Analysis of the role of RNA regulatory networks in cancer treatment: Mechanisms, applications and future prospects (Review)

WENHUA REN

College of Life Sciences, Nanjing Normal University, Nanjing, Jiangsu 210023, P.R. China

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Abstract. RNA regulatory networks play a central role in cancer development and progression, influencing key biological processes such as cell cycle control, proliferation, apoptosis and tumor microenvironment interactions. MicroRNA (miR) variants, specifically isomiRs, have emerged as a novel research focus due to their altered target specificity and cancer-specific expression patterns, highlighting their potential as diagnostic markers and therapeutic targets. Additionally, advanced technologies such as single-cell sequencing and CRISPR screening offer novel avenues to dissect the complexity of RNA networks and identify key regulators for personalized therapy. The present review provides an integrative overview of RNA regulatory mechanisms, with a particular focus on the functional dynamics of isomiRs and competing endogenous RNA networks. Finally, the translational potential of isomiRs in precision oncology is described and key challenges and future directions in the field are outlined.

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Correspondence to: Professor Wenhua Ren, College of Life Sciences, Nanjing Normal University, 1 Wenyuan Road, Nanjing, Jiangsu 210023, P.R. China
E-mail: 08162@njnu.edu.cn

Abbreviations: ceRNA, competitive endogenous RNA; isomiR, microRNA isoform; lncRNA, long non-coding RNA; miRNA, microRNA; scRNA-seq, single-cell RNA sequencing; scATAC-seq, single-cell assay for transposase-accessible chromatin; CRISPRi/a, CRISPR interference/activation; ASO, antisense oligonucleotide; siRNA, small interfering RNA; ddPCR, digital droplet PCR; LNP, lipid nanoparticle; TME, tumor microenvironment; MRE, microRNA response element; RISC, RNA-induced silencing complex; G4, G-quadruplex; CITE, cellular indexing of transcriptomes and epitopes

Key words: RNA regulatory network, ceRNA; isomiR, cancer treatment, prognostic marker, precision medicine

1. Introduction

Cancer remains one of the leading causes of morbidity and mortality worldwide, despite notable advances in diagnostic technologies and therapeutic interventions. Traditional treatments such as chemotherapy and radiotherapy often cause damaging side effects, exhibit limited specificity and frequently lead to therapeutic resistance, thus collectively undermining the quality of life of patients. For instance, ~40% of patients undergoing chemotherapy experience severe adverse effects, including gastrointestinal toxicity and myelosuppression (1,2), while ~50% of those with advanced solid tumors develop drug resistance (3,4). These limitations underscore an urgent need for more precise and effective therapeutic strategies.

Recent advances in molecular biology and computational analysis have revealed that RNA regulatory networks play pivotal roles in cancer progression by orchestrating key biological processes such as proliferation, apoptosis, angiogenesis and drug resistance (5,6). Non-coding RNAs (ncRNAs), including microRNAs (miRNAs or miRs), long non-coding RNAs (lncRNAs) and their variants, have emerged as central players in these networks (7,8). Notably, novel isoforms such as isomiRs and mechanisms such as the competitive endogenous RNA (ceRNA) network have introduced new layers of regulatory complexity, with notable implications for cancer diagnostics and treatment.

The present review provides a comprehensive and integrative perspective on RNA regulatory networks in oncology, with a particular focus on isomiRs and ceRNA interactions. Unlike previous reviews, this article highlights how multi-omics profiling, single-cell RNA sequencing (scRNA-seq), and CRISPR-based functional screening enable high-resolution

mapping of RNA interactions and support their translation into clinical applications. These tools offer the potential to address persistent challenges in the field, such as dynamic heterogeneity, spatiotemporal regulation and the inference of causality within complex networks (9-11).

By synthesizing recent findings, the present article highlights emerging molecular mechanisms, clinical relevance and the translational potential of RNA networks in cancer. In addition, barriers to their application were also discussed, particularly in multi-omics integration, and promising technological directions that may redefine precision oncology in the future were described.

Recent clinical experiences have underscored the need to align early clinical signals with mechanistic annotation in RNA oncology. Durable benefit appears to depend as much on delivery engineering and proactive mitigation of innate-immune sensing as on the RNA payload itself. Representative programs using optimized carriers and tissue-directed delivery (for example, lipid nanoparticles and ligand conjugates) illustrate how organ/lesion exposure, endosomal escape, and immune quieting can determine translational success (12,13). Specific trial details are summarized in 'Strategies and prospects', and failure modes with practical lessons are discussed in 'Existing problems'.

2. Basic composition and mechanism of action of RNA regulatory networks

Understanding the regulatory interplay among different RNA species is fundamental to deciphering cellular homeostasis and disease pathogenesis. In the following section, the basic functions of mRNA, miRNA and lncRNA were reviewed, the theoretical basis of the competitive ceRNA network was discussed and the generation of isomiRs and their unique regulatory roles were described.

Introduction to the basic functions of mRNA, miRNA and lncRNA. mRNAs are the key intermediates between DNA and proteins. They carry genetic information from the nucleus to the cytoplasm, where they serve as templates for protein synthesis. The translation of mRNAs into proteins is tightly regulated at multiple levels, ensuring that proteins are produced in response to the developmental stage of the cell and environmental cues. Previous studies have shown that alterations in mRNA expression patterns are not only reflective of cellular states, but also actively contribute to the progression of diseases, including cancer (5,7,11).

In contrast to mRNAs, miRNAs are short, ~22-nucleotide ncRNAs that function predominantly in post-transcriptional gene regulation. By binding to complementary sequences in the 3' untranslated regions (3' UTRs) of target mRNAs, miRNAs mediate mRNA degradation or inhibit translation. This mode of regulation is highly conserved and enables a single miRNA to target multiple mRNAs simultaneously, thus orchestrating complex gene expression networks (14,15).

lncRNAs are a diverse group of transcripts that are >200 nucleotides in length, and lack protein-coding potential. Despite their inability to code for proteins, lncRNAs are emerging as critical regulators of gene expression. They modulate chromatin structure, influence transcriptional activity and participate in post-transcriptional regulatory events. Notably, numerous lncRNAs function as molecular sponges for miRNAs by sequestering them away from their target mRNAs and thereby modulating downstream signaling pathways (16,17). Recent advances in single-cell sequencing have elucidated the cell type-specific expression and function of lncRNAs, reinforcing their importance in both physiological and pathological contexts (18).

