Review Article
DOI: https://doi.org/10.70749/ijbr.v3i11.2608

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://ijbr.com.pk
ISSN: 2960-2793/ 2960-2807

MicroRNAs as Oncogenes or Tumor Suppressors in Breast Cancer

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ARTICLE INFO

Keywords: Tumor, Oncogenic miRNAs, Exosomal miRNA, CRISPR, Breast Cancer.

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Declaration
Authors' Contribution
All authors equally contributed to the study and approved the final manuscript

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History
Received: 24-08-2025 Revised: 04-11-2025
Accepted: 13-11-2025 Published: 25-11-2025

ABSTRACT

MicroRNAs (miRNAs) are increasingly recognized as key regulators of gene expression in breast cancer as either an oncomir (oncogene) or a tumor suppressor. This article aims to provide an overview of the conflicting roles of miRNAs in breast cancer initiation and progression and response to treatment. Oncogenic miRNAs, such as miR-21, miR-155, and miR-221/222, promote tumorigenesis by targeting primary tumor suppressor pathways, while antitumor miRNAs (tumor suppressors),, including the miR-34 family, miR-200 family and miR-145, inhibit cancer progression by modulating oncogenic signaling networks. We will examine miRNA biogenesis pathways and miRNAs' context-dependent roles, including diagnostic biomarkers and therapeutic targets. We conclude with a discussion on subtype-specific signatures of miRNAs, exosomal miRNA content and emerging technologies, such as single-cell miRNA sequencing and CRISPR-based editing that will enhance understanding of the biology of miRNAs in breast cancer.

INTRODUCTION

As the second most common cause of cancer-related death and the most common cancer among women worldwide, breast cancer continues to pose a serious threat to global health. Recent global cancer statistics show that there are about 2.3 million new cases and 685,000 deaths per year, and that incidence rates are still rising, especially in developing countries that are rapidly modernizing (1). There are several molecular subtypes of the disease, each with unique clinical behaviors, treatment responses, and outcomes. The disease is remarkably heterogeneous. Despite significant advancements in early detection and therapeutic interventions, this heterogeneity, along with the emergence of therapy resistance and metastatic progression, remains a major barrier to successful treatment outcomes.

Our knowledge of cancer biology has been completely altered by the discovery of microRNAs (miRNAs), which unveil a completely new level of post-transcriptional gene regulation. These tiny, non-coding RNA molecules, which are usually 19-25 nucleotides long, bind to complementary sequences mainly found in the 3' untranslated regions (3'UTRs) of target mRNAs to act as master regulators of gene expression. Protein production from targeted transcripts is effectively adjusted by this interaction, which usually results in either translational repression or mRNA degradation (2). Thousands of miRNAs are encoded by the human genome, and over 60% of all protein-coding genes are thought to be regulated by miRNAs, underscoring their ubiquitous function in maintaining cellular homeostasis.

MiRNAs are remarkably dualistic in the context of breast cancer, acting as either important tumor suppressors that prevent malignant transformation or powerful oncogenes (oncomiRs) that propel tumor progression (3). This functional dichotomy has significant implications for the pathophysiology, progression, and

response to treatment of breast cancer, making it more than just an academic concept. When overexpressed, oncomiRs can target several tumor suppressor genes at once, resulting in coordinated oncogenic programs that stimulate angiogenesis, increase invasion and metastasis, prevent apoptosis, and promote cell proliferation. On the other hand, when tumor suppressor miRNAs are lost or downregulated, oncogenic pathways are stopped, allowing unchecked growth and progression (4).

Beyond their basic biological functions, the strong case for concentrating on miRNAs in breast cancer research encompasses a number of distinctive features that make them especially appealing for clinical translation. First, miRNAs are excellent candidates for biomarker development because of their exceptional stability in clinical specimens, such as formalin-fixed paraffin-embedded tissues, blood, urine, and other bodily fluids. Second, they show unique expression patterns among various molecular subtypes of breast cancer, offering chances for better categorization and individualized treatment strategies. Third, they are master regulators of cancer hallmarks due to their capacity to control several genes within biological pathways, providing opportunities for therapeutic intervention at crucial network nodes (5).

