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Dual targeting of miR-33 and miR-92a in atherosclerosis: mechanistic insights, therapeutic potential, and translational challenges

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Highlights:

- miR-33 targets ABCA1/ABCG1, reducing cholesterol efflux and promoting plaque growth.
- miR-92a impairs endothelial function by repressing KLF2, KLF4, and eNOS expression.
- Antisense oligonucleotide therapy shows promise in reversing atherosclerotic changes.
- AI tools can improve miRNA-based cardiovascular risk prediction and therapy design.

Abstract: Atherosclerosis remains a major contributor to cardiovascular morbidity and mortality, even with widespread use of lipid-lowering, antihypertensive, and anti-inflammatory therapies. The persistent residual risk and endothelial dysfunction highlight the need for targeted, mechanistically driven interventions. Among emerging regulators, microRNAs (miRNAs) offer precise control over gene networks implicated in atherogenesis. This review focuses on two well-characterized miRNAs with complementary roles: miR-33a/b, which impairs cholesterol efflux by targeting ABCA1 and ABCG1, and miR-92a, which disrupts endothelial homeostasis through suppression of KLF2, KLF4, and endothelial nitric oxide synthase (eNOS). We summarize their roles in key signaling pathways, including insulin and nitric oxide signaling, and examine recent advances in antisense oligonucleotide-based therapeutics and nanoparticle-mediated delivery. Together, these miRNAs represent promising precision targets for restoring lipid balance and vascular integrity, with early clinical trials supporting the translational potential of miRNA-based interventions for cardiovascular disease.

Keyword: atherosclerosis; microRNA; miR-33; miR-92a; cholesterol; endothelium; inflammation; insulin; therapeutics; nanoparticles

1. Introduction

Cardiovascular diseases remain the leading cause of death worldwide, accounting for approximately 17.9 million lives annually, representing nearly 31% of all global mortality [1]. Atherosclerosis, characterized by lipid-rich plaque accumulation in arterial walls, is a major contributor to this burden [2]. In the United States alone, it played a central role in the 702,880 deaths attributed to heart disease in 2022, accounting for one in every five deaths [3]. This chronic, multifactorial disease driven by lipid accumulation, inflammation, and endothelial dysfunction continues to pose a significant global health challenge [4].

Although existing therapies, including lipid-lowering agents, antiplatelet drugs, antihypertensives, glucose-lowering medications, and more recently, anti-inflammatory agents, have substantially reduced cardiovascular events, they often fail to fully address the intricate molecular mechanisms underlying atherogenesis. As a result, many patients continue to experience residual cardiovascular risk, emphasizing the need for innovative, mechanism-based interventions.

Advances in RNA biology have identified miRNAs as powerful post-transcriptional regulators of gene expression, playing crucial roles in cardiovascular physiology and disease [5,6]. In addition to their canonical function of mRNA silencing via 3′ untranslated region binding, miRNAs are increasingly recognized for their non-canonical roles, including nuclear gene activation, modulation of chromatin architecture, and functional crosstalk with long non-coding RNAs [7–10]. Importantly, atherosclerosis is now recognized to be governed not by individual miRNAs alone, but by interconnected regulatory networks of multiple miRNAs that act in concert to influence endothelial biology, inflammation, and cholesterol metabolism [11]. Among the growing list of miRNAs implicated in atherosclerosis, miR-33a/b and miR-92a have emerged as key regulators of cholesterol metabolism and endothelial function, respectively [12]. Their ability to modulate pathways involved in lipid homeostasis, vascular inflammation, and endothelial integrity highlights their therapeutic potential.

Unlike conventional therapies that address systemic risk markers, miRNA-based strategies offer the promise of targeting core molecular drivers of atherogenesis. This review outlines the complementary roles of miR-33a/b and miR-92a in atherosclerosis pathogenesis, with a focus on cholesterol transport and endothelial repair. We further summarize their modulation of classical pathways, including insulin and nitric oxide signaling, and discuss cutting-edge therapeutic approaches such as antisense oligonucleotides and nanoparticle delivery systems under investigation in preclinical models.

2. miR-33: master regulator of cholesterol metabolism

2.1. miR-33a/b-mediated control of cholesterol efflux

The elimination of excess cholesterol from peripheral tissues is a critical defense mechanism against atherosclerosis, facilitated by cholesterol efflux. This tightly regulated process depends on ATP-binding cassette transporters ABCA1 and ABCG1 which mediate the transfer of cholesterol to high-density lipoproteins (HDL), enabling its systemic clearance [13]. Dysfunction of this efflux pathway leads to intracellular cholesterol accumulation and foam cell formation, an early hallmark of atherosclerotic plaque development.

miR-33 acts as a molecular brake on cholesterol efflux. In humans, miR-33a and miR-33b are intronic microRNAs embedded in the SREBF2 and SREBF1 genes, respectively, whereas only miR-33a

is present in mice (within Srebf2). miR-33 targets the 3′ untranslated regions (3′UTRs) of ABCA1 and ABCG1, promoting their mRNA degradation and reducing protein expression, thereby impairing cholesterol efflux [14,15] (Figure 1). In humans, the combined activity of both isoforms exerts a synergistic effect on suppressing transporter expression. This not only reduces cholesterol export but also indirectly downregulates Liver X Receptor (LXR) activity, a transcriptional activator of ABCA1, leading to a dual suppression mechanism [16].

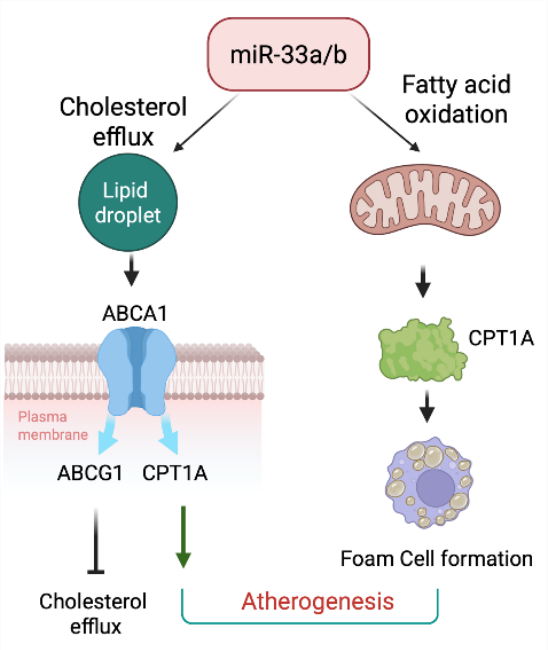

Figure 1. miR-33a/b represses cholesterol efflux by targeting ABCA1 and ABCG1 and inhibits fatty acid oxidation via CPT1A suppression, promoting foam cell formation and accelerating atherogenesis. “Created with BioRender.com”.

Given its critical role in cholesterol regulation, miR-33 has emerged as a compelling therapeutic target. In ApoE-deficient mouse models, miR-33 inhibition enhanced ABCA1 expression, increased HDL levels, and reduced foam cell formation by approximately 40%, significantly mitigating plaque progression [14]. Elevated miR-33 expression correlates with reduced ABCA1 levels and impaired cholesterol efflux in peripheral blood mononuclear cells from atherosclerotic patients [17]. Notably, in addition to its presence in circulating mononuclear cells, miR-33b has been observed to be markedly upregulated in human carotid atherosclerotic plaques under hypercholesterolemic conditions [18]. This finding further validates its function as a local regulator of ABCA1 expression *in vivo*. Genetic deletion of miR-33 in atherosclerosis-prone mice has demonstrated improved vascular function and marked reductions in plaque size and lipid content [19].