Collectively, the interplay between mRNAs, miRNAs and lncRNAs forms the backbone of the RNA regulatory network, ensuring precise control over gene expression in both normal cellular processes and in diseases such as cancer.

Theoretical basis of the ceRNA network. The ceRNA hypothesis, first proposed in 2011, has fundamentally reshaped the understanding of post-transcriptional gene regulation. This model posits that RNA molecules containing shared miRNA response elements (MREs) can compete for binding to miRNAs, thereby influencing the stability and translational efficiency of target mRNAs (16). In essence, RNA transcripts, including mRNAs, lncRNAs and pseudogene-derived RNAs, can function as 'molecular sponges', sequestering miRNAs and reducing their availability to other targets, thus indirectly modulating gene expression across a regulatory network.

Several factors determine the efficacy of ceRNA interactions. The foremost among these is the relative abundance of the competing RNAs, whereby highly expressed ceRNAs are more likely to effectively bind and sequester miRNAs. This concept is formalized in the 'miRNA sponge threshold hypothesis', which posits that only ceRNAs expressed above a certain quantitative threshold can exert functional competition. Mathematical models have substantiated this hypothesis, demonstrating non-linear regulatory outcomes in response to ceRNA concentration (19).

In addition to transcript abundance, the number and binding affinity of MREs within each RNA molecule shape ceRNA regulatory capacity. For instance, transcripts harboring multiple high-affinity sites for a given miRNA may be more susceptible to ceRNA-mediated modulation than those with fewer or weaker binding motifs (17,20). Furthermore, subcellular localization plays a critical role, as ceRNA interactions are spatially restricted to cellular compartments where miRNAs and their targets are co-localized.

Emerging evidence has reinforced the biological relevance of ceRNA networks, particularly in the context of cancer. These interactions have been linked to tumor proliferation, metastasis, immune evasion and therapeutic resistance. Context-specific ceRNA interactions have been identified in breast, liver, lung, pancreatic and brain cancers, demonstrating diverse regulatory roles across tumor types (17,21,22). A comprehensive overview of these lncRNA-miRNA interactions in various cancers further underscores their central regulatory role and translational potential (23).

CeRNA axes discovered in discovery sets have reproduced prognostic separation in independent patient cohorts, including proliferation-linked networks in lung adenocarcinoma (LUAD) and colon cancer (24,25).

Nevertheless, the generalizability of the ceRNA model across all cancer types remains under debate. Increasing evidence suggests that ceRNA network robustness is highly context-dependent, varying substantially between tumor

types. Factors such as tissue-specific RNA expression, tumor subtype heterogeneity and the tumor microenvironment (TME) can constrain or enhance ceRNA-mediated regulation. These foundational interactions within the RNA regulatory landscape are illustrated in Fig. 1.

Generation of isomiRs and their regulatory importance. Canonical miRNA loci produce multiple sequence variants, termed isomiRs, through imprecise cleavage by Drosha/Dicer and post-transcriptional tailing or trimming (such as adenylation/uridylation) (17,20,23). These edits alter the length and sequence at the 5' or 3' ends. Alterations at the 5' end are particularly consequential because they shift the seed (nts 2-8), thereby redefining the target repertoire and potentially rewiring regulatory programs. By contrast, numerous 3' variants primarily influence Argonaute loading, stability or binding affinity without altering seed identity (20).

In cancer, isomiRs show context-specific expression relative to canonical miRNAs and to matched normal tissues, implicating them in tumor-type-dependent pathway control (17,20). They may also reshape ceRNA competition by engaging distinct MREs, modifying post-transcriptional regulation in a manner that depends on both sequence and tissue context. This principle is illustrated in Fig. 2, using 5'-isomiRs with notable tumor-normal differences in three TCGA cohorts, including breast (BRCA), colorectal (COAD/READ) and liver (LIHC), displayed as z-scored log2(RPM+1) heatmaps. The cancer-type-specific patterns oppose a pan-cancer stereotype and instead support selective deployment of 5'-isomiRs across tumor contexts.

Despite their promise, the functional independence of numerous isomiRs remains debated. Some 5'-shifted variants exhibit distinct targetomes and phenotypes, whereas others appear largely redundant with their canonical counterparts (20,26). Most evidence to date is correlative or prediction-based. Rigorous validation such as isoform-resolved CLIP/CLASH, seed-swap reporters, rescue assays and locus-specific perturbations are still needed to establish causal mechanisms and to assess biomarker or therapeutic value at isoform resolution.

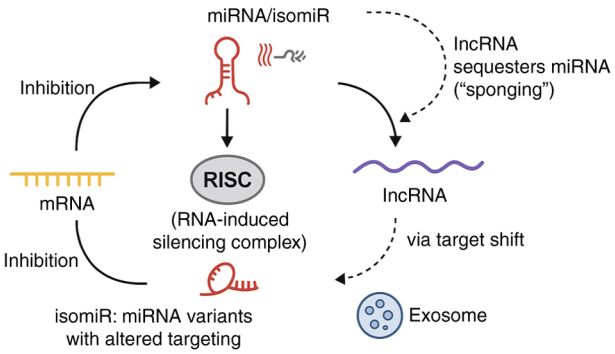

Figure 1. Core components of RNA regulatory networks in cancer. miRNAs and isomiRs bind to the RISC, which guides them to complementary mRNA targets. This interaction results in either translational repression or mRNA degradation. lncRNAs act as miRNA sponges, and RNA species can be exported via exosomes for intercellular communication. RISC, RNA-induced silencing complex; miRNA, microRNA; isomiR, microRNA isoform; mRNA, messenger RNA; lncRNA, long non-coding RNA.

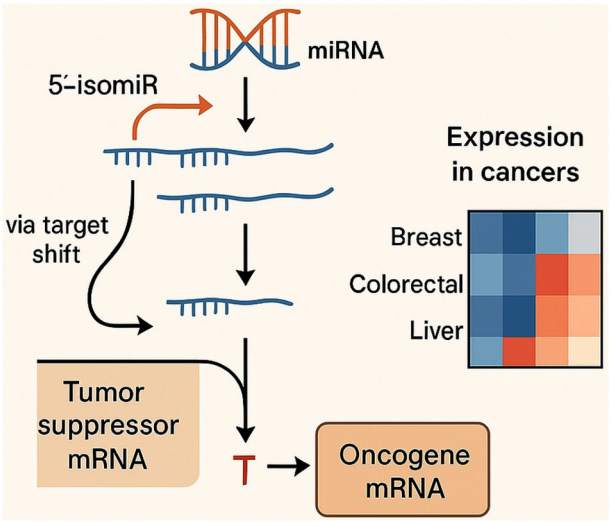

Figure 2. Functional impact of 5'-isomiRs in tumorigenesis. The 5'-end variation shifts the miRNA seed, retargeting transcripts. Heatmap shows z-scored log2(RPM+1) of representative 5'-isomiRs in TCGA BRCA, COAD/READ and LIHC (cancer-type-specific). IsomiR, microRNA isoform; miRNA, microRNA; TCGA, The Cancer Genome Atlas; BRCA, breast invasive carcinoma; COAD/READ, colon/rectum adenocarcinoma; LIHC, liver hepatocellular carcinoma.

