulsatile laminar flow leads to a decrease in miR-92a expression, whereas disturbed flow conditions result in its upregulation. Notably, miR-92a interacts with the 3'-UTR of KLF2, a transcription factor sensitive to flow changes [16], highlighting its potential role in modulating laminar blood flow. Additionally, in vivo analyses reveal that miR-92a is significantly upregulated in atherosclerosis-prone areas of the aortic arch compared to less affected regions. In laboratory settings, enhanced miR-92a expression has been found to inhibit KLF2 and KLF4 through their respective 3'-UTRs. Moreover, the ability of miR-92a to reduce TNF-α-induced cytokine release and leukocyte adhesion may largely rely on the activity of KLF4 siRNAs [17]. This indicates a partial dependence on KLF4. In a microchip profiling study conducted by Loyer et al., In experiments where Endothelial Cells (ECs) were subjected to oxLDL and low shear stress, it was discovered that the upregulation of miR-92a and pro-inflammatory markers (MCP-1 and IL-6) occurs, reliant on STAT3 [18]. An increase in miR-92a levels within Endothelial Cells (ECs) leads to a decrease in the expression of flow-regulated transcription factors KLF2 and KLF4. In contrast, reducing miR-92a levels correlates with lower EC inflammation and a significant decrease in phospho-p65 expression. Functionally, miR-92a targets SOCS5 in ECs under conditions of oxidized LDL (oxLDL) and low shear stress. The role of SOCS5 in the development of atherosclerosis in vivo remains uncertain [19]. On the other hand, silencing SOCS5 with siRNA resulted in increased levels of MCP-1 and IL-6 in ECs, but did not alter the expression of eNOS, KLF2, or KLF4. Importantly, experiments in mice showed that inhibiting miR-92a in LDLR−/− mice led to reduced EC inflammation and slowed the development of atherosclerotic lesions [20]. Additionally, mice with endothelial cell-specific deletion of miR-92a, induced by Tie2, demonstrated resistance to neointimal formation after mechanical arterial injury, which was partly attributed to enhanced re-endothelialization. Given the positive effects of miR-92a deficiency in processes such as re-endothelialization following arterial injury and angiogenesis in response to myocardial or peripheral ischemia, targeting miR-92a may prove beneficial for a range of cardiovascular conditions [21].

MiR-126

miR-126 is predominantly expressed in Endothelial Cells (ECs) and is essential for regulating both inflammation and angiogenesis, particularly in response to variations in fluid flow. Initial research indicated that miR-126 interacts in vitro with the 3'-UTR of VCAM-1, thereby reducing leukocyte adhesion. Numerous mouse studies have shown that a lack of miR-126 results in compromised vascular integrity, as well as reduced endothelial cell proliferation and angiogenesis. Additionally, it has been found that KLF2 can activate miR-126 to influence angiogenesis in a manner dependent on blood flow conditions [22]. Regarding atherosclerosis, studies by researchers like Zernecke et al. identified miR-126 as the most abundant miRNA in apoptotic bodies released from endothelial cells [23]. In this context, miR-126 enhances CXCL12 expression by targeting RGS16, which negatively regulates SDF-1/CXCR4 signaling and the mobilization of precursor cells. As a result, the intravenous administration of apoptotic endothelial cells prompts the mobilization of progenitor cells into the bloodstream, improving their integration into aortic plaques [24]. Due to this, the progression of atherosclerosis was suppressed in a manner dependent on miR-126. Schuber et al. conducted a study focusing on the functional role of miR-126-5p, a passenger chain found within the vascular endothelium. They discovered that miR-126-5p is sensitive to miRNA flow and its expression is suppressed by D-flow. Interestingly, this suppression contributes to lesion development by activating delta-like 1 homologue (Dlk-1), a negative regulator of endothelial cell proliferation [25]. In contrast, the systemic administration of miR-126-5p enhances endothelial cell proliferation in areas at risk and helps to inhibit lesion progression. Additionally, pro-inflammatory transcription factors Ets-1 and Ets-2 are involved in the upregulation of miR-126 expression. This allows the miR-126 duplex to function as a feedback regulator, reducing pro-inflammatory factors such as TNF-α and Ang-II within the vascular endothelium. Notably, when endothelial cells are subjected to L-flow, they display KLF2-dependent expression of pri-miR-126, but not of miR-126-3p [26]. Moreover, miR-126-5p expression levels were found to be lower in the ApoE−/− aorta, unlike miR-126-3p. Additionally, the administration of miR-126-5p antagomers, unlike those for miR-126-3p, resulted in increased damage after endothelial cell denudation in ApoE−/− mice. These findings suggest that miR-126-3p may not play a role in vessel protection in response to flow. The presence of atheroprotective laminar flow stimulates the release of miR-126, which