However, clinical translation requires overcoming the challenges of achieving targeted delivery. Emerging platforms such as lipid nanoparticles and exosome-based systems show promise in delivering miR-33 inhibitors to atherosclerotic plaques while minimizing off-target effects [20]. A summary of key preclinical and *in vitro* studies investigating miR-33 expression, its target genes, and their role in atherosclerosis progression is presented in Table 1.

Table 1. Summary of experimental studies on miR-33.

Study	miR-33 Expression	Sample Type	Species	Target Genes	Method	Outcome	Contextual Considerations
Zhang *et al.*, 2023 [21]	Downregulated	Macrophages	Mouse	COL2A1, COL3A1, COL1A2, FN1, TIMP3, MMP12, ABCA1	pH-Low Insertion Peptides	Atheroprotective	Use of targeted peptide may increase cellular uptake specificity, murine model with metabolic stress context.
Price *et al.*, 2020 [22]	Downregulated	Hepatocytes	Mouse	SREBP2, ABCA1, ABCG1	Not Applicable	Mixed: atherogenic lipid effects but metabolic improvement	Model focused on hepatic steatosis; metabolic outcomes improved while lipid-related targets remained altered.
Phu *et al.* 2022 [23]	Downregulated	THP-1 macrophages	Mouse	TNF, IL1-B, CD86, CD80, CD206, CD163, SREBP1, SREBP2, UCP1, PPARGC1A	Not Applicable	Atheroprotective	Complex co-culture system modeling inflammation, strong anti-inflammatory marker suppression.
Sun *et al.*, 2018 [24]	Upregulated	THP-1 monocytes, blood, aorta	Mouse	ABCA1, CD36, ABCG1, SR-BI	Not Applicable	Pro-atherogenic	Upregulation likely due to atherogenic stimulus, murine aortic tissue + monocyte activation context.
Price *et al.*, 2017 [25]	Upregulated	Arterial macrophages	Mouse	ABCA1, HADHβ, CROT	Not Applicable	Atheroprotective	Suggests context-specific gene reprogramming in macrophages under vascular stress conditions.
Rayner *et al.*, 2011 [26]	Downregulated	Liver tissue	Mouse	ABCA1, ABCG1	Anti-miR33 oligo	Atheroprotective	Strong *in vivo* validation, supports classic anti-miR33 lipid efflux role.
Ouimet *et al.*, 2017 [27]	Overexpression	Peritoneal macrophages	Mouse	APOA1, ABCA1, ATG5, LAMP1, PRKAA1, FOXO3	Overexpression	Pro-atherogenic	Excess overexpression may disrupt autophagy-lipid handling, supports tight regulation of miR-33 levels.
Yuan *et al.*, 2021 [28]	Downregulated	RAW264.7 cell line	N/A	-	Lipid microbubbles	Targeted delivery efficacy	Focused on delivery platform; no direct atherogenesis data, delivery vehicle validated.
Martino *et al.*, 2015 [29]	Upregulated	Plasma	Human	-	Not Applicable	Associated with metabolic markers	Observational clinical study, miR-33 level correlated with metabolic markers, not causality.
Goedeke *et al.*, 2014 [30]	Downregulated	Plasma	Mouse	NFYC, ACC, FAS	Not Applicable	Atheroprotective	Confirmed miR-33 inhibition reduces lipogenesis, systemic metabolic benefits.
Marquart *et al.*, 2014 [31]	Downregulated	Plasma	Mouse	ABCA1, CPT1α, ABCB11	Anti-miR-33	No atherosclerosis change	Suggests dose, timing, or model-specific thresholds may blunt therapeutic response.
Zhang *et al.*, 2021 [32]	Upregulated	HUVEC	Mouse	NR4A	Overexpression	Pro-inflammatory	miR-33 may modulate vascular inflammation pathways in endothelium under high-glucose stress.
Xie *et al.*, 2021 [33]	Upregulated	Endothelial cells	Human	ABCA1, CS	oxLDL exposure	Pro-atherogenic	oxLDL-induced miR-33 upregulation linked to endothelial dysfunction, oxidative stress model.
Afonso *et al.*, 2021 [34]	Downregulated	Blood monocytes, aorta	Mouse	ABCA1, NCOA1, CROT	Anti-miR-33	Atheroprotective	Robust *in vivo* study using anti-miR therapy, lipid efflux genes restored in vascular macrophages.

2.2. Impact of miR-33 on lipid metabolism

Beyond its role in cholesterol efflux, miR-33 significantly affects fatty acid metabolism, thereby exacerbating atherosclerotic pathology. It targets carnitine palmitoyltransferase 1A (CPT1A) a key enzyme in mitochondrial fatty acid β-oxidation, resulting in decreased fatty acid breakdown and increased lipid deposition in vascular tissues [35]. This metabolic reprogramming fosters foam cell formation and plaque development.

Inhibition of miR-33 upregulates CPT1A, CROT, and HADHB while downregulating lipogenic genes such as SREBF1 and FASN, promoting lipid oxidation and improving metabolic profiles [36,37]. These adaptations contribute to decreased circulating VLDL-triglyceride levels and enhanced lipid handling in vascular macrophages.

miR-33 also induces pro-inflammatory responses. Macrophages treated with miR-33 mimics (or overexpressing miR-33) exhibit increased lipid droplet formation, reduced mitochondrial respiration, and elevated expression of inflammatory mediators such as TNF-α and IL-6 [27,38]. Conversely, antisense oligonucleotides (ASOs), locked nucleic acid (LNA) inhibitors, and small molecule antagonists of miR-33 reduce lipid accumulation, increase HDL, and improve systemic lipid profiles in non-human primates [39,40] . Initial non-human primate (NHP) studies using a subcutaneous 8-mer LNA antimiR against miR-33a/b achieved robust derepression of targets such as ABCA1, elicited a ~40% rise in circulating HDL-C, and showed no evidence of hepatic or renal toxicity over a 108-day treatment course [41]. Long-term murine studies of miR-33 inhibition under diet-induced obesity regimens demonstrated species-specific metabolic effects, including impaired lipid metabolism [42]. These conflicting results underscore the species-specific nature of miR-33 biology and caution against direct extrapolation of preclinical findings to humans.

3. miR-92a: guardian of endothelial health

The regulation of endothelial homeostasis involves multiple microRNAs, among which miR-92a has received particular attention due to its roles in angiogenesis, endothelial repair, and vascular inflammation. miR-92a is part of the highly conserved miR-17~92 cluster, known for its dual roles in cell proliferation and vascular biology. Within this cluster, miR-92a stands out for its consistent antiangiogenic effects and its ability to repress KLF2, KLF4, and eNOS, key regulators of endothelial function. Inhibition of miR-92a has demonstrated therapeutic benefits in various models of ischemia and atherosclerosis [43,44], and a locked nucleic acid inhibitor of miR-92a (MRG-110) has already progressed to Phase I clinical testing [45]. This review emphasizes miR-92a because of its well-characterized repressive effects on vascular regeneration and its emerging status as a clinically actionable target in cardiovascular disease.