3. Role of the RNA regulatory network in cancer occurrence and progression

RNA regulatory networks play a pivotal role in tumorigenesis by modulating important cellular processes such as cell cycle control, proliferation, apoptosis and the TME. The interplay among various RNA species, including mRNAs, miRNAs, lncRNAs and other ncRNAs, provides a complex regulatory framework that influences cancer initiation, progression and therapeutic response. A comprehensive understanding of these networks not only elucidates the molecular mechanisms driving cancer but also highlights potential targets for intervention.

RNA regulatory networks in cell cycle, proliferation and apoptosis. Dysregulation of the cell cycle and proliferation pathways is a hallmark of cancer. Under physiological conditions, cyclins, cyclin-dependent kinases (CDKs) and their inhibitors coordinate orderly progression through the cell cycle. Disruptions in these circuits can lead to uncontrolled cell division. RNA regulatory networks, particularly those involving ceRNA interactions, modulate the expression of these critical regulators. For example, miRNAs targeting cyclin genes (such as CCNA2 and CCND1) or CDK inhibitors (such as p21 and p27) can alter cell cycle dynamics, thereby promoting or inhibiting tumor growth (27).

In parallel, these RNA networks also regulate apoptosis by balancing the expression of pro- and anti-apoptotic factors such as BCL2, BAX and CASP9. Dysregulation of RNA-mediated controls may tip this balance toward survival, enabling cancer cells to evade programmed cell death and develop therapeutic resistance. Certain lncRNAs have been shown to stabilize mRNAs encoding anti-apoptotic proteins, further contributing to tumor progression (28).

MiRNA signatures that regulate these circuits (for example, miR-21 and miR-145) correlate with recurrence and survival in retrospective/prospective cohorts and in liquid-biopsy studies, underscoring translational relevance (29,30-33).

Moreover, emerging evidence suggests that isomiRs, which are functionally diverse miRNA isoforms, can fine-tune RNA regulatory effects by altering target specificity, thereby influencing processes such as proliferation and apoptosis in a context-dependent manner (29).

RNA interactions and regulation of the TME. Beyond their roles in intrinsic tumor cell functions, RNA regulatory networks profoundly influence the TME. ncRNAs, including miRNAs and lncRNAs, are not only active intracellularly, but can also be secreted via extracellular vesicles (such as exosomes) to mediate intercellular communication with immune cells, fibroblasts and endothelial cells (34,35). These interactions affect immune infiltration, cytokine production, angiogenesis and extracellular matrix remodeling, creating a microenvironment conducive to tumor progression (36).

For instance, some miRNAs suppress T-cell activation or promote the polarization of macrophages toward an immunosuppressive M2 phenotype, facilitating immune escape (28). Similarly, lncRNAs can act as decoys or scaffolds that sequester transcription factors and chromatin remodelers involved in immune regulation. This multilayered regulatory network underscores the potential of targeting RNA interactions to enhance immunotherapy and overcome resistance. Ongoing clinical studies are exploring strategies to disrupt oncogenic RNA circuits or restore tumor-suppressive RNAs to therapeutically reprogram the TME (37).

These RNA-mediated mechanisms of immune modulation are schematically summarized in Fig. 3.

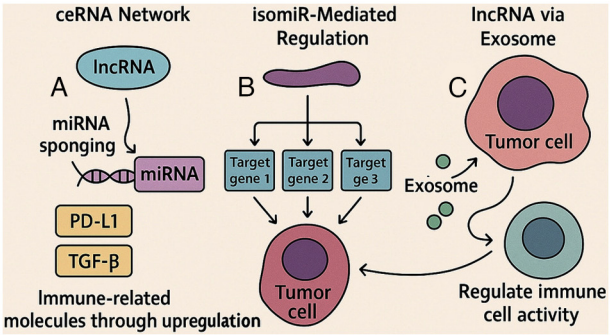

Figure 3. Integrated mechanisms of RNA regulatory networks in tumor immune modulation. (A) ceRNA networks: lncRNAs act as miRNA sponges, upregulating immune checkpoint genes such as PD-L1 and TGF-β. (B) isomiRs redirect gene targeting through altered seed sequences, promoting immune evasion. (C) Exosomal lncRNAs regulate immune cell activity in the TME. ceRNA, competitive endogenous RNA; lncRNA, long non-coding RNA; miRNA, microRNA; PD-L1, programmed death-ligand 1; TGF-β, transforming growth factor-β; TME, tumor microenvironment.

Case studies in various cancer types

LUAD: ceRNA networks in cell cycle regulation and prognosis. In LUAD, ceRNA networks have been implicated in regulating cell cycle-related gene expression and tumor proliferation, with prognostic importance. A representative ceRNA axis involved the lncRNA VPS9D1-AS1, miR-30a-5p and downstream targets such as CCNA2, KIF11 and MKI67 (24). VPS9D1-AS1 acts as a molecular sponge for miR-30a-5p, thereby upregulating key mitotic regulators and proliferation markers. This dysregulation contributes to unchecked tumor growth. Notably, large-scale bioinformatics analyses have highlighted these ceRNA circuits as potential biomarkers for patient stratification and therapeutic targeting.

Cholangiocarcinoma: SNHG6/miR-101-3p/E2F8 axis. In cholangiocarcinoma, the lncRNA *SNHG6* serves as a ceRNA that sequesters miR-101-3p, leading to derepression of the transcription factor *E2F8*, which promotes cell cycle progression. This ceRNA axis enhances proliferation, migration and angiogenesis in cholangiocarcinoma cells, supporting its role in aggressive tumor phenotypes (38). The *SNHG6/miR-101-3p/E2F8* pathway exemplifies the mechanisms by which ceRNA dysregulation facilitates oncogenic processes through post-transcriptional control.