is associated with Ago2, into extracellular microvesicles [27]. Extracellular microvesicles serve as a mediator for increasing VSMC turnover by targeting genes (e.g., BCL2, FOXO3, and IRS1) that are involved in maintaining the atheroprotective phenotype of VSMC contraction. In this study, the absence of miR-126 inhibited neointimal formation in the carotid arteries of mice following blood flow cessation. However, this effect was reversed when miR-126 mimics or conditioned media from static endothelial cell cultures were administered [28]. Recent findings imply that while miR-126 has an atheroprotective role in Endothelial Cells (ECs), it may compromise the stability of Smooth Muscle Cells (SMCs) within fibrous plaques, potentially leading to their destabilization. Future research is needed to explore the specific roles of the miR-126-5p/miR-126-3p duplex in relation to various biochemical and biomechanical stimuli during different stages of atherosclerosis [29].

MiR-712/MiR-205

The biogenesis of miRNAs generally occurs through the classical pathway that includes the enzymes Drosha/DGCR8 and Dicer. However, research suggests the significance of alternative pathways for miRNA maturation. One such example is miR-712 (equivalent to miR-205 in humans), which was found to be a responsive miRNA derived from non-canonical pre-ribosomal RNA derived from the RN45S gene. miR-712 induced by d-flow targets TIMP3, an effect that increases MMPs and ADAMs to stimulate pro-inflammatory EC reactions [30]. Thus, in a study on mice, it was found that due to the neutralization of miR-712, the levels of TIMP3 are reduced, which facilitates the advancement of atherosclerosis. Additional comprehensive investigations are necessary to evaluate the direct in vivo impact of miR-712 neutralization on various pertinent cell types, including immune cell subsets and vascular smooth muscle cells [31].

MiR-143/MiR-145

The transfer of miRNA between cells within tissues has introduced a new concept of intercellular communication. An intriguing example of this phenomenon is observed in Endothelial Cells (ECs), where the overexpression of L-flow or KLF2 triggers the release of extracellular microvesicles containing miR-143 and miR-145. These microvesicles, in turn, confer atheroprotective properties to neighboring Vascular Smooth Muscle Cells (VSMCs) [32]. Moreover, the administration of these extracellular vesicles via intravenous delivery has been shown to impede the progression of atherosclerotic lesions in a miR-143/145-dependent manner. In vitro research has shown that miR-143 and miR-145, which are expressed at greater levels in Vascular Smooth Muscle Cells (VSMCs) than in Endothelial Cells (ECs) under typical conditions, play a role in intercellular communication between VSMCs and ECs through specialized tubes known as Tunnel Nanotubes (TNT) [33]. This transition from VSMCs to ECs is supported by experimental evidence demonstrating that targeted deletion of the miR-143/miR-145 cluster in smooth muscle cells effectively inhibits the expression of miR-143/miR-145 in coronary artery endothelial cells. Notably, this phenomenon occurs in response to pressure overload caused by transaortic constriction may be influenced by the activation of the TGF-β signaling pathway. Furthermore, the targets of miR-143/miR-145 include hexokinase II and integrin-β8 are implicated in this process [34]. Taken together, these results raise a provocative question about distorted, preferred intercellular “miRNA shifts” that wax during the progression or regression of atherosclerosis, as well as in response to pharmacological therapy. However, it is yet to be determined whether there is a bidirectional transfer of extracellular miRNA between Smooth Muscle Cells (SMCs) and Endothelial Cells (ECs) in the context of atherosclerosis [35]. MiR-143 and miR-145, which are closely linked, are transcribed together as a single primary transcript. These microRNAs are predominantly found in Vascular Smooth Muscle Cells (VSMCs) and the medial layer of blood vessel walls. Their expression levels decline in response to vascular injury or atherosclerosis. Current studies indicate that miR-143/miR-145 are essential for regulating the contractile activities of VSMCs [36]. Mice deficient in miR-143/miR-145 demonstrate reduced expression and impaired function of contractile markers in VSMCs, disrupted actin stress fibers and cytoskeletal organization, as well as a thinner medial vessel wall. Furthermore, these mice demonstrate lower baseline blood pressure and reduced responsiveness to vasopressors, this reduction can be linked to decreased expression of the target gene, Angiotensin-Converting Enzyme (ACE) [37]. Conversely, delivering miR-145 to ApoE−/− mice via lentivirus controlled by the Smooth Muscle Cell (SMC)-specific promoter SM22a has been shown to significantly diminish the size of atherosclerotic plaques. This treatment promotes a more favorable plaque composition, which includes an enlarged fibrous cap area, increased collagen levels, a smaller necrotic core, and a reduced presence of macrophages [38]. Given that miR-145 is crucial for enhancing the contractile phenotype of VSMCs, these plaque-stabilizing outcomes are mechanistically linked to a decrease in KLF4 alongside an increase in myocardine expression within the vessel walls of ApoE−/− mice. Similar protective effects of miR-145 have also been noted in the formation of neointimal vascular lesions. In addition to KLF4 and myocardine, miR-143/miR-145 targets other important transcriptional regulators, such as ELK-1 and KLF5, which are involved in modulating VSMC differentiation [39]. The expression of miR-143/miR-145 is partly influenced by factors like Serum Response Factor (SRF), Nkx2.5, and myocardine,