The intricate and varied roles of miRNAs in the pathophysiology of breast cancer are methodically examined in this thorough review, with a focus on their mechanisms of action, clinical uses, and potential future treatments. We will examine the complex biogenesis pathways that control the maturation of miRNAs, the molecular mechanisms that underlie their role as tumor suppressors or oncogenes, and the mounting evidence that supports their use as predictive, prognostic, and diagnostic biomarkers. We will also go over the fascinating possibilities of miRNA-based treatments and the technological developments such as single-cell analysis, spatial transcriptomics, and artificial intelligence techniques that are advancing this field. We hope to provide a comprehensive understanding of how these tiny but potent regulatory molecules are changing how we diagnose, treat, and prevent breast cancer through this in-depth analysis.

2. Biogenesis and Function of Micrornas

2.1 Canonical miRNA Biogenesis Pathway

The precise maturation of functional miRNA molecules is ensured by the intricate multi-step canonical miRNA biogenesis pathway. RNA polymerase II-mediated transcription of primary miRNA transcripts (pri-miRNAs), which can have lengths ranging from hundreds to thousands of nucleotides and frequently contain hairpin structures, is the first step in this pathway. The RNase III enzyme Drosha and its necessary cofactor DGCR8 (DiGeorge syndrome critical region gene 8) make up the microprocessor complex, which recognizes and processes these pri-miRNAs. The pre-miRNA, which has a stem-loop structure and is about 70 nucleotides long, is released when this complex cleaves the pri-miRNA (6).

Exportin-5 then exports the pre-miRNA in a Ran-GTP-dependent fashion from the nucleus to the cytoplasm. After entering the cytoplasm, Dicer, another RNase III enzyme, cleaves the terminal loop of the pre-miRNA to create a temporary miRNA duplex with about 22 nucleotides. The passenger strand (miRNA), which is usually degraded, and the guide strand (the future mature miRNA) make up this duplex. Target recognition and silencing are made easier by the core Argonaute (Ago) proteins in the RNA-induced silencing complex (RISC), where the guide strand is specifically loaded (7).

2.2 Non-canonical miRNA Biogenesis Pathways

The complexity of miRNA regulation has increased with the discovery of multiple non-canonical miRNA biogenesis pathways. Mirtrons are a significant class of non-canonical miRNAs that come from intronic sequences and are produced by debranching and splicing processes that totally avoid Drosha processing. Like canonical pre-miRNAs, these pre-mirtrons are exported by Exportin-5 and processed by Dicer (8).

Other non-canonical pathways include endogenous short hairpin RNAs (endo-shRNAs), which are transcribed from particular genomic loci and processed similarly to canonical miRNAs but with unique characteristics, and Dicer-independent miRNAs, which depend on Ago2-mediated cleavage for maturation. The variety of miRNA biogenesis mechanisms is further increased by the fact that some miRNAs are produced from small nucleolar RNA (snoRNA) precursors. These alternative pathways may have particular implications for the pathophysiology of breast cancer and demonstrate the evolutionary flexibility in miRNA production (9).

2.3 Regulation of miRNA Biogenesis

Breast cancer can cause disruptions to the multiple layers of regulation that govern the miRNA biogenesis pathway. Several transcription factors directly control the expression of particular miRNA genes, such as MYC, p53, and estrogen receptor alpha. MiRNA expression patterns are strongly influenced by epigenetic mechanisms, specifically DNA methylation and histone modifications. For example, oncomiR promoters are overexpressed when they are hypomethylated, whereas tumor suppressor miRNA promoters are silenced when they are hypermethylated (10).

In breast cancer, the fundamental microprocessor components themselves are controlled. Aggressive breast cancer subtypes have been shown to have decreased Dicer and Drosha expression, which is associated with a poor prognosis and increased metastatic potential. The regulatory network is further complicated by the fact that different RNA-binding proteins can alter miRNA processing and stability. Processing efficiency and specificity can also be affected by post-translational modifications of biogenesis machinery components and the presence of particular co-factors (11).

2.4 Mechanisms of miRNA-Mediated Gene Silencing

Through imperfect base pairing with target mRNAs, mainly in the 3'UTR, miRNAs control gene expression. The mechanism of silencing is determined by the degree of complementarity between the target and the miRNA seed region (nucleotides 2-8). Ago2-mediated endonucleolytic cleavage and target mRNA degradation result from perfect or nearly perfect complementarity; this mechanism is

more prevalent in plants but can also occur for certain mammalian miRNA targets. More frequently, imperfect complementarity causes translational repression via a variety of mechanisms, such as degradation of the developing polypeptide, inhibition of translation initiation, or prevention of ribosomal subunit joining (12).