Other cancer types: Breast, colorectal and liver cancer. RNA regulatory networks are also perturbed in other cancer types. In breast cancer, lncRNAs and miRNAs jointly modulate the PI3K/AKT/mTOR signaling pathway, contributing to metastasis and therapy resistance (39-41). In colorectal cancer, the *LCMT1-AS2/miR-454-3p/RPS6KA5* ceRNA axis functions as a prognostic indicator of poor survival (25). In hepatocellular carcinoma, ncRNAs orchestrate PI3K/AKT signaling cascades to influence angiogenesis and immune evasion (42,43).

These case studies underscore the context-specific architecture and functional diversity of ceRNA networks across different malignancies (44). Despite shared mechanisms, such as lncRNA sponging and miRNA-mediated repression, the biological outcomes are highly dependent on tumor type, target gene context and network topology. Understanding these disease-specific ceRNA circuits is important for developing targeted RNA-based interventions in precision oncology. These differences in ceRNA network strength, biological relevance and experimental validation across cancer types are systematically summarized in Table I.

4. Application of RNA regulatory networks in cancer diagnosis and prognosis

As the understanding of RNA regulatory networks improves, their clinical implications in oncology have become increasingly evident. From identifying prognostic markers to guiding molecular classification and precision medicine, RNA-based tools hold promise for improving cancer diagnosis, treatment and patient outcomes.

Screening of prognostic markers based on RNA networks. RNA molecules, including mRNAs, miRNAs, lncRNAs and circRNAs, have gained traction as diagnostic and prognostic biomarkers due to their tissue-specific expression patterns, stability in biofluids and critical regulatory roles in tumorigenesis (45). In particular, panels of differentially expressed RNAs have been correlated with disease stages and patient survival across various cancer types (30,31). For instance, specific lncRNAs have been linked to metastasis and recurrence in breast and colorectal cancers, while

Table I. Comparative effectiveness of ceRNA networks across cancer types.

First author/s, year	Cancer type	Regulatory strength	Key influencing factors	Representative ceRNA axis	Functional validation method	(Refs.)
Zhang *et al*, 2014	Breast cancer	High	High lncRNA and miRNA abundance	HOTAIR/ miR-331-3p/ HER2	*In vitro* knockdown and luciferase reporter assays	(89)
Poliseno *et al*, 2010	Glioblastoma	Low	Low miRNA levels, high heterogeneity	PTENP1/ miR-19b/ PTEN	Bioinformatics analysis, low experimental confirmation	(90)
Chen *et al*, 2021	Hepatocellular carcinoma	Medium-High	High lncRNA expression, immune complexity	LINC00160/ miR-132/AKT1	Animal models and expression rescue experiments	(91)
Xie *et al*, 2020	Pancreatic cancer	Low	Immunosuppressive tumor microenvironment, scarce lncRNAs	MALAT1/ miR-200c/ZEB1	Limited functional studies	(92)

ceRNA, competitive endogenous RNA; miR, microRNA; lncRNA, long non-coding RNA.

unique miRNA signatures have been proposed as early detection markers for lung and pancreatic cancers (32). Multi-center studies of circulating RNAs report diagnostic AUCs in the ~0.80-0.90 range for several malignancies, and tissue lncRNA/miRNA panels track stage, recurrence and survival (30,31,33,45-47).

Moreover, circulating RNAs, those detectable in plasma, serum and exosomes, offer a minimally invasive means of cancer detection and monitoring (33). By quantifying changes in RNA expression, clinicians can potentially identify high-risk patients, track tumor progression and evaluate therapeutic response. Despite these advances, larger, multicenter clinical studies are needed to validate the robustness, reproducibility and clinical utility of RNA-based prognostic panels before they can be widely implemented in routine practice (48).

Role of RNA molecules in molecular typing and precision medicine. RNA signatures have proven invaluable for the molecular classification of tumors, which is a key step in precision medicine. For example, gene expression profiling has led to the identification of distinct subtypes in breast cancer (such as luminal A, luminal B, HER2-enriched and basal-like) and the stratification of diffuse large B-cell lymphoma into molecularly defined categories (49). These classifications guide therapeutic decisions, such as the use of targeted therapies (such as HER2 inhibitors in HER2-enriched breast cancer) and immunotherapies for tumors harboring specific expression profiles.

Additionally, RNA-based molecular typing extends beyond mRNA to include ncRNAs. LncRNAs and miRNAs have emerged as critical regulators of oncogenic pathways and have been associated with tumor subtypes, therapeutic resistance and disease outcomes (46). Integrating RNA signatures into diagnostic frameworks enables clinicians to tailor treatment regimens to the unique molecular features of tumors of individual patients, improving efficacy and minimizing toxicity (47).

Clinical detection technology and its limitations. Various technologies have been developed to harness RNA molecules for cancer diagnosis and prognosis. High-throughput sequencing (RNA-seq) enables comprehensive transcriptome profiling, allowing detection of novel transcripts and splicing variants (50). Microarray-based platforms and reverse transcription-quantitative PCR are widely used for validating candidate RNA biomarkers due to their ease of use and cost-effectiveness (51). More recently, digital droplet PCR (ddPCR) has gained popularity for its high sensitivity in detecting low-abundance circulating RNAs (52).

Despite these advances, challenges persist. Variability in sample collection, RNA extraction and data analysis can affect the sensitivity and reproducibility of RNA-based diagnostics. Standardization of protocols and optimization across pre-analytical steps are important (53). Furthermore, tumor heterogeneity and the dynamic nature of RNA expression require repeated sampling to capture the evolving transcriptome. Large, multicenter clinical studies and robust bioinformatics pipelines are crucial to ensure the clinical utility of RNA-based tests.

Clinical applications and translational progress. Despite the growing body of evidence supporting RNA-based biomarkers and regulatory networks in cancer biology, their clinical translation remains limited. However, several RNA-targeted therapeutics have made notable strides. For instance, Givosiran and Inclisiran, two small interfering RNA (siRNA) drugs approved by the FDA, have demonstrated the feasibility of RNA-based therapy in clinical settings, although outside oncology. In the cancer field, MRX34, a liposomal mimic of the tumor-suppressive miR-34a, was the first miRNA

therapeutic to enter clinical trials, marking a milestone despite its early termination (54).

