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Transcending the proteomic ceiling: regulatory networks, translational barriers, and the AI-assisted opportunities of microRNA therapeutics

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Highlights:

- miRNAs regulate disease gene-networks inaccessible to antibodies or small molecules.
- Mimics, antagomiRs, and antisense oligonucleotides directly modulate these networks.
- Clinical candidates reveal roadblocks: delivery, immunogenicity, off-target effects.
- miRNA dysregulation links cancer, cardiovascular disease, and renal pathology.
- AI-assisted target and biomarker discovery is promising but clinically unproven.

Abstract: MicroRNAs (miRNAs) are short non-coding RNAs (ncRNAs) that regulate gene expression post-transcriptionally, guiding Argonaute-containing RNA-Induced Silencing Complexes (RISC) to complementary messenger RNA (mRNA) sequences to repress translation and promote degradation. Because a single miRNA can influence hundreds of transcripts, these molecules offer access to regulatory nodes, including transcription factors and protein-protein interaction interfaces, that conventional antibodies and small-molecule inhibitors cannot reach, constrained as these are by target "druggability". This has driven sustained interest in miRNA-driven therapeutics—mimics, antagomiRs, and antisense oligonucleotides—as a route to modulating disease networks directly at the RNA level. This narrative review synthesizes current understanding of miRNA biogenesis, delivery platforms such as lipid nanoparticle (LNP) encapsulation and chemically modified oligonucleotides, as well as miRNA dysregulation across oncological, cardiovascular, and renal pathologies. We also examine the clinical trajectory of representative candidates (Cobomarsen, Remlarsen, Miravirsen) and the roadblocks—off-target effects, delivery inefficiency, and immunogenicity—that have limited their translation. Finally, we review emerging machine learning (ML) and deep learning (DL) applications to miRNA target prediction and biomarker discovery, including tools that outperform established prediction algorithms, considering whether these computational approaches address, rather than merely reframe, the field's translational bottlenecks. We conclude that miRNA therapeutics remain constrained by delivery and safety challenges, while artificial intelligence (AI)-assisted discovery represents a plausible, yet clinically unproven, avenue of progress.

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Keywords: miRNA; non-coding RNA therapeutics; lipid nanoparticles; gene silencing; oligonucleotide delivery; miRNA biomarkers; post-transcriptional regulation

1. Introduction

MicroRNAs (miRNAs) are 21–23 nucleotides long non-coding RNAs (ncRNAs) regulating post-transcriptional gene expression. Their genesis begins with the miRNA genes being transcribed by RNA Polymerase II to form the longer nuclear hairpin shaped precursors, primary-microRNA (pri-miRNA). The pri-miRNAs are then cleaved by Drosha-DiGeorge syndrome critical region 8 (DGCR8), a nuclear microprocessor complex to form shorter precursor sequences known as precursor-miRNAs (pre-miRNAs) exported further into the cytoplasm by Exportin-5. The cytoplasm plays the role of being the site of incipient development where miRNA specific nuclease called Dicer enters the act to cleave the precursor and leads to the formation of mature miRNAs duplex/double stranded miRNAs, 21–23 nucleotide in length [1] (Figure 1). They then direct the Argonaute (AGO) proteins to bind to and repress complementary messenger RNA (mRNA) targets. Numbering an estimated 2300 or more miRNAs in humans, a widespread regulatory role is played by miRNAs across the human genome with repression of hundreds of target genes [2].

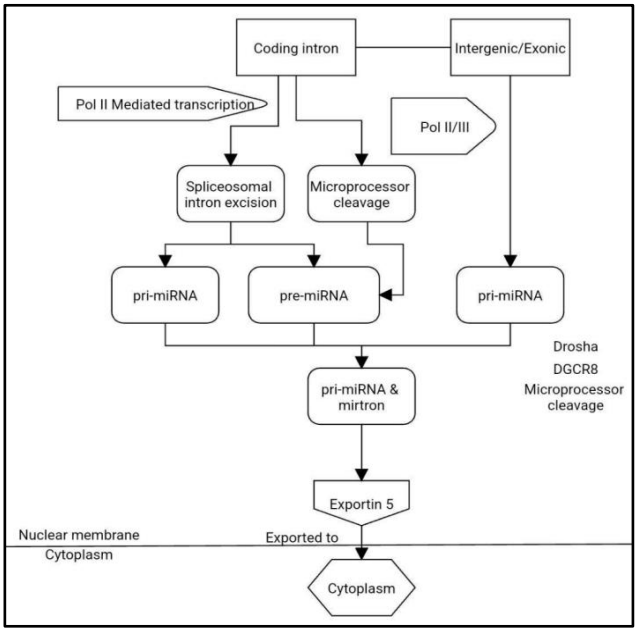


Figure 1. Canonical and Non-Canonical Pathways of MicroRNA Biogenesis. **(a)** Schematic representation of nuclear microRNA (miRNA) transcription and processing pathways. Transcription of genomic loci, originating from intergenic, and exonic or coding intron regions, is driven by RNA Polymerase II or III; **(b)** In the canonical pathway, primary transcripts (pri-miRNAs) undergo nuclear processing by the Microprocessor complex, comprising the RNase III enzyme Drosha and its cofactor DiGeorge syndrome critical region 8 (DGCR8), yielding hairpin pre-miRNAs; **(c)** Alternatively, in non-canonical pathways (mirtrons), coding introns undergo spliceosomal excision to generate pre-miRNA hairpin precursors directly, bypassing Drosha cleavage; **(d)** The resulting pre-miRNAs and mirtrons are recognized by the nuclear transport receptor Exportin-5 (XPO5) and active Ran-GTP, facilitating translocation across the nuclear membrane into the cytoplasm. Abbreviations: pri-miRNA, primary microRNA; pre-miRNA, precursor microRNA; Pol II/III, RNA Polymerase II/III; DGCR8, DiGeorge syndrome critical region 8.

1.1. Search strategy and scope

This narrative review synthesizes literature retrieved primarily from PubMed/MEDLINE, supplemented by ClinicalTrials.gov for verification of clinical trial status, phase, and outcome data for the therapeutic candidates discussed (Cobomarsen, Remlarsen, TargomiRs, Miravirsen, and MRX34). Search terms combined core concepts including "microRNA therapeutics", "miRNA mimics", "antagomiRs", "antisense oligonucleotides", "miRNA sponges", "small-molecule miRNA inhibitors (SMIRNs)", "artificial miRNA (amiRNA)", "miRNA delivery systems", "lipid nanoparticle" and "viral vector delivery" combined with disease-specific terms spanning oncology (pancreatic, colorectal and lung carcinoma), cardiovascular disease, and renal pathology (diabetic nephropathy, IgA nephropathy and polycystic kidney disease), as well as terms relevant to computational applications ("machine learning (ML) miRNA target prediction", "deep learning (DL) miRNA" and "artificial intelligence (AI) biomarker discovery").

Given the narrative design of this review, no formal PRISMA-based (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) screening protocol was applied, articles were selected for relevance, mechanistic depth, and citation within the field rather than through a fixed inclusion/exclusion criteria. Foundational mechanistic studies establishing core miRNA biology and early oncomiR/tumor-suppressor miRNA associations were included irrespective of publication date, given their continued relevance as primary evidentiary sources for canonical mechanisms. More recent literature spanning developments in delivery chemistry, clinical trial outcomes and artificial intelligence/machinelearning (AI/ML)-based target prediction was prioritized to reflect the current state of the field.

1.2. Temporal cutoff

The literature search and clinical trial status verification were current as of September 2026, with included references spanning publication dates from 2000 to 2026.

2. Principal role in the body

Subsequently integrated into AGO-protein-containing RNA Induced Silencing Complexes (RISC), single miRNA strands in the human body then facilitate imperfect binding of ribonucleoprotein complexes to target sequences in the 3' untranslated regions (UTRs) of the mRNAs consequently directing post-transcriptional repression of mRNA targets and their eventual degradation. Reverse complementary binding also takes place in the 7–8 nt region of mature RNA sequence called the seed region at the 5' end of miRNAs [3] (Figure 2). Some miRNAs also bind to Open Reading Frames (ORFs) of mRNA transcripts and subsequently repress translation [4]. The other unselected miRNA strand undergoes an uneventful fate and degenerates [5].

Estimated that at least a third of the protein-encoding genes may be subject to miRNA based post-transcriptional regulation, miRNAs thus are major contributors towards pleiotropic fine tuning of the human body as each miRNA is predicted to modulate almost 200 mRNA targets. Involved in ample cellular pathways and disease mechanisms, miRNAs are therefore of great significance in terms of therapeutic applications having a wide array of fields of operation ranging from synergistic modulation of deranged biological pathways in the diseased, treating both rare and commonplace genetic disorders to even acting as useful biomarkers in the forensic sciences [5,6].

