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# COMPARATIVE ANALYSIS OF MIRNA REGULATORY PATHWAYS IN ATHEROSCLEROSIS: UNRAVELLING SIMILARITIES AND DIVERGENCES ACROSS DIFFERENT STAGES AND PHENOTYPES

*Original Article*

**Sameen Shahid[1], Farah Abid[2], Nimra Shahzadi[1], Tufail Ahmad[3], Muhammad Hasnain Ijaz[4], Hafiz Sharjeel Ahmed[5], Amina Farrukh Alavi[6], Izza Rafique[7], Ghosia Noreen[8], Safdar Ali[9]***

[1]Center for Applied Molecular Biology, University of the Punjab, Lahore, Pakistan.
[2]Department of Pharmacy, University of South Asia, Pakistan.
[3]Department of Pharmacy, University of Swabi, Swabi.
[4]Department of Biochemistry, Bahaudin Zakaria University, Multan, Pakistan.
[5]Faculty of Pharmacy, Hamdard University, Pakistan.
[6]Department of Microbiology, Quaid-E-Azam University, Islamabad, Pakistan.
[7]Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan.
[8]Department of Zoology, Government College University, Lahore, Pakistan.
[9]Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan.

**Corresponding Author:** Safdar Ali, Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan, safdarali5065012@gmail.com

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## ABSTRACT

**Background:** Atherosclerosis remains a leading cause of cardiovascular morbidity and mortality worldwide, contributing to life-threatening complications such as coronary artery disease, myocardial infarction, stroke, and peripheral vascular disease. Despite well-established risk factors such as hyperlipidemia, hypertension, oxidative stress, and inflammation, the molecular underpinnings of plaque development and progression are still being elucidated. Among the emerging regulators, microRNAs (miRNAs)—short, non-coding RNAs—have gained recognition for their ability to post-transcriptionally modulate gene expression and influence vascular cell behaviour and immune responses.

**Objective:** This review aims to explore the regulatory role of miRNAs in the initiation, progression, and phenotypic diversification of atherosclerotic plaques, and to evaluate their potential as diagnostic biomarkers and therapeutic targets.

**Main Discussion Points:** The review discusses the involvement of miRNAs in endothelial dysfunction, lipid accumulation, foam cell formation, and plaque instability. It highlights the differential expression of key miRNAs such as miR-92a, miR-126, miR-155, and miR-221 across various stages and phenotypes of atherosclerosis. Methodological approaches including next-generation sequencing, in vivo animal models, and integrative omics analyses are reviewed. The role of genetic polymorphisms and environmental stimuli in modulating miRNA expression is also addressed.

**Conclusion:** miRNAs serve as central regulators in the complex pathophysiology of atherosclerosis, with distinct expression profiles correlating with disease stage and vascular phenotype. Their diagnostic and therapeutic potential warrants further investigation through well-designed clinical studies to enable their translation into clinical practice.

**Keywords:** Atherosclerosis, microRNAs, Cardiovascular Disease, Endothelial Dysfunction, Inflammation, Gene Regulation.

## INTRODUCTION

Atherosclerosis, a progressive and multifactorial cardiovascular disease, remains the leading cause of mortality globally, accounting for a significant proportion of deaths related to ischemic heart disease, stroke, and peripheral arterial disorders. According to the World Health Organization, cardiovascular diseases are responsible for approximately 17.9 million deaths each year, with atherosclerosis playing a central role in the pathogenesis of most cases. Characterized by the formation and progression of lipid-rich plaques within arterial walls, atherosclerosis evolves through distinct but interconnected stages—initiation, progression, and eventual plaque rupture—each of which contributes to the clinical manifestations of the disease. Central to its development is endothelial dysfunction, a critical event that promotes lipid infiltration, inflammatory cell recruitment, and neovascularization within the intimal layer of arteries (1,2). The initiation of atherosclerosis is often driven by a combination of well-documented risk factors including hypertension, hyperlipidemia, diabetes mellitus, smoking, and oxidative stress, which collectively compromise endothelial integrity. This impairment disrupts vascular homeostasis, diminishing nitric oxide bioavailability, and enhancing the endothelium's pro-inflammatory and pro-thrombotic state (3). Modified low-density lipoprotein (LDL), particularly oxidized LDL, accumulates beneath the endothelium and serves as a potent stimulus for monocyte recruitment and their differentiation into macrophages. These macrophages, upon ingesting oxidized LDL, transform into foam cells, marking the formation of fatty streaks, a hallmark of early atherogenesis. Over time, persistent inflammation, driven by both innate and adaptive immune responses, promotes smooth muscle cell proliferation, extracellular matrix remodeling, and plaque neovascularization, thereby advancing the lesion toward instability (4,5).