3.1. miR-92a-Induced endothelial dysfunction

Endothelial dysfunction is a critical, early, and reversible event in the pathogenesis of atherosclerosis. miR-92a has been identified as a critical contributor to endothelial dysfunction, acting via repression of the Krüppel-like factors KLF2 and KLF4 [46]. Mechanistically, it binds to conserved sequences in the 3′ untranslated regions (3′UTRs) of KLF2 and KLF4 mRNAs, resulting in their translational repression. This silencing promotes endothelial activation, leukocyte adhesion, and upregulation of adhesion

molecules such as VCAM-1, E-selectin, and MCP-1, creating a pro-atherogenic environment [46]. Notably, miR-92a expression is spatially enriched in athero-susceptible arterial regions, such as branch points and curvatures. These regions are subjected to disturbed flow, which suppresses KLF2/KLF4 expression and promotes endothelial dysfunction [46–48]. This spatial localization indicates the mechanosensitive nature of miR-92a in mediating regional vulnerability to atherogenesis (Figure 2). Also, in addition, miR-92a directly targets tight junction proteins such as claudin-5 and occluding, disrupting endothelial barrier integrity and facilitating inflammatory cell infiltration [49]. Inhibition of miR-92a restores endothelial junctions, reduces macrophage and T-cell recruitment, and suppresses vascular inflammation in experimental models [50,51].

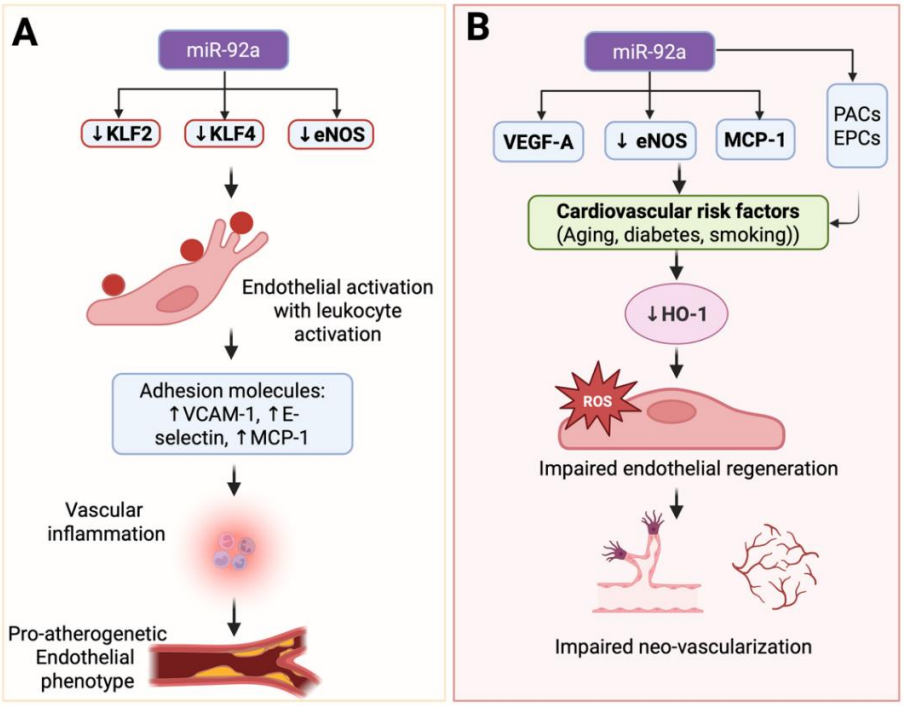

Figure 2. Mechanistic role of miR-92a in endothelial dysfunction and impaired vascular repair. **(A)** miR-92a-Induced Endothelial Dysfunction and Inflammation; **(B)** miR-92a-Mediated Inhibition of Angiogenesis and Endothelial Regeneration. "Created with BioRender.com".

3.2. Role of miR-92a in angiogenesis and vascular repair

miR-92a disrupts angiogenic signaling and vascular repair by directly targeting key regenerative mediators, including vascular endothelial growth factor A (VEGFA) and eNOS. These factors are critical for endothelial regeneration, proliferation, migration, and perfusion recovery in ischemic tissues [52,53].

Cardiovascular risk factors such as aging, diabetes, smoking, and hypercholesterolemia induce miR-92a expression in proangiogenic cells (PACs) and endothelial progenitor cells (EPCs), impairing their regenerative capacity. This leads to reduced capillary density, impaired endothelial migration and proliferation, and diminished neovascularization in ischemic tissues [54,55].

miR-92a also suppresses heme oxygenase-1 (HO-1), a cytoprotective enzyme involved in oxidative stress mitigation and nitric oxide preservation [56,57]. In diabetic mouse models, miR-92a inhibition restored HO-1 levels, decreased reactive oxygen species (ROS) generation, and improved endothelium-dependent vasodilation [56,58].

Advanced delivery systems such as lipid nanoparticles (LNPs) and engineered extracellular vesicles (EVs) have significantly improved the stability, endothelial specificity, and therapeutic potential of miR-92a inhibitors. Targeted delivery to endothelial cells has led to substantial reductions in vascular inflammation and improved tissue revascularization in ischemic models [59–61].

This evidence indicates that miR-92a plays a multifaceted role in driving endothelial dysfunction, barrier breakdown, and impaired vascular regeneration, reinforcing its therapeutic value as a modifiable target in atherosclerosis. Relevant findings on miR-92a's expression levels, experimental models, molecular targets, and vascular effects are summarized in Table 2.

Table 2. Summary of experimental studies on miR-92a.

Study	miR-92a Expression	Sample Type	Species	Target Genes	Method	Outcome
Wang *et al.*, 2019 [62]	Upregulated	Blood	Human	-	Not Applicable	Represses cholesterol efflux
Loyer *et al.*, 2014 [63]	Upregulated	HUVEC	Mouse	-	Not Applicable	Promotes endothelial activation
Fang *et al.*, 2012 [46]	Upregulated	Arterial endothelial cells	Swine	KLF2, KLF4	Not Applicable	Promotes endothelial inflammation and atherogenesis
Grosse *et al.*, 2021 [64]	Slightly upregulated	Blood and carotid plaques	Human	-	Not Applicable	Associated with carotid pathology
Zhou *et al.*, 2021 [65]	Overexpression	Arterial endothelial cells	Mouse	VCAM1	Overexpression	Promotes endothelial activation
Jiang *et al.*, 2014 [66]	Downregulated	Blood	Human	-	Not Applicable	Associated with lower atherosclerosis risk
Chabowski *et al.*, 2023 [67]	Upregulated	Resistance arterioles	Human	LPP3	Not Applicable	Involved in vascular regulation
Zhang *et al.*, 2014 [50]	Overexpression	HUVEC	N/A	PTEN	Overexpression	Protective against oxidative stress-induced apoptosis
Eren *et al.*, 2022 [68]	Upregulated	Plasma	Human	APOA-I, PON1, CP, CRP	Not Applicable	Associated with inflammation and lipid markers
Wang *et al.*, 2019 [60]	Downregulated	Thoracic aorta, A7r5 cells	Mouse	APOE, MLCK	Not Applicable	Promotes vessel wall thickening
Lin *et al.*, 2021 [69]	Downregulated	Coronary artery endothelial cells	Human	KLF2	Not Applicable	Atheroprotective effects
Barbalata *et al.*, 2020 [70]	Downregulated	Femoral artery plaques	Human	-	Not Applicable	Downregulated in plaque tissue
Xiaoling *et al.*, 2016 [71]	Upregulated	THP-1 cells, Blood, Thoracic aorta	Mouse	H3K27me3, EZH2	Not Applicable	Promotes homocysteine-related atherosclerosis
Wu *et al.*, 2011 [72]	Downregulated	HUVEC	Mouse	KLF2	Not Applicable	Atheroprotective
Gou *et al.*, 2018 [56]	Upregulated	Diabetic aortic endothelial cells	Mouse	HO-1	Not Applicable	Linked to oxidative stress
Stojkovic *et al.*, 2018 [73]	Upregulated	Main/proximal aorta	Mouse	KLF2, KLF4	Not Applicable	Suppresses endothelial protective factors
Liu *et al.*, 2017 [74]	Overexpression	Thoracic aorta	Rat	MMP9, TIMP3, SIRT1	Overexpression	Inhibits smooth muscle cell migration
Huang *et al.*, 2017 [75]	Upregulated	Unspecified	Human	-	Not Applicable	Promotes atherogenesis