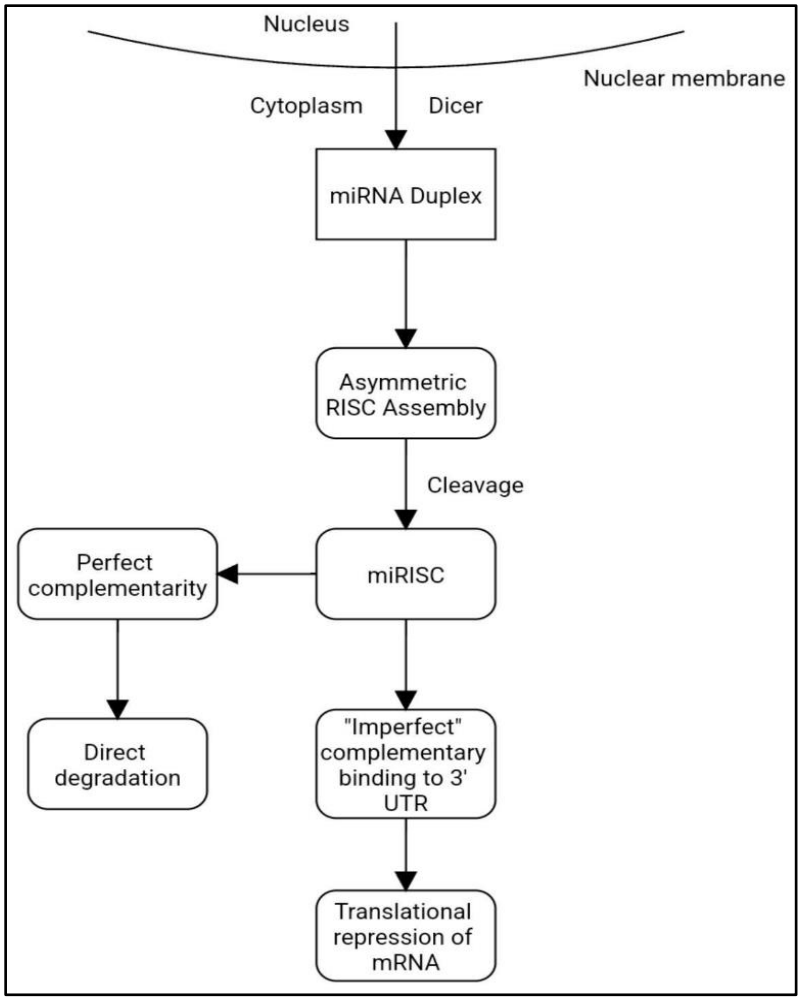


Figure 2. Cytoplasmic processing and regulatory mechanisms of microRNAs. **(a)** Precursor microRNAs (pre-miRNAs) exported across the nuclear membrane into the cytoplasm undergo cleavage by the RNase III endonuclease Dicer, producing a double-stranded miRNA duplex; **(b)** The duplex is loaded into the RNA-induced silencing complex (RISC) during asymmetric RISC assembly, where the passenger strand is cleaved and degraded to generate the functional microRNA-induced silencing complex (miRISC); **(c)** When the guide strand exhibits perfect complementarity with the target mRNA, miRISC induces direct endonucleolytic cleavage and mRNA degradation; **(d)** Alternatively, "imperfect" complementary binding to the 3' untranslated region (3' UTR) of target mRNAs results in translational repression, mediating post-transcriptional gene silencing. Abbreviations: miRNA, microRNA; RISC, RNA-induced silencing complex; miRISC, microRNA-induced silencing complex; UTR, untranslated region; mRNA, messenger RNA.

3. The strategic imperative

The foundational architecture of modern drug discovery remains fundamentally based on a classical, reductionist paradigm; the deployment of low molecular weight (LMW) chemical compounds or bioengineered monoclonal antibodies (mAbs) designed to bind isolated, downstream proteomic targets [7]. While these modalities have revolutionized medicine, they are increasingly hitting their biological ceilings, leaving extensively unaddressed areas of pathological landscape [8]. Thus, a contemporary pivot towards miRNA therapeutics represents a departure from this "one lock, one key" doctrine

currently in practice, born out of an urgent strategic necessity to transcend the roadblocks that hamstring conventional pharmacology.

Biological datasets reveal that approximately 80%–90% of the disease-associated proteome lacks these precise active site configurations [9]. Instead many critical disease-driving proteins such as transcription factors and scaffolding proteins consist of protein-protein interaction interfaces or intrinsically disordered regions, rendering them functionally exceptionally difficult to target via traditional pharmacology [9,10]. In stark contrast, miRNA therapeutics completely bypass the topology of the translated, tertiary protein altogether. By executing targeted, sequence specific base-pairing with the linear nucleotide sequence of the mRNA transcripts at the pre-translational level, they truly redefine the concept of targetability [8,11].

mAbs exhibit extraordinary target specificity and affinity. However, their massive molecular weight (~150 kDa) severely restricts their bioactivity almost exclusively to extracellular ligands or cell surface receptors. Due to size and hydrophilic nature, they are physically incapable of permeating the hydrophobic phospholipid bilayer of the mammalian cells, thus preventing them from modulating intracellular cascades. Conversely, synthetic oligonucleotide therapeutics including miRNA mimics and antagomiRs possess a dramatically smaller molecular footprint (~7–14 kDa). When adequately complexed within advanced biomimetic delivery vehicles or lipid nanoparticle (LNPs), these constructs readily penetrate into the intracellular cascades and aberrant cytoplasmic processes that remain entirely unreachable by antibody mediated interventions [8,12].

The primary driver of clinical attrition and drug resistance for traditional drug therapies, particularly within heterogeneous oncological and cardiovascular pathologies is adaptive cellular redundancy [8]. Inactivating an isolated cell-surface receptor or downstream tyrosine kinase frequently triggers the immediate compensatory up-regulation of parallel, redundant survival pathways or downstream mutations, rendering the monotherapy ineffective over time [7,8].

miRNAs circumvent this roadblock via a distributed multi-target architecture rather than a strict 1:1 stoichiometry. Leveraging a highly conserved nucleotide seed region, a single miRNA can bind with imperfect complementarity to the 3’-UTRs of hundreds of discrete mRNAs simultaneously. Some studies estimate that miRNAs collectively regulate greater than 60% of the human protein-coding transcriptome [13]. This multi-valent targeting capacity empowers a single therapeutic miRNA molecule to coordinate the systemic, concurrent downregulation of an entire network of pathologically interconnected nodes, fundamentally blocking the compensatory cellular mechanisms that drive acquired drug resistance [8,13].

4. Therapeutic modalities and delivery platforms

The above genomic insights clearly pointing towards the significance of the biological role played by miRNAs has contributed a great deal towards emergence of multiple approaches towards utilizing their immense potential in therapeutics and continues to do so in the ever-evolving world of pharmaceuticals with newer approaches put to the test on a daily basis.

To date, three mainstream approaches have emerged (Figure 3)—(a) miRNA sponges (decoys); (b) Small-molecule miRNA inhibitors; (c) Antisense oligonucleotides (ASOs). miRNA sponges, also known as decoys are RNA constructs reckoning on mRNA expression with multiple tandem, complementary miRNA binding sites acting as “miRNA binding sponges/decoys” facilitating the

selective segregation of endogenous miRNAs hence allowing target mRNA expression. Small molecule inhibitors achieve this objective via transcriptional regulation rather than inhibition of target miRNAs. Their therapeutic potential is limited by their high half-maximal effective concentration (EC_{50}) meanwhile ASOs are single-stranded oligonucleotides which induce inhibition via hybridization with endogenous RNA, although demonstrate poor utility *in vivo* applications and thus are chemically modified in practice to display increased resistance towards serum nucleases and enhance affinity towards their target. Furthermore, naked oligonucleotides also require modification/encapsulation to achieve penetration into negatively charged cell membranes which they are incapable of achieving of their own accord [14].

Other forms include synthetic miRNAs, also known as miRNA mimics, which are nucleic acids to modify miRNA levels and facilitate reconstitution or enhancement of endogenous miRNAs leading to target gene expression, small interfering RNAs (siRNAs), consisting of a group of small RNAs synthesized specifically for a select few/single target genes which may also be combined with miRNAs via recombinant plasmids. The same may be combined with primary scaffolds as members of an artificial miRNA construct (amiRNA) hence showing both target specificity and processing capacity in their therapeutic applications. Furthermore, strategies have also been adopted to combine the use of miRNA therapeutics with conventional drugs with subsequent improvement in the efficacy of drug based therapeutics when combined with interventions targeting specific cellular mechanisms [3] (Figure 3).

Despite their therapeutic utility, the translational deployment of these miRNA-modulating modalities is held back via intracellular, pharmacological and biophysical constraints. miRNA sponges necessitate continuous, high copy-number stoichiometry relative to endogenous miRNA levels. This relies on strong viral promoters, which can lead to transcriptional silencing or saturation of endogenous nuclear export machinery. Due to their rapid degradation, naked sponge transcripts also heavily rely on viral delivery systems. These vectors carry risks of host immune activation, neutralization, or integration into host genomic DNA, risking oncogenic mutagenesis. Long transcripts with tandem binding sites also tend to have unintended RNA-binding proteins with non-target miRNAs cultivating safety risks [15,16]. SMIRNs also generally display poor binding affinity compared to base-pairing oligonucleotides, requiring high working concentrations including the EC_{50} that increase non-specific cytotoxicity and off-target activity. SMIRNs acting indirectly via transcription factor modulation, enzyme biogenesis or upstreaming of signalling pathways results in reduction of specificity for a single miRNA family. Rapid hepatic clearance also poses a major bottleneck towards organ-specific targeted delivery of small molecules [17,18]. ASOs are limited by their hydrophilic, polyanionic phosphodiester backbones, which severely impair passive transmembrane bioavailability and render them susceptible to rapid 3'-5' exonucleolytic degradation, and even though, locked nucleic acid (LNA) and 2'-o-methoxyethyl (2'-MOE) mediated chemical modifications resolve nuclease sensitivity, high modification densities frequently induce sequence-independent hepatotoxicity, nephrotoxicity and Toll-like receptor (TLR)-mediated innate immune activation [19]. miRNA mimics risk supra-physiological RISC loading, leading to widespread off-target transcript downregulation driven by seed sequence microhomology, furthermore, asymmetric strand selection failures allow functional loading of the non-targeting passenger strand, while cationic carrier complexes remain predominantly sequestered within endo-lysosomal compartments [20–22]. Lastly, amiRNAs rely on endogenously expressed primary scaffolds (pri-miRNA) that compete with native transcripts for rate limiting biogenesis processing complexes (Drosha-DGCR8,

Dicer-TRBP), risking global miRNA dysregulation, while imprecise RNase III cleavage generates heterogeneous cleavage isoforms (isomiRs) with altered target specificity [23].