Recent advances in molecular biology have expanded the understanding of atherosclerosis by uncovering the regulatory roles of non-coding RNAs, particularly microRNAs (miRNAs). These small, non-coding RNA molecules, typically 20–24 nucleotides in length, exert gene-silencing effects post-transcriptionally through mRNA degradation or translation inhibition. miRNAs are highly conserved across species and have emerged as pivotal modulators of various physiological and pathological processes, including angiogenesis, inflammation, lipid metabolism, and endothelial function—all central to atherosclerotic disease. Endothelial cells, being highly responsive to hemodynamic forces and metabolic cues, undergo functional alterations under stress conditions such as hypoxia, hyperglycemia, oxidative stress, and exposure to inflammatory cytokines. These stressors influence the expression of specific miRNAs, many of which are now recognized to modulate key pathways in endothelial homeostasis and plaque development (6,7). For instance, miR-221 and miR-222, both implicated in the regulation of endothelial progenitor cell (EPC) activity, have shown elevated expression levels in patients with coronary artery disease. Their overexpression reduces EPC proliferation and impairs endothelial repair, partly by targeting the c-kit receptor and negatively regulating endothelial nitric oxide synthase (eNOS), a key enzyme responsible for nitric oxide production. Nitric oxide is essential for vasodilation, inhibition of platelet aggregation, and suppression of smooth muscle proliferation. Its deficiency is a defining feature of dysfunctional endothelium in atherosclerosis. In contrast, miR-126, an endothelial-specific miRNA, has demonstrated vasculoprotective effects by enhancing angiogenesis and maintaining vascular integrity through inhibition of negative regulators like Spred-1. Conversely, overexpression of miR-92a impairs endothelial cell migration and angiogenesis, while miR-129-1 and miR-133 suppress key angiogenic receptors such as FGFR1 and VEGFR2, thereby hindering neovascularization in plaque microenvironments (8,9).

While these mechanistic insights into miRNA-mediated regulation are promising, the clinical application of miRNAs extends beyond pathogenesis. Circulating miRNAs have emerged as potential non-invasive biomarkers for the diagnosis and prognosis of atherosclerotic cardiovascular diseases. Unlike traditional protein biomarkers, which often lack sensitivity and specificity, miRNAs exhibit stable expression profiles in blood and other body fluids, making them attractive candidates for early detection. For example, miR-423-5p levels are elevated in heart failure patients and appear to be independent of age and sex, suggesting its utility as a robust diagnostic marker. Similarly, decreased levels of miR-126 and miR-145 have been reported in individuals with coronary artery disease, whereas altered expression of miR-133a/b and miR-1 in myocardial infarction patients highlights their potential in risk stratification and disease monitoring (10,11). Despite growing evidence supporting the clinical relevance of miRNAs, several knowledge gaps persist. Most existing studies are limited by small sample sizes, heterogeneity in analytical methodologies, and lack of longitudinal validation. Furthermore, while preclinical models have elucidated the functional roles of many miRNAs in atherosclerosis, translating these findings into therapeutic interventions remains challenging due to issues related to delivery specificity, off-target effects, and miRNA stability. Therefore, there is a pressing need to consolidate current knowledge and critically evaluate the functional and diagnostic implications of miRNAs in atherosclerosis within a coherent framework (12,13).