4. Crosstalk between miRNAs and classical signaling pathways

4.1. miR-33 and miR-92a regulation of insulin signaling

Among the classical signaling pathways influencing atherogenesis, insulin signaling represents a critical intersection between metabolic and vascular dysfunction. Dysregulation of this pathway drives lipid abnormalities and compromises endothelial integrity, thereby providing a key axis through which miR-33 and miR-92a exert their pathogenic effects. Insulin resistance, a hallmark of metabolic syndrome, plays a key role in the progression of atherosclerosis by disrupting both lipid metabolism and endothelial homeostasis [76–78]. Among miRNAs involved in these processes, miR-33 is centrally implicated due to its suppression of insulin receptor substrate 2 (IRS2), a key adaptor in insulin signaling.

miR-33 overexpression in insulin-resistant models leads to IRS2 downregulation, reduced PI3K activity, and decreased AKT phosphorylation, ultimately impairing insulin signaling [35,79]. Conversely, miR-33 inhibition restores IRS2 levels, enhances PI3K-AKT activation, and improves metabolic outcomes [79].

Similarly, miR-92a aggravates endothelial dysfunction under insulin-resistant conditions by reducing eNOS activity, a key mediator of insulin's vasoprotective effects. This results in decreased nitric oxide (NO) availability, impaired vasodilation, and heightened endothelial inflammation [77,80].

These converging pathways underscore how miR-33 and miR-92a mediate an integrated axis of metabolic and vascular dysfunction, suggesting that their inhibition may offer a dual therapeutic strategy for insulin resistance and atherosclerosis.

4.2. miR-33 and miR-92a Regulation of Nitric Oxide signaling

NO is a critical regulator of vascular homeostasis, acting to suppress leukocyte adhesion, smooth muscle proliferation, platelet aggregation, and endothelial dysfunction [81].

miR-92a directly suppresses eNOS, reducing NO production and promoting a pro-inflammatory endothelial phenotype [52]. In parallel, miR-33 disrupts NO signaling indirectly by inhibiting cholesterol efflux, which alters plasma membrane lipid rafts the microdomains where eNOS localizes and becomes activated [82].

This dual suppression of NO synthesis, through transcriptional repression (miR-92a) and membrane remodeling (miR-33) further amplifies endothelial dysfunction. Elevated miR-92a also represses HO-1, increasing oxidative stress and impairing NO-mediated vasodilation. Inhibition of miR-92a has been shown to reverse these effects, restoring HO-1 levels, reducing ROS production, and improving endothelial relaxation [56].

Together, miR-33 and miR-92a form a deleterious feedback loop that reduces NO bioavailability, exacerbates vascular injury, and accelerates atherogenesis. Their simultaneous targeting may yield synergistic benefits by restoring lipid homeostasis and vascular integrity (Figure 3).

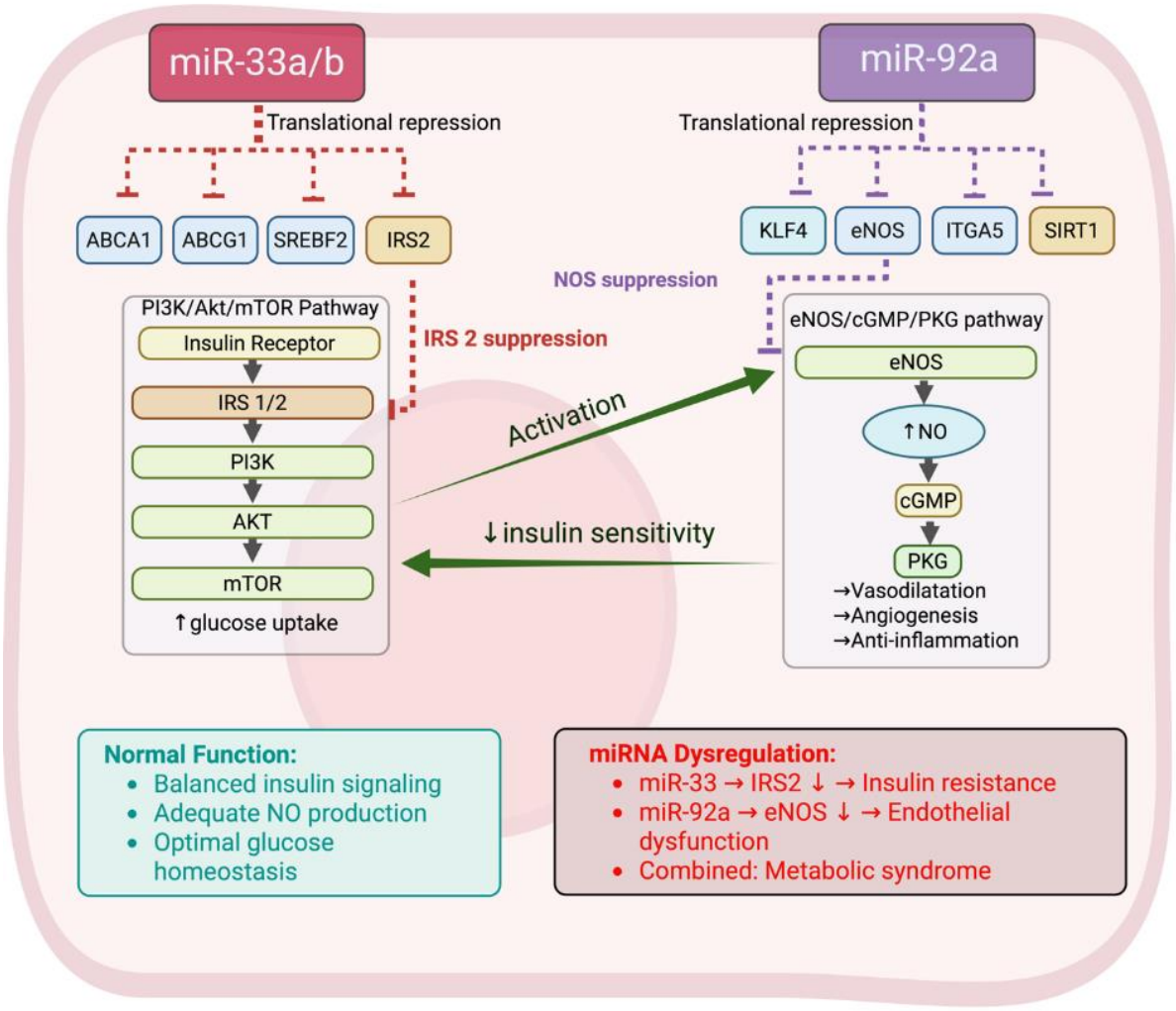

Figure 3. Crosstalk between miR-33 isoforms (miR-33a and miR-33b) and miR-92a in regulating insulin signaling and NO pathways. miR-33a and miR-33b (intronic in SREBF2 and SREBF1, respectively) suppresses IRS2 and cholesterol transporters (ABCA1, ABCG1), impairing PI3K/AKT signaling and insulin sensitivity. miR-92a represses eNOS, KLF4, and SIRT1, reducing NO production and endothelial function. Dysregulation of both miRNAs contributes to metabolic syndrome by impairing glucose homeostasis and vascular health. "Created with BioRender.com".