Additional mechanisms of miRNA-mediated regulation, such as binding to coding sequences, 5'UTRs, or gene promoters, have been identified by recent research. Through deadenylation, which is facilitated by the CCR4-NOT complex and results in quick degradation, miRNAs can also affect the stability of target mRNA. Moreover, miRNAs have the ability to separate target mRNAs into stress granules or processing bodies (P-bodies), where they may be stored, broken down, or eventually released for translation under particular circumstances (13).

2.5 Factors Affecting miRNA Expression and Function in Breast Cancer

In breast cancer, aberrant miRNA expression and function are caused by a number of different mechanisms. MiRNA expression levels can be significantly changed by genetic changes, such as amplification, deletion, or mutation of miRNA genes or their regulatory elements. Another important regulatory layer is epigenetic modifications; in breast cancer cells, oncomiRs are activated by hypomethylation and tumor suppressor miRNAs are often silenced by DNA hypermethylation (14).

Through a number of mechanisms, the tumor microenvironment has a significant impact on miRNA expression. Stromal-epithelial interactions result in reciprocal miRNA regulation, inflammatory cytokines can alter miRNA expression via NF-κB signaling, and hypoxia induces particular miRNA subsets through HIF-1α activation. Another important mechanism is exosomal miRNA exchange, in which breast cancer cells release and absorb miRNA-containing exosomes to alter the behavior of nearby or distant cells, promoting the development of pre-metastatic niches and the spread of metastases (15).

Furthermore, miRNA function and breast cancer susceptibility may be impacted by single nucleotide polymorphisms (SNPs) in miRNA genes, their binding sites, or biogenesis machinery components. Another level of regulatory complexity in the pathophysiology of breast cancer can be created by altered expression of competing endogenous RNAs (ceRNAs), such as circular RNAs and long non-coding RNAs, which can sequester miRNAs and stop them from interacting with their natural targets (16).

2.6 miRNA Target Recognition and Specificity

Beyond seed region pairing, several other factors control the specificity of miRNA targeting. Targeting efficiency can be affected by additional pairing in the 3' region of the miRNA, target site accessibility, local RNA structure, and the existence of RNA-binding proteins. Furthermore, the functional results of miRNA-mediated regulation are strongly influenced by the abundance of both miRNAs and their targets as well as the cellular context (17).

The intricacy of miRNA target networks in breast cancer cells has been made clear by recent developments in high-throughput techniques, such as CLIP-seq and related methods. These studies show that a single miRNA can control hundreds of targets, frequently within coordinated biological pathways, and that the same miRNA can perform a variety of roles based on target availability, cellular context, and cooperating regulatory factors(18). Understanding the complex roles of miRNAs in the biology of breast cancer and creating successful miRNA-based treatments depend on this network-level knowledge (19).

3. Oncogenic microRNAs (OncomiRs) in Breast Cancer

3.1 Criteria Defining an OncomiR

OncomiRs are characterized by a number of important traits: (1) their expression is higher in tumor tissues than in normal counterparts; (2) experimental inhibition results in decreased proliferation, increased apoptosis, or impaired metastasis; (3) their overexpression in normal or premalignant cells encourages transformation; and (4) they target genuine tumor suppressor genes or pathways. Gain-of-function and loss-of-function methods are usually needed for functional validation across several model systems (20).

3.2 Major OncomiRs and Their Targets

Targeting several tumor suppressors, such as PDCD4, PTEN, and TPM1, miR-21 is the most thoroughly researched oncomiR in breast cancer. Across all molecular subtypes, its overexpression is associated with poor survival, metastasis, and advanced stage [19]. The BIC gene contains miR-155, which promotes inflammation and cell survival by targeting SOCS1, SHIP1, and TP53INP1. Targeting p27/Kip1 and PTEN, the miR-221/222 cluster increases proliferation and therapy resistance, especially in triple-negative breast cancer (TNBC). By inhibiting HOXD10, miR-10b promotes metastasis by allowing pro-metastatic genes like RHOC to be expressed. By focusing on CD44 and LATS2, miR-373 encourages migration and invasion; its effects are especially potent in breast cancers that are estrogen receptor-negative (21).