This narrative review aims to provide a comprehensive overview of the regulatory role of microRNAs in the development, progression, and destabilization of atherosclerotic plaques. The review synthesizes evidence from recent molecular and clinical studies to highlight

 

the mechanistic pathways modulated by key miRNAs and explores their potential as diagnostic and therapeutic targets. It focuses primarily on literature published within the last five years, drawing upon experimental models and clinical observations to bridge the gap between bench research and bedside applications. By delineating the interplay between miRNA expression and vascular pathophysiology, this review seeks to contribute to the growing field of RNA-based diagnostics and therapeutics in cardiovascular medicine. The intent is not only to summarize the existing body of work but also to identify promising avenues for future investigation that may ultimately enhance the management of atherosclerotic disease.

## THEMATIC DISCUSSION

### Methodological Approaches in Studying miRNA Pathways

The study of microRNA (miRNA) pathways has evolved significantly due to the advancement of transcriptomic and sequencing technologies. Initially, the detection of miRNAs through cloning and in vitro sequencing was hindered by the small size and methylation characteristics of miRNAs, making the process laborious and time-intensive (14). The development of bioinformatics-based in silico methods has revolutionized this field by enabling high-throughput and automated analysis of small RNAs (15). Among modern techniques, next-generation sequencing (NGS) has become a cornerstone for identifying novel miRNAs, quantifying their expression, and predicting target genes. Platforms like Illumina's HiSeq and NovaSeq demonstrate high resolution and cost-effectiveness, making them the preferred options for miRNA profiling (16). Studies show that these sequencing tools, when combined with computational pipelines, offer robust insights into miRNA expression, precursor prediction, and gene target analysis (17). The comparison of hybridization versus sequencing methods confirms the superior specificity of RNA-seq for short transcripts like miRNAs (18). However, even with advances like machine learning models integrated into miRNA discovery, challenges remain in standardizing cross-platform validation and annotating functionally relevant miRNAs, especially across different cell types and pathological conditions.

### miRNA Regulatory Pathways in Early vs. Late-stage Atherosclerosis

miRNAs contribute to atherosclerosis from its earliest stages to the final stages of plaque rupture. In the initiation phase, miRNAs such as miR-122 and miR-185 regulate hepatic lipid metabolism by targeting sterol regulatory elements, ultimately modulating plasma cholesterol levels (19). Downregulation of miR-122 via antisense oligonucleotides has shown reductions in low-density lipoprotein (LDL) and total cholesterol in murine models, suggesting a therapeutic potential in lipid-related vascular diseases (20). During progression, miR-92a plays a pivotal role in inhibiting anti-atherogenic transcription factors like KLF2 and KLF4, contributing to endothelial dysfunction and inflammation (21). It further exacerbates atherosclerosis by being activated under oxidative stress and high-fat diet conditions, fostering vascular permeability and immune cell recruitment (22). Conversely, miR-126 supports endothelial integrity by enhancing angiogenic pathways and suppressing apoptotic signals, particularly through CXCL12/CXCR4 and Notch 1 signaling axes (23). Importantly, miR-181b, regulated through NF-κB signaling, exhibits dual roles—downregulated during acute inflammation yet overexpressed in a feedback-protective anti-inflammatory loop, illustrating the complex dynamics of miRNA involvement across disease stages (24).

### Plaque Formation

Atherosclerotic plaque formation is deeply intertwined with miRNA-mediated modulation of lipid transport, immune activation, and endothelial cell dynamics. Plaque formation begins with excessive LDL accumulation in the subendothelial space, which triggers macrophage infiltration and foam cell generation. These immune responses are orchestrated by miRNAs such as miR-223, which modulates cholesterol efflux by targeting enzymes like methylsterol mono-oxygenase 1 and HMG-CoA synthase (25). miRNAs like miR-33, miR-144, and miR-10b also suppress ABCA1/G1 transporters, impairing reverse cholesterol transport and aggravating lipid accumulation (26). This cascade leads to the formation of fatty streaks and progressive fibrous plaques. Furthermore, miR-92a and miR-155 have been associated with upregulation of adhesion molecules such as VCAM-1 and ICAM-1, enhancing leukocyte adhesion and exacerbating local inflammation (27). Thus, multiple miRNAs act synergistically to advance plaque formation via cellular infiltration, inflammation, and disruption of endothelial function.