5. Clinical translation: from bench to bedside

5.1. Clinical trials and translational feasibility

The transition of microRNA-based therapeutics from preclinical proof-of-concept to clinical application is progressing, though with varying momentum across different miRNA targets. Anti-miR-33 therapy, which has shown considerable promise in preclinical models by increasing HDL-C and promoting reverse cholesterol transport through upregulation of targets such as ABCA1 and ABCG1, has yet to reach clinical trials in humans. Studies in mice and non-human primates demonstrated that miR-33 inhibition led to favorable lipid modulation and regression of atherosclerotic lesions [14,83]. However, the long-term safety profile of miR-33 inhibition remains debated: while chronic dosing in mice has

been associated with hepatic lipid accumulation and hypertriglyceridemia [42], these adverse effects were not observed in long-term studies in non-human primates [41]. This species-dependent divergence underscores unresolved toxicity concerns that have delayed translation. As of 2025, no registered Phase I trial exists for anti-miR-33 in dyslipidemia or cardiometabolic disease, reflecting ongoing caution.

In contrast, anti-miR-92a therapy has successfully advanced into early-phase clinical evaluation. MRG-110, a locked nucleic acid (LNA)-based anti-miR-92a compound developed by miRagen Therapeutics (now part of Viridian Therapeutics), has undergone two Phase I clinical trials (NCT03603431 and NCT03494712). These placebo-controlled studies enrolled healthy volunteers to assess safety, pharmacokinetics, and target engagement. Results published by a study demonstrated dose-dependent reductions in miR-92a levels and miR-92a targets such as ITGA5 and KLF2 in peripheral blood mononuclear cells, with no serious adverse events reported [84]. These findings support the feasibility and safety of anti-miR-92a inhibition in humans, setting the stage for future studies in cardiovascular patients.

Despite these early promising findings, multiple barriers must be addressed for full clinical implementation. First, optimal dosing remains uncertain due to variable tissue distribution and pharmacokinetics of oligonucleotide therapies. Second, patient selection is evolving, with evidence suggesting that genetic polymorphisms within miRNA binding sites may influence therapeutic response [85]. Third, the prolonged treatment duration required for cardiovascular risk modification necessitates robust long-term safety data, which are currently lacking. Table 3 summarizes the major challenges associated with clinical translation of miRNA therapeutics.

Table 3. Major challenges associated with clinical translation of miRNA therapeutics.

Challenge category	Specific issue	Example
Delivery specificity	Preferential liver/kidney uptake	Limits vascular tropism: LNPs functionalized with VCAM-1 or plaque-homing ligands demonstrate endothelial-selective biodistribution [86,87]
Off-target effects	Partial homology to unintended mRNAs	CRISPR–Cas12a–based transcriptomic biosensors enable unbiased mapping of miRNA–mRNA interactions with enhanced specificity [88,89]
Immune activation	TLR stimulation, especially with phosphorothioate backbones	Ionizable and PEGylated LNP formulations attenuate immunostimulation; TLR-inert backbone chemistries are under active investigation [90,91]
Long-term safety	Organ accumulation, compensatory network shifts	Chronic administration perturbs miRNA-mRNA homeostasis in non-human primates; longitudinal transcriptomic surveillance is recommended [92,93]
Inter-individual variability	Genetic polymorphisms in miRNA or target UTRs	Genome-wide association studies (GWAS) reveal UTR single nucleotide polymorphisms (SNPs) that modulate miRNA binding affinity; AI-guided dose optimization platforms are in early clinical evaluation [94,95]
Regulatory complexity	Hybrid classification of RNA therapeutics	2024 FDA and EMA draft guidance documents designate oligonucleotide therapeutics as hybrid biologic–small molecule entities, necessitating dual regulatory pathways [96,97]
Cost and scalability	High GMP-grade oligonucleotide production costs	Continuous-flow oligonucleotide synthesis strategies reduce per-gram costs; self-amplifying RNA systems are being evaluated for scalable clinical deployment [98,99]

5.2. Regulatory hurdles and safety considerations

The regulatory framework for microRNA-based therapeutics poses distinct challenges that differ from traditional drug development approaches. Both the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have established specialized guidelines for RNA-based therapies, emphasizing the need to evaluate hybridization-dependent and independent toxicities, immune activation potential, and organ-specific accumulation profiles [86]. These requirements reflect the complex pharmacodynamic behavior of oligonucleotide-based agents and their broad biological activity.

Long-term safety concerns remain a critical barrier to clinical translation. First, off-target effects are a significant risk due to partial sequence homology between therapeutic miRNAs and unintended mRNA transcripts. While newer oligonucleotide chemistries have improved target specificity, multi-tissue transcriptomic profiling is now mandated to detect off-target interactions [88]. Second, tissue accumulation, particularly in the liver and kidneys, has been observed with chronic dosing of phosphorothioate-modified oligonucleotides. For instance, anti-miR-33 therapy induced mild vacuolation in renal tubular cells in non-human primates after 12 months, though without functional impairment [92]. Third, systemic biological effects must be considered, as miRNAs such as miR-33 influence diverse pathways beyond lipid metabolism, including glucose regulation, hematopoiesis, and neuronal function. As a result, longitudinal multi-omics monitoring (e.g., metabolomics, lipidomics) is now standard practice in early-phase trials [94].

Adding further complexity, genetic and immunological interindividual variability influences safety and efficacy. Polymorphisms in miRNA processing enzymes (e.g., DICER1, DROSHA) can alter oligonucleotide pharmacokinetics, while pre-existing inflammatory states may amplify TLR-mediated immune responses, underscoring the need for personalized safety assessments [90].

6. Comparison with existing therapies

miRNA-based therapeutics offer several potential advantages over current lipid-lowering and anti-inflammatory strategies, although direct head-to-head clinical data remain limited.

6.1. Lipid-lowering therapies

Beyond their canonical role in lowering LDL-C, statins also regulate miR-33 expression, with evidence showing both suppression of ABCA1-mediated cholesterol efflux [100] and contribution to hepatotoxicity reversible by anti-miR-33 [101], while high-dose rosuvastatin has been reported to increase ABCA1 in human plaques independent of cholesterol levels [102]. Compared to statins, anti-miR-33 therapy exerts complementary effects. While statins inhibit HMG-CoA reductase to lower LDL-C by 30–50%, miR-33 inhibition enhances reverse cholesterol transport, increasing HDL-C and improving cholesterol efflux mechanisms that may address residual cardiovascular risk unmitigated by statins [26].