However significant strides may miRNA-based therapeutics have taken in the past few years, safety and efficacious delivery remains a noticeable roadblock. The clinical promise of miRNA-based therapeutics to directly rectify the gene-regulatory dysfunctions underlying oncology, cardiovascular diseases, neurodegeneration, and infectious pathologies face the primary translational bottleneck of targeted, intact delivery to the desired tissue. Nuclease mediated degradation, rapid blood elimination, immunotoxicity and decreased tissue permeability remain fundamental obstacles. Chemical modifications to the miRNA backbone may mitigate nuclease susceptibility but they do not intrinsically resolve challenges related to biological barrier permeability or systemic clearance, warranting the use of specialized delivery platforms. These platforms are broadly classified into two distinct: Viral systems, which leverage evolutionary infection machinery for optimal transduction, and synthetic non-viral vehicles, which prioritize a favorable safety and immunogenicity profile over absolute delivery efficacy [24].

4.1. Virus-derived delivery platforms

Engineered via attenuation of pathogenic viral genomes, these vectors retain the molecular machinery required for efficient cellular entry and transgene expression while the pathogenic determinants are eliminated resulting in consequent reduction in toxicity [24].

4.1.1. Retroviral vectors

Tracing their origins to the Moloney murine leukemia viruses (MomLVs), Retroviral vectors (RVs) remain integral for mediating safe and efficacious transfer of therapeutic genes into dividing cells [25,26]. These vectors reverse-transcribe their RNA payload into dsDNA, integrating it permanently into the host chromosomes, thus RVs are imparted a “Janus-faced” character. This permanent integration presents a biological trade-off: while it guarantees sustained, long-term expression of the therapeutic miRNA, the quasi-random nature of the insertion site carries a persistent risk of insertional mutagenesis followed by consequent oncogenesis, potentially silencing essential cellular genes or dysregulating adjacent regulatory elements. Furthermore, since integration requires the dissolution of the nuclear envelope during mitosis, RVs are strictly limited to transducing actively dividing cells [27,28].

4.1.2. Lentiviral vectors

A specialized subclass of retroviruses (encompassing strains such as HIV-2, SIV, FIV, EIAV, and BIV), lentiviral vectors overcome the mitotic dependency of their predecessors. Their integration machinery can successfully translocate through an intact nuclear pore, enabling the transduction of both quiescent and actively dividing cell populations. Additionally, lentiviruses exhibit a propensity to integrate within actively transcribed genomic regions, which statistically reduces, though does not abolish, the probability of insertional oncogenesis compared to standard retroviral vectors. Consequently, the fundamental risk of integration-induced mutagenesis is attenuated, rather than entirely eliminated [24,29].

4.1.3. Adenoviral and adeno-associated viral vectors

In contrast to integrating retroviruses, adenoviral and adeno-associated viral (AAV) vectors maintain their genetic material without host chromosome integration, thereby circumventing insertional mutagenesis risks while preserving the ability to transduce both dividing and non-dividing cells. Adenoviral vectors offer a substantial packaging capacity (up to ~38 kb) compared to retroviral systems (~8 kb), whereas AAVs are more constrained (approximately 4.8 kb), though this remains sufficient for standard miRNA expression cassettes. The primary limitation of this platform, however, is pronounced host immunogenicity. Extensive clinical and large-animal models have documented immune activation against AAV capsids, the severity of which is dictated by a complex interplay of variables: transgene characteristics, vector dose, specific AAV serotype, route of administration, recipient species, and the presence of pre-existing neutralizing antibodies. To mitigate off-target expression (predominantly hepatic) and attenuate this immune response, current engineering strategies focus on utilizing highly specific, tissue-restricted promoters and embedding miRNA target sequences within the vector's untranslated regions [25,30–32].

4.1.4 Bacteriophage-derived virus-like particles

Constructed from bacteriophages, these particles naturally lack tropism for human cells and were developed specifically to bypass the toxicity, tumorigenicity, and severe immunogenicity associated with eukaryotic viral vectors. However, the limitation here lies in technological maturity since virus-like particles (VLPs) represent an early-stage modality. Prior to clinical translation, comprehensive mapping of their immunogenic profile and the establishment of robust, scalable pharmaceutical production protocols remain critical prerequisites [24].

4.2. Non-viral delivery platforms

Synthetic nanocarriers were engineered fundamentally to circumvent the immunogenic and cytotoxic liabilities inherent to viral vectors. This paradigm generally accepts a reduction in delivery efficiency in exchange for a significantly enhanced safety and tolerability profile.

4.2.1. Lipid-based carriers

Lipid-based nanocarriers, the most widely used non-viral delivery platforms are cationic lipid formulations (e.g. Invitrogen, Bio-Rad and Dharmacon *etc.*) that function via complex formation with anionic nucleic acids, leading to a non-immunogenic lipolex formation, followed by the fusion of the resultant lipoplex with cellular membranes [24]. These lipolexes are easy to manufacture, however, this approach encounters a dual challenge: sub-optimal *in vivo* delivery efficiency, and the requisite cationic surface charge promoting non-specific interactions with confounding proteins. This non-specific binding leads to particle instability and adverse systemic effects. Strategies to mitigate these issues and enhance tissue biodistribution include surface PEGylation (polyethylene glycol conjugation) to elude phagocytosis or transitioning towards neutral liposomes which has resulted in resolution of the issue of carrier instability and unwanted carrier-protein interactions. Systemic accumulation of miRNA also remains a valid concern thus warranting attempts to enhance tissue-specificity of miRNA delivery

via employment of target cell receptor-specific ligands in liposomal carrier formulations. Recently, aptamers have also been employed for delivery of miRNA and siRNA lipid nanoparticles [33–35].

4.2.2. Polymer-based carriers

Polyethylenimine (PEI) remains the most extensively studied polymer in this sub-category, securing its RNA payload via charge-mediated complex formation with anionic RNA. Its primary limitation manifests as a rigid molecular-weight-dependent paradox: low-molecular-weight PEIs exhibit minimal cytotoxicity but poor transfection efficacy. This trade-off has largely precluded unmodified PEI from late-stage clinical advancement [36]. Strategies including PEGylation and ligand conjugation have been used to address this via increased circulation time, reduction in immunogenicity and higher target tissue accumulation [37]. Conversely, the related copolymer PLGA (polylactic-co-glycolic acid) encounters a distinct biophysical barrier, its highly hydrophobic nature natively impedes delivery effectiveness, which the deployment of polymeric micelles attempts to address [38]. Alternative polymeric platforms include PAMAM (polyamidoamine) dendrimers [39,40] and protamine-derived cell-penetrating peptides [41]. Natural polysaccharides such as chitosan, offer more favorable biocompatibility profiles, however, their transfection efficiencies have yet to rival those of viral vectors [42–44].

4.2.3. Inorganic nanoparticle carriers

Inorganic nanocarriers including gold, iron oxide (Fe_3O_4) and mesoporous silica nanoparticles are typically functionalized with thiol or amino groups to bind miRNA payloads for delivery. These platforms offer distinct biophysical advantages, notably thermal stability, biocompatibility, and high surface-area-to-volume ratios for maximized loading. The primary limitation resides in their clinical maturity. Moreover, PEG-conjugated gold nanoparticles have also demonstrated high delivery efficiency and lower cytotoxicity [24,45,46].

4.2.4. Extracellular vesicle-based (exosome) carriers

Exosomes and analogous extracellular vesicles (EVs) (e.g. microvesicles, apoptotic bodies) possess intrinsic therapeutic advantages, chiefly minimal cytotoxicity and low immunogenicity, facilitated in part by native surface marker CD47 that assists in evading mononuclear phagocyte clearance. For EVs, the translational barriers are primarily logistical rather than strictly biological. Scalable manufacturing of exosomes to clinical-grade yields remains highly technically demanding. Furthermore, extensive biological characterization is still required to standardize biogenesis parameters, identify optimal cellular sources for large-scale production, establish optimal administration routes, and comprehensively profile recipient immunomodulatory responses prior to widespread clinical deployment [24,47].

The prevailing landscape of miRNA delivery is characterized by a fundamental dichotomy: synthetic non-viral platforms offer superior safety and modularity but often underperform regarding intracellular delivery kinetics, whereas viral vectors provide robust transfection at the cost of significant immunogenicity and toxicity risks that remain difficult to entirely resolve. Contemporary engineering efforts aimed at bridging this translational gap largely converge on two methodologies: the biochemical modification of carriers to prolong systemic circulation and attenuate cytotoxicity, and the functionalization of carrier surfaces with active targeting ligands. This active targeting aims to

facilitate precise tissue homing, thereby maximizing the therapeutic index while concurrently minimizing the off-target systemic exposure inherent to passive delivery strategies.