indicating their role in fine-tuning the SMC-specific transcriptional program. This fine-tuning is essential for regulating the contractile state of VSMCs [40].

MiR-221/MiR-222

Unlike miR-143 and miR-145, the microRNAs miR-221 and miR-222 exhibit elevated expression levels in response to damage associated with neointimal lesions. Studies conducted in vitro have demonstrated their role in modulating VSMC proliferation triggered by PDGF. Upon exposure to PDGF, the expression of miR-221 and miR-222 was upregulated. This led to a decrease in the expression of their target genes, Kip2 and p27Kip1, as well as a reduction in the expression of the SMC-specific contractile gene [41]. Reducing the levels of miR-221 and miR-222, which target p27 (Kip1) and p57 (Kip2), resulted in decreased proliferation of VSMCs and reduced neointimal damage following mechanical injury.

In humans, atherosclerotic lesions displayed lower levels of miR-221 and miR-222 in the plaque shoulder of patients who had undergone Carotid Endarterectomy (CEA) within five days post-acute neurological events [42]. This observation strongly links diminished expression of miR-221 and miR-222 to unstable lesions, which also correlates with a notable increase in their target gene, p27 (Kip1). Additionally, the expression of miR-222 was found to decrease in isolated human Endothelial Cells (ECs) as they progressed from early lesions to advanced plaques [43]. Overall, these findings suggest that miR-221 and miR-222 may contribute to the destabilization of atherosclerotic lesions.

MiR-21

MiR-21 has been linked to the promotion of VSMC proliferation in several models of mechanical vascular injury. However, its specific role in the development of atherosclerotic lesions remains unclear. Following mechanical damage caused by a balloon catheter, the inhibition of miR-21 led to a decrease in neointimal lesion formation [44]. Additionally, the expression of miR-21 was found to be significantly higher in isolated "dedifferentiated" VSMCs compared to mature, differentiated VSMCs. In accordance with these results for miR-21 with vascular damage in rodents, delivery of a stent coated with anti-miR-21 into the internal mammary arteries of a person damaged by a balloon Using a rat model that mimics human physiology, researchers observed beneficial effects on the prevention of neointimal damage [45]. The mechanistically proliferative properties of miR-21 may be due to its ability to target PTEN in Vascular Smooth Muscle Cells (VSMCs) and indirectly enhances the expression of the anti-apoptotic gene Bcl-2. Moreover, miR-21 plays a role in promoting the contractile phenotype of VSMCs. The communication of TGF-β and BMP signals during specific post-transcriptional processes seems to influence Smad proteins regulating the maturation of miRNAs controlled by DROSHA. To determine if inhibiting miR-21, which has antiproliferative effects, can also slow down the advancement of atherosclerosis in undamaged blood vessels, further studies are needed [46].