In preclinical models, combined statin and anti-miR-33 treatment produced synergistic effects, reducing plaque burden and improving lipid profiles beyond either agent alone. Although head-to-head clinical trials directly comparing PCSK9 inhibitors and miR-33–targeting therapies have not been conducted, existing evidence highlights distinct mechanisms and outcomes. PCSK9 inhibitors substantially reduce LDL-C levels by ~50–60% through stabilization of LDL receptors on hepatocytes, as shown in both preclinical

and clinical studies [103–105]. In contrast, miR-33 inhibition produces a more modest LDL-C reduction of ~10–15% in animal models, largely mediated via derepression of ABCA1 and enhanced reverse cholesterol transport [26,106,107]. Importantly, miR-33 inhibition also provides systemic benefits, including improved insulin sensitivity, enhanced fatty acid oxidation, and reduced triglyceride levels effects not typically observed with PCSK9 blockade [100,108]. PCSK9 inhibitors remain highly effective for lowering LDL-C, yet their benefits are largely confined to this single pathway and do not fully mitigate residual cardiovascular risk. In comparison, inhibition of miR-33 has the potential to exert broader effects by promoting cholesterol efflux and elevating HDL-C levels., Findings from long-term studies in mouse models have raised important safety concerns, including hepatic steatosis and dyslipidemia [42]. These observations underscore the translational barriers that must be addressed before miR-33 inhibition can be considered a viable therapeutic strategy. Although PCSK9 inhibitors achieve substantial LDL-C reductions of 50–60%, their use is limited by annual costs of approximately $14,000–18,000. Economic models suggest that miRNA-based therapeutics could reach comparable cost-effectiveness if manufacturing efficiencies are realized and their broader cardiometabolic benefits are confirmed in Phase III clinical trials [109].

6.2. Anti-inflammatory therapies

Anti-inflammatory therapies targeting miRNAs, including miR-92a and miR-33, are under study for their potential to reduce vascular inflammation, restore endothelial homeostasis, and modulate lipid metabolism in atherosclerosis. miR-92a inhibition has been shown to promote endothelial repair and protect against vascular injury. In experimental models, inhibition of miR-92a enhanced re-endothelialization and reduced neointima formation following vascular damage [52], while also promoting angiogenesis and reducing ischemia/reperfusion injury by attenuating oxidative stress and inflammatory signaling in the myocardium [110]. Beyond the cardiovascular system, inducible anti-miR-92a approaches accelerated wound healing through SIRT1-mediated keratinocyte proliferation in diabetic mouse models [111]. Importantly, in the context of atherosclerosis, Loyer *et al.* demonstrated that anti-miR-92a treatment in ApoE-deficient mice reduced atherosclerotic lesion burden and restored endothelial function by preserving KLF2/KLF4-dependent protective pathways [63]. Collectively, these findings establish miR-92a inhibition as a therapeutic strategy with both endothelial-specific and regenerative benefits.

miR-33 inhibition has been extensively investigated for its dual effects on lipid metabolism and inflammation. A study reported that antagonism of miR-33 upregulated ATP-binding cassette transporter A1 (ABCA1), thereby enhancing cholesterol efflux, promoting reverse cholesterol transport, and attenuating atherosclerosis progression [26]. Complementary studies showed that miR-33 inhibition reduces pro-inflammatory cytokine secretion, including TNF-α and IL-6, in lipopolysaccharide-stimulated macrophages [112], while also improving mitochondrial function, fatty acid oxidation, and lipid handling within macrophages, thereby limiting foam cell formation and inflammatory activity [107].

Taken together, these results highlight the complementary therapeutic potential of targeting miR-92a and miR-33. Whereas miR-92a inhibition primarily restores endothelial integrity and vascular repair, miR-33 inhibition improves cholesterol metabolism and attenuates macrophage-driven inflammation. Their combined modulation may therefore provide a synergistic strategy to simultaneously address endothelial dysfunction and lipid-driven inflammation in atherosclerosis.

6.3. Dual-target approaches

The concept of dual-target oligonucleotides targeting multiple miRNAs simultaneously represents an emerging "polypill" approach in cardiovascular therapeutics. While individual inhibition of miR-33 and miR-92a has shown promise in preclinical models with miR-33 inhibition improving cholesterol efflux pathways and miR-92a inhibition enhancing endothelial function comprehensive studies evaluating their combined therapeutic potential remain limited. The rationale for dual targeting stems from the complementary mechanisms of these miRNAs, where miR-33 primarily affects lipid metabolism, whereas miR-92a influences endothelial health and inflammatory responses (Table 4).

Table 4. Comparison of miRNA-based therapies with existing cardiovascular treatments.

Therapy type	Target/Mechanism	Primary benefit	Limitations	miRNA Therapy complementarity
Statins [26]	HMG-CoA reductase inhibition	↓LDL-C by 30%–50%	Limited HDL impact; residual risk persists	miR-33 inhibition ↑ HDL, ↑ cholesterol efflux
PCSK9 inhibitors [109]	PCSK9-LDLR pathway inhibition	↓LDL-C by 50%–60%	High cost; no direct effect on HDL or insulin	miR-33 inhibition improves insulin sensitivity
Canakinumab [63]	IL-1β monoclonal antibody	↓ systemic inflammation	Expensive; not endothelial-specific	miR-92a inhibition restores endothelial health
Anti-miR-33 [26]	ABCA1/ABCG1 derepression	↑ HDL, ↓ foam cells	Still preclinical; delivery challenges	Targets lipid handling and inflammation
Anti-miR-92a [63]	KLF2/KLF4/eNOS derepression	↑ endothelial repair, ↓ inflammation	Early-phase trials; tissue specificity required	Synergistic with statins, anti-inflammatories
Dual miR-33/92a inhibition [113]	Lipid and endothelial pathways together	~70% plaque reduction in preclinical models	Delivery complexity	Potential "polypill" strategy

Economic modeling studies predict that while miRNA therapeutics currently have higher initial costs, anticipated reductions in cardiovascular events, coupled with advancements in scalable production, could yield favorable cost-effectiveness within 5–7 years of clinical implementation [114].

6.4. Evaluating miRNA therapeutics versus siRNA-based interventions: efficacy, durability, and clinical translation

RNA-targeting therapeutics, encompassing small interfering RNAs (siRNAs) and miRNA-based inhibitors, may present distinct yet complementary advantages for the management of atherosclerotic cardiovascular disease (ASCVD). Inclisiran, a hepatocyte-targeted siRNA that silences PCSK9, has demonstrated substantial and sustained ~50% reductions in LDL-C in Phase III ORION trials (e.g., ORION-10 and ORION-11), facilitated by a biannual dosing regimen that enhances adherence and durability of response [115,116]. Its mechanism is characterized by high target specificity and limited off-target effects, making it a clinically attractive option for patients with familial hypercholesterolemia or established ASCVD. In contrast, miRNA therapeutics such as anti-miR-33 and anti-miR-92a regulate broader gene networks and impact multiple pathways, including cholesterol efflux, vascular inflammation, and endothelial repair [26]. This pleiotropic modulation offers the potential for disease modification beyond

lipid lowering but presents challenges in delivery precision and off-target risk, particularly in extrahepatic tissues [117,118]. From a cost-effectiveness perspective, real-world analyses and health economic models suggest that RNA-based therapies like inclisiran may offer acceptable value in high-risk populations if long-term cardiovascular benefits are realized [119,120]. Although miRNA therapeutics are still in earlier stages of development, their translational promise remains strong, particularly as advances in delivery technology and manufacturing scalability continue to reduce barriers to clinical implementation [121]. Overall, while siRNA therapies currently lead in clinical maturity and cost modeling, miRNA-based approaches may provide broader therapeutic value in the future, contingent upon overcoming current limitations in safety, durability, and delivery efficiency.