### Initiation

In the initiation of atherosclerosis, miRNAs regulate cholesterol biosynthesis and immune recruitment. miR-122, comprising a substantial proportion of hepatic miRNAs, plays a critical role in regulating lipid metabolism by modulating genes like SREBP2 and fatty acid synthase (28). Genome-wide association studies have highlighted miR-301b and miR-128-1 as regulators of LDL receptor

 

pathways, supporting their relevance in systemic lipid control (29). At the cellular level, miR-223 reduces intracellular cholesterol by targeting critical biosynthetic enzymes, and its absence in murine models results in elevated cholesterol levels and HDL-C (30). These findings align with miRNA expression profiling studies showing early alterations in liver and vascular tissues before morphological evidence of plaque development, positioning miRNAs as early biomarkers and potential therapeutic targets for atherosclerosis.

**Progression**

As the disease progresses, miRNAs mediate the transition from fatty streaks to more complex lesions. The activation of miR-92a under low shear stress and oxidative conditions leads to the repression of protective transcription factors and triggers endothelial dysfunction (31). In contrast, miR-126-3p and -5p are protective, enhancing endothelial repair via VEGF and Notch pathways (32). Inflammatory regulation is notably influenced by miR-181b, whose administration has been shown to attenuate plaque size in ApoE-deficient mice through its suppression of NF-κB translocation (33). Likewise, miR-155, initially atheroprotective, adopts pro-inflammatory properties in late-stage lesions by promoting TNF-α stabilization and macrophage polarization (32,33). This biphasic role of miRNAs underlines the need for stage-specific therapeutic strategies that consider disease chronology.

**Differential Expression in Vitro and in vivo**

Expression profiles of miRNAs differ significantly between in vitro models and in vivo disease conditions. For instance, miR-126 enhances angiogenesis and endothelial repair in vitro but is found to be downregulated in atherosclerotic plaques in vivo, particularly in unstable regions (27,28). Similarly, miR-92a shows consistent inhibitory effects on endothelial proliferation in vitro and is upregulated in diseased aortic tissue exposed to disturbed flow (29). Discrepancies in miRNA profiles may stem from environmental stressors, tissue heterogeneity, or differential immune cell infiltration in vivo. These findings emphasize the necessity of validating in vitro findings within relevant in vivo models to fully understand the pathological implications of miRNAs in vascular biology.

**Differential miRNA Regulation in Various Atherosclerosis Phenotypes**

The heterogeneity of atherosclerotic lesions across vascular beds is mirrored by distinct miRNA expression patterns. Microarray studies comparing carotid and coronary arteries revealed that only a limited number of miRNAs (e.g., miR-146a, miR-155, miR-221) are commonly dysregulated in both sites (17,25). Others, such as miR-100, miR-127, and miR-133, show phenotype-specific upregulation, particularly in unstable carotid plaques (18,29). Furthermore, expressions of miR-24, miR-29b, and let-7f varies between studies, reflecting inconsistencies likely due to sample origin, disease severity, and methodology (27). The identification of co-expression clusters among these miRNAs offers a new direction in understanding the complex molecular crosstalk within the atherosclerotic microenvironment (22,23).

**Role of Genetic and Environmental Factors in miRNA Regulation in Atherosclerosis**

Genetic polymorphisms and environmental stressors like shear stress profoundly affect miRNA regulation in endothelial cells. Variants such as rs2910164 in miR-146a have been associated with coronary artery disease in different ethnic populations (10,15). Experimentally, shear-sensitive miRNAs like miR-21, miR-23b, and miR-663 are upregulated under unidirectional flow, promoting endothelial cell survival and anti-inflammatory responses through KLF-2 and PI3K/Akt signaling (24,25). Conversely, oscillatory flow enhances miR-92a expression, triggering inflammation and apoptosis (23,27). These biomechanical cues act via mechano-transduction pathways to fine-tune endothelial responses, demonstrating that both genetics and hemodynamic forces shape miRNA activity and contribute to vascular homeostasis or dysfunction.

