

Nanoparticle-Based miRNA Therapeutics in Breast Cancer Highlighting Design Strategies and Translational Potential

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Abstract: Breast cancer remains one of the leading causes of cancer-related mortality worldwide, with its marked molecular heterogeneity and therapeutic resistance continuing to limit long-term clinical success. Although advances in targeted therapies have improved patient outcomes, tumor recurrence, systemic toxicity, and drug resistance remain major clinical challenges. MicroRNAs (miRNAs) have emerged as promising therapeutic molecules because they regulate multiple oncogenic pathways involved in proliferation, apoptosis, epithelial–mesenchymal transition, metastasis, and therapy resistance. Preclinical studies have demonstrated that restoring tumor-suppressive miRNAs or inhibiting oncogenic miRNAs can suppress tumor growth, reduce metastatic potential, and enhance treatment sensitivity. However, their clinical application is hindered by poor stability, rapid enzymatic degradation, limited cellular uptake, and inefficient intracellular delivery. Recent advances in nanomedicine have enabled the development of multifunctional nanoparticle platforms that effectively address these limitations. Lipid nanoparticles, polymeric nanoparticles, dendrimers, and inorganic nanocarriers have demonstrated the ability to protect miRNAs from degradation, prolong systemic circulation, enhance tumor-specific accumulation, facilitate cellular uptake, and promote endosomal escape for efficient cytoplasmic release. Moreover, targeted and stimuli-responsive nanocarriers, as well as combination strategies integrating miRNAs with conventional therapeutics, have shown encouraging therapeutic efficacy in preclinical breast cancer models. This review summarizes recent advances in nanoparticle-mediated miRNA delivery systems for breast cancer, highlighting the biological roles of therapeutic miRNAs, the design and performance of current nanocarriers, and their translational potential. Current challenges and future perspectives for the clinical implementation of miRNA-based nanomedicine are also discussed. Overall, nanoparticle-enabled miRNA therapeutics represent a promising platform for advancing precision medicine and next-generation personalized treatment strategies for breast cancer.

Keywords: miRNA, nanoparticle, nanocarrier-mediated miRNA delivery, breast cancer

Introduction

The Molecular Heterogeneity of Breast Cancer

Breast cancer is the most common malignancy in women and a leading cause of cancer-related deaths worldwide.¹ Although survival rates have increased thanks to the widespread use of early diagnosis and screening programs, diagnosis is often made at an advanced stage, and mortality rates remain high. Breast cancer is a major cause of financial, social, and personal difficulties. The disease, which is particularly prevalent in women of reproductive age, leads to both workforce loss and high treatment costs in healthcare systems.¹ The heterogeneous nature of breast cancer, meaning the coexistence of genetic, epigenetic, and microenvironmental differences, is a key factor determining the clinical course and response to treatment. Therefore, instead of a single treatment approach, personalized and molecularly targeted therapies are becoming increasingly important.²

Breast cancer is characterized by a high degree of molecular heterogeneity, which significantly influences tumor behavior, therapeutic response, and clinical outcomes. This heterogeneity arises from diverse genetic, epigenetic, and transcriptomic alterations that occur during tumor initiation and progression. Based on gene expression profiles and receptor status, breast cancer is commonly classified into several major molecular subtypes, including luminal A, luminal B, Human Epidermal Growth Factor Receptor 2 (HER2)-enriched, and triple-negative breast cancer (TNBC). These subtypes differ markedly in terms of their underlying signaling pathways, proliferative capacity, metastatic potential, and sensitivity to specific therapies. For instance, hormone receptor-positive tumors often respond well to endocrine therapies, whereas HER2-positive cancers benefit from HER2-targeted treatments. In contrast, TNBC lacks these therapeutic targets and is associated with a more aggressive clinical course and limited treatment options. In addition to intertumoral heterogeneity, intratumoral heterogeneity, referring to the coexistence of multiple genetically distinct cell populations within the same tumor, further complicates treatment strategies and contributes to therapeutic resistance and disease recurrence. Consequently, understanding the molecular heterogeneity of breast cancer is critical for the development of more precise and personalized therapeutic approaches.^{1,2}

The Limitations of Conventional Therapeutic Strategies

For many years, surgical resection, chemotherapy, radiotherapy, and hormone therapy have been the basic approaches in breast cancer treatment. While these approaches have advantages in reducing tumor size and preventing metastasis, they frequently fail to demonstrate adequate effectiveness, especially in late-stage or treatment-resistant cancers.³ Chemotherapy relies on cytotoxic agents that target rapidly dividing cells. However, because these drugs also damage healthy cells, they cause serious side effects such as myelosuppression, neurotoxicity, nausea, and hair loss.³ Furthermore, the development of drug resistance to chemotherapy limits the long-term effectiveness of the treatment.⁴ Radiotherapy is effective in local tumor control, but damage to surrounding tissues and the emergence of radiation-resistant subpopulations are significant disadvantages.⁵ Hormone therapy is effective in estrogen receptor-positive subtypes; however, it is ineffective in estrogen receptor (ER)-negative or TNBC. For these reasons, conventional treatments cannot adequately address the heterogeneous nature of the disease, and high recurrence rates and low survival times are observed, especially in aggressive subtypes.¹ Advances in molecular biology have enabled cancer treatment to be targeted at the level of cell surface receptors, signaling pathways, and gene expression regulation. Targeted therapies offer the advantage of higher selectivity and lower toxicity compared with conventional treatments.⁵ HER2-targeted therapies have significantly improved survival in patients with HER2 amplification.⁴ CDK4/6 inhibitors have shown efficacy in ER-positive metastatic cases by arresting the cell cycle.^{6,7} PARP inhibitors have provided tumor selectivity by targeting DNA repair pathways in cases with BRCA1/2 mutations.⁸ Nevertheless, during the development and clinical application of these targeted therapies, resistance to treatment is inevitable. It is evident that cancer cells have the capacity to evade treatment by developing new mutations over time or by activating alternative signaling pathways.⁶ Consequently, research has been directed toward investigating epigenetic regulatory mechanisms, with a particular emphasis on small regulatory RNAs, such as microRNAs (miRNAs).

The Development of RNA-Based Therapeutics

RNA-based therapeutics have emerged as a promising strategy in modern molecular medicine due to their ability to precisely regulate gene expression. Advances in genomics and RNA biology have revealed that non-coding RNAs play critical roles in controlling many cellular processes, including proliferation, differentiation, and apoptosis. Among these molecules, miRNAs have attracted particular attention because of their capacity to modulate multiple target genes simultaneously. Dysregulation of miRNA expression has been strongly associated with the initiation and progression of various cancers.⁹ Therefore, understanding the biological functions of miRNAs has opened new opportunities for both diagnostic biomarker development and targeted therapeutic approaches in cancer treatment.

miRNAs are non-coding RNA molecules approximately 19–25 nucleotides in length and regulate cellular functions by suppressing gene expression at the post-transcriptional level.¹⁰ miRNAs generally suppress translation or trigger messenger RNA (mRNA) degradation by binding to the 3' untranslated region (3' UTR) of the target mRNA. A significant percentage of genes in the human genome are thought to be regulated by miRNAs.¹⁰ In cancer biology,

miRNAs function in two main groups. By inhibiting tumor suppressor genes, oncomiRNAs promote cell division, invasion, and metastasis. For instance, miR-21 activates the PI3K/AKT pathway by targeting *PTEN* and *PDCD4*.¹¹ Tumor suppressor miRNAs regulate cell death pathways, such as apoptosis and ferroptosis, by suppressing oncogenes. For example, miR-34a regulates the p53-mediated apoptosis pathway by suppressing *BCL2* and *SIRT1* expression. In breast cancer, the miRNA expression profile shows a strong correlation with tumor subtype, invasive capacity, and treatment response.¹² It has been demonstrated that miR-10b increases metastatic potential, while miR-200c limits metastasis by suppressing epithelial–mesenchymal transition.¹³ Consequently, miRNAs are regarded not only as bio-markers but also as therapeutic agents.

The Importance of miRNA Delivery by Nanoparticles in Breast Cancer

miRNA-based therapeutic approaches have attracted growing interest as a promising strategy for precision oncology. However, the successful clinical implementation of these therapies requires effective delivery systems capable of preserving miRNA stability and ensuring their efficient transport to tumor tissues.¹⁴ Within this framework, nanoparticle-mediated delivery platforms have gained increasing attention as a powerful technological solution for improving the therapeutic performance of miRNA-based interventions in breast cancer.

Nevertheless, a major limitation to the clinical use of miRNAs is their intrinsic instability. Nanoparticle-based delivery systems have therefore emerged as essential platforms to overcome these limitations. By enhancing miRNA stability in systemic circulation and protecting them from nuclease-mediated degradation, these carriers improve their therapeutic potential. In addition, targeted delivery to cancer cells and efficient cellular uptake further optimize treatment efficacy while minimizing off-target effects on healthy tissues.¹⁵ Taken together, nanoparticle-mediated miRNA delivery represents a promising strategy to fully realize the clinical potential of miRNA-based treatments. However, a comprehensive analysis integrating miRNA therapeutic strategies with nanoparticle engineering principles, specifically in breast cancer subtypes, remains limited (Figure 1).

This review aims to investigate and optimize these nanoparticle-mediated miRNA delivery strategies, providing a novel and clinically relevant approach to improve the efficacy and safety of breast cancer therapy. This review highlights the translational potential of miRNA therapeutics and establishes a framework for future precision nanomedicine interventions.

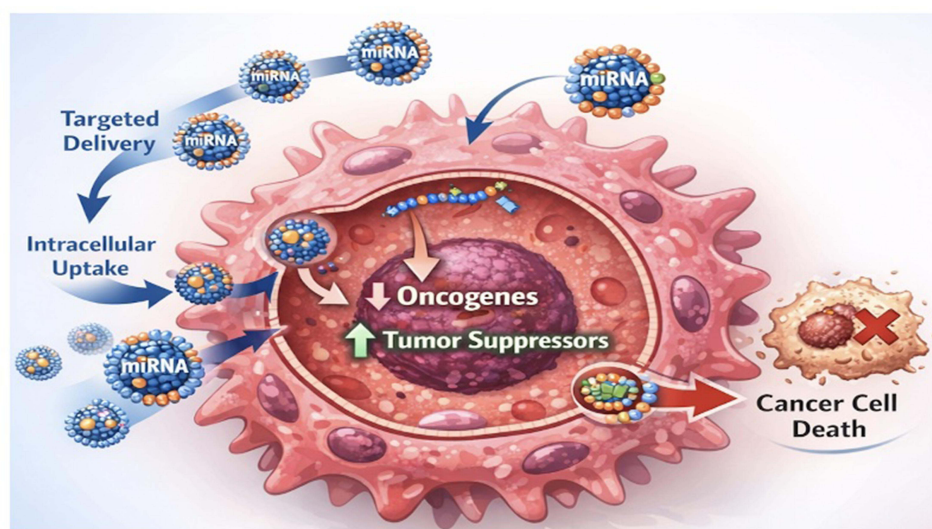


Figure 1 Schematic illustration of the mechanistic pathway underlying miRNA-mediated cancer cell therapeutics. The diagram depicts the intracellular delivery of therapeutic miRNAs into cancer cells using nanocarrier-based delivery systems, followed by cellular uptake through endocytosis and subsequent endosomal escape. After release into the cytoplasm, the therapeutic miRNA is incorporated into the RNA-induced silencing complex (RISC), where it specifically recognizes and binds to complementary sequences within the 3'-untranslated region (3'-UTR) of target messenger RNAs (mRNAs). This interaction results in mRNA degradation or translational repression, leading to the downregulation of oncogenic genes and upregulation of tumor suppressor genes and cause modulation of key signaling pathways involved in tumor progression, including cell proliferation and apoptosis.

The Biology and Therapeutic Potential of miRNAs

miRNA Biogenesis and Mechanisms of Gene Regulation

miRNAs are small, non-protein-coding RNA molecules, approximately 18–25 nucleotides in length, that are involved in the post-transcriptional regulation of gene expression.¹⁰ miRNAs, which were discovered in 1993 in the *Caenorhabditis elegans* model with the *lin-4* gene, have since been proven to be conserved in almost all eukaryotic organisms.¹⁰ The human genome contains around 2000 mature miRNAs, and it is expected that these molecules directly or indirectly influence the translation of many genes.¹⁰ Because individual miRNAs can simultaneously regulate multiple transcripts within interconnected signaling pathways, they function as key modulators of complex biological networks rather than as single-gene regulators. Consequently, even subtle alterations in miRNA expression may produce widespread downstream effects on cellular homeostasis and disease progression.¹⁶

miRNA biogenesis is an extensive procedure that occurs in both the nucleus and the cytoplasm. In the nucleus, miRNA genes are transcribed by RNA polymerase II to form primary miRNAs. These long precursor RNA molecules have a hairpin structure containing double-stranded regions. The microprocessor complex, consisting of Drosha and DGCR8 proteins, cleaves the pri-miRNA into a precursor miRNA approximately 70 nucleotides in length.¹⁰ The efficiency of this processing step is tightly regulated by RNA-binding proteins and epigenetic mechanisms, thereby contributing to tissue-specific miRNA expression profiles and dynamic responses to physiological and pathological stimuli.¹⁷ This pre-miRNA is then transported from the nucleus to the cytoplasm via Exportin-5 and Ran-GTP.¹⁸ In the cytoplasm, the Dicer enzyme cleaves the ends of the pre-miRNA, creating a double-stranded RNA 20–22 base pairs in length. One of these two strands is selected as the guide strand and incorporated into the RNA-Induced Silencing Complex (RISC). The other strand is usually degraded. The RISC complex recognizes the target mRNA through Argonaute proteins and suppresses translation or cleaves the mRNA.^{10,18} Accumulating evidence indicates that alterations in Drosha, Dicer, Exportin-5, or Argonaute proteins can impair miRNA maturation and contribute to the development of numerous human diseases, particularly cancer.¹⁷ Thus, miRNAs play an important regulatory role in inhibiting cellular protein production.

miRNAs regulate gene expression by binding to the 3' UTRs of their target mRNAs, resulting in either translational repression or mRNA degradation. A single miRNA can influence many genes, while several different miRNAs can regulate the same gene. This complex regulatory network enables miRNAs to play pivotal roles in critical cellular processes, including cell cycle progression, proliferation, differentiation, apoptosis, stress responses, and metabolic homeostasis.¹⁸ Under normal physiological conditions, miRNA expression is precisely regulated; however, this homeostasis is frequently perturbed in cancer. Dysregulation of miRNAs disrupts the balance between oncogenes and tumor suppressor genes, thereby facilitating tumor initiation and progression.¹⁸ In breast cancer, aberrant expression of oncogenic miRNAs (oncomiRs) and tumor-suppressive miRNAs has been associated with tumor heterogeneity, epithelial–mesenchymal transition, metastasis, therapeutic resistance, and poor clinical outcomes, highlighting their importance as both diagnostic biomarkers and therapeutic targets.¹⁹ Thus, miRNAs have emerged as critical biomarkers and potential therapeutic targets.

miRNAs are small, non-protein-coding RNA molecules that play a critical role in the post-transcriptional regulation of gene expression. Altered miRNA expression profiles have been observed in many pathological processes, such as cancer, cardiovascular diseases, and neurodegenerative disorders. These altered profiles suggest that miRNAs can be used as biomarkers and potential therapeutic targets. Current therapeutic strategies are based on two main mechanisms. A therapeutic strategy aimed at limiting cancer cell development and inducing tumor regression involves increasing the levels of tumor suppressor miRNAs with low expression profiles and inhibiting the activity of overexpressed oncogenic miRNAs (Figure 2).²⁰

miRNA-Targeted Therapeutic Strategies

The miRNA repair strategy aims to restore the function of miRNAs whose expression is decreased or completely eliminated under pathological conditions. This therapeutic approach is particularly relevant for tumor suppressor miRNAs that are frequently downregulated in various malignancies. miRNA mimics are synthetically designed double-