7. Future directions and remaining knowledge gaps

7.1. Harnessing artificial intelligence and big data for miRNA therapeutic development

Artificial intelligence (AI) and machine learning (ML) are increasingly being applied to accelerate the development of miRNA-based therapies [122–124]. By integrating multi-omics datasets, AI models can map disease-specific regulatory networks [125], predict cooperative effects between miRNAs [126], and stratify patients most likely to benefit from targeted interventions [127]. Advanced deep learning frameworks, such as graph neural networks and transformer architectures, refine the prediction of non-canonical miRNA interactions, revealing crosstalk between lipid metabolism, endothelial repair, and inflammatory pathways [128,129]. Machine learning algorithms are increasingly applied to large-scale miRNA expression datasets from thousands of patients, enabling the identification of subtle regulatory patterns undetectable by traditional analysis. These tools have uncovered novel functional roles for miR-33 and miR-92a in specific patient subgroups, paving the way for precision-targeted therapies [122]. These approaches can also predict compensatory miRNA responses (e.g., miR-34 or miR-126 activation) following miR-33/92a inhibition, guiding rational combination strategies. Natural language processing (NLP) further accelerates discovery by mining literature and integrating heterogeneous data, while federated learning frameworks enable biomarker discovery across decentralized cohorts without sharing raw data, preserving privacy while achieving high model performance.

These computational insights can be prospectively validated in patient-derived vascular tissues or high-throughput perturbation screens, creating a translational bridge from in silico modeling to precision-guided therapeutic strategies [130]. Digital twin models virtual replicas of patient cardiovascular systems that are now being explored to simulate the effects of miRNA inhibition in silico, allowing individualized prediction of therapeutic efficacy and adverse effects before clinical application. Such patient-specific simulations could refine dosing strategies, optimize dual-target regimens, and inform clinical trial design [131,132].

Looking ahead, quantum computing offers the theoretical potential to simulate complex RNA-RNA and RNA-protein interactions. Prototype quantum simulations of miRNA seed-sequence binding reveal secondary binding sites within metabolic networks, suggesting novel targets that are missed by conventional algorithms. However, the advantages of quantum computing over traditional high-performance computing in drug discovery remain mostly theoretical. Practical applications are still in their early stages, with initial efforts to integrate quantum methods into drug design workflows [133–137]. When integrated with digital twin frameworks, quantum-enabled models may accelerate the rational design of targeted

miRNA inhibitors with minimized off-target effects, moving closer to truly personalized miRNA therapeutics development.

7.2. Epigenetic modulation of miRNA expression

Epigenetic regulation, both endogenous and engineered, represents a critical mechanism for modulating miRNA expression relevant to atherosclerosis. CRISPR-based epigenome editing tools, such as dCas9-KRAB, enable the selective silencing of miRNA promoters without altering DNA sequences. In preclinical models, targeted repression of miR-33 using this approach led to sustained ABCA1 upregulation and improved lipid profiles for over six months post-treatment [138].

Conversely, epigenetic activation of vasculoprotective miRNAs may offer complementary therapeutic benefits. Importantly, the miR-17–92 cluster, which includes miR-92a, is subject to endogenous epigenetic repression by histone deacetylase 9 (HDAC9). In endothelial cells, HDAC9 silencing derepresses the cluster and impairs angiogenic signaling, demonstrating a key regulatory checkpoint relevant to vascular homeostasis [43]. Dietary components can also shape epigenetic landscapes governing miRNA transcription. Compounds such as resveratrol and epigallocatechin gallate (EGCG) modulate DNA methylation at the miR-33 and miR-92a loci, offering potential adjunctive strategies to boost therapeutic efficacy [139].

Beyond being regulated epigenetically, certain miRNAs also function as regulators of the epigenome, such as, miR-29 and miR-21 directly target key epigenetic enzymes such as DNA methyltransferases (DNMTs) and histone deacetylases (HDACs), thereby exerting second-order effects on vascular gene expression programs [140]. Nonetheless, the therapeutic potential of epigenetic editing is tempered by concerns about off-target effects. For example, dCas9-KRAB can inadvertently silence genes adjacent to the targeted promoter due to chromatin spreading, and imperfect gRNA specificity may repress unrelated loci. Such risks are particularly important in long-term cardiovascular applications, where even subtle transcriptional perturbations could impact vascular homeostasis. Rigorous off-target profiling (e.g., CUT&Tag, ATAC-seq) and the development of inducible or reversible editing platforms will be critical for safe translation into the clinic. Collectively, these insights highlight the bidirectional relationship between miRNAs and epigenetic machinery, offering both mechanistic clarity and therapeutic leverage.

7.3. Clinical translation challenges

Delivery specificity

Achieving cell- and tissue-specific delivery remains the foremost challenge in miRNA therapy. Most delivery systems exhibit preferential hepatic targeting, limiting utility for vascular-focused interventions. However, third-generation LNPs with tissue-penetrating peptides have shown an 8-fold increase in plaque accumulation compared to earlier designs [141]. Engineered exosomes derived from endothelial cells have demonstrated 60% greater uptake in vascular tissue relative to synthetic carriers [142]. Ligand–oligonucleotide conjugates, such as GalNAc for hepatocyte targeting and peptide-based motifs for endothelium, are being refined for clinical use [143]. These platforms may allow for lower therapeutic dosing, reduced off-target exposure, and enhanced safety.

Beyond single-target delivery, dual inhibition of miR-33 and miR-92a introduces additional translational barriers. miR-33 is primarily expressed in hepatocytes and macrophages, whereas miR-92a is enriched in vascular endothelium exposed to disturbed flow. This compartmentalized biology complicates co-targeting strategies, since most current vehicles preferentially deliver to the liver. Achieving simultaneous therapeutic levels in both the hepatic/macrophage compartments (for miR-33) and vascular endothelium (for miR-92a) may require engineered dual-target nanoparticles with tailored tropism, sequential or compartment-specific dosing regimens, or "polypill" oligonucleotides incorporating modular targeting ligands. Balancing dosing ratios is also a critical challenge: disproportionate inhibition of one miRNA could blunt synergy, exacerbate compensatory network activation, or increase toxicity. While proof-of-principle studies suggest nanoparticle composition and administration route substantially shape biodistribution, no existing platform has yet demonstrated efficient and safe co-delivery of both miRNAs *in vivo*. Overcoming these barriers will be essential for translating dual miRNA inhibition into a clinically viable therapy.

Long-term safety concerns

The chronic use of oligonucleotide-based miRNA therapeutics presents distinct toxicological challenges that must be rigorously addressed prior to widespread clinical adoption. First, while chemical modifications such as phosphorothioate backbones improve stability and therapeutic half-life, they may also lead to progressive accumulation in organs including the liver, kidneys, and spleen. Long-term preclinical studies involving 12 months of continuous dosing have reported vacuolation in hepatocytes and renal proximal tubular cells, though notably without overt functional impairment [144]. Second, immunogenic responses remain variable and poorly predictable. Although newer oligonucleotide chemistries have reduced activation of toll-like receptors (TLRs), patients with underlying autoimmune conditions or genetic polymorphisms in immune sensing pathways may still exhibit exaggerated inflammatory responses, emphasizing the need for pre-treatment immunogenetic screening protocols [145]. Third, the chronic inhibition of miRNAs can lead to target derepression and compensatory shifts in miRNA-mRNA networks, potentially reducing efficacy or eliciting unanticipated physiological effects. Longitudinal transcriptomic profiling in ongoing trials has revealed dynamic alterations in competing regulatory circuits, highlighting the importance of intermittent dosing strategies or combinatorial regimens to preserve therapeutic durability [146]. Addressing these concerns through robust preclinical modeling, patient stratification, and real-time monitoring will be essential for ensuring the long-term safety and clinical viability of miRNA therapeutics.