In parallel, RNA expression profiling has become a valuable tool in molecular subtyping and precision medicine. For example, the PAM50 RNA panel informs hormone and HER2-targeted therapies in breast cancer based on intrinsic molecular subtypes (49). However, several technological barriers still hinder the routine clinical adoption of RNA-based diagnostics. Although ddPCR offers high sensitivity for detecting low-abundance RNAs in liquid biopsies, its limited multiplexing capability and cost pose challenges for large-scale implementation (52). Similarly, while RNA sequencing provides comprehensive transcriptomic data, issues such as batch effects, variable library preparation and complex data analysis reduce inter-laboratory reproducibility (51).

To address these challenges, emerging strategies focus on standardizing workflows, minimizing RNA input requirements and developing FDA-aligned diagnostic guidelines. These developments represent a critical transition from bench to bedside, underscoring the translational promise of RNA regulatory networks in precision oncology.

5. Strategies and prospects of RNA regulatory networks in cancer treatment

RNA regulatory networks have become attractive therapeutic targets in oncology. By modulating miRNAs, lncRNAs and their downstream effectors, it may be possible to slow tumor progression, overcome drug resistance and improve outcomes (55,56).

Therapeutic strategies targeting RNA networks. miRNA/lncRNA mimics and inhibitors restore tumor-suppressive functions or block oncogenic ncRNAs, using synthetic oligonucleotides such as mimics, antagomiRs and ASOs (57-59). Modulating ceRNA interactions represents another avenue: Downregulating oncogenic lncRNAs or other competing RNAs can liberate tumor-suppressive miRNAs to repress their targets (60).

Clinical status and delivery lessons. The liposomal miR-34a mimic MRX34 reached first-in-human testing but was terminated for immune-related toxicities, highlighting the need to minimize innate immune activation and to optimize delivery and preclinical toxicology (61). By contrast, siRNA agents have demonstrated that careful carrier design enables tissue-specific delivery with manageable safety, as illustrated by patisiran in non-oncology indications (12) and by RNA nanoparticles with favorable biodistribution and conditional endosomal escape (13). Oncology-focused programs span locoregional depots such as siG12D-LODER for KRAS^G12D pancreatic cancer (62,63), systemically delivered LNP siRNAs such as DCR-MYC (64,65), and newer platforms exemplified by the GSTP-targeting NBF-006 evaluated in KRAS-mutant NSCLC (66,67). Collectively, these data emphasize the centrality of delivery engineering, target and context selection, and early immune-risk mitigation, which are elaborated below.

6. Existing problems and limitations

While RNA regulatory networks present promise in cancer research and treatment, several challenges remain in understanding their full potential. Data integration, technical limitations and the need for more refined experimental approaches hinder the progress in this field. Addressing these issues will be crucial for optimizing RNA-based therapeutic strategies and improving the clinical application of RNA regulatory networks in oncology.

Challenges of data integration and network construction in existing studies. Integrating high-throughput RNA sequencing data remains a major challenge due to the complexity of transcriptomic interactions. RNA molecules, including mRNAs, miRNAs, lncRNAs and isomiRs, form intricate, context-dependent networks that vary across tissue types, disease stages and microenvironments. A single RNA species may regulate or interact with multiple targets in diverse ways. However, most current reconstruction methods rely on statistical correlations rather than causal or directional relationships, resulting in oversimplified or incomplete network models (68). While numerous computational tools have been developed to predict miRNA-lncRNA interactions, such as correlation-based and sequence-complementarity methods, these tools often produce inconsistent outputs and require further experimental validation (69).

The lack of standardized computational pipelines and network inference frameworks further impedes data integration and cross-platform comparability. Although numerous tools have been developed, few can integrate multi-omics datasets (such as RNA-seq with ATAC-seq) at the single-cell level to construct cell type-specific regulatory maps (70). Emerging tools such as IReNA and pySCENIC attempt to address these issues by incorporating transcription factor motifs, chromatin accessibility and regulatory logic into network inference (71). However, robust integration of large-scale omics data and reproducibility across computational platforms remain unresolved challenges.

Moreover, scRNA-seq faces limitations in detecting low-abundance RNAs such as miRNAs and lncRNAs. These datasets are also prone to batch effects and technical noise, affecting reproducibility and interpretation. Although multi-omics integration tools are advancing, fully capturing dynamic RNA regulatory networks at single-cell resolution remains an ongoing area of research (10).

Technical and biological problems in isomiR research. IsomiRs, the variant forms of miRNAs, have garnered increasing attention for their ability to expand the miRNA regulatory landscape. However, several technical and biological challenges hinder their accurate identification and functional characterization. A major limitation lies in the resolution of current sequencing technologies, which may fail to detect low-abundance isomiRs or differentiate between closely related isoforms, particularly those with 5' seed shifts, which can alter target specificity (26). Furthermore, incomplete or inconsistent annotation in public databases hampers reliable mapping and interpretation (20).

Biologically, the roles of isomiRs in cancer remain only partially understood. Variants that differ at the 5' end may possess novel seed sequences, leading to distinct mRNA targeting and potentially divergent effects on oncogenic

signaling pathways (17). Functional validation of such isomiRs is still limited, and systematic studies across different tissues and tumor types are required to determine their contribution to tumorigenesis, metastasis and therapy resistance. Advances such as dual-index library construction for reproducible isomiR sequencing (26), along with isomiR-specific databases [such as isomiRTar (72) and TIE], are beginning to address some of these limitations and offer promising platforms for future isomiR discovery and clinical application.

In the present review, three recurrent challenges that have hampered the clinical success of RNA therapeutics in oncology (delivery efficiency, innate immune activation, and off-target effects) were highlighted, and each paired with evidence-informed mitigation strategies.

Delivery. Effective delivery to tumor tissue remains a major obstacle. For systemically administered agents, the composition of lipid nanoparticles (LNPs), including the pK_a of ionizable lipids, helper lipids, and PEG-lipids, critically influences stability, cellular uptake and endosomal escape (73-75). Clinical experience demonstrates that rational LNP design can successfully translate to humans, as exemplified by patisiran, which achieved robust hepatic gene silencing using an MC3-based LNP and exhibited a manageable safety profile (12). Tumor selectivity can be further enhanced through ligand-mediated targeting (for example, GalNAc, antibodies, or aptamers) or via locoregional depot systems. Oncology case studies (for example, siG12D-LODER and DCR-MYC) have been described in Section 5, illustrating how delivery mode, namely local depot versus systemic LNP, critically shapes tumor exposure and clinical feasibility (62-65). Practical strategies to improve delivery include refining ionizable lipid structures, modulating PEG content, incorporating validated targeting ligands, utilizing localized administration where possible, and prioritizing the assessment of tumor exposure and endosomal escape during early development (68-78).