**Therapeutic Potential of Targeting miRNA Pathways**

Targeting miRNA pathways presents a promising therapeutic avenue due to their ability to regulate multiple genes simultaneously. Anti-miRs and miRNA mimics have been used in preclinical studies to modulate atherosclerotic pathways. For instance, inhibition of miR-92a restores endothelial function and reduces lesion size, while miR-181b mimics decrease NF-κB-driven inflammation (19,21). The miR-143/145 cluster has demonstrated efficacy in promoting VSMC stability and reducing atherosclerosis in animal models by modulating myocardin and KLFs (19,20). Furthermore, miR-221 and miR-222 exhibit dual activity, promoting VSMC proliferation while inducing EC apoptosis, highlighting the need for cell-type-specific targeting strategies (27). Although challenges remain regarding delivery systems and off-target effects, the comprehensive control offered by miRNA-based therapies holds considerable potential for integrated vascular disease management.

 

**Table 1. Different miRNAs are involved in various atherosclerotic pathways.**

| miRNAs | Stimuli | Targets | Affected Signaling Pathway | Effect on Atherosclerosis | References |
|---|---|---|---|---|---|
| miRNA-19b | Unknown | ATP Binding Cassette A1 | Unknown | Increase | (8) |
| miRNA-24 | Macrophage Colony Stimulating factor | Chitinase 3-Like-1, Matrix Metallopeptidase 14 | Extracellular signal-regulated kinase | Decrease | (9) |
| miRNA-33-5p | Low cholesterol levels, insulin | ATP Binding Cassette A1, ATP Binding Cassette G1 | Unknown | Increase | (10,11) |
| miRNA-92a | Low shear stress; High Fat Diet | Krüppel-like factor 2/4, Suppressor of cytokine signaling 5 | Nuclear Factor κB | Increase | (12) |
| miRNA-126-3p | Apoptosis | Regulator of G-protein signaling 16 | chemokine (C–X–C motif) ligand 12 | Decrease | (13) |
| miRNA-126-5p | High shear stress | Delta-like homologue 1 | Notch | Decrease | (14) |
| miRNA-144-3p | Unknown | ATP Binding Cassette A1 | Unknown | Increase | (15) |
| miRNA-155 | Inflammation | B cell lymphoma 6 | Nuclear Factor κB | Increase | (16) |
| miRNA-181b | High shear stress | Importin $\alpha_3$ | Nuclear Factor κB | Decrease | (7) |
| miRNA-342-5p | Differentiation | Serine/threonine protein kinase 1, bone morphogenetic protein receptor type II | Nuclear Factor κB | Increase | (17) |
| miRNA-467b | Unknown | Lipoprotein lipase | Unknown | Decrease | (18) |
| miRNA-205/712 | Low shear stress | Metalloproteinase inhibitor 3 | Nuclear Factor κB | Increase | (19) |

**Table 2. Gene polymorphism of miRNAs in different cardiovascular diseases in various populations.**

| MiRNA | rs Number | Disorder | Population | References |
|---|---|---|---|---|
| MiR-149 | rs2292832 | Coronary artery disease | Korean | (24) |
| Pre-MiR-27a | rs895819 | Myocardial infarction | Chinese Han population | (25) |
| Pre-MiR-146a | rs2910164 | Acute coronary syndrome | Chinese | (26,27,28) |
| | | Coronary artery disease | Korean | |
| | | Coronary artery disease | Iranian | |
| MiR-196a | rs11614913 | Cardiovascular disease | Polish | (29) |
| MiR-499 | rs3746444 | Coronary artery disease | Chinese | (29-32) |
| | | Ischemic stroke | Chinese | |
| | | Coronary artery disease | Chinese | |
| | | Myocardial infarction | Chinese | |
| MiR-4513 | rs2168518 | Coronary artery disease | Chinese | (32) |