7.4. Regulatory and cost barriers

miRNA therapies challenge existing regulatory frameworks. Neither small molecule nor biologic guidelines fully accommodate the hybrid nature of these compounds. Agencies are developing specialized approval pathways for oligonucleotide drugs, yet inter-regional inconsistencies pose hurdles for global development [96]. Manufacturing is currently expensive. Current GMP-grade oligonucleotide synthesis yields are low, resulting in estimated annual costs of $50,000–$150,000 per patient. Innovations in continuous-flow and enzymatic synthesis could reduce these costs by up to 80%. Health technology assessment models are also lagging. Most frameworks are ill-suited to evaluate preventive

therapies with lifelong administration. New models incorporating QALYs, long-term cardiovascular event reduction, and system-level cost offsets are essential to guide reimbursement [147].

8. Future of personalized medicine

8.1. Patient-specific miRNA profiling

The heterogeneous nature of atherosclerosis progression suggests that personalized miRNA-based therapies could yield superior clinical outcomes compared to one-size-fits-all approaches. Recent advances in plasma-based miRNA sequencing enable high-throughput profiling of individualized miRNA signatures [148].

In a prospective cohort of 749 patients with coronary artery disease, baseline miRNA expression profiles predicted statin responsiveness with 78% accuracy, identifying a subset (~15%) with poor response to statins who subsequently showed significant benefit from targeted miRNA therapies [149]. Importantly, these miRNA signatures exhibit greater longitudinal stability than traditional protein biomarkers, providing consistent risk stratification across multiple clinical time points.

Emerging evidence supports the use of specific miRNA biomarkers to guide therapy in atherosclerosis and broader cardiovascular disease. For example, polymorphisms in the ABCA1 3′UTR, such as rs4149339, alter the binding affinity of miR-33, a key regulator of cholesterol efflux. Carriers of this variant may require 1.5–2-fold higher doses of anti-miR-33 therapy to achieve comparable therapeutic responses, underscoring the importance of genotype-informed dosing strategies [150]. Similarly, elevated circulating miR-92a levels have been linked to impaired endothelial or cardiac repair mechanisms; notably, inhibition of miR-92a has been shown to confer protection against ischemia reperfusion injury in large-animal models [110]. In addition, circulating miRNA panels incorporating miR-33, miR-126, and miR-146a have demonstrated value in stratifying cardiovascular risk and predicting responsiveness to lipid-lowering or anti-inflammatory therapies, reinforcing their potential as theranostic tools in precision cardiovascular medicine [151,152]. When integrated with genomic, metabolomic, and clinical data via machine learning, miRNA-based models outperform conventional risk calculators in predicting cardiovascular events [153].

8.2. miRNAs as dual biomarkers and therapeutic targets

miRNAs possess a unique theranostic potential, functioning simultaneously as biomarkers and therapeutic targets. This enables the real-time monitoring of treatment engagement, efficacy, and patient-specific responses. In early clinical studies, circulating miR-33 levels changed within 72 hours of therapy initiation, and these changes accurately predicted enhanced cholesterol efflux capacity in responders [26]. Such dynamic monitoring supports rapid treatment adaptation in non-responders. In addition, genotype-guided therapy is gaining relevance. For example, polymorphisms in the ABCA1 3′UTR (e.g., rs4149339) affect miR-33 binding affinity, necessitating dose adjustments-with carriers requiring 1.5–2 × higher doses to achieve therapeutic effects [154].

8.3. Next-generation delivery platforms

The future success of miRNA therapeutics will rely heavily on advanced delivery technologies that enhance their precision, bioavailability, and safety. pH-responsive nanoparticles are designed to remain inert in circulation but release therapeutic cargo in the acidic microenvironment of atherosclerotic plaques, increasing target specificity by four-fold [155]. Aptamer-guided delivery vehicles use molecular recognition elements to selectively bind endothelial or macrophage surface markers. In preclinical models, this strategy achieved 70% greater plaque regression compared to untargeted formulations [156]. CRISPR-mediated miRNA regulation represents a transformative paradigm. Rather than relying on transient inhibition, these tools allow for permanent editing or epigenetic silencing of miRNA loci. For instance, CRISPR suppression of miR-33 resulted in sustained HDL-C elevation for over six months following a single treatment [157].

8.4. Knowledge gaps and research priorities

Despite substantial advances in miRNA-based therapeutics, several critical knowledge gaps must be addressed to ensure safe and effective clinical translation. First, the tissue-specific functions of miRNAs remain incompletely characterized. While systemic inhibition of miR-33 and miR-92a demonstrates vascular benefit, emerging data suggest these miRNAs also play pivotal roles in adipose tissue, the central nervous system, and hematopoiesis, raising concerns about unintended effects in non-target organs [158]. Second, the long-term consequences of sustained miRNA modulation are poorly understood, as most studies have focused on short-term outcomes despite the need for lifelong cardiovascular prevention strategies [159]. Third, gene–environment interactions, including dietary patterns, physical activity, and environmental exposures, can significantly influence both miRNA expression and therapeutic response. Clarifying these modulatory factors is essential for designing personalized, durable, and effective miRNA-based treatment regimens.

Call to action

Bridging the existing knowledge and translational gaps in miRNA-based cardiovascular therapy necessitates a coordinated, multidisciplinary research effort. This includes the development of tissue-specific knockout models to elucidate organ-specific effects, longitudinal population studies to assess biomarker dynamics and treatment response over time, and the optimization of advanced delivery platforms to enhance therapeutic precision. Equally important is the integration of miRNA therapies with existing pharmacologic regimens and the generation of real-world clinical and health economic data to inform regulatory and reimbursement decisions. Addressing these priorities systematically will be essential to accelerate the clinical implementation of miRNA therapeutics and unlock their full potential in precision cardiovascular medicine.

9. Conclusion

The integration of microRNA (miRNA)-based therapeutics in cardiovascular medicine signifies a significant advancement in our ability to target complex disease mechanisms at the molecular level. Among the miRNAs involved in atherosclerosis, miR-33 and miR-92a have been identified as pivotal

regulators of cholesterol homeostasis and endothelial function, which are critical components of atherogenesis. In contrast to traditional therapies that address systemic biomarkers like LDL cholesterol and inflammation, miRNA-targeted interventions provide pathway-specific modulation, allowing for nuanced regulation of gene networks related to lipid metabolism, nitric oxide signaling, and vascular repair. Promising preclinical findings indicate that antisense oligonucleotides, nanoparticle delivery systems, and CRISPR-based methodologies effectively reverse atherosclerosis-related pathologies, while early clinical trials suggest acceptable safety profiles alongside potential functional improvements. However, several challenges must be addressed for successful clinical implementation. Issues such as tissue-specific delivery, long-term safety, inter-patient variability, and scalable production require comprehensive research and innovative solutions. Additionally, regulatory frameworks and the economic models governing the approval and reimbursement of these RNA-based therapies will need adaptation to reflect their distinctive properties and implications.

Authors' contributions

D.S.: writing—original draft, review & editing, methodology, prepared figures, conceptualization. P.D.: writing—original draft, review & editing, methodology, prepared figures, conceptualization. T.H.: writing—original draft, review & editing, conceptualization. A.C.: writing—review & editing, writing—original draft, supervision, resources, methodology, conceptualization. All authors have read and approved the manuscript.

Conflicts of interests

The authors declare no conflict of interest.

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