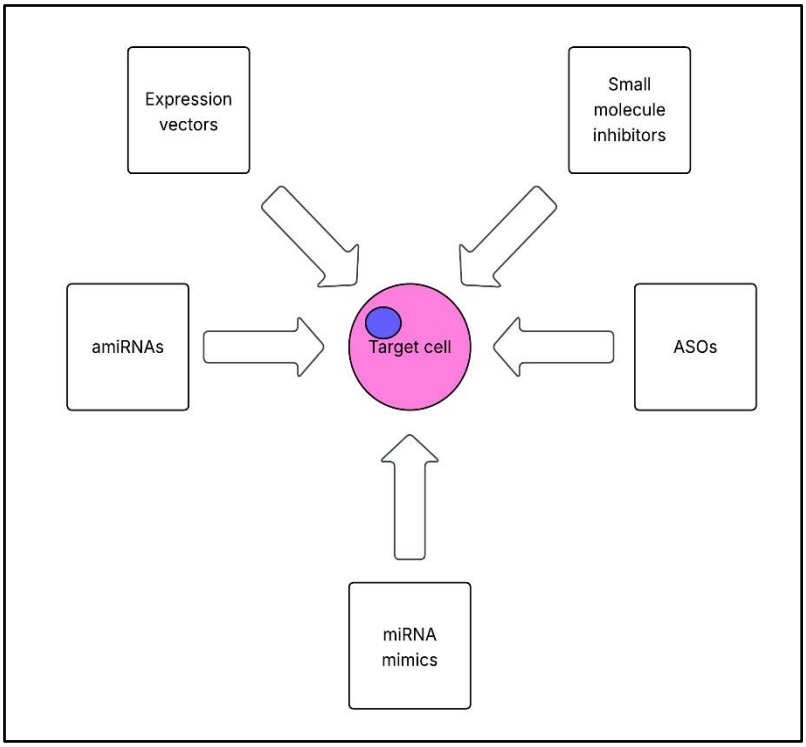


Figure 3. Therapeutic modalities and delivery forms for microRNA targeting. **(a)** Schematic representation of major pharmacological strategies utilized to target dysregulated microRNA (miRNA) pathways within a target cell; **(b)** Restoration of down-regulated or tumor-suppressor miRNAs (miRNA replacement therapy) is achieved using synthetic double-stranded miRNA mimics, artificial miRNAs (amiRNAs), or viral/non-viral expression vectors; **(c)** Inhibition of overexpressed oncogenic miRNAs (miRNA inhibition therapy) is driven by single-stranded antisense oligonucleotides (ASOs) that sterically block target miRNAs; **(d)** Small molecule inhibitors modulate miRNA biogenesis transcriptional or processing stages, offering multiple targeted therapeutic avenues. Abbreviations: miRNA, microRNA; amiRNA, artificial microRNA; ASO, antisense oligonucleotide.

5. Dysregulation in systemic pathologies and future potential

The recent successful application of RNA-based therapeutic and management approaches as a viable intervention strategy during the SARS-CoV-2 pandemic (evident consequently as clinical successes of the Pfizer-BioNTech and Moderna mRNA vaccines) demonstrated broader feasibility of large-scale RNA manufacturing and delivery, lending indirect support to evidence pointing towards the potential of miRNAs as therapeutic agents and their relevance to the molecular pathology of countless diseases, including but not limited to, cancer, cardiovascular and kidney diseases [48–50].

miRNAs with their gene targeting effects while also resulting in subsequent enhancement of the multi-target regulation place them at an immense advantage as pharmacological agents since this makes it possible for us to manipulate a whole array of genes playing important regulatory roles in the respective cellular pathway. This ability to regulate multiple genes simultaneously offers a mechanistic advantage over single-target drugs. Conversely, this broad regulatory capacity also inherently increases the risk of

off-target effects, where unintended cross-reactivity with non-target transcripts induces undesirable effects and off-mechanism cellular toxicity [8].

There are also multiple other factors placing miRNA based approaches in an advantageous position over small molecule approaches. Eminently standing out, is the endogenous nature of miRNAs, which imparts them with improved therapeutic effects as compared to their synthetic counterparts [51].

In the near forthcoming future, an increasingly important role as an adjunctive and in precision-based treatment strategies is expected of miRNA therapeutics, especially in diseases where conventional therapeutic strategies show limited efficacy or have their application faced with significant resistance.

ncRNAs such as miRNAs, transcribed from non-coding regions which constitute about 98% of the human genome, play a noteworthy role in normal physiological processes and tumorigenesis as well as progression, even as most of the research in oncology keeps highlighting coding RNAs and proteins as its center of attention, hence aptly accentuating the importance of miRNAs and other ncRNAs in oncological pathologies [52,53].

Cancers are frequently characterized as complex and heterogeneous diseases, governed by genetic anomalies facilitating subsequent tumorigenesis. The alterations often result in genomic aberrations, chromosomal translocations, insertions, deletions, amplifications and epigenetic mutations, leading to activation of oncogenes and suppression of tumor suppressor genes [54]. Present evidence strongly suggests that miRNA dysregulation, whether loss or gain, tumor suppressors (onco-suppressor miRs) and even facilitate oncogenic processes as oncomirs due to their significant role in regulation of expression of genes involved in cellular responses to environmental stressors such as DNA damage, oxidative stress, nutrient deprivation and hypoxia, as also supported by the recent evidence identifying the role of miRNAs in multiple malignant neoplasms [55–57].

The following are a few examples of miRNAs identified acting as oncogenes and tumor suppressors.

5.1. Pancreatic cancer

miR-103, miR-107, miR-21 and miR-155 have been recorded to have an oncogenic effect in pancreatic cancer by studies involving microarray analysis to compare miRNA expression in pancreatic tumors and normal individuals. The above studies also found evidence of higher expression of miR-103, miR-107 in primary pancreatic tumors [58,59].

Other studies also report evidence of a frequent absence of miR-34a from pancreatic cancer cells. miR-34a induction is facilitated by p53 tumor suppressor protein and plays a significant role in cell-cycle regulation, DNA repair, angiogenesis and induction of apoptosis and is observed being silenced in several cancers due to aberrant CpG methylation [60,61].

5.2. Colorectal cancer

Evaluation of association between chemosensitivity and tumorigenesis for miRNAs in colon cancer showed increased levels of expression of let-7g, miR-181b and miR-200c. It has also been demonstrated that all members of the Mir-17-92 family show upregulation in microsatellite stability in colon cancer suggesting oncogenic function. A study on 22 colorectal cancer patients also recorded evidence of inverse correlation between miR-21 and PDCD4-protein, a tumor suppressor, shown to be negatively

regulated by miR-21. In addition, miR-21 was also evidenced for induction of invasion, intravasation and metastasis of Colo206f-cells [62–64].

In other studies, reduced levels of expression of miR-143 and miR-145 were also reported and transfection of the same in cells with lower levels of expression inhibited cell growth [65–69]. A similar pattern was observed in let-7 miRNAs with let-7a-1 transfection leading to growth suppression indicated tumor suppressing effects [70,71].

5.3. Lung cancer

MiR-17-92 clusters have also been observed to be overexpressed in lung cancers. The oncogenic effects of MiR-17-92 seem to be exerted via inhibition of cancer cell apoptosis [72,73].

let-7 family members, in contrast, have been recorded as being downregulated in lung cancer and associated with decreased survival rates. The tumor suppression abilities of the let-7 family being credited to RAS inhibition and their ability to alter cell cycle progression. miR-34a, in addition, has also shown evidence of reduced expression in non-small lung cancers with its restoration inhibiting tumor cell growth [74–78].

Dysregulated circulating miRNAs have also shown to harbor associations with disease origin, progression, prognosis and survival with their distinctive tissue specificity also construing them their well-deserved status as potential biomarkers for diagnosing unknown primaries [79–83].

miRNA dysregulation across pancreatic, colorectal and lung carcinoma converges on a few recurring circuits, even as individual miRNAs diverge by tissue. miR-21 and the miR-17-92 cluster consistently act as oncomiRs across all three cancers, driving proliferation and apoptosis evasion via repression of tumor suppressors like PDCD4 and PTEN [84–86]. miR-34a functions predominantly as a p53 dependent tumor suppressor whose silencing recurs across these malignancies, though p53-independent regulatory mechanisms complicate the view of a single universal pathway [87]. The let-7 family shows the clearest tissue-dependent divergence, canonically tumor-suppressive in lung cancer, yet elevated let-7g expression linked to poorer survival in colorectal cancer, a functionally inverted role in the same miRNA family [88,89]. This mixture of conserved oncogenic circuits and tissue-specific exception is what sustains miRNA profiles as viable biomarkers for cancer diagnosis, prognosis and identifying tumors of unknown origin.

5.4. miRNAs & the cardiovascular system

miRNAs demonstrate an important role in development and functioning of the cardiovascular system thus granting them a promising role as biomarkers and therapeutic targets in cardiovascular diseases.

They contribute to key processes essential to cardiovascular homeostasis, including [90] (Table 1):

Table 1. Key roles of miRNAs in biological processes essential to cardiovascular homeostasis.

Biological process	Key role
Angiogenesis	Regulate endothelial cell proliferation and new blood vessel formation
Cardiac contractility & rhythm	miRNAs influence proteins involved in excitation-contraction coupling as well as regulation of electrical conductance
Lipid metabolism & vascular integrity	Facilitate modulation of lipid handling and endothelial barrier function
Cardiac growth & differentiation	Participate in cardiomyocyte development and adaptive growth responses

Abbreviations: miRNA, microRNA.

miRNAs demonstrate multifaceted role in the cardiovascular physiology with several miRNAs including but not limited to miR-1, miR-195, miR-133, miR-136, miR-126, miR-143 & miR-15a/b participating in cardiovascular development, cardiogenesis, cell proliferation & differentiation and even cardiac ischemia or cell cycle arrest, others such as miR-26, miR-9, miR-208a/b & miR-98 were also found involved processes of cardiac hypertrophy of both physiological as well as pathological origin. Atheroprotective effects are observed in association with extracellular miR-126a-3p, miR-143-3p/miR-145-3p whereas miR-143-3p, miR-222-5p, miR-92a-3p confer proangiogenic effects. In contrast the opposite effect is conferred by miR-320-3p. miR-21, as secreted by the endometrial mesenchymal cells also performs a cardioprotective function [90–93].