MiR-10a, MiR-663, MiR-155, MiR-30-5p

Various profiling techniques have helped uncover additional miRNAs (such as miR-10a, miR-663, and miR-155) that are responsive to mechanical forces. However, the specific roles of miR-10a and miR-663 in the regulation of experimental atherosclerosis in mice have not yet been established. Additionally, the function of miR-155 is still not well understood. It is worth noting that they are noticeably manifested in places of predisposition in the macrovascular network [47]. To illustrate, a study conducted on miRNA expression in ECs from various regions of the porcine aorta (internal arch and descending thoracic aorta) revealed a significant decrease in miR-10a levels within atherosclerotic regions. This reduction in miR-10a was found to impact the regulation of key factors involved in NF-kB signaling, specifically MAPK kinase kinase 7 and the β-transducin repeat-containing gene. Notably, the overexpression of miR-10a demonstrated its ability to inhibit the canonical transmission of NF-kB signals in vitro EC models [48]. To comprehend the role of miR-10a in Endothelial Cell (EC) function and its effects on atherosclerosis in mice, therapeutic interventions will be necessary. These interventions will help evaluate miR-10a's contribution to EC activity in areas prone to lesion formation and disease progression [49]. It is worth noting that miR-10b, a member of the miR-10a family, has been associated with facilitating tumor invasion and metastasis by targeting similar genes. Therefore, the administration of miR-10 mimetics as a potential therapeutic approach should be carefully studied to ensure its beneficial effects [49].

miR-663, was referred as miRNA, which is stimulated by D-flow in the ECs. The generation of miR-663 and miR-712 in humans is linked to the ribosomal RNA gene, RN45S [50]. Interestingly, the spacer regions that give rise to these RNAs can be rapidly degraded by the XRN1 exonuclease. Son et al. conducted a study to explore how the suppression of XRN1 affects the expression levels of miR-663 and miR-712. They discovered that reducing XRN1 levels using D-flow in the predisposition

regions of the mouse carotid artery and aortic arch resulted in decreased XRN1 expression. Additionally, when XRN1 was lacking in Endothelial Cells (ECs) in vitro, there was a notable rise in the expression of miR-663 and miR-712. These results underscore the unique regulatory pathways and the potential for therapeutic intervention involving these specific mechanosensitive miRNAs within the vascular endothelium [50].

Compared to the lower aortic arch, miR-155 increases due to L-flow in the ECs and in the thoracic aorta. In terms of mechanisms, miR-155 has the ability to bind to both pro-inflammatory targets (such as MYLK, RhoA, SOCS-1, and Bcl6) and anti-inflammatory targets (such as eNOS). Research examining the role of miR-155 in mouse models of experimental atherosclerosis has uncovered information that highlight its dual nature in promoting and inhibiting atherosclerosis, depending on the particular circumstances [51]. For example, LDLR−/− mice that had bone marrow deficient in miR-155 showed heightened atherosclerosis and lower plaque stability. These results highlight the intricate influence of miR-155 on atherosclerosis and its involvement in both pro- and anti-atherosclerotic processes. Conversely, the absence of miR-155 in macrophages led to a reduction in the size of atherosclerotic plaques in ApoE−/− mice. This effect is attributed to miR-155's ability to target BCL6, which decreases the macrophage recruitment mediated by CCL2 [52]. Additionally, when delivered in vivo, miR-155 targets MAP3K10, resulting in a reduction of atherosclerotic lesion formation.

Moreover, since miR-155 also affects Myosin Light Chain Kinase (MYLK) in endothelial cells, and MYLK deficiency in ApoE−/− mice has been shown to reduce atherosclerosis by enhancing endothelial barrier function and inhibiting monocyte migration, miR-155 derived from the endothelium may be viewed as a potentially advantageous miRNA within the vascular wall. This idea is supported by the observation that miR-155 expression is significantly higher in the thoracic aorta, which experiences unidirectional shear stress, compared to the lower curvature of the aortic arch, where shear stress is lower [53]. Nonetheless, despite these findings, several questions remain about the exact role of miR-155 in vascular inflammation, highlighting the need for further research to clarify its specific contributions in endothelial cells and macrophages during atherogenesis.

In addition, the presence of laminar flow shear stress or the overexpression of BCL2 in endothelial cells triggers the expression of members of the miR-30-5p family. These miRNAs subsequently decrease the levels of anti-inflammatory markers such as VCAM-1 and ICAM-1 by targeting angiopoietin-2. However, further studies are required to investigate the specific roles of miR-30-5p family members in modulating endothelial inflammation and atherosclerosis in mouse models [54].