Innate immune activation. The case of MRX34, which was discontinued despite evidence of target engagement, highlights the clinical consequences of uncontrolled innate immune stimulation, often mediated through endosomal Toll-like receptors (for example, TLR7/8) (61). Mitigation approaches incorporate sequence 'de-immunization' using chemically modified nucleotides (such as 2'-OMe, 2'-F, or LNA), optimized PEGylation strategies, and early *in vitro* screening using human whole-blood assays or PBMC cultures to quantify cytokine release (79,80). These measures reduce TLR-dependent immune recognition, dampen excessive cytokine production, and help establish safer starting doses and premedication regimens for first-in-human trials (63,79,80).

Off-target effects. siRNA candidates can induce seed region-mediated off-target silencing via interactions with 3' UTRs, leading to unintended transcriptome-wide effects (81). Comprehensive off-target profiling involves coupling AGO2-centered methods (for example, HITS-CLIP or PAR-CLIP) with dose-responsive transcriptomics (RNA-seq) and proteomics. Computational filtering and CRISPR-based validation help establish causal relationships between observed effects and off-target binding (82,83). Design-based solutions include modifying the seed sequence, introducing chemical modifications that reduce miRNA-like activity, and selecting guide strands with minimal predicted off-target potential in relevant cellular models (81-83).

Functional verification of isomiRs. Demonstrating function requires evidence of seed dependence, direct target engagement and a measurable cellular consequence in the relevant context. To establish robust evidence for isomiR-mediated targeting, a multi-tier framework can be applied. Initial screening with dual-luciferase reporter assays comparing wild-type versus seed-mutant 3'UTRs, including canonical and 5'-shifted isomiRs, provides seed-specific activity, while seed-reversion rescues confirm specificity (61). Genetic perturbation at the pri-miRNA locus (CRISPRi/a or base editing) modulates isomiR production and can be linked to phenotypic outcomes using pooled single-cell transcriptomics such as Perturb-seq or CROP-seq (79,80). Direct binding is then established by AGO-CLIP (HITS-CLIP or PAR-CLIP) (61), and functional repression verified by dose-responsive RNA-seq and quantitative proteomics (61). At higher resolution, these steps can be integrated with single-cell multi-omics to assign effects to specific cell states (68,69,73).

Seed specificity can be assessed by comparing canonical and 5'-shifted variants in dual-luciferase assays and by reversing the altered seed (81). Causality in living cells can be tested via CRISPR-based perturbation of isomiR production or processing, with single-cell transcriptomic readouts when cell-state resolution is required. Physical binding to predicted sites is evaluated with AGO-centered CLIP (HITS-CLIP or PAR-CLIP), and downstream impact is quantified under therapeutically relevant exposures using dose-ranged RNA-seq and quantitative proteomics (82,83).

Implications and new directions for future cancer treatment strategies. Despite ongoing challenges, RNA-based therapeutics are advancing rapidly, driven by innovations in molecular design, delivery systems and target specificity. An increasing number of therapeutic agents, including miRNA mimics, lncRNA inhibitors and small molecules that modulate RNA-RNA or RNA-protein interactions, are under active development. These approaches offer fine-tuned control over key pathways such as cell proliferation, apoptosis and immune regulation, particularly in tumors resistant to traditional treatments (60).

At the same time, progress in RNA drug delivery is expanding the clinical applicability of these strategies. LNP formulations, ligand-targeted conjugates and chemically modified oligonucleotides are being optimized to enhance stability, reduce off-target effects and achieve tumor-specific accumulation. The success of RNA-based drugs in non-cancer diseases, such as Patisiran for transthyretin-mediated amyloidosis, provides valuable insights for oncology applications.

In the future, RNA therapeutics are hypothesized to integrate more deeply into combination regimens alongside immunotherapies, targeted therapies and radiotherapy. Such synergistic approaches may improve treatment efficacy while minimizing systemic toxicity. Moreover, advances in biomarker discovery, particularly RNA-based expression signatures, will facilitate patient stratification and response monitoring, supporting a shift toward more personalized cancer care.

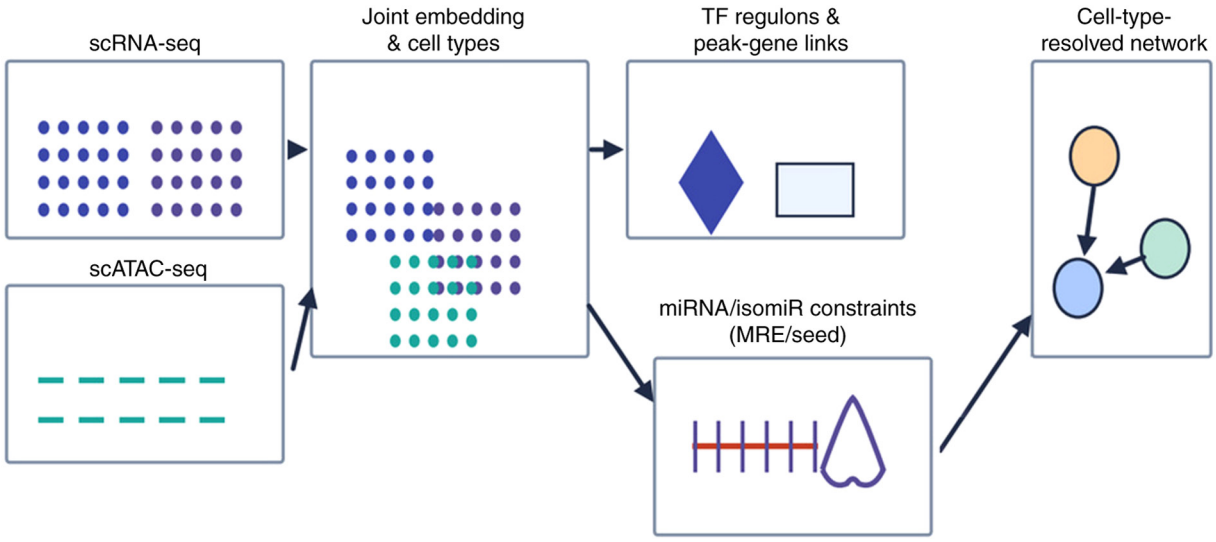

Figure 4. Single-cell multi-omics integration to resolve cell type-specific RNA interaction networks. scRNA-seq and scATAC-seq are jointly embedded to define cell types (colored clusters). Peak-gene links and TF regulons are derived from chromatin accessibility and motif/footprint analysis, while microRNA/isomiR constraints (seed/MRE) restrict candidate edges. Optional spatial data prioritize edges co-localized in the TME. Output: Cell type-resolved ceRNA/isomiR networks and prioritized targets. scRNA-seq, single-cell RNA sequencing; scATAC-seq, single-cell assay for transposase-accessible chromatin; isomiR, microRNA isoform; MRE, microRNA response element; TME, tumor microenvironment; ceRNA, competitive endogenous RNA; TF, transcription factor.