**Table 3: MicroRNAs Involved in Cellular Processes Across Different Stages of Atherosclerosis**

| miRNA | Cell type | Process | Stage of the Disease |
|---|---|---|---|
| miR-24 | Hepatocytes | Biosynthesis of Cholesterol | Homeostasis and transport of cholesterol |
| miR-122 | | | |
| miR-185 | | | |
| miR-223 | | | |
| miR-486 | | | |
| miR-10b | Macrophages | Cholestrol Efflux | |
| miR-20a/b | | | |
| miR-23a/b | | | |
| miR-30e | | | |
| miR-33a/b | | | |
| miR-92a | | | |
| miR-101 | | | |
| miR-144 | | | |
| miR-148 | | | |
| miR-302a | | | |
| let-7g | Endothelial Cells | Inflamamation | Atherosclerotic plaque (initiation and progression) |
| miR-17-3p | | | |
| miR-31 | | | |
| miR-146a | | | |
| miR-155 | | | |
| miR-181a-3p/-5p | | | |
| miR-181b | | | |
| miR-221 | | | |
| miR-222 | | | |
| let-7a/b | Endothelial Cells | Oxidative stress | |
| miR-19b | | | |
| miR-20a | | | |
| miR-34 | | | |
| miR-98 | | | |
| miR-142-3p | | | |
| miR-199a-3p/-5p | | | |
| miR-200c | | | |
| miR-221 | | | |
| miR-222 | | | |
| miR-328 | | | |
| miR-155 | Endothelial Cells | Endothelial barrier integrity Modification | |
| let-7g | Endothelial Cells | Senescence | |

| miRNA | Cell type | Process | Stage of the Disease |
|---|---|---|---|
| miR-216a | | | |
| miR-21 | Monocytes | Recruitment of monocytes | |
| miR-22 | Monocytes | Differentiation of Monocytes to macrophages | |
| miR-27a/b | | | |
| miR-33 | | | |
| miR-34a | | | |
| miR-155 | | | |
| miR-155 | Macrophages | Polarization of macrophages | |
| miR-33 | | | |
| miR-23a-5p | Macrophages | Foam cell formation | |
| miR-27 | | | |
| miR-34 | | | |
| miR-212 | | | |
| miR-590 | | | |
| miR-758-5p | | | |
| miR-1 | Smooth Muscle Cells | Vascular smooth muscle cell proliferation and differentiation | |
| miR-21 | | | |
| miR-143/145 | | | |
| miR-21 | Smooth Muscle Cells | Fibrous cap thinning | Atherosclerotic plaque rupture |
| miR-29b | | | |
| miR-124-3p | | | |
| miR-133b | | | |
| miR-181b | | | |
| miR-362 | | | |
| miR-155 | Macrophages | Necrotic core formation | |
| miR-378a | | | |

## CRITICAL ANALYSIS AND LIMITATIONS

The existing body of literature investigating the role of microRNAs (miRNAs) in the pathogenesis, progression, and potential treatment of atherosclerosis presents a promising yet incomplete picture. While numerous studies have demonstrated mechanistic links between specific miRNAs and key molecular pathways in lipid metabolism, inflammation, endothelial dysfunction, and plaque instability, several limitations within the current evidence base constrain the clinical applicability of these findings. A recurrent issue across many studies is the limited sample size, which undermines the statistical power required to draw robust conclusions, particularly in human-based

research. Small cohorts restrict subgroup analysis by demographic or clinical variables, resulting in findings that may not reflect broader patient populations (1,2). Moreover, most studies to date have been observational or preclinical in nature, with a notable scarcity of randomized controlled trials (RCTs) to confirm therapeutic efficacy or prognostic utility in atherosclerotic disease (3). Methodological inconsistencies further complicate the interpretation of miRNA-related research in this field. The reliance on differing protocols for RNA extraction, quantification techniques, and normalization strategies introduces significant variability across studies. While high-throughput sequencing technologies like NGS have improved sensitivity, discrepancies persist due to batch effects, inconsistent bioinformatic pipelines, and variability in miRNA annotation across databases (4). In many cases, miRNA expression is evaluated without consideration of confounding variables such as medication use, comorbidities, or environmental factors, all of which can modulate miRNA levels independently of atherosclerotic pathology (5). Additionally, many studies rely on in vitro models or animal systems, which do not fully recapitulate the complexity of human atherosclerotic disease. The controlled nature of these models may also mask interactions present in heterogeneous human populations, limiting translational relevance (6).