Other miRNAs such as miR-21-5p are often observed upregulated in heart failure as well as pathologies of the kidney and lung due to fibrosis acting as the shared common denominator [94].

Several other miRNAs have shown upregulation as well as downregulation in various animal models giving rise to the development of mimics to serve as therapeutic targets in human cardiovascular disease, including but not limited to miR-29, miR-92a-3p and miR-155-5p [95].

miRNAs, thus play a conclusive and determining role in molecular regulation, disease processes and clinical application, offering therapeutics promises across a wide spectrum of diseases not limited merely by the references aforementioned with an expanding body of evidence underscoring their potential to redefine diagnostic as well as therapeutics paradigms, especially in complex, multifactorial diseases like cancer and cardiovascular disorders.

5.5. miRNA and the kidneys

miRNAs play a demonstrably significant and specific role in the kidneys with multiple miRNAs expressed specifically in the kidney and their absence resulting in expression of multiple target proteins. miRNAs including but not limited to miR-192, miR-194, miR-204, miR-215 and miR-216 have been found to be present in higher abundance in the kidney over other organ systems [96].

Animal studies suggesting the role of miR-192 as TGF-β (Transforming Growth Factor-beta) effectors have also emerged, showing miR-192 controlled regulation and expression of TGF-β induced extracellular matrix proteins, Col1a1 and Col1a2. Col1a1 and Col1a2 were also shown to accumulate during diabetic nephropathy. Loss of TGF-β has also shown to be associated with tubulointerstitial fibrosis and decrease in eGFR (estimated Glomerular filtration rate) as observed in renal biopsies of patients diagnosed with diabetic nephropathy [97]. Furthermore, PTEN downregulation on chromosome 10, targeted by miR-216a and miR-217 was also found to have activating effect on Akt kinase, an important mediator of diabetic nephropathy. These miRNAs were further found to be regulated by TGF-β in mesangial cells of mice. An overexpression of miR-377 was also reported in both human and mice mesangial cells when exposed to high glucose levels, miR-377 reduces PAK1 and MnSOD expression resulting in enhanced fibronectin production, a significant characteristic of mesangial cells in diabetic nephropathy [98–100].

miRNAs have also been observed to play a role in expression of PKD (Polycystic Kidney Disease) genes. miR-17 has been demonstrated targeting 3’UTR of PKD2 leading to its post-transcriptional repression; overexpression of the aforementioned was also observed promoting cell proliferation via repression of PKD2 [101,102].

Multiple studies have also found associations between differential expression of miRNAs and kidney diseases, including but not limited to miR-200c, miR-141, miR-205 and miR-192 in IgA (Immunoglobulin A) nephropathy. Dai and colleagues in a study also identified 132 miRNA in both patients of IgA nephropathy and control samples of renal tissue, 35 were found to be upregulated while 31 downregulated in the diseased tissue [103,104]. Differential expression of multiple miRNAs has also been observed among patients of lupus nephritis and systemic lupus erythematosus when compared with healthy subjects [105–107].

Similar patterns have also started emerging associating miRNAs expression with viral infections and even highlighting their possible role as diagnostic biomarkers in renal diseases due to their stability and tissue-specificity. They may also prove advantageous in early detection and prediction of acute kidney injury incidents and predict progression of chronic renal diseases highlighting the indeed significant role miRNAs play in our kidneys and the immense potential they harbour that is yet to be harnessed.

To provide a consolidated overview of the regulatory landscape discussed above, Table 2 summarizes the validated target genes, dysregulation patterns and pathological associations of each cited miRNA:

Table 2. Dysregulated miRNAs in systemic pathologies: System categories, target pathways, proposed biological roles, and level of experimental evidence.

miRNA	System/Disease category	Dysregulation	Disease	Principal target/Pathway	Proposed biological role	Level of evidence
miR-103	Pancreatic	↑ Up	Pancreatic cancer	Not specified*	Oncogenic	Microarray, tumor *vs.* normal [58]
miR-107	Pancreatic	↑ Up	Pancreatic cancer	Not specified*	Oncogenic	Microarray, tumor *vs.* normal [58]
miR-21	Pancreatic	↑ Up	Pancreatic cancer	PDCD4 (Inversely correlated)	Oncogenic	Microarray, tumor *vs.* normal [58]
	Colorectal	↑ Up	Colorectal cancer	PDCD4 (Inversely correlated)	Oncogenic - invasion, intravasation, metastasis	Clinical cohort and cell-line study [62–64]
miR-155	Pancreatic	↑ Up	Pancreatic cancer	Not specified*	Oncogenic	Microarray, tumor *vs.* normal [58]
miR-34a	Pancreatic	↓ Down	Pancreatic cancer	p53 dependent induction, cell-cycle, DNA repair, angiogenesis, apoptosis	Tumor suppressor, silenced via CpG methylation	Expression and methylation studies [60,61]

Table 2. *Cont.*

miRNA	System/Disease category	Dysregulation	Disease	Principal target/Pathway	Proposed biological role	Level of evidence
	Lung	↓ Down	Non-small cell lung cancer	p53 dependent induction	Tumor suppressor - restoration inhibits tumor growth	Expression and functional restoration [74–78]
let-7g	Colorectal	↑ Up	Colon cancer	p53 dependent induction	Chemosensitivity/tumorigenesis association	Association study [62–64]
miR-181b	Colorectal	↑ Up	Colon cancer	p53 dependent induction	Associated with tumorigenesis	Association study [62–64]
miR-200c	Colorectal	↑ Up	Colon cancer	p53 dependent induction	Associated with tumorigenesis	Association study [62–64]
miR-17-92 cluster	Colorectal	↑ Up	Colon cancer	Inhibition of apoptosis	Oncogenic	Expression study [62–64]
miR-17-92 cluster	Lung	↑ Up	Lung cancer	Inhibition of apoptosis	Oncogenic	Expression/functional studies [72,73]
miR-143	Colorectal	↓ Down	Colorectal cancer	ERK5/c-myc	Tumor suppressor- restored expression inhibited growth	In vitro transfection [65–68]
miR-145	Colorectal	↓ Down	Colorectal cancer	IRS-1 downregulation	Tumor suppressor- restored expression inhibited growth	In vitro transfection [65–68]
let-7a-1	Colorectal	↓ Down	Colorectal cancer	RAS, c-myc	Tumor suppressor- transfection caused growth suppression	In vitro transfection [65–68]
let-7 family	Lung	↓ Down	Lung cancer	RAS, cell-cycle progression	Tumor suppressor - linked to decreased survival	Expression and survival correlation [74–78]
miR-1	Cardiovascular	Not specified*	Cardiovascular development	Excitation-contraction coupling proteins	Cardiogenesis, proliferation/differentiation, ischemia response	Review [90]
miR-195	Cardiovascular	Not specified*	Cardiovascular development	Not specified*	Cardiogenesis, proliferation/differentiation	Review [90]
miR-133	Cardiovascular	Not specified*	Cardiovascular development	Not specified*	Cardiogenesis, cell-cycle arrest	Review [90]

Table 2. *Cont.*

miRNA	System/Disease category	Dysregulation	Disease	Principal target/Pathway	Proposed biological role	Level of evidence
miR-143	Cardiovascular	Not specified*	Cardiovascular development	Not specified*	Cardiogenesis, proliferation/differentiation	Review [90]
miR-15a/b	Cardiovascular	Not specified*	Cardiovascular development	Not specified*	Cardiogenesis, cell-cycle arrest	Review [90]
miR-26	Cardiovascular	Dysregulated	Cardiac hypertrophy	Transcription factor Gata4	Regulator of hypertrophic signalling	Review synthesis [91]
miR-9	Cardiovascular	Dysregulated	Cardiac hypertrophy	Myocardin expression	Regulator of hypertrophic signalling	Review synthesis [91]
miR-208a/b	Cardiovascular	Dysregulated	Cardiac hypertrophy	Myh7 activation	Regulator of hypertrophic signalling	Review synthesis [91]
miR-98	Cardiovascular	Dysregulated	Cardiac hypertrophy	Trx1, cyclin D2	Regulator of hypertrophic signalling	Review synthesis [91]
miR-143-3p	Cardiovascular	Context-dependent (protective and proangiogenic)	Atherosclerosis	Not specified*	Atheroprotective and separately proangiogenic	Circulating miRNA study [92]
miR-145-3p	Cardiovascular	Extracellular, protective	Atherosclerosis	Not specified*	Atheroprotective	Circulating miRNA study [92]
miR-222-5p	Cardiovascular	Opposing effect	Angiogenesis	STAT5	Anti-angiogenic	Review synthesis [91]
miR-92a-3p	Cardiovascular	Proangiogenic, ↑ Up	Angiogenesis, CVD therapeutic target (Heart failure)	Not specified*	Proangiogenic, candidate mimic-based target	Review [90]
miR-320-3p	Cardiovascular	Opposing effect	Atherosclerosis/angiogenesis	Not specified*	Opposes atheroprotective/ proangiogenic miRNAs	Review synthesis [91]