Ultimately, the continued refinement of RNA therapeutics, along with robust clinical validation and regulatory frameworks, will be important to fully realize their potential in precision oncology.

Emerging frontiers: Hot topics in RNA regulatory network research

Single-cell multi-omics for high-resolution regulatory mapping. A compact workflow for single-cell multi-omics integration [scRNA-seq + single-cell assay for transposase-accessible chromatin (scATAC-seq) ± spatial] is provided in Fig. 4, from joint embedding and TF/peak-gene linking to MRE-constrained edge inference and cell type-resolved networks.

Concise description of single-cell multi-omics integration. ScRNA-seq and scATAC-seq datasets were quality-controlled and batch-corrected to obtain a joint embedding and a reproducible cell-type map (68,69). Chromatin peaks are linked to genes by correlating accessibility with expression within cell types and by motif/footprint analysis to define transcription-factor regulons with per-cell activity scores (71). Integrated regulatory networks are inferred with methods that couple scRNA-seq and chromatin-accessibility constraints (such as IReNA) (70). Candidate ceRNA/isomiR relationships are then filtered by the presence of microRNA-response elements and, where available, orthogonal support from isomiR-target resources (72). When spatial transcriptomics are available, edges that co-localize within the TME are prioritized. Robustness is assessed by stability across donors/batches and held-out performance (68,73).

Single-cell sequencing technologies, particularly when integrated with transcriptomic, epigenomic and proteomic modalities (such as scRNA-seq, scATAC-seq and CITE-seq), are transforming the current understanding of RNA regulatory networks in cancer. These tools enable the resolution of cell type-specific expression profiles and uncover spatial and temporal heterogeneity in ceRNA and isomiR interactions, especially within the TME (10,84). Such fine-grained insights are crucial for identifying rare subpopulations that drive therapy resistance, metastasis or immune escape.

Previous efforts have also applied single-cell spatial transcriptomics to validate ceRNA networks *in situ*, revealing topological constraints and microenvironmental influences on RNA-based regulation (16,19,23). These approaches provide unprecedented opportunities to reconstruct RNA interaction maps at a cellular resolution.

Emerging multi-omics integration strategies are moving beyond single-cell approaches to provide robust, pan-cancer insights into post-transcriptional regulation. Representative cases illustrate this evolution:

In cholangiocarcinoma, integrated transcriptomic and proteomic profiling, combining RNA-seq with quantitative mass spectrometry, has uncovered functional isomiR-mediated networks. Predictions of isomiR-target interactions were corroborated by corresponding protein-level changes, highlighting cross-regulatory RNA crosstalk and prioritizing mechanistically relevant edges for experimental validation (21).

Pan-cancer integrative frameworks have further enabled the identification of consensus ceRNA networks across large cohorts. By aggregating miRNA-lncRNA-mRNA data from multiple tumor types, these approaches derive robust cross-cancer regulatory edges, which are subsequently evaluated through dataset stability tests and survival analysis, offering a reproducible template for bulk multi-omics synthesis (23,68,69).

Curated multi-omics databases now systematically consolidate single-cell, spatial and bulk genomic data to enhance ceRNA inference. These resources support cross-modality validation, such as confirming co-expression in bulk datasets, spatial co-localization in tissue maps, and recurrence of edges across cancer types, enabling more reliable triage of candidate interactions (10).

Additionally, constraint-guided methods that incorporate chromatin features, such as motif accessibility, foot-printing data, and open chromatin states, are increasing the specificity of regulatory network inference. Although often applied in single-cell analyses, these priors are equally valuable in bulk multi-omics integration, where they help reduce false-positive predictions and refine edges based on functional genomic constraints (70,71).

CRISPR-based functional validation and network causality. Traditional studies of RNA networks rely heavily on correlational analyses, which limit causal inference. CRISPR-based screening technologies, particularly CRISPR interference and activation (CRISPRa), now offer a powerful means of interrogating RNA function. When combined with single-cell transcriptomics (such as Perturb-seq and CRISPRa-Perturb-seq), these tools allow high-throughput, context-specific validation of lncRNAs, miRNAs and ceRNA nodes (9,85). These screens have successfully identified key regulatory elements that contribute to drug resistance and tumor progression, enhancing the biological credibility of proposed networks. As RNA-targeted therapies continue to develop, such functional genomics approaches will play a pivotal role in bridging predictive models and therapeutic translation.

RNA structural biology and non-canonical regulatory layers. Beyond sequence-level regulation, emerging evidence points to the importance of RNA secondary and tertiary structures, especially G-quadruplexes (G4s), in shaping RNA regulatory landscapes. G4 motifs are highly stable, guanine-rich structures found in both coding and non-coding regions, where they influence miRNA binding, splicing and translation (17,86). These structures can modulate ceRNA stability or alter the seed-region recognition capacity of isomiRs, introducing novel dimensions of regulatory control. Structure-based targeting of RNA is gaining traction as a therapeutic strategy. Molecules that selectively bind or stabilize RNA G4s have shown antiproliferative effects in cancer models, offering new possibilities for intervention (87,88). Future work may focus on programmable modulation of such structures using CRISPR-guided systems or synthetic ligands.

These emerging technologies, including single-cell multi-omics, CRISPR-based functional validation and RNA structure-focused regulation, are redefining the landscape of RNA network research. Their integration will unlock novel therapeutic avenues and deepen the mechanistic understanding of cancer biology at single-cell and molecular resolutions.

Dynamic heterogeneity in RNA regulatory networks and its regulatory mechanisms. Recent studies have revealed that cellular heterogeneity within tumors and their microenvironments influence the construction and function of RNA regulatory networks (10,20,33). First, distinct cell types, such as immune cells versus tumor cells, exhibit fundamentally different RNA network regulatory mechanisms. Immune cells, which play key roles in antigen presentation, cytokine secretion and immune modulation, tend to have ceRNA networks tailored to regulating immune responses. By contrast, tumor cells often display ceRNA networks that primarily promote proliferation, evade immune surveillance and drive metabolic reprogramming. For example, immune cell ceRNA interactions are closely linked to immune response regulation, whereas in tumor cells these networks are more frequently involved in cell cycle and metabolic pathway regulation (37).