The effects of miRNAs on various cell types involved in atherogenesis are summarized in Table 1.

Table 1: Effects of miRNAs on various cell types in atherogenesis

miRNA	Target	Effect	Cells	Reference
MiR-181b	importin-α3	inhibits the transmission of NF-kB signals	endothelial cells	[6]
MiR-146a	HuR	inhibits NF-kB and MAPK signaling pathways	endothelial cells	[13]
MiR-146a	TRAF6 and IRAK1	suppresses the induction of EC adhesion molecules; inhibits the transmission of NF-kB signals	endothelial cells, macrophages	[14]
miR-92	KLF4	inhibition of TNF-α-induced cytokines and leukocyte adhesion	endothelial cells	[17]
miR-126	3'-UTR VCAM-1	limit leukocyte adhesion	endothelial cells	[22]
MiR-712	TIMP3	increases MMPs and ADAMs to stimulate pro-inflammatory EC reactions	endothelial cells	[30]
miR-143/miR-145	hexokinase II and integrin-β8	activation of the TGF-β signaling pathway	coronary artery endothelial cells	[35]
miR-145	KLF4 and myocardine	stimulating the contractile phenotype in VSMCs	vascular smooth muscle cells	[39]
miR-145	ELK-1 and KLF5	the modulation of VSMC differentiation	vascular smooth muscle cells	[39]
miR-221/miR-222	p27(Kip1) and p57 (Kip2)	reduces VSMC proliferation and the formation of neointimal damage after mechanical injury	vascular smooth muscle cells	[42]
MiR-21	PTEN	indirectly increase the anti-apoptotic Bcl-2 gene	vascular smooth muscle cells	[46]
MiR-21		induction of the contractile phenotype of VSMC	vascular smooth muscle cells	[46]

miR-10a	3′ UTRs of MAPK kinase kinase 7 and the β-transducin repeat-containing gene	10a inhibits canonical transmission of NF-kB signals	endothelial cells	[48,49]

Circulating MiRNA as Potential Biomarkers

Atherosclerosis patients and non-atherosclerosis individuals show distinct miRNA profiles, highlighting the significant impact of miRNA signatures. These miRNAs have gained attention as potential biomarkers due to their rapid release and stability in the plasma. Within the diverse range of miRNAs, miR-126 plays a crucial role in inhibiting VCAM-1 during inflammation, effectively preventing the development of atherosclerotic lesions. This highlights the possible therapeutic importance of miR-126 in the treatment of atherosclerosis [55]. miR-221 and miR-222 are also considered protective miRNAs. They facilitate both growth and mobility of endothelial cells to facilitate the development of neointima. Studies conducted in living organisms have revealed a decrease in the levels of miR-221/222 among individuals with peripheral artery diseases, while miR-21 and miR-30 exhibited contrasting results when compared to healthy control groups. Conversely, after experiencing a heart attack, miR-122, 221, and 222 were found to increase [56].

Research indicates that miR-92a contributes to protection against atherosclerosis by modulating Krüppel-like factors 2 and 4 within the arterial endothelium, resulting in lower levels among patients with Coronary Artery Disease (CAD). In contrast, miR-130a showed a significant increase in individuals with atherosclerosis and a reduction in those with pre-atherosclerosis. Moreover, intima samples from patients with atherosclerosis revealed elevated levels of miR-130a, miR-27b, let-7f, and miR-210 [57]. Similar increases for miR-130a, miR-27b, and miR-210 were also detected in serum samples. Consequently, serum levels of miR-210, miR-130a, and miR-27b could potentially serve as biomarkers for atherosclerosis. In a separate in vivo study, the roles of miR-21-5p, miR-361-5p, and miR-519e-5p were examined in the context of acute myocardial infarction. The findings showed a significant rise in circulating miR-21-5p and miR-361-5p, while miR-519e-5p levels decreased significantly compared to healthy individuals. Additionally, an increase in circulating miR-519e-5p during cardiac ischemia was also validated [58].

MiRNA as Diagnostic Tool

Recent studies suggest that miRNAs may act as valuable diagnostic and prognostic biomarkers for various health conditions (for example: Rheumatoid arthritis, diabetes, cardiovascular diseases, cancer and kidney diseases). Diagnostic markers need to be improved, as protein-based biomarkers cannot meet diagnostic criteria for the diagnosis of heart disease [59].