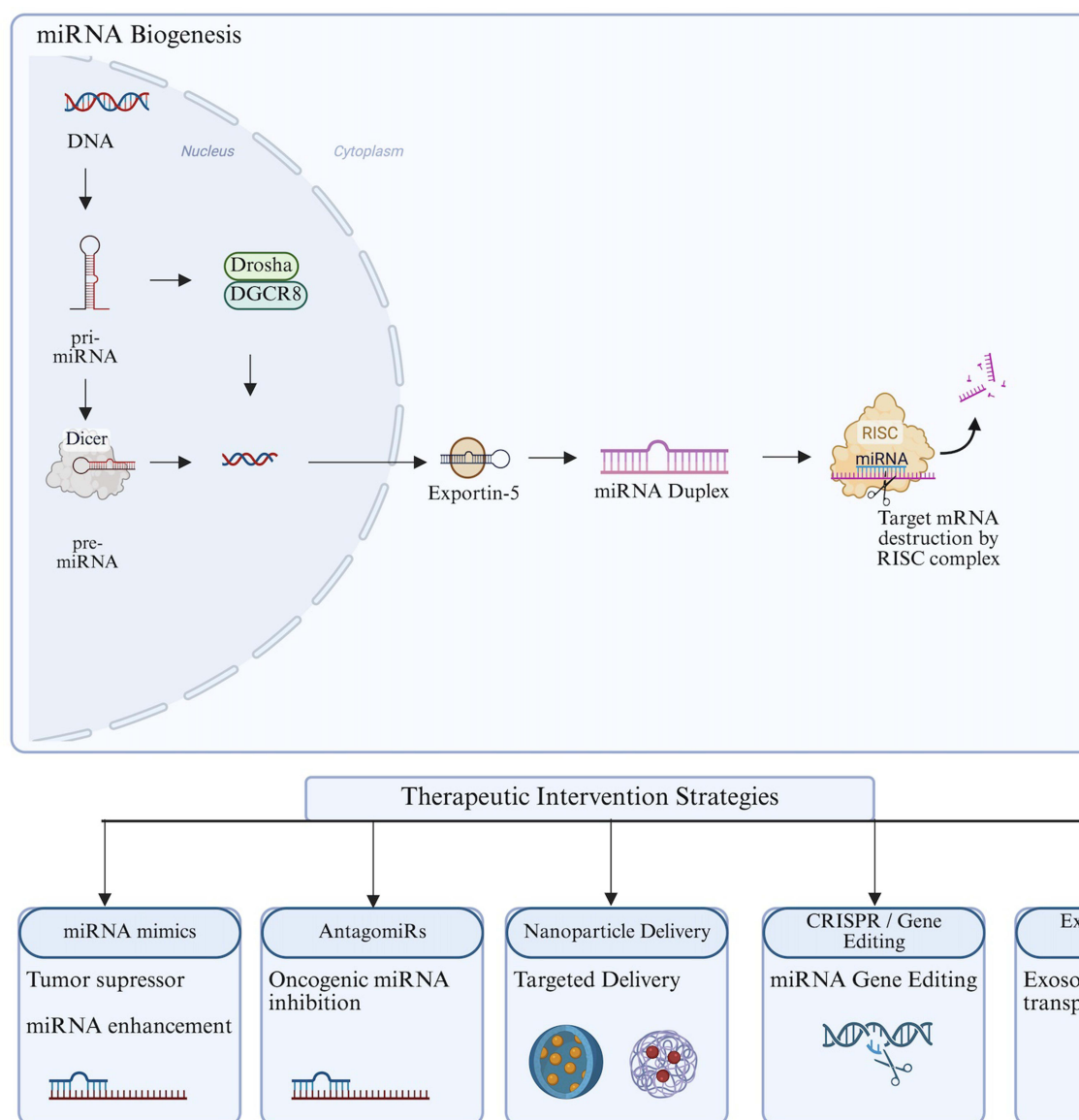


Figure 2 Schematic representation of miRNA biogenesis and therapeutic intervention strategies. miRNA biogenesis is initiated in the nucleus, where primary miRNA (pri-miRNA) transcripts are synthesized by RNA polymerase II and processed by the Drosha–DGCR8 microprocessor complex into precursor miRNAs (pre-miRNAs). Pre-miRNAs are subsequently exported to the cytoplasm by Exportin-5 in a Ran-GTP-dependent manner and further cleaved by Dicer to generate mature miRNA duplexes. The guide strand is selectively incorporated into the RNA-induced silencing complex (RISC), while the passenger strand is typically degraded. The mature miRNA-loaded RISC recognizes complementary sequences within the 3'-untranslated region (3'-UTR) of target mRNAs, resulting in translational repression and/or mRNA degradation, thereby regulating diverse cellular processes including proliferation, apoptosis, differentiation, angiogenesis, epithelial–mesenchymal transition (EMT), metastasis, and immune responses. Therapeutic strategies targeting miRNA signaling include the restoration of tumor-suppressive miRNAs using synthetic miRNA mimics, inhibition of oncogenic miRNAs through antisense oligonucleotides (antagomiRs), locked nucleic acids (LNAs), miRNA sponges, or small-molecule modulators. To improve therapeutic stability, targeted delivery, and intracellular bioavailability, these agents are commonly incorporated into nanocarrier-based delivery platforms thereby enhancing therapeutic efficacy while minimizing off-target effects and systemic toxicity.

stranded RNA oligonucleotides that structurally resemble endogenous miRNA duplexes. Following cellular uptake, these synthetic molecules bypass the canonical miRNA biogenesis pathway and are directly incorporated into the RISC, enabling them to regulate target gene expression.¹⁸ Within the miRNA mimic structure, the guide strand plays a central role in mediating the therapeutic effect by recognizing and binding complementary sequences on target mRNAs, thereby promoting post-transcriptional gene silencing. In contrast, the passenger strand, often referred to as the mediator strand, is typically chemically modified to prevent its incorporation into the RISC and is subsequently degraded within the intracellular environment.²¹

Among tumor suppressor miRNAs, the miR-34 family represents one of the most extensively studied examples due to its critical involvement in the p53 signaling pathway. The expression of miR-34 is frequently suppressed in a wide range of cancer types, contributing to tumor progression and resistance to apoptosis. In preclinical studies, restoring miR-34a levels with synthetic miR-34a mimics has shown promising anticancer effects, including tumor growth suppression and apoptosis induction. Despite these encouraging findings, significant challenges remain in translating miRNA restoration therapies into clinical applications. In particular, efficient and targeted delivery to diseased tissues, as well as the mitigation of unintended immune activation, represent major obstacles. To address these limitations, recent research has focused on the development of nanoparticle-based delivery systems and chemical modifications designed to enhance mimic stability, improve cellular uptake, and minimize off-target effects.²²

miRNA silencing strategies are designed to inhibit the activity of miRNAs that are aberrantly upregulated under pathological conditions and contribute to disease progression. The primary objective of this approach is either to prevent endogenous miRNAs from binding to their target mRNAs or to promote their degradation. In most cases, miRNA silencing is achieved through the use of single-stranded antisense oligonucleotides that are fully complementary to the target miRNA sequence, thereby enabling highly specific inhibition of miRNA function.²³

Several molecular platforms have been developed to achieve efficient miRNA silencing. Antagomirs are chemically modified RNA molecules, often conjugated with cholesterol to enhance cellular uptake and stability, that bind target miRNAs with high affinity and prevent their incorporation into the RISC.²⁴ Locked nucleic acids (LNAs) represent another widely used strategy; these ribose-modified oligonucleotides possess a conformationally “locked” structure that significantly increases nuclease resistance and enhances binding affinity to complementary miRNA sequences.²⁵ In addition, miRNA sponges consist of artificially engineered transcripts containing multiple miRNA-binding sites, enabling the simultaneous sequestration of multiple members of a miRNA family rather than a single miRNA, thereby broadening their regulatory capacity.²⁶

Recent evidence suggests that the biological functions of miRNAs extend beyond their canonical role in post-transcriptional gene silencing. Chen et al introduced the concept of Nuclear Activating miRNAs (NamiRNAs), a subset of nuclear-localized miRNAs that activate gene transcription through enhancer interactions rather than repressing target mRNAs. According to the proposed NamiRNA-enhancer regulatory network, these miRNAs bind enhancer regions in a sequence-specific manner, recruit AGO2, promote H3K27ac enrichment, and facilitate RNA polymerase II-dependent transcriptional activation. This mechanism provides a conceptual framework for understanding how miRNAs can function as both gene repressors and transcriptional activators in a context-dependent manner. From a therapeutic perspective, these findings broaden the potential applications of miRNA-based interventions by suggesting that selected miRNAs could be exploited not only to inhibit oncogenic pathways but also to restore the expression of tumor suppressor genes through enhancer-mediated gene activation. Although the NamiRNA model requires further validation across different disease settings, it offers an important mechanistic perspective that may guide the future design of more precise and functionally diverse miRNA therapeutics, including nanoparticle-mediated delivery strategies for cancer treatment.²⁷

Tumor Suppressor miRNAs in Breast Cancer

Tumor suppressor (TS) miRNAs are key regulatory molecules that control cell cycle progression and proliferation by restraining oncogene overactivation under normal conditions. In cancer, these miRNAs are frequently downregulated or silenced via epigenetic mechanisms.²⁸ Loss of TS-miRNAs confers a proliferative advantage to cells, allows evasion of apoptosis, and promotes the acquisition of metastatic characteristics.²⁸ The miR-34 family is one of the most well-characterized TS-miRNAs. miR-34a, a direct transcriptional target of p53, regulates cell cycle arrest and apoptosis through activation of p21.²⁹ Its loss contributes to chemotherapy resistance and poor prognosis across multiple tumor types, including breast cancer.²⁹ Notably, synthetic miR-34a advanced to Phase I clinical trials as the first miRNA-based anticancer therapeutic; although development was halted due to immune-related adverse effects, the study underscored the clinical potential of TS-miRNAs.²⁹ Another important group is the miR-200 family, which suppresses metastasis by regulating the epithelial–mesenchymal transition (EMT), a critical process that enhances cellular migratory capacity. The miR-200 family targets the ZEB1 and ZEB2 transcription factors, maintaining E-cadherin expression and preserving epithelial characteristics.³⁰ In breast cancer, reduced miR-200 expression is particularly associated with TNBC subtypes

characterized by high metastatic potential (Table 1).³¹ In summary, decreased expression of TS-miRNAs promotes tumor growth by increasing proliferative, invasive, and anti-apoptotic characteristics. As a result, methods aimed at increasing TS-miRNA expression provide a potential therapeutic strategy for next-generation breast cancer therapies.³⁰

Breast cancer is a highly heterogeneous disease, with various molecular subtypes such as ER-positive, HER2-positive, and TNBC, each characterized by a distinct genomic landscape, signaling pathways, and therapeutic limitations.³ Consequently, the development of miRNA-based therapeutic strategies requires a subtype-specific perspective that considers the differential miRNA expression profiles associated with each tumor subtype.⁴² Several studies have demonstrated that particular miRNAs exhibit subtype-dependent dysregulation and contribute to key oncogenic processes such as proliferation, metastasis, and therapy resistance.⁴³

In ER-positive breast cancer, dysregulation of miRNAs involved in estrogen receptor signaling pathways, including miR-221/222 and miR-206, has been associated with endocrine therapy resistance and tumor progression.⁴⁴ Therapeutic strategies aimed at restoring tumor-suppressive miRNAs or silencing oncogenic miRNAs within this signaling network may enhance sensitivity to hormonal therapies such as tamoxifen or aromatase inhibitors.⁴⁵ In contrast, HER2-positive breast cancers exhibit distinct miRNA signatures that regulate HER2-driven signaling pathways, including the PI3K/AKT and MAPK pathways.⁴⁶ Targeting these miRNAs using nanoparticle-based delivery systems could potentially improve the efficacy of HER2-targeted therapies and mitigate resistance mechanisms.

Table 1 miRNAs Play Key Roles in Breast Cancer Pathogenesis by Regulating Molecular Targets and Biological Functions

miRNA	Target Genes	Biological Function and Role in Breast Cancer	Reference
let-7 family	RAS HMGA2 c-Myc	It suppresses cancer stem cell (CSC) characteristics. Its loss leads to chemotherapy resistance and aggressive tumor growth.	[32]
miR-34a	Bcl-2 SIRT1 c-MET CD44	It is activated by the p53 pathway. It initiates apoptosis, halts the cell cycle in the G1 phase, and prevents metastasis.	[33]
miR-200c	ZEB1 ZEB2	It is the most important factor that inhibits epithelial-mesenchymal transition (EMT). Its loss leads to cells gaining motility and metastasis.	[34]
miR-205	HER3 ZEB1	It is particularly reduced in TNBC. It suppresses EMT and tumor invasion.	[35]
miR-206	NOTCH3 ER α	It regulates estrogen receptor (ER α) signaling. It inhibits cell migration and proliferation.	[36]
miR-126	VEGF PI3K/AKT	It suppresses angiogenesis (new blood vessel formation). Its loss accelerates the nourishment and growth of the tumor.	[37]
miR-145	MUC1 c-Myc RTKN	It regulates the cytoskeleton. It is one of the miRNAs whose expression level is most frequently decreased in breast cancer tissues.	[38]
miR-31	RhoA Radixin	It is a potent “anti-metastatic” miRNA that blocks every stage of metastasis (local invasion, entry into blood vessels).	[39]
miR-125b	ERBB2 (HER2) ETS1	It limits cell growth. It is frequently silenced in HER2-positive breast cancers.	[40]
miR-335	SOX4 TNC	It is a metastasis suppressant. Its loss increases the risk of breast cancer metastasizing to the lungs and bones.	[41]

TNBC, which lacks expression of the estrogen receptor, progesterone receptor, and HER2, represents the most aggressive breast cancer subtype and is frequently associated with poor clinical outcomes and limited therapeutic options. Several oncogenic miRNAs, such as miR-21 and miR-155, are frequently overexpressed in TNBC and contribute to enhanced invasion, metastasis, and chemoresistance.⁴⁷ In this context, nanoparticle-mediated delivery of anti-miRNA therapeutics or tumor-suppressive miRNA mimics has emerged as a promising strategy to selectively modulate dysregulated miRNA networks in TNBC tumors.

Importantly, the design of nanoparticle delivery platforms should also incorporate subtype-specific targeting strategies to improve tumor selectivity and therapeutic efficacy. For example, ligand-functionalized nanoparticles targeting receptors that are overexpressed in specific breast cancer subtypes, such as HER2 receptors in HER2-positive tumors or epidermal growth factor receptor (EGFR) in TNBC, may significantly enhance the precision of miRNA delivery.⁴⁸ Therefore, integrating subtype-specific miRNA signatures with rationally engineered nanoparticle delivery systems represents a critical step toward the development of personalized miRNA-based therapeutic strategies for breast cancer.

The molecular heterogeneity of breast cancer presents significant barriers to the development of universally successful therapeutic protocols, necessitating the creation of subtype-specific delivery platforms. In recent years, nanoparticle-based drug delivery systems have emerged as highly adaptable carriers capable of incorporating targeting ligands that recognize receptors selectively overexpressed in distinct breast cancer subtypes.⁴⁹ Such subtype-oriented targeting strategies not only enhance the selective accumulation of therapeutic cargo in tumor tissues but also reduce off-target toxicity in normal organs. For instance, nanoparticles functionalized with ligands or antibodies against human epidermal growth factor receptor 2 (HER2) can facilitate preferential uptake in HER2-positive tumors, thereby improving the intracellular delivery efficiency of miRNA mimics or anti-miRNA oligonucleotides.⁵⁰ Similarly, TNBC, which frequently exhibits elevated expression of EGFR and other surface receptors associated with aggressive tumor phenotypes, represents an attractive target for ligand-mediated nanoparticle delivery systems.⁴⁹ Aptamer- or peptide-modified nanoparticles targeting EGFR have demonstrated enhanced tumor specificity and improved therapeutic responses in preclinical TNBC models.⁴⁹ In hormone receptor-positive breast cancers, targeting strategies may focus on receptors and signaling pathways associated with estrogen receptor signaling, potentially enabling the selective modulation of miRNAs involved in endocrine resistance.

The integration of molecular subtype information with rationally engineered nanoparticle delivery systems represents a promising strategy to enhance the therapeutic precision of miRNA-based interventions in breast cancer. Distinct breast cancer subtypes exhibit unique miRNA dysregulation patterns that can be selectively targeted using nanoparticle platforms functionalized with subtype-specific ligands.⁵⁰ For instance, HER2-targeted nanoparticles have demonstrated enhanced delivery efficiency in HER2-positive tumors, while EGFR-targeted nanocarriers have shown considerable potential in TNBC models. These subtype-adapted delivery strategies not only improve tumor selectivity but also enhance intracellular delivery efficiency and therapeutic outcomes.⁵¹ Consequently, the development of subtype-specific nanoparticle platforms capable of delivering miRNA mimics or anti-miRNA therapeutics may represent a critical step toward realizing precision nanomedicine approaches in breast cancer treatment.