The literature also reveals a significant publication bias, with a strong emphasis on positive findings that highlight statistically significant associations between miRNAs and atherosclerotic endpoints. Studies that report negative or inconclusive results are less frequently published, which may skew the perceived importance of certain miRNAs or overestimate their potential as diagnostic or therapeutic targets (7). This selective reporting may foster an incomplete understanding of the miRNA landscape in atherosclerosis, particularly if functional redundancy or compensatory mechanisms are at play among different miRNA families. Another layer of complexity arises from the heterogeneity in outcome measures used to assess miRNA effects. For instance, some studies assess circulating miRNA levels as diagnostic biomarkers, while others focus on tissue-specific expression, functional assays of endothelial cell proliferation, or inflammatory markers. The lack of standardized endpoints complicates cross-study comparisons and meta-analytic syntheses. Inconsistent definitions of disease stages—such as what constitutes early versus advanced plaque—also pose challenges in determining how miRNA regulation differs across the atherosclerosis continuum (8).

Furthermore, the generalizability of current findings is limited due to underrepresentation of diverse populations. Many studies have been conducted in restricted ethnic or geographical cohorts, particularly in East Asian or European populations, where genetic and environmental profiles may differ significantly from those in other regions. Genetic polymorphisms in miRNA genes or their target sites may also lead to differential expression or activity, making it inappropriate to extrapolate findings universally without validation in varied populations (9). The influence of age, sex, and comorbid conditions like diabetes and hypertension on miRNA profiles has not been uniformly explored, further narrowing the clinical scope of interpretation. Despite these limitations, the role of miRNAs in atherosclerosis remains a promising field that merits continued exploration. However, future studies should prioritize rigorous experimental design, adequate sample sizes, longer follow-up durations, and standardization of measurement techniques to reduce bias and improve reproducibility. Moreover, integrating multi-omics approaches and real-world patient data could enhance understanding of miRNA interactions in the broader context of cardiovascular disease.

## IMPLICATIONS AND FUTURE DIRECTIONS

The expanding body of evidence surrounding microRNAs (miRNAs) in the pathogenesis and regulation of atherosclerosis holds transformative potential for clinical practice, particularly in the realms of early diagnosis, personalized therapy, and disease monitoring. The differential expression of specific miRNAs across various stages and phenotypes of atherosclerotic disease offers an opportunity to redefine biomarker-based diagnostic strategies. For instance, the downregulation of miR-126 and miR-145 in coronary artery disease and the upregulation of miR-155 in inflammatory monocytes could inform more precise risk stratification and enable clinicians to tailor interventions before significant arterial damage occurs (1,2). Moreover, the use of circulating miRNAs as minimally invasive biomarkers can reduce the reliance on traditional imaging or histopathological assessments, making atherosclerosis detection more accessible and patient friendly. In clinical therapeutics, the ability of miRNAs to simultaneously regulate multiple gene networks involved in lipid metabolism, endothelial function, and inflammatory cascades provides a compelling rationale for their integration into treatment regimens. miRNA mimics and anti-miRs have shown preclinical efficacy in modulating pathways implicated in plaque formation and rupture, such as KLF2/4 inhibition by miR-92a or foam cell regulation by miR-33 and miR-223 (3,4). The potential for personalized medicine is particularly promising, as interindividual variability in miRNA expression suggests that miRNA profiles could one day guide individualized treatment decisions. However, the clinical translation of miRNA-based therapies will require the development of standardized delivery systems, with extracellular vesicle-mediated miRNA transfer emerging as a feasible and physiologically relevant option (5).

 

From a policy and guideline perspective, the clinical utility of miRNA diagnostics and therapeutics warrants inclusion in future cardiovascular care frameworks. To realize this, regulatory bodies must support the development of clinical guidelines that address the validation, safety, and ethical implications of using genetic and epigenetic data in routine practice. The integration of miRNA assays into cardiovascular screening protocols could greatly enhance predictive accuracy, especially when combined with conventional risk assessments such as lipid profiles and imaging. This necessitates robust policymaking that prioritizes funding and infrastructure for molecular diagnostics within public healthcare systems (6). Despite the progress made, several critical research gaps remain. Many of the currently studied miRNAs have not undergone longitudinal clinical validation, and the long-term effects of modulating these molecules remain unknown. Moreover, the majority of investigations have focused on a narrow subset of well-characterized miRNAs, leaving a vast array of novel or tissue-specific miRNAs unexplored. There is also limited understanding of how lifestyle and environmental factors modulate miRNA expression and their consequent impact on atherosclerotic risk. Exploring these factors could unveil modifiable risk determinants and guide preventive strategies (7). Furthermore, the interplay between miRNAs and other non-coding RNAs, including long non-coding RNAs and circular RNAs, represents an underexplored but potentially synergistic domain of investigation.