Research on clinical samples has revealed that miRNAs have emerged as a crucial biomarker for accurate diagnosis. Furthermore, they hold potential therapeutic value in addressing various cardiovascular conditions, such as atherosclerosis, hypertension, acute myocardial infarction, heart failure, stroke, and cancer [60].

For instance, in a heart failure study, miR-423-5p demonstrated elevated expression levels irrespective of gender and age, making it a sensitive indicator for heart-related conditions. In contrast, individuals with coronary heart disease showed reduced levels of miR-126 and miR-145. Furthermore, levels of miR-133a/b and miR-1 were decreased, whereas miR-208 was found to be increased in those with myocardial infarction. Overall, these findings underscore the potential of miRNAs as important biomarkers for the diagnosis, prognosis, and detection of atherosclerosis [61].

Therapeutic Opportunities and Challenges

Because miRNAs can influence gene expression, they offer a potential strategy for treating diseases through gene modulation. However, since a single miRNA can target multiple genes, there is a risk of unintended side effects on other genes. Nevertheless, this strategy might effectively address complex conditions like atherosclerosis, which involves multiple pathways in its development [62].

To successfully implement this type of therapy, it is crucial to identify the key pathways and genes involved in atherosclerosis pathogenesis. Research has demonstrated the significant role of miRNAs in both the onset and progression of atherosclerosis. However, further research is required to develop new candidate drugs based on miRNA, since miRNAs-imitators and inhibitors can affect the genes responsible for the pathogenesis of atherosclerosis [63].

Antisense oligonucleotides offer the potential to downregulate miRNA expression or modulate specific pathways affected by the disease. To enhance the effectiveness of antisense oligonucleotide therapy, various techniques have been explored to improve tissue absorption, increase miRNA stability, and enhance target binding affinity chemically. A thorough assessment of chemical modifications will be required. This is necessary in order to minimize unforeseen toxicity and potential side effects. Single-stranded oligonucleotides aimed at miRs can be delivered without the need for lipid-based delivery systems [64]. They can be prepared in a saline solution suitable for intravenous or subcutaneous injection. Following systemic delivery, miRNAs are efficiently taken up by multiple organs, including the bone marrow, kidneys, liver, spleen, and adipose tissue. Upon entering the cell, antisense oligonucleotides form a stable and robust connection with the targeted miRNA, obstructing its ability to interact with the corresponding mRNA [65]. Promising results were obtained from preclinical studies on non-human primates, indicating that the use of unmodified anti-miR oligonucleotides can effectively target miRNAs, including miR-33 and miR-122, with a particular emphasis on liver-related applications. Cholesterol analogues have been used to increase the uptake of anti-MIRS by cells. This led to a higher incorporation into Low-Density Lipoproteins (LDL) and High-Density Lipoproteins (HDL). Another approach to inhibiting miRNA is miRNA decoy or sponge transcripts. These RNA transcripts act as competitive inhibitors for the target miRNA. Referred to as miRNA sponges, they feature numerous binding sites that include sequences complementary to the original miRNA. By interacting with the miRNA, these sponges effectively hinder its function [66]. To deliver miRNA sponges, viral vectors can be employed. Their expression can be triggered at certain phases or in particular cell lines by utilizing specific promoters. Observations indicate that after cell transplantation using viral vectors that deliver miRNA sponges, the associated miRNAs show reduced expression levels [67].

The first purpose of using miRNA mimic is to restore miRNA with reduced regulation. The second objective is to minimize the expression of genes associated with the disease's pathogenesis. For example, the expression of miR-181b in the Endothelial Cells (ECs) of patients with Coronary Artery Disease (CAD) can be restored through the use of miRNA mimics [68]. To effectively deliver therapeutic oligonucleotides to their targets, various delivery systems, including lipoprotein-based formulations, polymer micelles, and liposome carriers, have been thoroughly investigated. Research has shown that miRNAs can be linked to High-Density Lipoprotein (HDL) particles for intercellular transfer, suggesting the potential efficacy of HDL infusion for miRNA delivery. The targeted delivery of miRNA to specific cells or targets, as well as the challenge of achieving optimal repression with multiple doses of miRNA, remain significant issues confronted by researchers [69].