A notable clinical application of miRNA silencing involves targeting miR-122 for the treatment of Hepatitis C virus infection. LNA-based anti-miR therapeutics, such as miravirsin, inhibit viral replication by functionally inactivating miR-122, a host factor required for efficient viral RNA stability and replication.⁵² In oncology, miRNA silencing has also demonstrated significant therapeutic potential. For example, Zhang et al targeted the oncogenic miR-21, which is frequently overexpressed in TNBC, a highly aggressive and treatment-resistant subtype. In their study, RNA nanoparticles loaded with anti-miR-21 were selectively delivered to tumor cells using EGFR-targeting aptamers. This targeted delivery system effectively silenced miR-21, suppressed tumor cell migration and invasion, and restored sensitivity to doxorubicin in previously resistant cancer cells (Table 2). These findings highlight the considerable promise of anti-miRNA therapeutics not only in inhibiting tumor progression but also in enhancing the therapeutic efficacy of conventional chemotherapeutic agents.⁵³

These approaches highlight the importance of integrating molecular subtype profiling with rational nanoparticle engineering to achieve the precise delivery of miRNA therapeutics. Future advances in this field are expected to involve multifunctional nanoplatfoms capable of simultaneously incorporating subtype-specific targeting ligands, stimuli-responsive release mechanisms, and combination therapeutic cargos, thereby paving the way toward personalized nanomedicine approaches for breast cancer treatment.

Table 2 Subtype-Specific Nanoparticle-Mediated miRNA Therapeutic Strategies in Breast Cancer

Breast Cancer Subtype	Therapeutic miRNA	Nanoparticle Strategy	Main Therapeutic Outcome	Reference
TNBC	miR-34a mimic	Hyaluronic acid/chitosan (HA-CS) nanoparticles	Restored tumor suppressor miR-34a, inhibited proliferation and migration, enhanced apoptosis, and significantly suppressed tumor growth in vivo.	[54]
TNBC	anti-miR-21 + anti-miR-10b	uPAR-targeted PLGA-b-PEG polymeric nanoparticles	Simultaneous inhibition of two oncomiRs reduced tumor growth and metastatic potential with enhanced tumor targeting.	[55]
TNBC	miR-203 mimic + vinorelbine	Liposomal nanoparticles	Suppressed epithelial-mesenchymal transition through the SLUG/TGF- β pathway and produced synergistic antitumor activity with chemotherapy.	[56]
TNBC	miR-603 mimic	PEGylated liposomal nanoparticles	Downregulated eEF2K, inhibited proliferation and tumor growth, and improved therapeutic efficacy in orthotopic models.	[57]
HER2-positive / TNBC	anti-miR-21	PAMAM-modified gold nanoparticles	Reduced cancer stem cell characteristics, inhibited tumor growth, and restored tumor suppressor signaling.	[58]
HER2-positive	miR-125b (tumor suppressor)	Lipid-polymer hybrid nanoparticles	Downregulation of HER2 signaling and reduced tumor progression	[59]
HER2-positive	miR-21 (oncogenic)	Gold nanoparticles	Inhibition of tumor cell proliferation and enhanced response to HER2-targeted therapy	[59]
ER-positive	miR-221/222 (oncogenic)	Polymeric nanoparticles (PLGA)	Restoration of endocrine therapy sensitivity and inhibition of tumor proliferation	[59]
ER-positive	miR-206 (tumor suppressor)	Lipid nanoparticles	Suppression of estrogen receptor signaling and reduced tumor growth	[59]
TNBC	miR-205 mimic + anti-miR-221	RNA triple-helix hydrogel nanoscaffold	Simultaneously restored tumor suppressor miR-205 and inhibited oncogenic miR-221, markedly reducing tumor growth.	[60]
TNBC (immune-active)	let-7b mimic	Poly(β -amino ester) nanoparticles	Reprogrammed the tumor immune microenvironment through TLR7 activation and significantly inhibited tumor progression.	[61]

Biological Limitations to the Clinical Translation of miRNA Therapeutics

Despite their considerable potential, particularly in oncology, there are significant biological limitations to the clinical translation of miRNA-based therapeutics. Systemically administered synthetic miRNA mimics or anti-miRNA oligonucleotides are rapidly recognized as foreign nucleic acids by endogenous endonucleases, which have evolved as a defense mechanism against viral RNA, and are enzymatically degraded within seconds. Pharmacokinetic analyses indicate that the plasma half-life of unmodified RNA molecules is typically only a few minutes, which is insufficient for therapeutic accumulation in target tumor tissues.⁶² Molecules capable of surviving enzymatic degradation are rapidly cleared by the kidneys due to their small molecular size (7–15 kDa), which is substantially below the approximately 50 kDa glomerular filtration threshold, resulting in urinary elimination. For the few molecules that reach the tumor site, efficient intracellular transport represents another critical limitation. The negatively charged phosphate backbone and hydrophilic nature of miRNAs cause strong electrostatic repulsion from the lipophilic cell membrane, thereby limiting passive diffusion.⁶³ Furthermore, a significant fraction of unmodified miRNAs internalized through endocytosis becomes trapped and degraded within acidic lysosomes, preventing efficient endosomal escape. Beyond these physical challenges, safety

concerns remain paramount. Untargeted miRNAs may aberrantly silence genes in healthy tissues or activate Toll-like receptors on immune cells, provoking severe immune responses (Figure 3). A notable example is the Phase I clinical trial of liposomal miR-34a mimics conducted by Hong et al, which was terminated prematurely due to uncontrolled immune activation and severe adverse events despite demonstrable tumor suppression.⁶⁴

Consequently, these limitations underscore the critical need for multifunctional nanoparticle-based delivery systems that protect miRNAs from nuclease-mediated degradation, increase their hydrodynamic size to reduce renal clearance, facilitate intracellular uptake by mitigating charge repulsion, and, most importantly, enable tumor-specific targeting while minimizing immune stimulation. Such strategies are not optional but essential for translating miRNA therapeutics from preclinical models to clinical applications.

Nanoparticle-Based Delivery Systems for miRNA Therapeutics

Nanotechnology research represents one of the most promising strategies for overcoming the biological challenges associated with the delivery of nucleic acid-based therapeutics. Compared with traditional chemotherapeutic agents, nanoparticle-based systems alter the pharmacokinetic properties of therapeutic agents, thereby improving their therapeutic efficacy.

Advantages and Design Criteria of Nanoparticle-Based in miRNA Delivery

Nanoparticles not only physically protect sensitive molecules such as miRNAs, but also optimize their biological behavior. Nanoparticle-based formulations provide numerous benefits for miRNAs, including targeted delivery, increased stability, controlled release, and enhanced therapeutic efficacy within the tumor microenvironment. In targeted delivery, nanoparticle systems accumulate the therapeutic agent selectively in tumor tissue through passive and active targeting strategies. This minimizes systemic toxicity and side effects by reducing exposure to healthy tissues while increasing the concentration of the therapeutic agent in the tumor region.⁶⁵ To maintain long-term stability, nanoparticles efficiently encapsulate miRNAs, protecting them from enzymatic degradation by serum nucleases. This increases the stability of free miRNAs from minutes to hours or even days.⁶⁶ In controlled release, nanoparticles are designed to release their cargo according to a pre-programmed kinetic process rather than through rapid or uncontrolled release. This maintains a stable therapeutic concentration of miRNAs in the bloodstream.⁶⁷ To create tumor microenvironment-responsive platforms, nanoparticles are engineered to respond to the specific pathophysiological characteristics of the tumor microenvironment. For instance, pH-sensitive polymers remain stable

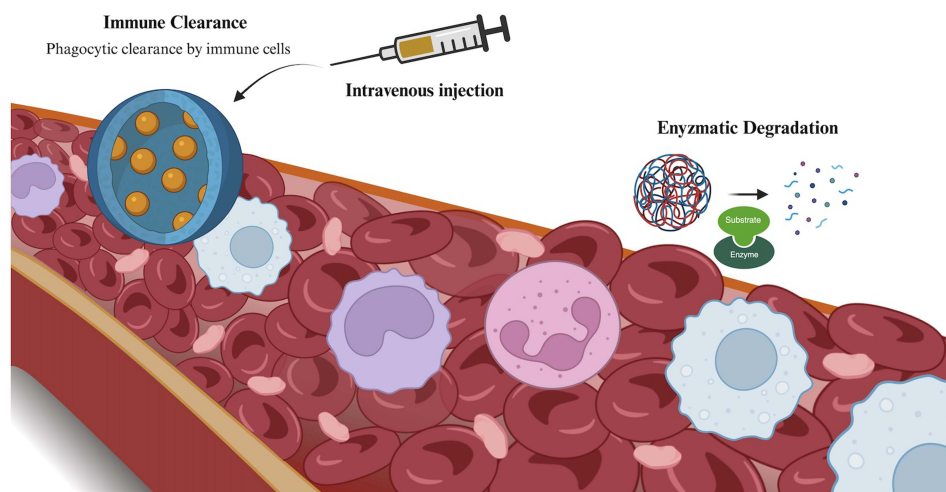


Figure 3 Schematic illustration of the major biological barriers limiting the systemic delivery of therapeutic miRNAs. Following systemic administration, naked miRNAs are rapidly degraded by circulating ribonucleases (RNases) and are susceptible to renal clearance due to their small molecular size, resulting in a short circulation half-life. Interactions with plasma proteins and uptake by the mononuclear phagocyte system (MPS), particularly macrophages in the liver and spleen, further reduce their bioavailability. Following extravasation from the bloodstream, therapeutic miRNAs must penetrate the dense extracellular matrix and elevated interstitial fluid pressure within the tumor microenvironment to reach target cells. Efficient cellular internalization is hindered by the negatively charged plasma membrane, while endosomal entrapment following endocytosis substantially limits cytoplasmic delivery.

at the neutral pH of blood (7.4) but degrade in acidic environments such as tumor tissue (pH ~6.5) or endosomes (pH ~5.0). This enables the controlled release of miRNAs precisely at the desired site.⁶⁸

The design of nanoparticle-based miRNA delivery systems is subject to specific criteria to optimize therapeutic performance and safety. To develop an effective system, the physicochemical properties of the nanoparticles need to be optimized using precise engineering techniques. Several parameters play important roles in the development of such systems, including nanoparticle size, surface charge, biocompatibility, biodegradability, polyethylene glycol (PEG)ylation, and ligand addition. The size of a nanoparticle is the most critical parameter in determining biodistribution. Particles smaller than 10 nm are rapidly filtered by the kidneys, whereas particles larger than 200 nm are retained by the liver and spleen. Therefore, the 20–100 nm size range is generally targeted to achieve optimal accumulation in tumor tissue. A positive surface charge on the nanoparticle facilitates intracellular uptake by increasing interactions with the negatively charged cell membrane. However, an excessive positive charge can lead to serum protein binding and toxicity. Therefore, mildly positive or neutral surface charges are generally preferred.⁶⁹

Nanoparticles with high biocompatibility and biodegradability should be preferred. The nanomaterials used should be biodegradable without leaving toxic metabolites in the body and should be non-immunogenic.⁷⁰ PEGylation involves coating the nanoparticle surface with polyethylene glycol to create a hydrophilic shield that prevents serum proteins from adhering to the particle surface. This prolongs the circulation time of the nanoparticles by preventing their recognition and clearance by macrophages.⁷¹ Ligand attachment involves conjugating ligands specific to receptors that are over-expressed on cancer cells to the nanoparticle surface. This enables the nanoparticle to bind to the target cell in a lock-and-key manner, triggering receptor-mediated endocytosis (Figure 4).⁷¹

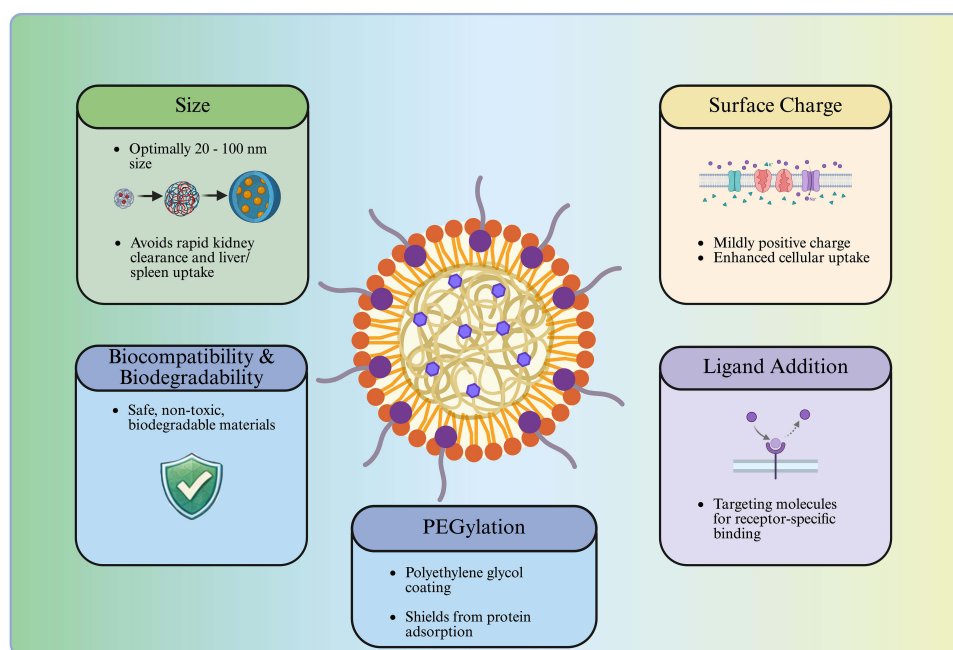


Figure 4 Key design criteria for the development of optimized nanoparticle-based miRNA delivery systems. The schematic summarizes the principal physicochemical and biological parameters that govern the therapeutic performance of nanoparticle-based miRNA delivery platforms. Nanoparticle size should be optimized to ensure prolonged systemic circulation, efficient tumor accumulation through the enhanced permeability and retention (EPR) effect, and effective cellular internalization while minimizing rapid renal clearance and uptake by the mononuclear phagocyte system (MPS). The use of biocompatible and biodegradable materials is essential to reduce cytotoxicity, minimize long-term tissue accumulation, and enable safe metabolic degradation and elimination. Surface PEGylation enhances colloidal stability, prolongs blood circulation by reducing protein adsorption and opsonization, and decreases premature clearance by the immune system. The incorporation of targeting ligands, including antibodies, peptides, aptamers, carbohydrates, or small molecules, facilitates receptor-mediated uptake and selective delivery to target cells, thereby improving therapeutic specificity and reducing off-target effects. Surface charge should be carefully balanced to promote efficient miRNA complexation and cellular uptake while limiting nonspecific interactions with serum proteins, hemolytic activity, and systemic toxicity.