Table 2. *Cont.*

miRNA	System/Disease category	Dysregulation	Disease	Principal target/Pathway	Proposed biological role	Level of evidence
miR-21 (secreted)	Cardiovascular	Secreted/Up↑	Cardiac hypertrophy	Pten, subsequently AKT/mTOR	Regulator of hypertrophic signalling	Review [92]
miR-21-5p	Cardiovascular	Up↑	Heart failure, renal/pulmonary fibrosis	Fibrosis pathway (shared across organs)	Pro-fibrotic, common denominator across heart/lung/kidney	Cross-organ expression study [94]
miR-29	Cardiovascular	Dysregulated (mimic developed)	Cardiovascular disease	Wnt pathway	Candidate mimic-based therapeutic target	Animal model and therapeutic development [95]
miR-155-5p	Cardiovascular	Dysregulated (antimiR) developed	Cardiovascular disease	Enhances NF-κB expression, promotes arteriogenesis	Therapeutic target	Animal model and therapeutic development [95]
miR-192	Kidney	Kidney-enriched, dysregulated in disease	Diabetic nephropathy, IgA nephropathy	TGF-β effector—regulates Col1a1/Col1a2 (ECM)	Pro-fibrotic—ECM accumulation, ↓eGFR	Animal and biopsy correlation [96,97], Expression study [103,104]
miR-194	Kidney	Kidney-enriched	Baseline tissue specificity	Not specified*	Kidney-specific regulatory role (Tissue specification)	Tissue distribution study [96]
miR-204	Kidney	Kidney-enriched	Baseline tissue specificity	Not specified*	Kidney-specific regulatory role (Tissue specification)	Tissue distribution study [96]
miR-215	Kidney	Kidney-enriched	Baseline tissue specificity	Not specified*	Kidney-specific regulatory role (Tissue specification)	Tissue distribution study [96]
miR-216	Kidney	Kidney-enriched	Baseline tissue specificity	Not specified*	Kidney-specific regulatory role (Tissue specification)	Tissue distribution study [96]
miR-216a	Kidney	Up↑ (functional)	Diabetic nephropathy	PTEN (chromosome 10)→ Akt activation	Pro-pathogenic—Akt-driven nephropathy progression	Mesangial cell (mouse) TGF-β regulated [98–100]

Table 2. *Cont.*

miRNA	System/Disease category	Dysregulation	Disease	Principal target/Pathway	Proposed biological role	Level of evidence
miR-217	Kidney	Up↑ (functional)	Diabetic nephropathy	PTEN (chromosome 10)→ Akt activation	Pro-pathogenic—Akt-driven nephropathy progression	Mesangial cell (mouse) TGF-β regulated [98–100]
miR-377	Kidney	Up↑	Diabetic nephropathy	PAK1, MnSOD (reduced)	Profibrotic—enhances fibronectin production	Human and mouse mesangial cell [98–100]
miR-17	Kidney	Up↑ (functional)	Polycystic kidney disease	PKD2 (3'UTR repression)	Promotes cell proliferation via PKD2 repression	Gene regulation/functional study [101,102]
miR-200c	Kidney	↓ Down	IgA nephropathy	ZEB1/ZEB2 inhibition	Disease-associated dysregulation	Expression study, patient *vs.* control [103,104]
miR-141	Kidney	↑ Up	IgA nephropathy	ZEB1/ZEB2 repression	Disease-associated dysregulation	Expression study, patient *vs.* control [103,104]
miR-205	Kidney	↑ Up	IgA nephropathy	ZEB1/ZEB2 repression	Disease-associated dysregulation	Expression study, patient *vs.* control [103,104]

*Note: Entries designated as "Not specified" reflect parameters that were not explicitly evaluated or reported in the primary cited references. Abbreviations: miRNA, microRNA; ↑, upregulated; ↓, downregulated; CpG, cytosine-phosphate-guanine dinucleotide; PDCD4, programmed cell death 4; p53, tumor protein p53; ERK5, extracellular signal-regulated kinase 5; c-myc, MYC proto-oncogene; IRS-1, insulin receptor substrate 1; RAS, rat sarcoma viral oncogene homolog; Gata4, GATA-binding protein 4; Myh7, myosin heavy chain 7; Trx1, thioredoxin 1; STAT5, signal transducer and activator of transcription 5; Pten/PTEN, phosphatase and tensin homolog; AKT, protein kinase B; mTOR, mechanistic target of rapamycin; Wnt, wingless/integrated signalling pathway; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; CVD, cardiovascular disease; TGF-β, transforming growth factor-beta; Col1a1/Col1a2, collagen type I alpha 1/alpha 2 chains; ECM, extracellular matrix; eGFR, estimated glomerular filtration rate; IgA, immunoglobulin A; PAK1, p21-activated kinase 1; MnSOD, manganese superoxide dismutase; PKD2, polycystin-2 (polycystic kidney disease 2 gene); UTR, untranslated region; ZEB1/ZEB2, zinc finger E-box-binding homeobox 1/2; SIP1, Smad-interacting protein 1 (also known as ZEB2).

6. Current role in therapeutics

The trajectory of miRNA therapeutics resembles a classic clinical crucible *i.e.*, an initial era of hyperacceleration, followed by strict regulatory hurdles and roadblocks, now seen to be culminating in a multi-billion dollar race for precision control. Moving beyond the speculative promises from laboratory benches, the operational reality of miRNA therapeutics is being written in the rigorous language of intellectual property (IP) and clinical endpoints. To map this rapidly advancing frontier, one must analyze the field through a tripartite lens: the select, pioneering agents currently navigating clinical deployment and the patents defining modern delivery chemistry. The active human trials that are setting the stage for the next generation of genetic interventions must also be carefully peered upon.

Despite this potential, significant roadblocks remain before miRNA-based therapeutics can be widely adopted, including but not limited to identification of administration routes, achieving *in-situ* stability and attainment of intended therapeutic effects via specific tissue targeting. Thus, only a few miRNA-based therapeutic agents have entered clinical testing phase as of now, including (Table 3):

The clinical journey of these pioneering drugs has also exposed steep biological roadblocks, early trials, such as the MRX34, revealed that the systemic administration of double-stranded RNA can trigger potentially fatal immunological reactions. Mechanistically, MRX34, a liposomal miR-34a mimic produced a pattern of late-onset serious adverse events (sepsis, hepatic failure, colitis/pneumonitis, and grade 4 cytokine release syndrome) consistent with strong innate immune activation, likely arising from recognition of the synthetic double-stranded RNA cargo or its LNP carrier by pattern-recognition receptors. The trial was voluntarily halted in 2016 after five such immune-related SAEs, several of which were fatal [108]. Furthermore, since a single miRNA regulates hundreds if not more of downstream genes, complete silencing or forced overexpression of a target may also disrupt vital, parallel cellular networks and pathways. A similar pleiotropic property was also demonstrated by RG-101 resulting in blockade of proteins essential for hepatic clearance causing adverse events of severe hyperbilirubinemia in patients [109]. Cobomarsen (MRG-106), an anti-miR-155 locked nucleic acid oligonucleotide developed for cutaneous T-cell lymphoma, illustrates a related but distinct failure mode rooted in the same pleiotropy, because miR-155 concurrently regulates the Janus kinase/signal transducer and activator of transcription (JAK/STAT), mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) and phosphoinositide 3-kinase/protein kinase B (AKT) (PI3K/AKT) survival pathways [110], its clinical effect on skin lesions in the Phase 2 SOLAR trial was diffuse rather than decisive. Cobomarsen was well tolerated with no toxicity-driven discontinuations, but it failed to produce a statistically compelling four-month objective skin response *versus* the active comparator vorinostat, and the trial was discontinued for futility rather than safety in 2020 [111]. In addition, molecular delivery to target sites without destruction via blood enzymes also remains a significant challenge as seen in the stalled trials for TargomiRs. However, a distinct problem is observed, the EGFR-targeted bacterial minicells encapsulating the miR-16 mimic were largely protected from nuclease degradation by their bacterial envelope, but this same bacterial origin triggered dose-dependent innate immune responses severe enough to lead to the abandonment of the planned 5–9 × 10^9 minicell starting doses in favor of a much lower 1 × 10^9 dose with added cardiac monitoring, constraining the achievable systemic exposure and, ultimately, therapeutic benefit [112].

Table 3. Clinical trial landscape of miRNA-based therapeutics: Target miRNAs, delivery modalities, clinical trial status, and key trial outcomes.

Drug name [Reference]	Targeted miRNA	Mode of action	Background Disease	Body application/Cellular uptake	Clinical trial number(s)	Current Trial status	Clinical Outcome/ Cause for discontinuation	Verification date (DD/MM/YY)
Cobomarsen [113]	miR-155	Inhibition (AntimiR)	Cutaneous T-Cell Lymphoma	Naked LNA Oligonucleotide (IV/SC)	NCT03713320	Phase 2 (Terminated)	Sponsor strategic reprioritization (non-safety)	15/09/26
Remlarsen [114]	miR-29	Mimic	Keloids/Fibrosis	Local ID injection	NCT03601052	Phase 2 (Completed; Discontinued)	Corporate pipeline restructuring (non-safety)	15/09/26
TargomiRs [115]	miR-16	Mimic	Mesothelioma/NSCLC	Anti-EGFR Bacterial minicells EDV	NCT02369198	Phase 1 (Completed; Discontinued)	Dose-limiting immune toxicities	15/09/26
Miravirsen [116]	miR-122	Inhibition (AntimiR)	Chronic Hepatitis C	Naked LNA Oligonucleotide (SC)	NCT01200420	Phase 2a (Completed)	Demonstrated dose-dependent viral reduction, discontinued due to commercial displacement by DAAs	15/09/26
MRX34 [108]	miR-34a	Mimic	Advanced Solid Tumors	LNP	NCT01829971	Phase 1 (Terminated)	Severe immune-mediated toxicities leading to 5 treatment-related SAEs and 4 patient deaths	15/09/26

Abbreviations: miRNA, microRNA; AntimiR, anti-miRNA (miRNA inhibitor); LNA, locked nucleic acid; IV, intravenous; SC, subcutaneous; ID, intradermal; NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; EDV, EnGeneIC delivery vehicle (bacterially derived minicell); LNP, lipid nanoparticle; DAAs, direct-acting antivirals; SAEs, serious adverse events; NCT, National Clinical Trial (ClinicalTrials.gov identifier); DD/MM/YY, day/month/year.