To address these knowledge gaps, future research must employ more comprehensive and integrative approaches. The use of single-cell RNA sequencing and CRISPR-based gene editing technologies can elucidate the cell-specific functions of miRNAs and their interactions with protein-coding genes across the cellular landscape of atherosclerotic plaques. Moreover, integrated multi-omics approaches encompassing genomics, proteomics, and metabolomics can uncover complex regulatory networks and yield insights into disease heterogeneity and treatment responses (8,9). Prospective cohort studies and randomized controlled trials are essential to determine the biosafety, dosage thresholds, and time-dependent effects of miRNA-targeted interventions. Importantly, global collaboration and data sharing should be promoted to ensure diverse population representation and to enhance the reproducibility and generalizability of findings. In parallel, there is a need for ethical frameworks that address the responsible use of miRNA data, especially in light of the sensitive nature of genetic information. Public trust in miRNA-based diagnostics and therapies will depend on transparent governance, informed consent protocols, and equitable access to emerging treatments. As the field advances, fostering interdisciplinary cooperation between molecular biologists, clinicians, bioinformaticians, and policymakers will be critical in translating laboratory discoveries into real-world cardiovascular solutions. Overall, the integration of miRNA research into the clinical landscape of atherosclerosis promises to reshape diagnostic accuracy, therapeutic precision, and disease prevention strategies. By addressing current limitations and investing in innovative research designs, the future of miRNA-guided cardiovascular care appears both achievable and impactful.

## CONCLUSION

This review underscores the pivotal regulatory function of microRNAs (miRNAs) across the entire continuum of atherosclerosis, from early endothelial dysfunction and lipid imbalance to inflammation, plaque progression, and eventual instability. The evidence consistently demonstrates that specific miRNAs, including miR-92a, miR-126, miR-155, and miR-221, modulate critical molecular pathways that influence vascular homeostasis, immune activation, and cellular remodeling. Comparative analyses highlight the dynamic expression and function of miRNAs across different atherosclerotic phenotypes, revealing both conserved and divergent patterns in response to genetic predisposition and environmental stimuli. While current findings are promising, the strength of the evidence remains moderate due to methodological limitations, such as small sample sizes, lack of standardized measurement protocols, and underrepresentation of diverse populations. Clinicians should remain cautious but aware of the diagnostic and therapeutic potential of miRNA profiling, while researchers are encouraged to prioritize large-scale, longitudinal studies using multi-omics approaches and standardized analytical pipelines. Continued exploration of miRNA-based interventions, especially with advances in bioinformatics and targeted delivery systems, is essential to translate these molecular insights into clinically meaningful strategies for atherosclerosis prevention and treatment.

 

**AUTHOR CONTRIBUTION**

| Author | Contribution |
|---|---|
| Sameen Shahid | Substantial Contribution to study design, analysis, acquisition of Data<br>Manuscript Writing<br>Has given Final Approval of the version to be published |
| Farah Abid | Substantial Contribution to study design, acquisition and interpretation of Data<br>Critical Review and Manuscript Writing<br>Has given Final Approval of the version to be published |
| Nimra Shahzadi | Substantial Contribution to acquisition and interpretation of Data<br>Has given Final Approval of the version to be published |
| Tufail Ahmad | Contributed to Data Collection and Analysis<br>Has given Final Approval of the version to be published |
| Muhammad Hasnain Ijaz | Contributed to Data Collection and Analysis<br>Has given Final Approval of the version to be published |
| Hafiz Sharjeel Ahmed | Substantial Contribution to study design and Data Analysis<br>Has given Final Approval of the version to be published |
| Amina Farrukh Alavi | Contributed to study concept and Data collection<br>Has given Final Approval of the version to be published |
| Izza Rafique | Writing - Review & Editing, Assistance with Data Curation |
| Ghosia Noreen | Writing - Review & Editing, Assistance with Data Curation |
| Safdar Ali* | Writing - Review & Editing, Assistance with Data Curation |

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