Targeting Mechanisms in Nanoparticle-Based Delivery

Passive Targeting: The Enhanced Permeability and Retention (EPR) Effect for Targeted Nanoparticle Delivery in Cancer Therapy

The EPR effect, which forms the basis of the passive targeting strategy, is a phenomenon arising from the unique anatomical and physiological characteristics of solid tumors. As tumor tissue grows rapidly, it requires the formation of new blood vessels. However, these newly formed vessels are structurally defective, with large gaps between the endothelial cells. At the same time, the lymphatic drainage system within tumor tissue is impaired.⁷² Rapidly proliferating cancer cells strongly stimulate the formation of new blood vessels to meet their nutritional and oxygen demands. In this process, vasoactive mediators such as vascular endothelial growth factor (VEGF) and nitric oxide, which are secreted in excessive amounts by tumor cells, lead to abnormalities in the vascular structure.⁷³ The endothelial layer in healthy tissues has a regular structure, whereas tumor vessels exhibit a chaotic architecture. Due to the disruption of tight junctions between endothelial cells and insufficient formation of the basement membrane, large openings occur in the vessel walls. These large pores enable macromolecules, nanoparticles, and small-molecule chemotherapeutics to diffuse from the bloodstream into the extracellular fluid of tumor tissue.⁷³ For a molecule to be effective, it must not only enter the tumor tissue but also remain within the tissue. In healthy tissues, waste products and macromolecules that leak into the extracellular fluid are collected via the lymphatic system and returned to the systemic circulation. However, the mechanical pressure generated by the rapidly growing tumor mass and the irregular organization of the tumor micro-environment cause the collapse or complete absence of lymphatic vessels. The absence of a functional lymphatic drainage system means that nanoparticles leaking from the vessels remain trapped within the tumor tissue.⁷⁴ Shi et al determined that the EPR effect is the primary mechanism enabling nanoparticles to accumulate in tumors.⁷⁵ However, due to tumor heterogeneity, the EPR effect is not always guaranteed. Therefore, the current approach to improving therapeutic efficacy is to complement the EPR effect with ligand-based active targeting.⁷⁵

Active Targeting

In nanoparticle-based miRNA delivery systems, active targeting is a strategy that prevents the random distribution of therapeutic agents, ensuring their delivery to the targeted site.⁷⁶ Passive targeting, which involves adjusting nanoparticle size to allow entry through leaky tumor vessels, is not always a sufficient strategy. Therefore, an active delivery strategy involving the addition of specific molecules to the carrier surface is preferred.⁷⁷ In active targeting, molecules called ligands are conjugated to the surface of nanoparticles. These ligands bind to specific receptors that are highly over-expressed on the surface of the target cell population through a “lock-and-key” mechanism. Following binding, the cells internalize the nanoparticles via receptor-mediated endocytosis. Antibodies, peptides, aptamers, and small molecules are among the main classes of ligands used for this purpose.⁷⁸ For example, cells require folate to synthesize new DNA. Cancer cells express significantly higher levels of folate receptors on their surface than normal cells to capture folate from the surrounding environment. Therefore, coating nanoparticles with folate molecules is an important strategy used in active targeting.⁷⁹

Significant Nanoparticle Platforms for the Delivery of miRNA

Organic Based Nanoparticles

Lipid-based nanoparticles (LNPs) are currently among the most advanced and clinically validated carrier platforms for delivering nucleic acids to target cells while maintaining their stability in systemic circulation. Although traditional liposomes have an aqueous inner cavity in which hydrophilic molecules are encapsulated, lipid nanoparticles have a dense, rigid core structure composed of nucleic acid–lipid complexes organized in an inverted micelle configuration. LNP formulations are generated by combining four essential lipid components in carefully controlled stoichiometric ratios.⁸⁰ The biological function of a nanoparticle is determined by the critical roles performed by each of these components. Ionizable lipids, the key component of the system, remain neutral at physiological pH (7.4), thereby minimizing systemic toxicity. However, in the acidic endosomal environment (pH 5.5), they become protonated and acquire a positive charge, enabling interaction with the endosomal membrane and facilitating the release of miRNA into the cytoplasm through the proton sponge effect. Cholesterol contributes to structural stability and enhances the integrity

of the nanoparticle. Helper phospholipids, such as 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), support membrane fusion by forming a stable lipid bilayer. PEG-lipids located on the particle surface create a steric barrier that prevents nanoparticle aggregation and confer a “stealth” property that delays macrophage recognition and prolongs circulation time.⁸¹

The global success of mRNA vaccines developed during the pandemic is one of the major reasons for the emergence of the LNP platform as the gold standard for nucleic acid delivery. These vaccines demonstrated that LNPs can safely and efficiently deliver nucleic acids into human cells, thereby accelerating regulatory approval pathways and large-scale production strategies for miRNA molecules, which share a similar chemical structure but are significantly smaller in size. In addition, microfluidic manufacturing technologies and safety data obtained from mRNA vaccine platforms enable the highly efficient encapsulation of miRNA mimics or anti-miRNAs into LNPs and facilitate scalable drug production.⁸² The translational implications of this technological adaptation in breast cancer therapy are increasingly evident in the current literature. For instance, Rui et al developed solid lipid nanoparticles designed to overcome chemotherapy resistance in breast cancer and generate a synergistic therapeutic effect by co-loading the chemotherapeutic agent doxorubicin and the tumor-suppressive miR-34a.⁸³ A particularly notable aspect of this study was the active targeting strategy achieved by coating the nanoparticle surface with fucose molecules, enabling recognition of lectin receptors that are overexpressed on cancer cells. The results demonstrated that fucose-modified SLNs selectively accumulated in tumor tissues, while miR-34a suppressed intracellular survivin expression, thereby sensitizing cancer cells to chemotherapy. This dual-delivery strategy produced a significantly stronger antitumor effect than treatment with the chemotherapeutic agent alone.⁸⁰

Despite these promising developments, the clinical translation of LNP-based miRNA delivery systems depends on achieving a careful balance between their advantages and limitations. One of the main strengths of this platform is its high encapsulation efficiency enabled by ionizable lipid technology, as well as its ability to overcome the challenge of endosomal escape, which represents a major barrier to nucleic acid delivery. Moreover, the availability of FDA-approved LNP formulations provides an important regulatory advantage that facilitates the transition toward clinical studies. Nevertheless, several limitations remain. Systemically administered LNPs tend to accumulate in hepatocytes because they readily adsorb apolipoprotein E (ApoE) from the bloodstream onto their surfaces. This phenomenon complicates the targeting of extrahepatic tissues, such as breast tumors, and often necessitates additional surface modifications.⁸¹ Furthermore, repeated administration of PEGylated nanoparticles may induce the formation of anti-PEG antibodies, which can lead to accelerated blood clearance and consequently reduce therapeutic efficacy during subsequent administrations. Therefore, future LNP design strategies should focus on minimizing immunological responses while improving tissue-specific targeting capabilities.⁸⁰

Polymeric nanoparticles represent a compelling alternative to lipid-based systems for miRNA and gene delivery due to their structural versatility, biodegradable nature, and the ease with which their surface chemistry can be modified. These systems are generally classified as either natural or synthetic according to their origin, and each type is designed to address specific barriers in the miRNA delivery process.⁸⁴ Compared with lipid-based nanocarriers, polymeric nanoparticles offer greater flexibility in controlling particle size, degradation kinetics, and surface functionalization, making them particularly suitable for the co-delivery of multiple therapeutic agents and stimuli-responsive drug release.⁶⁵

Poly(lactic-co-glycolic acid) (PLGA), one of the few polymers approved by the FDA for biomedical applications, exhibits excellent biocompatibility because it is hydrolyzed in the body into harmless monomers such as lactic acid and glycolic acid. However, PLGA possesses an inherent negative charge and therefore exhibits limited capacity to encapsulate negatively charged molecules such as miRNAs because of electrostatic repulsion. In addition, PLGA alone has limited endosomal escape capability, which necessitates the incorporation of cationic polymers into the delivery system.⁸⁵ Surface modification with polyethylene glycol (PEG), cationic polymers, or targeting ligands has therefore become a common strategy to improve circulation time, cellular uptake, and intracellular delivery efficiency while preserving the favorable biodegradability of PLGA.⁸⁶

Polyethylenimine (PEI), a synthetic cationic polymer, is widely recognized for its exceptional efficiency in condensing nucleic acids and facilitating their cellular uptake. Owing to its high density of amine groups, PEI induces proton influx into endosomes, resulting in osmotic swelling and subsequent endosomal rupture, thereby enabling the release of

miRNA into the cytoplasm. However, the same property that confers this high delivery efficiency also contributes to significant cytotoxicity. The high positive charge density of PEI can disrupt cellular membranes, and because it is not biodegradable, it may accumulate in the body.⁸⁵ Consequently, numerous studies have focused on reducing PEI-associated toxicity through chemical modification or by combining PEI with biodegradable polymers, thereby preserving its transfection efficiency while improving its biocompatibility.⁸⁷

To overcome this efficacy–toxicity trade-off, recent research has focused on next-generation polymers, particularly poly(beta-amino ester) (PBAE) systems, which combine the high transfection efficiency of PEI with a safety profile closer to that of PLGA.⁷⁸ PBAEs can rapidly degrade under physiological conditions due to the presence of ester bonds, generating non-toxic byproducts. Furthermore, these polymers exhibit pH responsiveness because their pKa values are close to the endosomal pH range (5.5–6.0), enabling more efficient intracellular release of nucleic acids.⁸⁵ The modular synthesis of PBAEs also allows precise optimization of polymer composition, molecular weight, and terminal functional groups, facilitating the development of customized carriers for different nucleic acid cargos and cancer types.⁸⁸

Chitosan, a naturally derived polymer, is another promising material owing to its low toxicity and strong mucoadhesive properties. However, when used alone, its transfection efficiency is generally lower than that of synthetic polymers, and it is therefore often incorporated as an auxiliary component in hybrid delivery systems.⁸⁵ Chemical modifications such as quaternization, PEGylation, and ligand conjugation have been shown to substantially improve the solubility, stability, and gene delivery efficiency of chitosan-based nanoparticles.⁸⁹

One of the major advantages of polymeric nanoparticle systems is their capacity to generate controlled release profiles that respond to the acidic conditions of the tumor microenvironment. A particularly notable and comprehensive example is the study conducted by Wang et al.⁹⁰ In this work, the researchers developed hyaluronic acid-coated PEI–PLGA nanoparticles designed to target CD44 receptors, which are overexpressed on tumor cells, for the treatment of TNBC, a highly aggressive subtype with a poor prognosis.⁹⁰ In this multilayered smart delivery system, the potent chemotherapeutic agent doxorubicin and the tumor-suppressive miR-542-3p were co-encapsulated. The hydrophobic doxorubicin was incorporated within the PLGA core, while the cationic PEI component formed an electrostatic complex with the negatively charged miR-542-3p, protecting it from nuclease-mediated degradation. The outer hyaluronic acid coating enhanced nanoparticle uptake through CD44 receptor-mediated targeting while simultaneously reducing the cytotoxic effects associated with PEI.⁹⁰ Following cellular internalization, the released miR-542-3p disrupted the cancer cell's defense mechanisms by activating the p53 signaling pathway and suppressing the apoptosis inhibitor survivin. This molecular intervention markedly enhanced the cytotoxic effect of the simultaneously released doxorubicin and significantly inhibited tumor growth in *in vivo* models. Collectively, these findings highlight the therapeutic potential of PEI–PLGA hybrid polymeric systems when combined with appropriate targeting ligands, even in the treatment of highly aggressive cancers such as TNBC.⁹⁰ More broadly, these findings illustrate that the integration of biodegradable polymers, active targeting ligands, and therapeutic miRNAs into multifunctional nanoplatforms represents a promising strategy for overcoming the biological barriers that continue to limit the clinical translation of RNA-based therapeutics.⁶⁵

Inorganic Nanoparticles

Inorganic nanoparticles, unlike the flexible and biodegradable structures of lipid- or polymer-based organic carriers, offer considerable potential for miRNA delivery and cancer theranostic applications due to their rigid core structures, atomically controllable sizes, and unique physicochemical properties. These platforms possess optical, magnetic, and electrical characteristics that cannot be achieved with organic systems, thereby transforming nanoparticles from passive carriers into multifunctional smart devices. Three major classes of inorganic nanoparticles have attracted particular attention in this context.⁹¹ Gold nanoparticles are widely used because they are biologically inert and can be easily modified at their surface. In addition, they exhibit a unique optical phenomenon known as surface plasmon resonance. This property enables them to convert incident near-infrared light into thermal energy, which can induce localized hyperthermia in tumor cells while simultaneously triggering the release of their miRNA cargo in a light-responsive manner.⁹¹ Mesoporous silica nanoparticles possess an exceptionally large surface area due to their highly ordered, honeycomb-like porous architecture. This extensive internal pore network allows for the encapsulation and protection of large quantities of miRNA molecules as well as chemotherapeutic drugs, enabling efficient co-delivery strategies.⁹²

Magnetic nanoparticles, typically composed of iron oxide cores, can be physically guided toward tumor tissues using an externally applied magnetic field. In addition to their targeting potential, they also function as T2 contrast agents in magnetic resonance imaging, enabling real-time monitoring of therapeutic responses. However, bare inorganic cores may exhibit colloidal instability in biological fluids or induce toxicity through the release of metal ions. Therefore, surface modification with materials such as PEG, silica shells, or biocompatible polymers is essential to achieve steric stabilization and improve their biological compatibility.⁹²

One of the most recent and comprehensive studies demonstrating the importance of surface engineering in gold nanoparticle-mediated gene restoration was conducted by Chaudhari et al.⁹¹ In this study, the researchers employed PEG-stabilized gold nanoparticles to restore the expression of miR-206, a tumor suppressor that plays a critical role in the pathogenesis of luminal A breast cancer. The synthesized gold nanoparticles were first functionalized with PEG chains to enhance biocompatibility and create a suitable interface for miRNA conjugation. Subsequently, miR-206 mimics were attached to the nanoparticle surface to generate a stable nanoconjugate system.⁹¹

Application of these nanoconjugates to MCF-7 breast cancer cells resulted in efficient cellular internalization of the nanoparticles, followed by successful release of the miR-206 cargo into the cytoplasm. The restored miR-206 markedly suppressed the expression of *NOTCH3*, a direct oncogenic target that promotes cancer progression, thereby disrupting critical intracellular signaling pathways. Phenotypic analyses further demonstrated that inhibition of the NOTCH3 pathway induced cell cycle arrest at the G0/G1 phase and triggered apoptosis in cancer cells.⁹¹ These findings highlight that properly engineered inorganic nanoparticles can provide potent therapeutic benefits in breast cancer treatment by not only protecting and delivering nucleic acids but also precisely modulating intracellular molecular pathways.

Hybrid Nanoparticle Systems

Lipid–polymer hybrid nanoparticles (LPHNPs), one of the most advanced forms of nanotechnology-based drug delivery systems, represent a superior platform that integrates the high drug-loading capacity and structural stability of a polymeric core with the biocompatibility and prolonged circulation advantages provided by a lipid shell within a single core–shell architecture. Developed to overcome multidrug resistance and systemic toxicity frequently encountered in breast cancer therapy, these systems enable the simultaneous delivery of agents with distinct mechanisms of action and allow their release under conditions specific to the tumor microenvironment.⁹³ One of the most recent and comprehensive examples of this strategy is the study conducted by Sarma et al.⁹⁴ In this study, the researchers co-loaded paclitaxel, an important chemotherapeutic agent used in breast cancer treatment, and curcumin, a natural polyphenolic compound with anticancer, anti-inflammatory, and resistance-modulating properties, into a single hybrid nanoparticle system. In this study, pH-sensitive LPHNPs composed of a biodegradable PLGA core that encapsulates the therapeutic agents and a surrounding soy lecithin/ 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine (DSPE)-PEG lipid layer were synthesized using the emulsification–solvent evaporation method.⁹⁴ A key engineering feature of this system is its intelligent release profile, which responds to the acidic conditions characteristic of tumor tissues. In vitro drug release studies demonstrated that the nanoparticles maintained minimal drug release under physiological pH conditions (7.4), whereas rapid and enhanced release of both drugs occurred under acidic conditions (pH 5.5) mimicking the tumor microenvironment due to destabilization of the lipid layer.⁹⁴ Cytotoxicity analyses performed in MCF-7 breast cancer cell lines revealed that curcumin sensitized cancer cells to paclitaxel by suppressing cellular resistance mechanisms. As a result, the simultaneous delivery achieved by the LPHNP system produced significantly higher levels of cancer cell death even at substantially lower doses compared with the free drug combination.⁹⁴ This study provides strong evidence that lipid–polymer hybrid systems can effectively address the solubility limitations of hydrophobic drugs, enable pH-responsive targeting, and offer an ideal platform for synergistic combination therapies.