Despite these roadblocks, the future scope of miRNA-based interventions appears to be harboring significant potential, driven by advances in precision engineering and active molecular homing keys [113]. The industry has largely transitioned from untargeted LNPs in favour of single-stranded inhibitors [117]. Via chemical shielding of the oligonucleotide backbone, modern candidates have also been observed to have far superior structural stability and binding affinity, thus requiring a fraction of the original toxic dosage [111,117]. Most notably, a delivery revolution is also underway through the application of direct ligand conjugation, such as attachment of sugar clusters (GalNAc) that display exclusive binding to hepatic receptors, or via utilisation of localized viral vectors (AAVs) to deliver artificial miRNAs directly to insulated tissues like the central nervous system, which is also under investigation in uniQure's AMT-130 trial for Huntington's disease [113,118].

However, several critical questions remain unanswered still before us before miRNAs can be put up for widespread clinical adoption. Foremost, is the long-term safety profile of permanently altering or silencing master genetic rheostats; since miRNAs regulate as well as dictate key components of cellular homeostasis, chronic suppression risks inadvertently switching on oncogenic pathways or induction of toxic metabolic imbalances in the patient [113]. Evaluation of reliable, non-invasive biomarkers to measure real-time tissue saturation and downstream gene knockdown effects also remains a major hurdle among clinicians [117]. Determining optimal, standardised dosing also remains an exceptionally complex task given the target specific micro-regulatory nature of miRNAs, meaning over-dosing may trigger toxicity while under-dosing may easily lead to failure in achieving clinical efficacy [112,113].

7. miRNAs and AI

Bridging the gap between the complex miRNA biology and a successful clinically efficacious therapeutics, requires navigating dense regulatory networks that may exceed the scope of classic bioinformatics. AI has emerged as a critical catalyst in this very domain, transforming how researchers analyze high-throughput sequencing data and navigate complex pharmacogenomic pipelines [119]. Integration of AI and ML architectures into the bioinformatics workflow, it is now possible to predictively model complex molecular interactions, streamline the identification of disease-specific miRNA signatures for drug targeting [120] and systematically leverage neural networks to be able to analyze miRNA expression profiles for precision medicine framework [121].

Multiple tools have been developed to predict candidate target sites (CTS) of miRNAs based on their already recorded ability to identify targets via highly conserved seed sequences [122]. These machine learning and DL prediction models rely on positive and negative miRNA target sites in the training dataset for learning [123,124]. Recent data has also demonstrated these machine learning models having a higher accuracy compared to traditional prediction algorithms used for miRNA target prediction [125]. Target prediction model mintRULS, when compared with the existing tools including miRDB (AUC: 0.73) (Area under the curve [AUC]), TargetScan (AUC: 0.77) and STarMir (AUC: 0.67) has also demonstrated superior accuracy and specificity, achieving a robust AUC metric of 0.93 by evaluating localized thermodynamic parameters like minimum free energy and target site accessibility [122]. Representative AI/ML studies across target prediction, delivery design, biomarker classification and subcellular localization are summarized in Table 4. Despite superior prediction capabilities, machine learning requires manual selection of target feature parameters leading to increased possibility of subjective errors [126].

Based on the immensely studied interactions between ncRNAs such as long non-coding RNA (lncRNA), circular RNA (circRNA) and miRNA [127,128], deep learning-based target prediction models have also made significant progress with prediction of miRNA:lncRNA and miRNA:circRNA interactions. ncRNAInter uses 191 feature parameters of miRNA:lncRNA interactions with the resultant prediction model demonstrating an accuracy of up to 93.1% along with a 26.7% higher Matthews Correlation Coefficient (MCC) for human network pathways [129] outperforming IncMirNet (based on convolutional neural network [CNN]) on several indicators including but not limited to accuracy, specificity and sensitivity [130]. Many deep learning models also demonstrably suffer from overfitting due to inordinate parameters and meager training samples, to avoid which, the Dropout algorithm is used by models such as miRAW and miTAR, both of which enforce a standardized 20% dropout rate configuration alongside a batch training layer size of 100 to stabilize model generation [131,132].

In addition, machine learning models have also been demonstrated to be suitable for mining biomarkers based on body fluid miRNA expression profiles [133]. These include blood, serum, urine, pleural effusion and exosomal miRNAs, which have shown promising potential as a class of non-invasive biomarkers [134,135], frequently yielding multi-marker diagnostic discrimination metrics with AUC scores exceeding 0.90 [125,136–138]. Furthermore, models based on tumor miRNA expression profiles may also be of significant benefit in improving efficiency of candidate drug target discovery as demonstrated by multiple researchers previously. Models including MirLocPredictor, miRNALoc, miRLoc and DAmiRLocGNet may also be utilised for predicting subcellular distribution of miRNA, specifically processing sequence-derived feature vectors to determine localization between the nucleus and cytoplasm, thus critically aiding novel drug target development [125].

AI model aided miRNA signature analysis are redefining precision oncology by helping to diagnose various cancers. 3- miRNA combination hsa-let-7i-3p, miR-362-3p, and miR-3651 identified using Support Vector Machine (SVM) is used to study cancer progression and to predict staging for 8 different cancers [120,139]. In addition to diagnostics AI-backed dual machine learning models (LASSO and RSF) integrated with miRNA have entered oncotherapeutics where prostate cancer cell-derived endoplasmic reticulum derived nanocarrier has successfully delivered miR-873 mimic and miR-20a inhibitors in prostate cancers which has shown promising results towards tumor suppression [140]. Such promising findings emphasises both the therapeutic synergy of rationally selected miRNA combinations and a highly versatile platform for targeted cancer treatment. Integration of artificial intelligence with miRNA technology has its own challenges scaling over computational, biological, and translational.

Lack of verified high quality data and transparency in AI models used in miRNA research often hinders the progress of its application leaving researchers in doubt,how an AI predicts therapeutic outcomes [141].

7.1. Accelerating delivery design and safety screening

AI has accelerated the miRNA target discovery but main concern now is to ensure safe, tissue-selective delivery of fragile oligonucleotides and avoiding off-target hybridization, immune activation, and hepatotoxicity once they enter the bloodstream. Thus integration of AI algorithms with miRNA research helps to tackle both problems in silico, narrowing the experimental search space before, and alongside wet-lab optimization [142–144]. By training deep-learning models on large LNP measurements, activity datasets can now propose chemically novel ionizable lipids and predict delivery efficiency and key biophysical properties (e.g., pKa, conformation) to prioritize leads before synthesis. Such approaches

have identified potent candidates that were subsequently validated *in vivo* (e.g., in ferret lung models), illustrating that in-silico screening can surface high-performing formulations earlier in the pipeline [145].

Now gradient-boosted trees like XGBoost, LightGBM coupled with Bayesian optimization are utilized to tune particle size, polydispersity, and encapsulation efficiency against quality-control benchmarks, achieving strong predictive performance ($R^2 > 0.87$) across hundreds of formulations [143,144].

Machine learning has extended miRNA towards exosome engineering thus optimizing cargo-loading strategies and selecting organ-targeting peptides, miRNA are loaded into Rabies virus glycoprotein (RVG)-tagged exosomes designed to cross the blood–brain barrier for glioblastoma as per workflow designed by AI [139,143].

7.2. AI for safety: predicting off-targets, toxicity, and immunogenicity

ML is used to triage candidate miRNA/siRNA sequences for risks such as seed-mediated off-target silencing, hybridization-independent toxicity, and innate immune activation prior to synthesis. Transformer-based models (e.g., OligoFormer) integrate sequence and thermodynamic features to jointly predict knockdown efficacy, toxicity liability, and off-target risk in a single pipeline. Self-attention and feature-based classifiers (e.g., AttSiOff and related models) further incorporate 3D structural and molecular fingerprint descriptors to improve joint efficacy/off-target prediction, reflecting the field's consensus that partial complementarity–driven off-targeting is a key safety bottleneck for oligonucleotide therapeutics [146–149].

Parallel efforts apply ML to preclinical immunogenicity assessment, scanning sequences for motifs and structural patterns linked to interferon/TLR activation before advancing leads, especially pertinent for liver-targeted strategies like GalNAc conjugation) that helps to deliver candidates directly to hepatocytes taking care of immunogenicity [141].

Despite these advances, reported model performance cannot always be equated with clinical benefit. miRNA-driven AI/ML requires a strong dataset training on corporas like miRTarBase, TarBase, Crosslinking, ligation, and sequencing of hybrids (CLASH)/Covalent ligation of endogenous argonaute-bound RNAs with crosslinking and immunoprecipitation (CLEAR-CLIP) sets, which differ in curation rules, cell types, and protocols, so accuracy can shift markedly when models are re-evaluated on differently curated data. Benchmarking practices sometimes allow leakage between training and test splits, especially data on shared sequences, miRNA families, or near-duplicate interactions, inflating apparent accuracy without proving true generalization [146].

Deep architectures with many parameters are prone to overfitting on the relatively small, curated datasets typical of miRNA research. Regularization strategies and corrections like Synthetic Minority Over-sampling Technique (SMOTE) have been adopted, but oversampling itself can induce overfitting, with strong training performance failing on independent test data. Class imbalance is another persistent issue: true positives, namely validated hairpins, target sites, or drug-responsive profiles are vastly outnumbered by negatives, and common remedies—under-sampling, cost-sensitive learning each introduce their own biases. Compounding this, "negative" miRNA–mRNA pairs are often inferred computationally rather than experimentally confirmed, risking systematic artifacts that models may learn instead of genuine non-binding biology. Consequently, cross-validation within a single dataset frequently does not hold up under external or independently generated data, and limited external validation remains a widely noted weakness [141].