Most oligonucleotide inhibitors and miRNA mimics predominantly accumulate in the liver. Consequently, a limited amount of these oligonucleotides reaches the vascular wall, resulting in a lower than optimal chance of success. Studies have shown that effectively targeting miRNAs to peripheral blood mononuclear cells and the endothelial cells of the vascular wall can enhance the success rate of treatments [70]. Essentially, delivering miRNA mimics or inhibitors directly to the affected tissues or cells, instead of relying on systemic delivery, offers a promising approach to prevent the onset and progression of atherosclerosis. It is extremely important to find a suitable dosage regimen with translation. To achieve optimal effectiveness with minimal dosages and side effects, it is essential to investigate targeted strategies. Currently, several miRNA-based therapies are in preclinical development, with two advancing to clinical trials. The first therapy under evaluation is Miravirsen, a Locked Nucleic Acid (LNA) designed to target miR-122 [71]. Its primary aim is to inhibit HEPATITIS C VIRUS (HCV) RNA. Studies conducted on non-human primates have shown that inhibiting miR-122 can significantly reduce HCV viremia without causing notable side effects or promoting viral resistance. The second therapy focuses on a miRNA mimic of miR-34, which enhances anti-tumor immune responses while suppressing various oncogenic pathways. Encouraging findings have emerged from trials of MRX34, a miR-34 mimic delivered in liposomal nanoparticles, in patients with hematological cancers or advanced solid tumors [72]. To date, significant progress has been made in the treatment of atherosclerosis. Statins (inhibitors of 3-hydroxy-3-methyl-glutaryl-coenzyme a reductase) were particularly important for the treatment of patients with CAD. Despite the fact that statins significantly reduce LDL levels and lead to an improvement in the cardiovascular system, the burden of the disease remains even in patients treated with CAD.

Limitations

Bias in Literature Selection: Despite employing a systematic search strategy, there is a possibility of selection bias in the included studies, which may limit the comprehensiveness of the review. Omission of potentially relevant articles could introduce bias and affect the generalizability of the findings.

Heterogeneity of Study Designs: The variability in study designs, methodologies, and patient populations across the included studies may hinder direct comparisons and limit the overall strength of the evidence synthesized in this review. This heterogeneity could impact the robustness of the conclusions drawn regarding the roles of miRNAs in atherosclerosis.

Publication Bias: The tendency for positive or significant results to be more readily published can introduce publication bias, potentially skewing the overall interpretation of the literature reviewed. Publication bias may lead to an overestimation of the role of miRNAs in atherosclerosis pathogenesis, affecting the validity of the conclusions.

Limited Clinical Translation: The majority of studies included in this review may have focused on preclinical or experimental models of atherosclerosis, potentially limiting the direct clinical relevance of the findings. The extrapolation of these results to clinical practice may be constrained by the lack of robust clinical evidence supporting the utility of miRNAs as diagnostic biomarkers or therapeutic targets in atherosclerosis.

Lack of Longitudinal Data: The cross-sectional nature of many studies investigating miRNA involvement in atherosclerosis may limit our ability to ascertain temporal relationships and causality between miRNA expression patterns and disease progression. The lack of longitudinal data may restrict the reliability of inferring predictive or prognostic value from the reviewed literature.

Interpretation Challenges: Given the intricate regulatory networks involving miRNAs in atherosclerosis, the complexity of molecular interactions and signaling pathways may present challenges in interpreting the functional implications of specific miRNAs. The multifaceted nature of miRNA biology in atherosclerosis warrants careful consideration and may pose challenges in deriving definitive conclusions.

Resource and Time Constraints: The scope of this review may have been limited by resource and time constraints, potentially restricting the depth of analysis and coverage of all relevant studies in the field of miRNA research in atherosclerosis. As a result, certain pertinent studies or data may not have been included, impacting the overall comprehensiveness of the review.

Conclusion

To date, a lot of evidence has been obtained, that various molecules from the microRNA class are involved in the initiation and development of atherosclerosis. New and complementary therapeutic approaches are required for the treatment of atherosclerosis. Considering the potential impact of miRNAs on gene expression, it is essential to acknowledge the possibility of effects on cellular functions, biological pathways, and overall balance in the peripheral areas, including the liver, and vascular walls when treating miRNA-related conditions. In addressing atherosclerosis and its associated complications, the utilization of kits for delivering miRNA simulators or inhibitors presents an appealing and practical approach for treatment.

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Author's Contributions

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