Lipid-based, polymeric, inorganic, and hybrid nanoparticle platforms represent complementary strategies for overcoming the major biological limitations associated with miRNA delivery in breast cancer therapy⁹⁵ (Figure 5). Each platform addresses distinct delivery barriers, including protection from nuclease degradation, prolonged systemic circulation, enhanced tumor accumulation, cellular internalization, and endosomal escape, which collectively determine the therapeutic efficacy of miRNA nanomedicines.⁶⁵

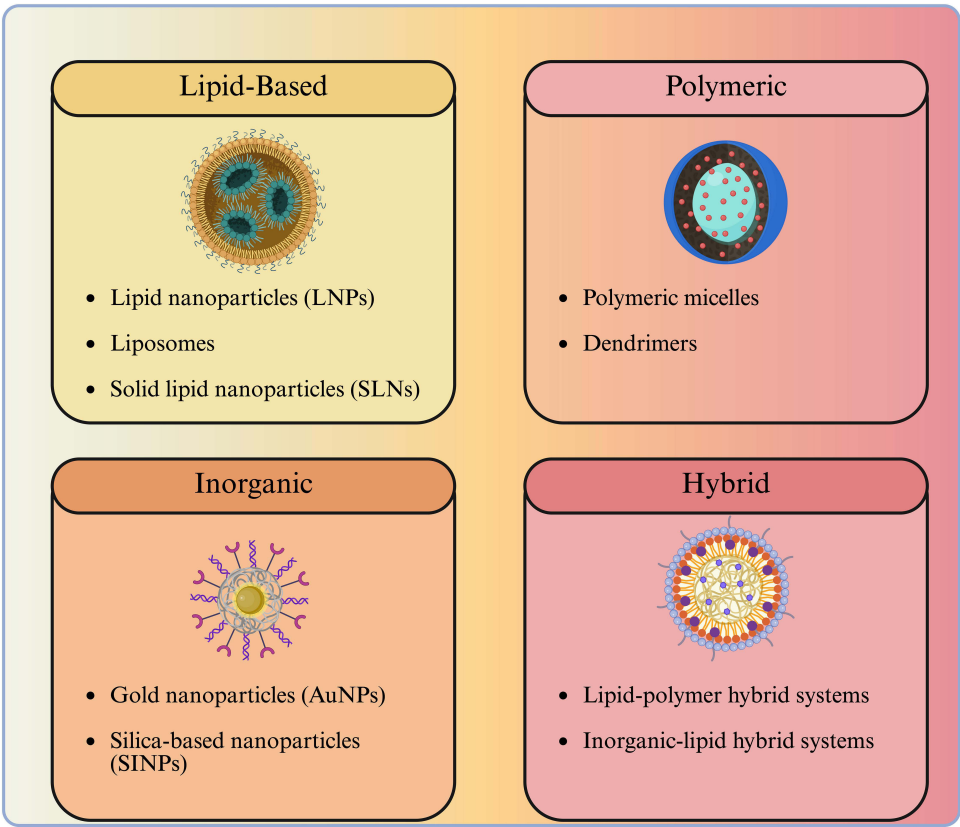


Figure 5 Schematic overview of nanoparticle-based miRNA delivery systems. The illustration summarizes the major classes of nanocarriers employed for therapeutic miRNA delivery, including lipid-based nanoparticles, polymeric nanoparticles, inorganic nanoparticles, and hybrid nanosystems. Lipid-based nanoparticles efficiently encapsulate miRNAs, protect them from nuclease-mediated degradation, and facilitate membrane fusion and endosomal escape. Polymeric nanoparticles, composed of either natural or synthetic polymers, enable tunable miRNA loading, controlled release, high structural stability, and versatile surface functionalization. Inorganic nanoparticles, such as gold, silica, magnetic, and calcium phosphate nanoparticles, provide unique physicochemical properties, including high loading capacity, imaging capability, and stimulus-responsive cargo release, while requiring careful optimization to ensure biocompatibility. Hybrid nanoparticles integrate the complementary advantages of multiple material platforms to enhance miRNA stability, circulation time, targeting specificity, intracellular delivery, and therapeutic efficacy.

Table 3 summarizes the primary advantages, inherent limitations, and current clinical potential of three major classes of nanocarriers: lipid-based, polymeric, and inorganic nanoparticles. The selection of a specific delivery system depends on the balance between transfection requirements, material stability, and the intended therapeutic or diagnostic application. Importantly, no single nanoparticle platform is universally optimal, and carrier selection should be guided by the physicochemical properties of the miRNA cargo, the molecular characteristics of the target tumor, and the desired pharmacokinetic profile.⁶²

Table 3 Comparative Analysis of Different Nanoparticle Systems for miRNA Delivery

System	Advantages	Limitations	Clinical Potential	Ref
Lipid nanoparticles	High transfection efficiency	Immunogenicity	High	[96,97]
Polymeric nanoparticles	Stability	Toxicity concerns	Moderate	[65]
Inorganic nanoparticles	Imaging capability	Poor biodegradability	Emerging	[98,99]
Hybrid nanoparticles (lipid–polymer)	Combined biocompatibility, stability, controlled release, and enhanced targeting efficiency	Complex manufacturing, reproducibility, and scale-up challenges	Emerging–Moderate	[100,101]

Lipid nanoparticles have emerged as clinically validated carriers due to their high nucleic acid encapsulation efficiency and favorable regulatory track record, while polymeric nanoparticles offer remarkable structural versatility and the ability to engineer stimuli-responsive release profiles tailored to the tumor microenvironment. The clinical success of lipid nanoparticle-based RNA therapeutics has established important manufacturing, quality-control, and regulatory frameworks that are expected to facilitate the future translation of miRNA nanomedicines, whereas polymeric systems continue to provide greater flexibility for multifunctional and combination therapeutic strategies.¹⁰²

Inorganic nanoparticles, in contrast, provide unique physicochemical properties, including optical, magnetic, and imaging capabilities, enabling theranostic applications that integrate diagnosis and therapy within a single platform. However, concerns regarding long-term biodegradability, tissue accumulation, and potential chronic toxicity remain significant barriers to their widespread clinical implementation, emphasizing the need for careful material selection and comprehensive biosafety evaluation.⁶⁹

Hybrid systems, particularly lipid-polymer hybrid nanoparticles, further bridge the advantages of organic and inorganic designs by combining structural stability, high drug-loading capacity, and improved biocompatibility within a unified architecture.⁶² Recent studies have further demonstrated that hybrid nanoparticles can simultaneously incorporate targeting ligands, imaging agents, and multiple therapeutic cargos, enabling personalized and multifunctional nanomedicine approaches for heterogeneous breast cancer subtypes.⁶⁵

Despite these advances, each platform still faces critical challenges, including immune recognition, off-target biodistribution, and the need for precise tumor-specific targeting. Future research should therefore focus on the rational integration of these nanotechnologies with advanced surface engineering, biomimetic coatings, and microenvironment-responsive mechanisms to develop next-generation delivery systems capable of achieving safe, efficient, and clinically translatable miRNA-based therapies for breast cancer.

The choice of nanocarrier critically influences the therapeutic performance of miRNA-based interventions in breast cancer, with each platform exhibiting distinct advantages and biological limitations. Lipid-based nanoparticles remain the most clinically advanced carriers because of their superior transfection efficiency and rapid cytosolic release of nucleic acids. For example, systemic delivery of liposomal T-VISA-miR-34a significantly inhibited orthotopic breast tumor growth, suppressed migration through downregulation of *E2F3*, *CD44*, and *SIRT1*, and prolonged survival without detectable systemic toxicity, highlighting the translational potential of lipid-based formulations.¹⁰³ Similarly, DOPC nanoliposomes carrying miR-200 efficiently restored epithelial characteristics by suppressing *IL-8* and *CXCL1*, thereby reducing tumor progression and metastatic behavior. Despite these encouraging outcomes, lipid nanoparticles remain susceptible to complement activation, rapid uptake by the mononuclear phagocyte system, and limited penetration into dense tumor stroma, which may reduce therapeutic efficacy after systemic administration.¹⁰⁴

Polymeric nanoparticles provide greater structural stability, prolonged circulation, and controlled miRNA release, making them particularly attractive for sustained gene regulation. Layer-by-layer PLGA/poly-L-lysine nanoparticles delivering miR-34a achieved efficient intracellular release and induced apoptosis in triple-negative breast cancer by suppressing *CCND1*, while PLGA-b-PEG nanoparticles co-delivering anti-miR-21 and anti-miR-10b simultaneously inhibited primary tumor growth and metastatic dissemination through dual oncogenic pathway blockade.¹⁰⁵ Chitosan- and hyaluronic acid-based nanoparticles further improved selective uptake through CD44-mediated targeting and restored tumor-suppressive miR-34a activity with reduced systemic toxicity compared with conventional cationic polymers.⁵⁴ Nevertheless, polymeric systems often exhibit lower transfection efficiency than lipid nanoparticles, and highly cationic polymers such as PEI or high-generation PAMAM dendrimers may induce membrane damage, inflammatory responses, and dose-dependent cytotoxicity, limiting their clinical applicability despite excellent endosomal escape via the proton sponge effect.¹⁰⁶

In contrast, inorganic and hybrid nanoplatforms have emerged to overcome the shortcomings of single-component carriers. Gold and mesoporous silica nanoparticles enable simultaneous miRNA delivery and imaging or photothermal therapy while exhibiting high loading capacity and facile surface functionalization. For instance, PEG-PLGA-coated mesoporous silica nanoparticles co-delivering miR-34a mimic and anti-miR-10b produced marked inhibition of both tumor growth and lung metastasis in TNBC through simultaneous restoration of tumor-suppressive signaling and

suppression of metastatic pathways.¹⁰⁷ These findings suggest that hybrid nanoplateforms combine the high transfection efficiency of lipid nanoparticles with the structural stability and controlled release properties of polymeric carriers, thereby addressing several key barriers associated with miRNA delivery. However, their complex manufacturing processes, batch-to-batch reproducibility, and large-scale production remain important challenges that must be resolved before widespread clinical translation can be achieved.

Mechanistic Aspects of Nanoparticle-Mediated miRNA Delivery

miRNA-based therapeutics possess remarkable potential to reprogram cellular gene expression in a highly targeted manner in numerous complex diseases, particularly cancer. However, the direct therapeutic application of miRNAs faces several major challenges, including rapid enzymatic degradation, poor bioavailability, limited ability to cross cellular membranes, and the risk of off-target effects.¹⁰⁸ To overcome these limitations, nanoparticle-based delivery systems have been developed to enhance the stability of miRNA therapeutics, regulate their biodistribution in vivo, and facilitate their efficient and selective uptake by target tissues.¹⁰⁹

Strategies for Improving miRNA Stability

miRNAs are small, single-stranded, negatively charged RNA molecules that are highly susceptible to rapid degradation by serum RNases. The circulating half-life of unmodified miRNAs is typically only a few minutes.¹¹⁰ Nanoparticle-based delivery systems provide effective protection against this degradation by physically encapsulating or electrostatically complexing miRNA molecules. One of the most widely employed strategies is electrostatic binding, which relies on attractive interactions between oppositely charged molecules. Under physiological conditions, the phosphate backbone of miRNAs imparts a strong negative charge, which spontaneously interacts with the positively charged amine groups of cationic polymers or lipids within nanoparticles, resulting in the formation of stable nanostructures known as polyplexes or lipoplexes. The nitrogen-to-phosphate (N/P) ratio, representing the molar ratio of positively charged nitrogen groups in the polymer to negatively charged phosphate groups in the miRNA, is a critical parameter for successful delivery. Optimizing the N/P ratio ensures complete miRNA condensation and protection from nucleases while maintaining a net positive surface charge that facilitates nanoparticle interaction with the negatively charged cell membrane and promotes endocytic uptake.¹¹⁰

Hydrophobic encapsulation is particularly relevant in combination therapies involving poorly water-soluble chemotherapeutic agents such as doxorubicin or paclitaxel. During nanoparticle formation in an aqueous environment, hydrophobic polymer segments fold inward, forming a dense core that minimizes water contact. Lipophilic drug molecules preferentially partition into this core and are retained through Van der Waals interactions. Hydrophilic miRNAs cannot naturally enter this core; however, chemical conjugation strategies, such as attaching hydrophobic moieties like cholesterol to the 3' end of miRNAs, render these molecules amphiphilic. This modification allows miRNAs to associate with the hydrophobic interface of nanoparticles, enhancing stability and reducing premature release.¹¹¹

The layer-by-layer (LbL) technique represents a highly sophisticated approach for programming release kinetics and overcoming multidrug resistance. In this strategy, nanoparticles are assembled sequentially, layer by layer, through the alternating adsorption of oppositely charged polyelectrolytes. Typically, a positively charged polymer is first coated onto a drug-loaded core, followed by the electrostatic attachment of negatively charged miRNA molecules, and finally, a protective or targeting outer polymer layer is applied. This design enables sequential release: upon entering the endosomal environment of tumor cells, the outer layers degrade first, releasing the miRNA to suppress resistance pathways, after which the inner chemotherapeutic payload is delivered, thereby maximizing cytotoxicity. This controlled, time-dependent release is crucial for achieving synergistic effects in breast cancer treatment.¹¹²

Beyond these strategies, steric shielding further enhances miRNA stability by physically isolating nucleic acids from RNase access.¹¹³ Hydrophilic surface coatings such as PEG additionally reduce plasma protein adsorption, prevent protein corona formation, and limit immune recognition.¹¹³ Some systems incorporate stimuli-responsive linkages, such as pH-sensitive or redox-sensitive bonds, which remain stable during circulation but are cleaved in the acidic or high-glutathione tumor microenvironment, ensuring precise, site-specific activation of miRNA release.

Cellular Uptake Pathways

Effective delivery of miRNA therapeutics requires nanoparticles that remain stable during circulation while efficiently entering target cells and releasing miRNA into the cytoplasm.^{62,69} This process involves three critical steps: targeted binding to the cell surface, internalization via endocytosis, and cytoplasmic release. Nanoparticles can be functionalized to interact with tumor-specific receptors, including folate receptor α (FR- α) for TNBC targeting via folic acid conjugation¹¹⁴ and the HER2 receptor for selective uptake in HER2-positive subtypes using trastuzumab⁴⁸ (Figure 6).

The internalization mechanism depends on nanoparticle size, surface charge, and composition. Clathrin-mediated endocytosis is commonly utilized by lipid nanoparticles, PEG-lipid systems, and some dendrimer-miRNA complexes with anionic or slightly cationic surfaces (50–150 nm). Ligand-receptor interactions induce clathrin-coated pit formation, followed by vesicle scission via dynamin. These vesicles transport nanoparticles to early endosomes, where pH-dependent protonation of ionizable lipids facilitates endosomal escape, allowing miRNAs to reach the cytosol without lysosomal degradation.¹¹⁵ Macropinocytosis is a non-selective pathway induced by growth factor signaling and actin remodeling, resulting in the formation of large vesicles (macropinosomes). High-surface-charge carriers, such as PAMAM or PEI complexes, often utilize this pathway, which is upregulated in highly proliferative tumor cells to enable bulk uptake of dendrimers, lipoplexes, and polymeric nanocomplexes.¹¹⁶

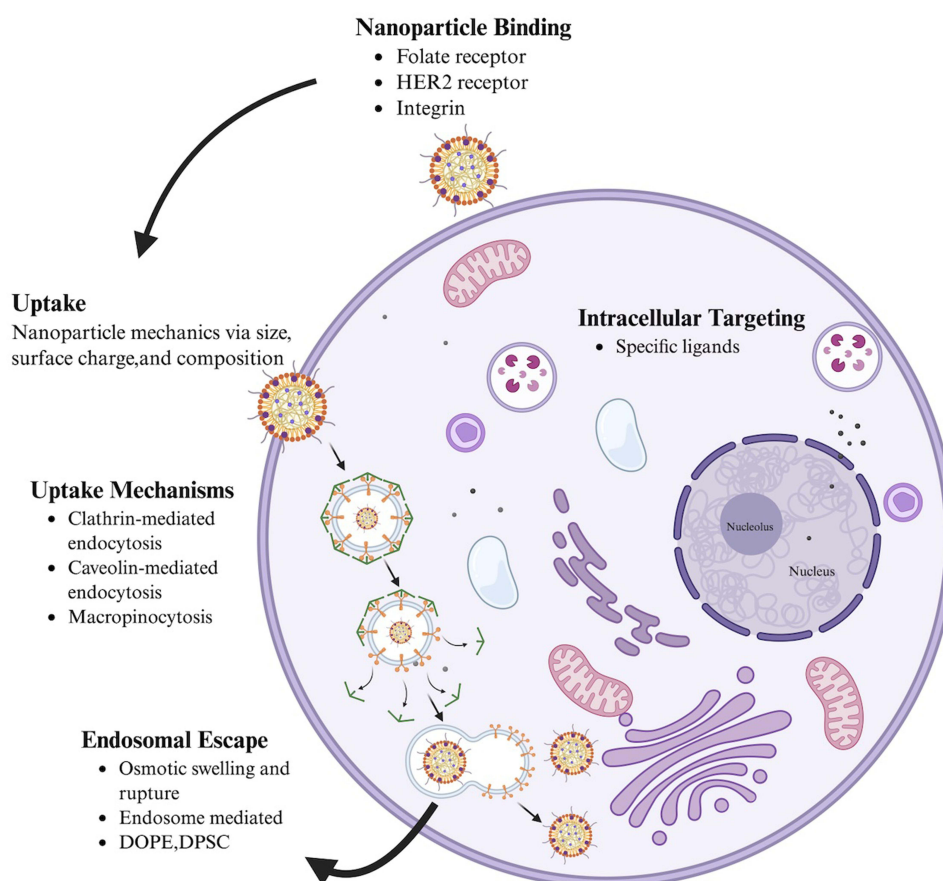


Figure 6 Schematic illustration of the cellular uptake mechanisms of optimized nanoparticle-based miRNA therapeutics. The diagram depicts the sequential intracellular trafficking of miRNA-loaded nanoparticles following their interaction with target cells. Initially, nanoparticles bind to the cell surface through electrostatic interactions or ligand-receptor recognition, enabling selective targeting and enhanced cellular association. Internalization occurs predominantly via endocytic pathways, including clathrin-mediated endocytosis, caveolae-mediated endocytosis, macropinocytosis, and clathrin/caveolae-independent mechanisms, depending on the physicochemical properties of the nanoparticles and the cellular context. Following endocytic uptake, nanoparticles are trafficked through early and late endosomes, where efficient endosomal escape is essential to prevent lysosomal degradation of the miRNA cargo. Various escape mechanisms, including the proton sponge effect, membrane destabilization, pH-responsive carrier disassembly, membrane fusion, and pore formation, facilitate the release of therapeutic miRNAs into the cytoplasm. Subsequently, mature miRNAs are incorporated into the RNA-induced silencing complex (RISC), where they mediate sequence-specific degradation or translational repression of target mRNAs.