Finally, high AUC, accuracy, or Matthews Correlation Coefficient on held-out computational tests demonstrate statistical discrimination but not clinical utility which additionally requires reproducibility across diverse patient populations, prospective validation, and evidence of impact on treatment decisions. Models such as mintRULS, ncRNAInter, miRAW, miTAR, MirLocPredictor, miRNALoc, miRLoc, DAmiRLocGNet, OligoFormer, AttSiOff, and the LNP/exosome ML frameworks have been evaluated only via in-silico cross-validation or held-out computational test sets. The closest exception is AI-guided LNP design validated in a preclinical ferret-lung model, which remains a preclinical, not a clinical benchmark. These tools are best viewed as candidate-prioritization aids that require downstream wet-lab and *in vivo* confirmation, not as clinically validated diagnostics or therapeutics. This suggests AI currently narrows and prioritizes candidates within these bottlenecks rather than resolving them outright and reframing, more than transcending, the field's central translational constraints [142].

Table 4. Summary of representative AI/ML studies in miRNA-target and interaction prediction, therapeutic delivery design, biomarker classification, and subcellular localization.

Prediction Task	Input Data	Model Type	Dataset	Validation Strategy	Comparator	External Validation	Main Limitation
miRNA candidate target site (CTS) prediction (mintRULS) AUC = 0.93	miRNA–target sequence pairs; thermodynamic features (MFE, site accessibility)	Regularized least squares–based kernel model	CLASH data: the miRNA interactome study in HEK293 cells, miRTarBase 8.0	Cross-validation (CV)	miRDB (0.73), TargetScan (0.77), STarMir (0.67)	PolymiRTS entries may overlap with miRTarBase	Manual feature selection increases risk of subjective error [122]
miRNA: target interaction prediction (miRAW/miTAR)	Whole miRNA/target transcript sequences	Deep neural network	Not specified in source	Random k-fold CV	TargetScan (context++ and PCT), miRanda, PITA, RNAhybrid, RNA22, DIANA-microT-CDS, miRDB, STarMir.	eCLIP-chimeric data and transfection expression data	Susceptible to overfitting given parameter count *vs.* sample size [126,131]
siRNA efficacy, toxicity, off-target prediction (OligoFormer)	siRNA/mRNA embeddings (RNA-FM) and thermodynamic parameters	Transformer	Curated siRNA efficacy sets; off-target via TargetScan/PITA	Microarray-based validation	OligoWalk, siRNAPred, DSIR, i-Score	Partial (microarray)	Dataset biases affecting generalization [148]
siRNA inhibition and off-target prediction (AttSiOff) PCC 0.81; SPCC 0.84; AUC 0.886	siRNA/mRNA embeddings (RNA-FM) and prior-knowledge features	Self-attention network	Aggregated validated siRNA sets	10-fold CV and cross-dataset validation	SVM/CNN/GNN-based methods	Yes—5 approved siRNA drugs + 1 novel gene	Broader generalizability beyond tested cases untested [149]
Ionizable lipid/LNP design (LiON)	LNP composition linked to activity data	Deep learning optimization framework	> 9000 LNP activity measurements	In vivo experimental testing	Conventional combinatorial/rational lipid screening	Yes— *in vivo*, ferret lung (preclinical)	Preclinical animal validation only; no human data [145]
miRNA binding-site prediction benchmarking (miRBench)	AGO2 CLASH/chimeric eCLIP interaction data	Benchmark dataset and baseline CNN models	> 2.5M class-balanced pairs (new) and reprocessed existing sets	Cross-dataset benchmarking of multiple SOTA tools	Multiple existing SOTA predictors	Built for external/cross-dataset testing	Shows most existing predictors underperform once frequency-class bias is removed [150]

Table 4. *Cont.*

Prediction Task	Input Data	Model Type	Dataset	Validation Strategy	Comparator	External Validation	Main Limitation
miRNA-target prediction with ranked output (MultiMiTar)	miRNA:target sequence-derived features	Multi-objective SVM ensemble	289 tissue-specific negatives (from TargetMiner)	Random stratified k-fold, Nested CV	miRAW, miTAR, DeepMirTar, TargetNet, and miRBind	eCLIP-chimeric, Transfection arrays/RNA-seq, pSILAC	Lack of gold-standard negative examples [151]
Pan-cancer stage classification (early *vs.* advanced) via compact miRNA signature panel (CancerSig) 84.27% accuracy; AUC = 0.80	miRNA expression profiles (avg. 474 miRNAs/sample)	SVM (LibSVM, RBF kernel) and IBCGA evolutionary feature-selection wrapper (CancerSig)	TCGA; 4667 samples across 15 cancer types (filtered from 6,578)	10-fold cross-validation, 30 independent IBCGA runs per cancer type for robust signature selection	Outperformed LightGBM, XGBoost, Random Forest, CatBoost, Extra Trees (72%–74% accuracy *vs.* 84.4%) on BLCA benchmark	None reported—TCGA only; authors state this explicitly as the method's limitation	Single-source (TCGA) data only, no independent cohort validation; the 3-miRNA subset (hsa-let-7i-3p, miR-362-3p, miR-3651) was significant in 8 of the 15 studied cancers specifically (BLCA, BRCA, ESCA, KIRC, LIHC, LUAD, STAD, UVM) [120]
Multi-label miRNA subcellular localization (7 compartments: cytoplasm, exosome, nucleolus, nucleus, extracellular vesicle, microvesicle, mitochondrion) (DAmiRLocGNet) mean AUC = 0.805	miRNA sequence (miRBase), miRNA–disease associations (HMDD v3.2), disease semantic similarity (MeSH-derived)	Graph convolutional network (GCN) and autoencoder (AE), with random-walk-with-restart preprocessing (DAmiRLocGNet)	1,041 miRNAs; RNALocate2.0-derived localization labels (2,930 locative entries); 15,547 miRNA–disease associations across 640 diseases	10-fold cross-validation; component-wise ablation study	MiRLoc (functional-similarity-network based); MirLocPredictor (CNN + k-mer); TextRNN (sequence-based)	None reported—internal 10-fold CV only, no independent test cohort	Performance depends on completeness/quality of the miRNA–disease association network; rare, less-studied miRNAs with sparse MDA data are likely underserved [152]

Abbreviations: AI/ML, artificial intelligence/machine learning; miRNA, microRNA; siRNA, small interfering RNA; mRNA, messenger RNA; CTS, candidate target site; AUC, area under the receiver operating characteristic curve; MFE, minimum free energy; CV, cross-validation; CLASH, crosslinking, ligation and sequencing of hybrids; HEK293, human embryonic kidney 293 cells; PCT, probability of conserved targeting; eCLIP, enhanced crosslinking and immunoprecipitation; pSILAC, pulsed stable isotope labelling by amino acids in cell culture; RNA-FM, RNA foundation model; PCC, Pearson correlation coefficient; SPCC, Spearman correlation coefficient; SVM, support vector machine; CNN, convolutional neural network; GNN, graph neural network; LNP, lipid nanoparticle; LiON, lipid optimization using neural networks; AGO2, Argonaute 2; SOTA, state-of-the-art; RBF, radial basis function; IBCGA, inheritable bi-objective combinatorial genetic algorithm; TCGA, The Cancer Genome Atlas; LightGBM, light gradient boosting machine; XGBoost, extreme gradient boosting; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; ESCA, esophageal carcinoma; KIRC, kidney renal clear cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; STAD, stomach adenocarcinoma; UVM, uveal melanoma; hsa, Homo sapiens; HMDD, Human microRNA Disease Database; MeSH, Medical Subject Headings; MDA, miRNA–disease association; GCN, graph convolutional network; AE, autoencoder. Tool and database names (e.g., mintRULS, miRAW, miTAR, OligoFormer, AttSiOff, MultiMiTar, DAmiRLocGNet, miRBench, TargetScan, miRDB, miRTarBase, miRBase, RNALocate) are proper names and are not expanded.

8. Conclusion

Ultimately, the trajectory of miRNA development truly underscores a fundamental departure from traditional pharmacology; it is no longer a question of whether we can manipulate genetic networks, but rather how precisely that control can be directed. While early clinical attrition served as a stark reminder of the immunological and structural complexities inherent to RNA interference, these setbacks have forced valuable design adaptations [112,113]. By merging complex chemical modifications with tissue-specific delivery vectors, the modern pipeline has been actively transforming these fragile molecules into resilient chemical assets [117]. As long-term safety profiles become clearer and delivery forms continue to mature further, miRNA-based interventions may increasingly find a role as adjunctive, precision-targeted therapies in diseases where conventional approaches fall short, though this depends on resolving the tissue-selective delivery, dosing, and safety challenges outlined above. In conclusion, the maturation of miRNA therapeutics represents more than just an expansion of the current drug pipeline; it marks a profound paradigm shift in modern medicine.

Declaration of generative AI and AI-assisted technologies

During the preparation of this manuscript, the authors used generative AI tools, only to improve language and readability. Specifically, the authors used Claude for polishing and reframing limited sections of the manuscript. The authors take full responsibility for the content of the manuscript.

Authors' contribution

Conceptualization, K.K. and V.K.M.; methodology, K.K.; validation, V.K.M.; investigation, K.K. and V.K.M.; writing—original draft preparation, K.K.; writing—review and editing, K.K. and V.K.M.; visualization, K.K.; supervision, V.K.M. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interests.

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