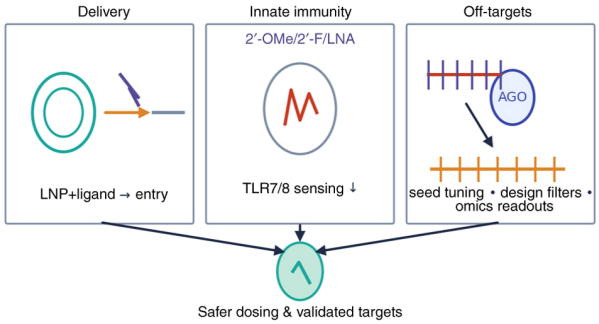

Figure 5. Translational constraints for RNA therapeutics. Schematic overview of delivery (LNP with ligand and tumor entry), innate immunity (endosomal TLR7/8 sensing and mitigation by chemical modifications) and off-target control (seed motif, AGO engagement and mRNA ladder), converging on safer dosing and robust target validation. LNP, lipid nanoparticle; TLR, Toll-like receptor; mRNA, messenger RNA; AGO, Argonaute.

Furthermore, there is mounting evidence that ceRNA networks undergo dynamic remodeling during tumor progression. In early-stage tumors, ceRNA networks may serve to maintain a balance between proliferation and apoptosis. However, as tumors advance, these networks can be restructured to support metastasis, drug resistance and immune evasion. In LUAD, key ceRNA components have been observed to undergo notable changes in both expression and interaction patterns as the disease progresses, indicating a dynamic adjustment of the network architecture over time (24).

Lastly, integrating time- and space-resolved expression data is crucial for reconstructing accurate RNA regulatory maps. Single-cell multi-omics and spatial transcriptomics technologies now enable researchers to capture RNA expression dynamics across different tumor regions and time points. For instance, the LnCeCell 2.0 database leverages such data to reveal the spatiotemporal specificity of ceRNA interactions among various cell types, providing powerful tools for dissecting the complex heterogeneity of tumor tissues (10).

Collectively, these insights underscore the importance of considering both cellular and temporal heterogeneity in RNA regulatory network analyses. A deeper understanding of these dynamic changes not only enriches the knowledge of tumor biology but also informs the development of personalized therapeutic strategies based on the spatiotemporal modulation of gene expression. These considerations are consolidated in Fig. 5, which links the major constraints to practical mitigation levers and validation readouts.

7. Future prospects and emerging directions

The rapid evolution of RNA regulatory research is opening new frontiers in cancer biology and therapy. While marked progress has been made in identifying the components and functions of RNA networks, several challenges remain. These include the need for robust, context-specific functional validation, improved delivery of RNA-targeted therapeutics and standardization in data integration across multi-omics platforms.

In the future, a key direction may be the convergence of mechanistic insight and clinical application. High-resolution

technologies such as single-cell multi-omics and CRISPR-based screening may continue to play a vital role in decoding network dynamics and identifying cell-specific vulnerabilities. However, their full translational potential depends on the development of clinically viable tools for manipulating RNA interactions *in vivo*, including programmable RNA editors, structure-targeted ligands and combination therapies that modulate multiple layers of regulation simultaneously.

Another promising avenue lies in integrating RNA-based approaches with other therapeutic modalities, such as immunotherapy and metabolism-targeting drugs. RNA biomarkers and regulatory signatures may enable more refined patient stratification and dynamic monitoring of treatment response, supporting the vision of adaptive, personalized oncology.

Ultimately, the future of RNA regulatory network research hinges on the ability of researchers to move from descriptive models to predictive, actionable frameworks. This may require not only technological innovation but also interdisciplinary collaboration across molecular biology, bioinformatics, structural biology and clinical oncology. With continued progress, RNA networks may shift from being viewed as passive readouts of gene activity to becoming active therapeutic blueprints that guide next-generation cancer treatment.

Design implications begin with a biology-driven approach: Prioritizing clinical evaluation in adjuvant or minimal residual disease settings, or other immune-permissive contexts, to maximize therapeutic responsiveness (12,13,79,80). When monotherapy cytotoxicity is insufficient, intentional combination strategies, such as pairing RNA-based agents with checkpoint inhibitors or pathway-sensitizing therapeutics, should be employed (66,67,79,80). Emphasis should be placed on target quality and dependency rather than tumor mutational burden alone, favoring high-quality neoantigens or functionally validated targets (79,80). Biomarker plans must be pre-specified, integrating diagnostic-enrichment-pharmacodynamic (D-E-P) chains and systematic tissue or ctDNA sampling into the trial's statistical design (12,13). Furthermore, innate immune sensing should be de-risked prior to first-in-human studies through optimized sequence chemistry, formulation, and delivery routes, as underscored by the clinical experience with MRX34, where immune-related toxicity led to discontinuation despite evidence of target engagement (61). By contrast, previous systemic siRNA programs demonstrate that careful carrier tuning and chemical modification can markedly improve uptake and endosomal escape while mitigating immunostimulatory risks (64,67).

8. Conclusion

RNA regulatory networks offer compelling opportunities to improve cancer diagnosis, prognosis and treatment, yet translation is constrained by challenges in data integration, context-specific network inference, delivery and the isoform-level biology of regulators such as isomiRs. New modalities including single-cell multi-omics and CRISPR-based functional genomics are enabling causal mapping and more reliable biomarkers and targets.

Real-world experience points to clear design rules for RNA therapeutics: Optimize delivery, proactively mitigate innate immune sensing, and build rigorous, isoform-aware validation. If these hurdles are addressed through integrated multi-omics and improved delivery platforms, RNA networks can realize their potential to transform precision oncology.

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Availability of data and materials

Not applicable.

Authors' contributions

WR conceived the topic area, conducted literature searches, evaluated all relevant literature, designed the research framework, wrote the manuscript, and critically revised the intellectual content. The author read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The author declares that he has no competing interests.

Use of artificial intelligence tools

During the preparation of this work, artificial intelligence tools were used to improve the readability and language of the manuscript, and subsequently, the authors revised and edited the content produced by the artificial intelligence tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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