Following internalization, endosomal escape is crucial for cytoplasmic delivery. Cationic polymers, including PAMAM and PEI, buffer endosomal protons, induce osmotic swelling, and promote rupture of the vesicle membrane.¹¹⁷ Lipids such as 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and DSPC promote membrane fusion under acidic conditions,⁸⁰ while histidine-containing polymers increase membrane permeability via protonation at low pH.¹¹⁸

Finally, intracellular targeting ensures that miRNA therapeutics reach the desired cellular compartments and minimizes off-target effects. Nanoparticles decorated with specific ligands are internalized through receptor-mediated endocytosis, allowing precise delivery to tumor cells and reducing systemic toxicity. For instance, mesoporous silica nanoparticles carrying miR-34a and anti-miR-10b via CD44 receptors demonstrate selective uptake in target cells.¹⁰⁷ This combination of targeted uptake, endosomal escape, and intracellular delivery maximizes therapeutic efficacy while minimizing systemic side effects.

Biodistribution and Pharmacokinetics of Nanoparticles

Biodistribution represents one of the most critical pharmacokinetic parameters determining the therapeutic efficacy and safety profile of nanoparticle-based miRNA delivery systems. It is largely governed by the dynamic interplay between the physicochemical characteristics of nanoparticles, such as particle size, surface charge, and surface chemistry, and the biological barriers encountered within the body.⁶⁹ Following intravenous administration, nanoparticles entering systemic circulation are frequently recognized as foreign entities and are rapidly sequestered by macrophages located in the liver and spleen, the primary organs of the reticuloendothelial system (RES), also referred to as the mononuclear phagocyte system (MPS). In addition, nanoparticles with a hydrodynamic diameter below the renal filtration threshold may be swiftly eliminated through renal clearance via the urinary system. To achieve efficient tumor accumulation, nanoparticles are generally engineered within a size range of approximately 10–200 nm. Moreover, surface modification with hydrophilic polymers such as PEG is widely employed to reduce serum protein adsorption and prevent rapid opsonization. This strategy prolongs systemic circulation by enabling nanocarriers to evade macrophage-mediated clearance.¹¹⁹ Consequently, nanoparticles can accumulate in tumor tissues either passively through the EPR effect, arising from abnormal and highly permeable tumor vasculature, or actively via the incorporation of specific targeting ligands on their surfaces. These approaches collectively aim to maximize therapeutic concentrations within the tumor microenvironment while minimizing off-target accumulation and cytotoxic effects in healthy tissues.

Future Perspectives and Translational Potential of Nanoparticle-Mediated miRNA Therapy

The therapeutic potential of nanoparticle-based miRNA delivery systems in breast cancer has been extensively explored in light of current preclinical evidence and emerging clinical findings. Various nanoparticle platforms, including lipid-based nanoparticles, polymeric systems, inorganic nanostructures, and hybrid formulations, offer significant advantages in enhancing miRNA stability, facilitating targeted tumor accumulation, and enabling controlled intracellular release.¹⁰⁸ Despite these promising features, several key challenges, such as formulation stability, immunogenic responses, off-target effects, and large-scale clinical manufacturing, continue to limit the widespread clinical adoption of these technologies.¹⁰⁸

Among the nanocarrier platforms, LNPs are generally considered the most clinically advanced owing to their high nucleic acid encapsulation efficiency and favorable translational profile, as demonstrated by the success of mRNA-LNP therapeutics. However, the clinical performance of miRNA-loaded LNPs may differ from that of mRNA formulations because miRNAs possess distinct intracellular trafficking requirements and may induce broader gene-regulatory effects. In contrast, polymeric nanoparticles, including PAMAM dendrimers and PEI-based systems, offer enhanced structural versatility and controlled release properties but may exhibit higher cytotoxicity and batch-to-batch variability. Inorganic nanoparticles, such as gold, silica, and magnetic nanoparticles, provide additional imaging and theranostic capabilities; nevertheless, concerns regarding biodegradability and long-term tissue accumulation remain important translational barriers. Consequently, hybrid nanoparticles that combine the biocompatibility of lipids with the stability and functionality of polymeric or inorganic components are increasingly viewed as a promising next-generation strategy for miRNA delivery.^{120–122}

Future research is therefore expected to focus on the development of multifunctional and personalized nanoparticle platforms capable of responding to specific features of the tumor microenvironment. Mechanistically, the therapeutic efficacy of miRNA nanomedicines is strongly influenced by several biological barriers encountered after systemic administration. Following intravenous injection, nanoparticles rapidly interact with plasma proteins, leading to protein corona formation, which can alter biodistribution, cellular uptake, and immune recognition. Subsequent clearance by the RES may substantially reduce tumor accumulation. Even after successful cellular internalization, efficient endosomal escape remains a major bottleneck because many nanoparticles become trapped within endolysosomal compartments, resulting in degradation of the miRNA cargo. Design strategies such as ionizable lipids, proton-sponge polymers, pH-responsive materials, and membrane-disruptive peptides have therefore been developed to enhance cytosolic delivery and improve RISC loading efficiency, which ultimately determines miRNA-mediated gene silencing.^{80,123}

Given the molecular heterogeneity of breast cancer, the therapeutic application of miRNA-loaded nanoparticles has been investigated across major molecular subtypes, including ER-positive, HER2-positive, and TNBC. Among these, TNBC represents a particularly compelling target due to its aggressive tumor biology, high metastatic potential, and limited treatment options.¹²⁴ Thus, the selective and efficient delivery of therapeutic miRNAs to tumor tissue by targeted nanoparticle systems emerges as a promising strategy. However, several translational limitations, including precise control of biodistribution, mitigation of immune activation, scalable production processes, and assessment of long-term safety, must be addressed before these approaches can be successfully integrated into clinical practice.

Importantly, subtype-specific targeting strategies have emerged as a critical consideration in nanoparticle design. In ER-positive breast cancer, miRNAs that modulate estrogen receptor signaling and endocrine resistance pathways, such as miR221/222-associated networks, have shown therapeutic potential. In HER2-positive tumors, active targeting approaches employing HER2 antibodies, peptides, or aptamers can enhance selective tumor uptake and may help overcome trastuzumab resistance.^{125–127} In TNBC, where actionable molecular targets are limited, nanoparticles functionalized with ligands against EGFR, CD44, integrin $\alpha v \beta 3$, folate receptor, or other cancer stem cell-associated markers have demonstrated improved tumor selectivity and reduced off-target exposure.^{128,129} These observations suggest that the optimal nanoparticle formulation may differ substantially across breast cancer subtypes, supporting a more personalized approach to miRNA nanotherapy.¹²⁵

Recent advances have focused on overcoming the limited intracellular delivery and inefficient endosomal escape that continue to restrict the clinical translation of miRNA therapeutics. In this context, a carrier-free supramolecular nanoengine (AMGA) has been developed to enhance the cytoplasmic delivery of anti-miRNAs through a nanomechanical endosomal escape mechanism without requiring external stimulation. By simultaneously delivering anti-miR-21 and anti-miR-10b, this platform achieved efficient gene silencing, resulting in reduced proliferation, migration, and invasion of TNBC cells. Furthermore, significant antitumor efficacy was demonstrated in an orthotopic TNBC model, and the combination of the nanoengine with chemotherapy produced synergistic chemo-gene therapeutic effects. These findings highlight the potential of stimulus-independent, carrier-free supramolecular nanoplatfroms as an emerging strategy to improve the intracellular delivery and therapeutic efficacy of miRNA-based cancer therapies, while addressing one of the major barriers limiting their clinical application.¹³⁰

Another important consideration is the long-standing assumption that nanoparticle accumulation in tumors is primarily mediated by the enhanced permeability and retention (EPR) effect. Recent evidence indicates that EPR alone may not sufficiently explain the heterogeneous and often limited tumor delivery observed in patients. Instead, active endothelial transport, transcytosis, and tumor-specific retention mechanisms appear to play substantial roles in nanoparticle accumulation. This evolving understanding has shifted the field toward the development of actively targeted and biologically responsive nanocarriers rather than relying solely on passive EPR-mediated delivery.¹³¹

Rapid advances in nanotechnology and RNA therapeutics are expected to significantly expand the clinical potential of miRNA-based treatment strategies for breast cancer in the coming years.¹²⁴ One of the most promising directions involves the integration of artificial intelligence (AI) and machine learning algorithms into nanoparticle design. AI-assisted nanoparticle engineering can facilitate the optimization of key physicochemical parameters, including particle size, surface charge, ligand density, and drug-loading capacity, thereby improving tumor-targeting efficiency and

minimizing off-target effects. Such computational approaches may accelerate the development of next-generation nanocarriers with enhanced stability, biodistribution profiles, and therapeutic performance.¹³²

Beyond conventional optimization, AI-based platforms are increasingly being explored for ligand screening, lipid composition prediction, protein corona modeling, biodistribution forecasting, and digital-twin-guided nanocarrier design. By integrating large-scale experimental datasets with machine learning algorithms, these approaches may substantially reduce empirical trial-and-error processes and accelerate the identification of clinically viable formulations with improved reproducibility and manufacturability.¹³³

Another important area of future development is the design of tumor microenvironment-responsive nanoparticles capable of releasing their therapeutic cargo in response to specific stimuli present within tumor tissues. Breast tumors are characterized by distinct microenvironmental conditions, including acidic pH, elevated reactive oxygen species levels, and the presence of tumor-associated enzymes. Nanoparticle systems engineered to respond to these stimuli can enable controlled and site-specific release of miRNA therapeutics, thereby improving therapeutic efficacy while reducing systemic toxicity.¹³⁴

Accordingly, pH-responsive, ROS-responsive, enzyme-cleavable, charge-switching, and biomimetic membrane-coated nanoparticles have emerged as particularly attractive design strategies. These systems remain relatively stable during circulation but undergo structural or physicochemical changes within the tumor microenvironment, thereby promoting enhanced cellular uptake, endosomal escape, and localized miRNA release. Such stimuli-responsive behavior may be especially valuable in TNBC, where intratumoral heterogeneity and an immunosuppressive microenvironment often limit conventional therapeutic efficacy.¹³⁵

Personalized miRNA therapy also represents a key emerging concept in precision oncology. Given the substantial molecular heterogeneity observed across breast cancer subtypes, patient-specific miRNA expression profiles may serve as valuable biomarkers for the design of individualized therapeutic strategies. Integrating genomic and transcriptomic data with targeted nanoparticle delivery systems could allow the development of tailored miRNA therapeutics that selectively modulate dysregulated signaling pathways in individual patients.⁴²

Despite the remarkable therapeutic efficacy demonstrated in preclinical breast cancer models, the successful clinical translation of nanoparticle-mediated miRNA therapy requires overcoming several biological, technological, and regulatory challenges. The clinical experience with MRX34, the first liposomal miR-34a mimic evaluated in patients with advanced solid tumors, highlighted both the promise and the limitations of miRNA therapeutics. Although pharmacodynamic analyses confirmed target gene modulation and preliminary antitumor activity, the phase I trial was terminated because of severe immune-related adverse events, emphasizing that safety depends not only on the therapeutic miRNA but also on carrier composition, dosing strategy, and patient selection.¹³⁶ In contrast, the EGFR-targeted bacterial minicell-based TargomiR (miR-16 mimic) demonstrated a more favorable safety profile together with early evidence of disease control, supporting the concept that rationally engineered targeted nanocarriers can substantially improve the therapeutic index of miRNA delivery.¹³⁷ These clinical experiences have shifted current research toward ionizable lipid nanoparticles, biomimetic and exosome-inspired carriers, and ligand-functionalized nanoplateforms designed to enhance tumor-specific accumulation while minimizing systemic toxicity. Equally important for clinical implementation is the identification of predictive biomarkers and subtype-specific miRNA signatures to enable patient stratification and personalized treatment.

Advances in single-cell sequencing, spatial transcriptomics, and multi-omics profiling further support this vision by enabling the identification of patient-specific miRNA signatures and resistant tumor cell populations. Rather than employing a single universal miRNA, future therapeutic strategies may utilize multi-miRNA panels designed according to the molecular characteristics of each patient's tumor, thereby improving therapeutic precision and reducing unnecessary exposure to ineffective treatments.

Furthermore, the development of multifunctional and multi-cargo nanoplateforms capable of co-delivering miRNAs alongside conventional chemotherapeutic agents, small molecule inhibitors, or immunomodulatory molecules may offer synergistic therapeutic benefits.¹³⁸ Such combinational nanotherapeutic strategies have the potential to simultaneously regulate multiple oncogenic pathways, overcome drug resistance, and enhance overall treatment outcomes.

Particularly promising combinations include miRNA plus doxorubicin or paclitaxel for chemosensitization, miRNA plus PARP inhibitors for BRCA-associated tumors, miRNA plus CDK4/6 inhibitors for hormone receptor-positive disease, and miRNA plus immune checkpoint inhibitors for immunologically active TNBC. By simultaneously modulating oncogenic signaling, apoptosis, DNA damage response, and immune evasion pathways, these combinational approaches may provide broader and more durable therapeutic responses than monotherapy alone.^{139–142}

These emerging technological advances demonstrate the potential for nanotechnology, systems biology, and computational design to transform the development of next-generation miRNA therapeutics. The translation of these innovations into clinically viable treatment strategies for breast cancer will be essential and will require continued interdisciplinary collaboration between nanotechnologists, molecular biologists, and clinicians.

Nevertheless, successful clinical translation will also depend on addressing several practical challenges that extend beyond biological efficacy. These include GMP-compliant large-scale manufacturing, formulation reproducibility, long-term storage stability, sterilization procedures, pharmacokinetic variability among patients, regulatory approval pathways, and comprehensive long-term safety evaluation. The experience with earlier RNA-based therapeutics, including immune-related toxicities observed in some clinical programs, underscores the importance of careful dose optimization and patient selection. Therefore, while nanoparticle-mediated miRNA delivery has demonstrated substantial promise in preclinical breast cancer models, its ultimate clinical success will likely require the convergence of advanced nanomaterial engineering, precision oncology, computational modeling, and rigorous translational research.

Conclusion

miRNA-based therapeutics have emerged as a promising strategy for breast cancer treatment owing to their ability to simultaneously modulate multiple oncogenic pathways. As discussed throughout this review, nanoparticle-based delivery systems provide effective protection against miRNA degradation while enhancing cellular uptake, tumor targeting, and intracellular delivery. The integration of subtype-specific miRNA signatures with rationally engineered nanocarriers offers considerable potential for improving therapeutic precision across diverse breast cancer subtypes. Despite these advances, several challenges continue to limit clinical translation, including off-target biodistribution, immunogenicity, long-term safety, and the complexity of large-scale, standardized manufacturing. Addressing these limitations will require rigorous preclinical validation, deeper understanding of nanoparticle–target interactions, and the establishment of robust regulatory and Good Manufacturing Practice (GMP)-compliant production strategies. The convergence of miRNA biology, nanotechnology, and precision medicine provides a strong foundation for the next generation of breast cancer therapeutics. Continued interdisciplinary research, together with advances in intelligent nanocarrier design and personalized treatment approaches, is expected to accelerate the successful clinical implementation of nanoparticle-mediated miRNA delivery systems.

Data Sharing Statement

By ordering from the corresponding author.

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References

1. Zhang X. Molecular classification of breast cancer: relevance and challenges. *Arch Pathol Lab Med.* 2023;147(1):46–51. doi:10.5858/arpa.2022-0070-RA
2. Polyak K. Heterogeneity in breast cancer. *J Clin Invest.* 2011;121(10):3786–3788. doi:10.1172/JCI60534
3. Waks AG, Winer EP. Breast cancer treatment: a review. *Jama.* 2019;321(3):288–300. doi:10.1001/jama.2018.19323

4. Lei Z, Tian Q, Teng Q, et al. Understanding and targeting resistance mechanisms in cancer. *MedComm*. 2023;4(3):e265. doi:10.1002/mco2.265
5. Barker HE, Paget JTE, Khan AA, Harrington KJ. The tumour microenvironment after radiotherapy: mechanisms of resistance and recurrence. *Nat Rev Cancer*. 2015;15(7):409–425. doi:10.1038/nrc3958
6. Kaneko MK, Suzuki H, Ohishi T, Nakamura T, Tanaka T, Kato Y. A cancer-specific monoclonal antibody against HER2 exerts antitumor activities in human breast cancer xenograft models. *Int J Mol Sci*. 2024;25(3):1941. doi:10.3390/ijms25031941
7. Tolaney SM, Jiang Z, Zhang Q, et al. Trastuzumab deruxtecan plus pertuzumab for HER2-positive metastatic breast cancer. *N Engl J Med*. 2026;394(6):551–562. doi:10.1056/NEJMoa2508668
8. Senkus E, Delaloge S, Domchek SM, et al. Olaparib efficacy in patients with germline BRCA-mutated, HER2-negative metastatic breast cancer: subgroup analyses from the Phase III OlympiAD trial. *Int J Cancer*. 2023;153(2):380–389. doi:10.1002/ijc.34525
9. Shahid U. Advances in RNA therapeutics: classes, innovations and clinical applications. *J Precision Med*. 2025;3:100016. doi:10.1016/j.premed.2025.100016
10. Slamon DJ, Diéras V, Rugo HS, et al. Overall survival with palbociclib plus letrozole in advanced breast cancer. *J Clin Oncol*. 2024;42(9):994–1000. doi:10.1200/JCO.23.00137
11. Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY. miR-21-mediated tumor growth. *Oncogene*. 2007;26(19):2799–2803. doi:10.1038/sj.onc.1210083
12. Imran K, Iqbal MJ, Ahmed MM, et al. Epigenetic dysregulation in cancer: mechanisms, diagnostic biomarkers and therapeutic strategies. *Med Oncol*. 2025;42(8):359. doi:10.1007/s12032-025-02905-z
13. Elgeshy KM, Abdel Wahab AHA. The role, significance, and association of MicroRNA-10a/b in physiology of cancer. *Microrna*. 2022;11(2):118–138. doi:10.2174/2211536611666220523104408
14. Wang Z, Peng Y, Zhou H, Zhang M, Ju D, Chen Z. MiRNA-based drugs: challenges and delivery strategies. *Appl Microbiol Biotechnol*. 2025;109(1):247. doi:10.1007/s00253-025-13620-0
15. Lee SWL, Paoletti C, Campisi M, et al. MicroRNA delivery through nanoparticles. *J Control Release*. 2019;313:80–95. doi:10.1016/j.jconrel.2019.10.007
16. Bartel DP. MicroRNA target recognition and regulatory functions. *Cell*. 2009;136(2):215–233. doi:10.1016/j.cell.2009.01.002
17. Ha M, Kim VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol*. 2014;15(8):509–524. doi:10.1038/nrm3838
18. Lin S, Gregory RI. MicroRNA biogenesis pathways in cancer. *Nat Rev Cancer*. 2015;15(6):321–333. doi:10.1038/nrc3932
19. Peng Y, Croce CM. The role of MicroRNAs in human cancer. *Signal Transduct Target Ther*. 2016;1:15004. doi:10.1038/sigtrans.2015.4
20. Diener C, Keller A, Meese E. Emerging concepts of miRNA therapeutics: from cells to clinic. *Trends Genet*. 2022;38(6):613–626. doi:10.1016/j.tig.2022.02.006
21. Otoukesh B, Abbasi M, o L GH, et al. MicroRNAs signatures, bioinformatics analysis of miRNAs, miRNA mimics and antagonists, and miRNA therapeutics in osteosarcoma. *Cancer Cell Int*. 2020;20:254. doi:10.1186/s12935-020-01342-4
22. S RES, Cristante J, Guyon L, Denis J, Chabre O, Cherradi N. MicroRNA therapeutics in cancer: current advances and challenges. *Cancers*. 2021;13(11):2680. doi:10.3390/cancers13112680
23. Chan SK, Steinmetz NF. microRNA-181a silencing by antisense oligonucleotides delivered by virus-like particles. *J Mater Chem B*. 2023;11(4):816–825. doi:10.1039/d2tb02199d
24. Preethi KA, Lakshmanan G, Sekar D. Antagomir technology in the treatment of different types of cancer. *Epigenomics*. 2021;13(7):481–484. doi:10.2217/epi-2020-0439
25. Samolovac S, Hinkel R. Locked nucleic acid AntimiR therapy for the heart. *Methods Mol Biol*. 2022;2573:159–169. doi:10.1007/978-1-0716-2707-5_12
26. Alkan AH, Akgül B. Endogenous miRNA sponges. *Methods Mol Biol*. 2022;2257:91–104. doi:10.1007/978-1-0716-1170-8_5
27. Chen L, Liang Y, Wei H, et al. NamiRNA-enhancer network: the changing era challenge and chance of miRNA. *Adv Sci*. 2025;12(39):e06830. doi:10.1002/advs.202506830
28. Saito Y, Jones PM. Epigenetic activation of tumor suppressor MicroRNAs in human cancer cells. *Cell Cycle*. 2006;5(19):2220–2222. doi:10.4161/cc.5.19.3340
29. Hermeking H. The miR-34 family in cancer and apoptosis. *Cell Death Differ*. 2010;17(2):193–199. doi:10.1038/cdd.2009.56
30. Kalinkova L, Nikolaieva N, Smolkova B, et al. miR-205-5p downregulation and ZEB1 upregulation characterize the disseminated tumor cells in patients with invasive ductal breast cancer. *Int J Mol Sci*. 2021;23(1):103. doi:10.3390/ijms23010103
31. Cavallari I, Ciccarese F, Sharova E, et al. The miR-200 family of microRNAs: fine tuners of epithelial-mesenchymal transition and circulating cancer biomarkers. *Cancers*. 2021;13(23):5874. doi:10.3390/cancers13235874
32. Yu F, Yao H, Zhu P, et al. let-7 regulates self renewal and tumorigenicity of breast cancer cells. *Cell*. 2007;131(6):1109–1123. doi:10.1016/j.cell.2007.10.054
33. Misso G, Di Martino MT, De Rosa G, et al. Mir-34: a new weapon against cancer? *Mol Ther Nucleic Acids*. 2014;3(9):e195. doi:10.1038/mtna.2014.47
34. Wen B, Zhu R, Jin H, Zhao K. Differential expression and role of miR-200 family in multiple tumors. *Anal Biochem*. 2021;626:114243. doi:10.1016/j.ab.2021.114243
35. Ouyang B, Bi M, Jadhao M, Bick G, Zhang X. miR-205 regulates tamoxifen resistance by targeting estrogen receptor coactivator MED1 in human breast cancer. *Cancers*. 2024;16(23):3992. doi:10.3390/cancers16233992
36. Maroni P, Bendinelli P, Ferraretto A, Lombardi G. Interleukin 11 (IL-11): role(s) in breast cancer bone metastases. *Biomedicines*. 2021;9(6):659. doi:10.3390/biomedicines9060659
37. Soofiyan SR, Hosseini K, Ebrahimi T, et al. Prognostic value and biological role of miR-126 in breast cancer. *MicroRNA*. 2022;11(2):95–103. doi:10.2174/1876402914666220428123203
38. Mallet JF, Shahbazi R, Alsadi N, Matar C. Polyphenol-enriched blueberry preparation controls breast cancer Stem cells by targeting FOXO1 and miR-145. *Molecules*. 2021;26(14):4330. doi:10.3390/molecules26144330
39. Qiu Z, Wang Y, Zhang Z, et al. Roles of intercellular cell adhesion molecule-1 (ICAM-1) in colorectal cancer: expression, functions, prognosis, tumorigenesis, polymorphisms and therapeutic implications. *Front Oncol*. 2022;12:1052672. doi:10.3389/fonc.2022.1052672
40. Ferracin M, Veronese A, Negrini M. Micromarkers: miRNAs in cancer diagnosis and prognosis. *Exp Rev Mol Diagnost*. 2010;10(3):297–308. doi:10.1586/erm.10.11

41. Tavazoie SF, Alarcón C, Oskarsson T, et al. Endogenous human microRNAs that suppress breast cancer metastasis. *Nature*. 2008;451(7175):147–152. doi:10.1038/nature06487
42. Di Martino MT, Tagliaferri P, Tassone P. MicroRNA in cancer therapy: breakthroughs and challenges in early clinical applications. *J Exp Clin Cancer Res*. 2025;44:126. doi:10.1186/s13046-025-03391-x
43. Qian H, Maghsoudloo M, Kaboli PJ, et al. Decoding the promise and challenges of miRNA-based cancer therapies: an essential update on miR-21, miR-34, and miR-155. *Int J Med Sci*. 2024;21(14):2781–2798. doi:10.7150/ijms.102123
44. Al Hashami ZS, van der Vegt B, Mourits MJE, Kluiver J, van den Berg A. miRNA-dependent resistance mechanisms to anti-hormonal therapies in estrogen receptor-positive breast cancer patients. *Mol Ther Oncol*. 2025;33(1):200941. doi:10.1016/j.omton.2025.200941
45. Howard EW, Yang X. microRNA regulation in estrogen receptor-positive breast cancer and endocrine therapy. *Biol Proced Online*. 2018;20:17. doi:10.1186/s12575-018-0082-9
46. Normann LS, Aure MR, Leivonen SK, et al. MicroRNA in combination with HER2-targeting drugs reduces breast cancer cell viability in vitro. *Sci Rep*. 2021;11(1):10893. doi:10.1038/s41598-021-90385-2
47. Berber U, Yilmaz I, Narli G, Haholu A, Kucukodaci Z, Demirel D. miR-205 and miR-200c: predictive micro RNAs for lymph node metastasis in triple negative breast cancer. *J Breast Cancer*. 2014;17(2):143–148. doi:10.4048/jbc.2014.17.2.143
48. Sitia L, Sevieri M, Signati L, et al. HER-2-targeted nanoparticles for breast cancer diagnosis and treatment. *Cancers*. 2022;14(10):2424. doi:10.3390/cancers14102424
49. Jahan S, MdE K, Chowdhury EH. Nanoparticles targeting receptors on breast cancer for efficient delivery of chemotherapeutics. *Biomedicines*. 2021;9(2):114. doi:10.3390/biomedicines9020114
50. Wu W, He Y, Zhang C. Emerging targeted and multimodal therapeutic strategies in breast cancer: a comprehensive review. *Breast Cancer*. 2026;18:575936. doi:10.2147/BCTT.S575936
51. Crintea A, Bocşan CI, Jianu EM, et al. Overcoming chemotherapy resistance in triple-negative breast cancer with nanocarrier-delivered siRNA therapeutics. *J Clin Med*. 2026;15(6):2311. doi:10.3390/jcm15062311
52. Panigrahi M, Thibault PA, Wilson JA. MicroRNA 122 affects both the initiation and the maintenance of hepatitis C virus infections. *J Virol*. 2022;96(4):e01903–21. doi:10.1128/jvi.01903-21
53. Zhang T, Wu Y, Yang D, Wu C, Li H. Preparation, characterization, and in vitro tumor-suppressive effect of anti-miR-21-equipped RNA nanoparticles. *Biochem Biophys Res Commun*. 2021;558:107–113. doi:10.1016/j.bbrc.2021.04.040
54. Deng X, Cao M, Zhang J, et al. Hyaluronic acid-chitosan nanoparticles for co-delivery of MiR-34a and doxorubicin in therapy against triple negative breast cancer. *Biomaterials*. 2014;35(14):4333–4344. doi:10.1016/j.biomaterials.2014.02.006
55. Devulapally R, Sekar NM, Sekar TV, et al. Polymer nanoparticles mediated codelivery of AntimiR-10b and AntimiR-21 for achieving triple negative breast cancer therapy. *ACS Nano*. 2015;9(3):2290–2302. doi:10.1021/nn507465d
56. Yan Y, Li XQ, Duan JL, et al. Nanosized functional miRNA liposomes and application in the treatment of TNBC by silencing Slug gene. *Int J Nanomed*. 2019;14:3645–3667. doi:10.2147/IJN.S207837
57. Bayraktar R, Pichler M, Kanlikilicer P, et al. MicroRNA 603 acts as a tumor suppressor and inhibits triple-negative breast cancer tumorigenesis by targeting elongation factor 2 kinase. *Oncotarget*. 2016;8(7):11641–11658. doi:10.18632/oncotarget.14264
58. Ren Y, Wang R, Gao L, et al. Sequential co-delivery of miR-21 inhibitor followed by burst release doxorubicin using NIR-responsive hollow gold nanoparticle to enhance anticancer efficacy. *J Control Release*. 2016;228:74–86. doi:10.1016/j.jconrel.2016.03.008
59. Aleixo DT, Cruz-Cazarim ELC, Ferreira KCB, et al. Breakthroughs in the nanoparticle-mediated delivery of siRNA for breast cancer treatment. *Nanomedicine*. 2025;20(22):2765–2789. doi:10.1080/17435889.2025.2567842
60. Conde J, Oliva N, Atilano M, Song HS, Artzi N. Self-assembled RNA-triple-helix hydrogel scaffold for microRNA modulation in the tumour microenvironment. *Nat Mater*. 2016;15(3):353–363. doi:10.1038/nmat4497
61. Huang Z, Gan J, Long Z, et al. Targeted delivery of let-7b to reprogramme tumor-associated macrophages and tumor infiltrating dendritic cells for tumor rejection. *Biomaterials*. 2016;90:72–84. doi:10.1016/j.biomaterials.2016.03.009
62. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov*. 2017;16(3):203–222. doi:10.1038/nrd.2016.246
63. Mollaei H, Safaralizadeh R, Rostami Z. MicroRNA replacement therapy in cancer. *J Cell Physiol*. 2019;234(8):12369–12384. doi:10.1002/jcp.28058
64. Hong DS, Kang YK, Borad M, et al. Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours. *Br J Cancer*. 2020;122(11):1630–1637. doi:10.1038/s41416-020-0802-1
65. Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. *Nat Rev Drug Discov*. 2021;20(2):101–124. doi:10.1038/s41573-020-0090-8
66. Qian X, Shi Z, Qi H, et al. A novel Granzyme B nanoparticle delivery system simulates immune cell functions for suppression of solid tumors. *Theranostics*. 2019;9(25):7616–7627. doi:10.7150/thno.35900
67. Herdiana Y, Wathoni N, Shamsuddin S, Muchtaridi M. Drug release study of the chitosan-based nanoparticles. *Heliyon*. 2022;8(1):e08674. doi:10.1016/j.heliyon.2021.e08674
68. Farhoudi L, Hosseinihah SM, Kazemi-Beydokhti A, et al. pH-sensitive polymeric micelles enhance the co-delivery of doxorubicin and docetaxel: an emerging modality for treating breast cancer. *Cancer Nano*. 2024;15(1):37. doi:10.1186/s12645-024-00275-1
69. Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat Biotechnol*. 2015;33(9):941–951. doi:10.1038/nbt.3330
70. Shrestha N, Xu Y, Prévoost JRC, et al. Impact of PEGylation on an antibody-loaded nanoparticle-based drug delivery system for the treatment of inflammatory bowel disease. *Acta Biomater*. 2022;140:561–572. doi:10.1016/j.actbio.2021.12.015
71. Bajracharya R, Song JG, Patil BR, et al. Functional ligands for improving anticancer drug therapy: current status and applications to drug delivery systems. *Drug Deliv*. 2022;29(1):1959–1970. doi:10.1080/10717544.2022.2089296
72. Shinde VR, Revi N, Murugappan S, Singh SP, Rengan AK. Enhanced permeability and retention effect: a key facilitator for solid tumor targeting by nanoparticles. *Photodiagn Photodyn Ther*. 2022;39:102915. doi:10.1016/j.pdpdt.2022.102915
73. Wu J. The enhanced Permeability and Retention (EPR) effect: the significance of the concept and methods to enhance its application. *J Pers Med*. 2021;11(8):771. doi:10.3390/jpm11080771

74. Golombek SK, May JN, Theek B, et al. Tumor targeting via EPR: strategies to enhance patient responses. *Adv Drug Deliv Rev.* 2018;130:17–38. doi:10.1016/j.addr.2018.07.007
75. Shi J, Kantoff PW, Wooster R, Farokhzad OC. Cancer nanomedicine: progress, challenges and opportunities. *Nat Rev Cancer.* 2017;17(1):20–37. doi:10.1038/nrc.2016.108
76. Xu Z, Xie Y, Chen W, Deng W. Nanocarrier-based systems for targeted delivery: current challenges and future directions. *MedComm.* 2025;6(9):e70337. doi:10.1002/mco2.70337
77. Singh D, Kumar A. Next-generation nanocarriers for precision antitumor therapy: from passive targeting to intelligent response. *Explor Target Antitumor Ther.* 2025;6:1002355. doi:10.37349/etat.2025.1002355
78. Spada A, Gerber-Lemaire S. Surface functionalization of nanocarriers with anti-EGFR ligands for cancer active targeting. *Nanomaterials.* 2025;15(3):158. doi:10.3390/nano15030158
79. Zhang X, Yan R, Wei Z, et al. Folate decorated multifunctional biodegradable nanoparticles for gastric carcinoma active targeting theranostics. *Int J Nanomed.* 2022;17:2493–2502. doi:10.2147/IJN.S348380
80. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. *Nat Rev Mater.* 2021;6(12):1078–1094. doi:10.1038/s41578-021-00358-0
81. Tenchov R, Bird R, Curtze AE, Zhou Q. Lipid nanoparticles—from liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. *ACS Nano.* 2021;15(11):16982–17015. doi:10.1021/acsnano.1c04996
82. Kulkarni JA, Cullis PR, van der Meel R. Lipid nanoparticles enabling gene therapies: from concepts to clinical utility. *Nucleic Acid Ther.* 2018;28(3):146–157. doi:10.1089/nat.2018.0721
83. Rui M, Qu Y, Gao T, Ge Y, Feng C, Xu X. Simultaneous delivery of anti-miR21 with doxorubicin prodrug by mimetic lipoprotein nanoparticles for synergistic effect against drug resistance in cancer cells. *Int J Nanomed.* 2016;12:217–237. doi:10.2147/IJN.S122171
84. Bhardwaj H, Jangde RK. Current updated review on preparation of polymeric nanoparticles for drug delivery and biomedical applications. *Next Nanotechnol.* 2023;2:100013. doi:10.1016/j.nxnano.2023.100013
85. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. *J Control Release.* 2012;161(2):505–522. doi:10.1016/j.jconrel.2012.01.043
86. Thomas M, Klibanov AM. Conjugation to gold nanoparticles enhances polyethylenimine's transfer of plasmid DNA into mammalian cells. *Proc Natl Acad Sci U S A.* 2003;100(16):9138–9143. doi:10.1073/pnas.1233634100
87. Garg T, Rath G, Goyal AK. Biomaterials-based nanofiber scaffold: targeted and controlled carrier for cell and drug delivery. *J Drug Targeting.* 2015;23(3):202–221. doi:10.3109/1061186X.2014.992899
88. Lynn DM, Langer R. Degradable poly(β -amino esters): synthesis, characterization, and self-assembly with plasmid DNA. *J Am Chem Soc.* 2000;122(44):10761–10768. doi:10.1021/ja0015388
89. Bernkop-Schnürch A, Dünhaupt S. Chitosan-based drug delivery systems. *Eur J Pharm Biopharm.* 2012;81(3):463–469. doi:10.1016/j.ejpb.2012.04.007
90. Wang S, Zhang J, Wang Y, Chen M. Hyaluronic acid-coated PEI-PLGA nanoparticles mediated co-delivery of doxorubicin and miR-542-3p for triple negative breast cancer therapy. *Nanomed Nanotechnol Biol Med.* 2016;12(2):411–420. doi:10.1016/j.nano.2015.09.014
91. Chaudhari R, Nasra S, Meghani N, Kumar A. MiR-206 conjugated gold nanoparticle based targeted therapy in breast cancer cells. *Sci Rep.* 2022;12(1):4713. doi:10.1038/s41598-022-08185-1
92. Lin G, Revia RA, Zhang M. Inorganic nanomaterial-mediated gene therapy in combination with other antitumor treatment modalities. *Adv Funct Mater.* 2021;31(5):2007096. doi:10.1002/adfm.202007096
93. Mukherjee A, Waters AK, Kalyan P, Achrol AS, Kesari S, Yenugonda VM. Lipid-polymer hybrid nanoparticles as a next-generation drug delivery platform: state of the art, emerging technologies, and perspectives. *Int J Nanomed.* 2019;14:1937–1952. doi:10.2147/IJN.S198353
94. Sarma H, Dutta A, Bharali A, et al. pH sensitive lipid polymeric hybrid nanoparticle (LPHNP) of paclitaxel and curcumin for targeted delivery in breast cancer. *Drug Dev Ind Pharm.* 2024;50(10):856–864. doi:10.1080/03639045.2024.2421198
95. Moraes FC, Pichon C, Letourneur D, Chaubet F. miRNA delivery by nanosystems: state of the art and perspectives. *Pharmaceutics.* 2021;13(11):1901. doi:10.3390/pharmaceutics13111901
96. Xu S, Hu Z, Song F, Xu Y, Han X. Lipid nanoparticles: composition, formulation, and application. *Mol Ther Meth Clin Develop.* 2025;33(2):101463. doi:10.1016/j.omtm.2025.101463
97. Lee Y, Jeong M, Park J, Jung H, Lee H. Immunogenicity of lipid nanoparticles and its impact on the efficacy of mRNA vaccines and therapeutics. *Exp Mol Med.* 2023;55(10):2085–2096. doi:10.1038/s12276-023-01086-x
98. Huang HC, Barua S, Sharma G, Dey SK, Rege K. Inorganic nanoparticles for cancer imaging and therapy. *J Control Release.* 2011;155(3):344–357. doi:10.1016/j.jconrel.2011.06.004
99. Wang X, Zhong X, Li J, Liu Z, Cheng L. Inorganic nanomaterials with rapid clearance for biomedical applications. *Chem Soc Rev.* 2021;50(15):8669–8742. doi:10.1039/d0cs00461h
100. Jain S, Kumar M, Kumar P, et al. Lipid-polymer hybrid nanosystems: a rational fusion for advanced therapeutic delivery. *J Funct Biomaterials.* 2023;14(9):437. doi:10.3390/jfb14090437
101. Rajana N, Mounika A, Chary PS, et al. Multifunctional hybrid nanoparticles in diagnosis and therapy of breast cancer. *J Control Release.* 2022;352:1024–1047. doi:10.1016/j.jconrel.2022.11.009
102. Cullis PR, Hope MJ. Lipid nanoparticle systems for enabling gene therapies. *Mol Ther.* 2017;25(7):1467–1475. doi:10.1016/j.ymthe.2017.03.013
103. Li L, Xie X, Luo J, et al. Targeted expression of miR-34a using the T-VISA system suppresses breast cancer cell growth and invasion. *Mol Ther.* 2012;20(12):2326–2334. doi:10.1038/mt.2012.201
104. Pecot CV, Rupaimoole R, Yang D, et al. Tumour angiogenesis regulation by the miR-200 family. *Nat Commun.* 2013;4:2427. doi:10.1038/ncomms3427
105. Kapadia CH, Ioele SA, Day ES. Layer-by-layer assembled PLGA nanoparticles carrying miR-34a cargo inhibit the proliferation and cell cycle progression of triple-negative breast cancer cells. *J Biomed Mater Res Part A.* 2020;108(3):601–613. doi:10.1002/jbm.a.36840
106. Akinc A, Thomas M, Klibanov AM, Langer R. Exploring polyethylenimine-mediated DNA transfection and the proton sponge hypothesis. *J Gene Med.* 2005;7(5):657–663. doi:10.1002/jgm.696

107. Ahir M, Upadhyay P, Ghosh A, et al. Delivery of dual miRNA through CD44-targeted mesoporous silica nanoparticles for enhanced and effective triple-negative breast cancer therapy. *Biomater Sci*. 2020;8(10):2939–2954. doi:10.1039/d0bm00015a
108. Bader AG, Brown D, Winkler M. The promise of MicroRNA replacement therapy. *Cancer Res*. 2010;70(18):7027–7030. doi:10.1158/0008-5472.CAN-10-2010
109. Kaczmarek JC, Kowalski PS, Anderson DG. Advances in the delivery of RNA therapeutics: from concept to clinical reality. *Genome Med*. 2017;9:60. doi:10.1186/s13073-017-0450-0
110. Mendes BB, Conniot J, Avital A, et al. Nanodelivery of nucleic acids. *Nat Rev Meth Primers*. 2022;2:24. doi:10.1038/s43586-022-00104-y
111. Ameta RK, Soni K, Bhattarai A. Recent advances in improving the bioavailability of hydrophobic/lipophilic drugs and their delivery via self-emulsifying formulations. *Colloids Interfaces*. 2023;7(1):16. doi:10.3390/colloids7010016
112. Passos Gibson V, Tahiri H, Yang C, Phan QT, Banquy X, Hardy P. Hyaluronan decorated layer-by-layer assembled lipid nanoparticles for miR-181a delivery in glioblastoma treatment. *Biomaterials*. 2023;302:122341. doi:10.1016/j.biomaterials.2023.122341
113. Deuker MFS, Mailänder V, Morsbach S, Landfester K. Anti-PEG antibodies enriched in the protein Corona of PEGylated nanocarriers impact the cell uptake. *Nanoscale Horiz*. 2023;8(10):1377–1385. doi:10.1039/d3nh00198a
114. Gangopadhyay S, Nikam RR, Gore KR. Folate receptor-mediated siRNA delivery: recent developments and future directions for RNAi therapeutics. *Nucleic Acid Therapeutics*. 2021;31(4):245–270. doi:10.1089/nat.2020.0882
115. Harush-Frenkel O, Debotton N, Benita S, Altschuler Y. Targeting of nanoparticles to the clathrin-mediated endocytic pathway. *Biochem Biophys Res Commun*. 2007;353(1):26–32. doi:10.1016/j.bbrc.2006.11.135
116. Zhao M, Zhou L, Zhai Y, Sun A, Shao G, Lin Q. Macropinocytosis: both a target and a tool for cancer therapy. *Biomolecules*. 2025;15(7):936. doi:10.3390/biom15070936
117. Hwang ME, Keswani RK, Pack DW. Dependence of PEI and PAMAM gene delivery on clathrin- and caveolin-dependent trafficking pathways. *Pharm Res*. 2015;32(6):2051–2059. doi:10.1007/s11095-014-1598-6
118. Hooshmand SE, Jahanpeimay Sabet M, Hasanzadeh A, et al. Histidine-enhanced gene delivery systems: the state of the art. *J Gene Med*. 2022;24(5):e3415. doi:10.1002/jgm.3415
119. Suk JS, Xu Q, Kim N, Hanes J, Ensign LM. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Adv Drug Deliv Rev*. 2016;99(Pt A):28–51. doi:10.1016/j.addr.2015.09.012
120. Serpico L, Zhu Y, Maia RF, Sumedha S, Shahbazi MA, Santos HA. Lipid nanoparticles-based RNA therapies for breast cancer treatment. *Drug Deliv Transl Res*. 2024;14(10):2823–2844. doi:10.1007/s13346-024-01638-2
121. Ahmed T, Liu FCF, Wu XY. An update on strategies for optimizing polymer-lipid hybrid nanoparticle-mediated drug delivery: exploiting transformability and bioactivity of PLN and harnessing intracellular lipid transport mechanism. *Expert Opin Drug Delivery*. 2024;21(2):245–278. doi:10.1080/17425247.2024.2318459
122. Gameiro M, Mano JF, Gaspar VM. Emerging lipid–polymer hybrid nanoparticles for genome editing. *Polym Chem*. 2024;15(34):3436–3468. doi:10.1039/d4py00298a
123. Kulkarni JA, Witzigmann D, Leung J, Tam YYC, Cullis PR. On the role of helper lipids in lipid nanoparticle formulations of siRNA. *Nanoscale*. 2019;11(45):21733–21739. doi:10.1039/c9nr09347h
124. Lou Y, Wang Y, Lu J, Chen X. MicroRNA-targeted nanoparticle delivery systems for cancer therapy: current status and future prospects. *Nanomedicine*. 2025;20(10):1181–1194. doi:10.1080/17435889.2025.2492542
125. Verma A, Patel K, Kumar A. Targeting drug resistance in breast cancer: the potential of miRNA and nanotechnology-driven delivery systems. *Nanoscale Adv*. 2024;6(24):6079–6095. doi:10.1039/d4na00660g
126. Bisht A, Bhowmik S, Patel P, Gupta GD, Kurmi BD. Aptamer as a targeted approach towards treatment of breast cancer. *J Drug Targeting*. 2024;32(5):510–528. doi:10.1080/1061186X.2024.2333866
127. Swain SM, Shastri M, Hamilton E. Targeting HER2-positive breast cancer: advances and future directions. *Nat Rev Drug Discov*. 2023;22(2):101–126. doi:10.1038/s41573-022-00579-0
128. Raj A, Chandran CS, Dua K, Kamath V, Alex AT. Targeting overexpressed surface proteins: a new strategy to manage the recalcitrant triple-negative breast cancer. *Eur J Pharmacol*. 2024;981:176914. doi:10.1016/j.ejphar.2024.176914
129. Ahmadi M, Ritter CA, Von Woedtke T, Bekeschus S, Wende K. Package delivered: folate receptor-mediated transporters in cancer therapy and diagnosis. *Chem Sci*. 2024;15(6):1966–2006. doi:10.1039/d3sc05539f
130. Yang Y, Ning H, Zhu H, et al. A supramolecular nanoengine generates nanomechanical force on demand for precise cytosolic delivery of Anti-miRNAs and synergistic TNBC therapy. *Adv Mater*. 2025;37(14):2419651. doi:10.1002/adma.202419651
131. Sindhvani S, Syed AM, Ngai J, et al. The entry of nanoparticles into solid tumours. *Nat Mater*. 2020;19(5):566–575. doi:10.1038/s41563-019-0566-2
132. Azimi S. Intelligent nanoparticle design: unlocking the potential of AI for transformative drug delivery. *Curr Opin Biomed Eng*. 2025;36:100625. doi:10.1016/j.cobme.2025.100625
133. Dorsey PJ, Lau CL, Chang TC, Doerschuk PC, D'Addio SM. Review of machine learning for lipid nanoparticle formulation and process development. *J Pharmaceut Sci*. 2024;113(12):3413–3433. doi:10.1016/j.xphs.2024.09.015
134. Sun H, Li Y, Xue M, Feng D. Tumor microenvironment-responsive nanoparticles: promising cancer PTT carriers. *Int J Nanomed*. 2025;20:7987–8001. doi:10.2147/IJN.S526497
135. Liu Y, Wang Y, Gemingnuer A, Wang H, Meng X. Tumor-responsive nanomedicines for cancer therapy: design principles and therapeutic advances. *Biomed Mater*. 2025;20(5):052001. doi:10.1088/1748-605X/adb387
136. Beg MS, Brenner AJ, Sachdev J, et al. Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. *Invest New Drugs*. 2017;35(2):180–188. doi:10.1007/s10637-016-0407-y
137. Van Zandwijk N, Pavlakis N, Kao SC, et al. Safety and activity of microRNA-loaded minicells in patients with recurrent malignant pleural mesothelioma: a first-in-man, phase 1, open-label, dose-escalation study. *Lancet Oncol*. 2017;18(10):1386–1396. doi:10.1016/S1470-2045(17)30621-6
138. Jayaraj S, Jayaprakash N, Devaraji M, Murugavel S, Robinson AX, Murugesan L. Nanocarrier-based strategies for advanced cancer drug delivery and therapeutics: design principles, biological interactions, and clinical potential. *Nano Trends*. 2026;14:100203. doi:10.1016/j.nwnano.2026.100203
139. Di Leva G, Garofalo M, Croce CM. microRNAs in cancer. *Annu Rev Pathol*. 2014;9:287–314. doi:10.1146/annurev-pathol-012513-104715

140. Gupta T, Vinayak S, Telli M. Emerging strategies: PARP inhibitors in combination with immune checkpoint blockade in BRCA1 and BRCA2 mutation-associated and triple-negative breast cancer. *Breast Cancer Res Treat.* 2023;197(1):51–56. doi:10.1007/s10549-022-06780-4
141. Pandey K, An H, Kim SK, et al. Molecular mechanisms of resistance to CDK4/6 inhibitors in breast cancer: a review. *Int J Cancer.* 2019;145(5):1179–1188. doi:10.1002/ijc.32020
142. Debien V, De caluwé A, Wang X, et al. Immunotherapy in breast cancer: an overview of current strategies and perspectives. *Npj Breast Cancer.* 2023;9(1):7. doi:10.1038/s41523-023-00508-3

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