3.3 Mechanisms by Which OncomiRs Promote Tumorigenesis

Through a variety of mechanisms, oncomiRs promote the development of breast cancer. By focusing on pro-apoptotic proteins (like BIM, which is targeted by miR-17-92) and cell cycle inhibitors (like p21, which is targeted by miR-106b), they increase cell survival and proliferation. Repression of E-cadherin transcriptional repressors (e.g., ZEB1 targeted by the miR-200 family) and activation of invasion programs promote the epithelial-mesenchymal transition (EMT) and metastasis. Targeting anti-angiogenic factors such as TSP1 (targeted by miR-194) and THBS1 (targeted by miR-17-92) stimulates angiogenesis. MiRNAs that alter the tumor microenvironment, such as miR-146a, which inhibits T-cell activation, and miR-222, which prevents natural killer cell cytotoxicity, aid immune escape (22).

3.4 Subtype-Specific OncomiRs

Molecular subtypes of breast cancer display unique oncomiR signatures. Estrogen signaling pathways are modulated by miR-342 and miR-199a, which are often overexpressed in luminal A/B cancers. MiR-4728 and miR-301a are enriched in HER2-positive tumors, which improve HER2 signaling and trastuzumab resistance. TNBC exhibits upregulation of miR-221/222, miR-155,

and miR-182, which contribute to its stem cell characteristics and aggressive phenotype (23). In addition to reflecting unique pathogenesis, these subtype-specific patterns present chances for subtype-selective therapeutic targeting.

3.5 OncomiR Crosstalk with Key Pathways

In breast cancer, oncomiRs have a significant impact on key signaling pathways. Many miRNAs control the PI3K/Akt/mTOR pathway, such as miR-21, which targets PTEN, and miR-155, which represses INPP5D. MiR-21 modulates MAPK signaling by targeting SPRY2, while miR-221 modulates it by repressing KIT. miR-181a (SMAD7) and miR-106b-25 cluster (CDKN1A) target elements of the TGF-β/Smad pathway. miR-206, which specifically targets ESR1, and the miR-17-92 cluster, which controls AIB1, both fine-tune estrogen receptor signaling (24). These complex networks produce feedback loops that strengthen cancer cells and enhance oncogenic signaling.

4. Tumour Suppressor microRNAs in Breast Cancer: Guardians of Cellular Integrity

4.1 Defining Features and Identification of Tumour-Suppressive miRNAs

Tumor suppressor miRNAs represent an important class of regulatory molecules that serve as molecular brakes on cell proliferation and transformation. They have several distinctive features: (1) their expression is significantly diminished or nonexistent in tumor specimens compared to normal counterparts; (2) their experimental re-expression in cancer cells diminishes malignant traits, promotes apoptosis or cell cycle arrest, and inhibits proliferation; (3) their deletion or downregulation promotes transformation in experimental models; and (4) they can target specific oncogenes or essential components of oncogenic pathways (25).

Finding true tumor suppressor miRNAs necessitates thorough validation using a variety of techniques. The genetic and epigenetic mechanisms underlying their downregulation are established through loss-of-heterozygosity studies, promoter methylation analyses, and copy number variation evaluations. In vitro and in vivo methods, such as miRNA mimic transfection, transgenic animal models, and xenograft studies, are commonly used for functional validation. New tumor suppressor miRNAs in breast cancer have been found more quickly thanks to high-throughput screening techniques like miRNA library transfection followed by phenotypic analysis (26).

4.2 Major Tumour-Suppressor miRNAs and Their Molecular Networks

The miR-34 family consists of miR-34a, miR-34b, and miR-34c, which is a pivotal tumor suppressor network in breast cancer, as they are direct transcriptional targets of p53. The miRNAs collectively regulate different oncogenic pathways by targeting BCL2 (anti-apoptotic), MET (receptor tyrosine kinase), CDK4/6 (cells cycle progression), SIRT1 (p53 deacetylase), and NOTCH1 (stemness pathway). The loss of miR-34 family members was implicated in the mechanism of drug resistance and increased metastatic capacity in triple-negative breast cancer (TNBC); however, expression of miR-34a in luminal breast cancers was shown to correlate with better response to endocrine therapy (27).

The miR-200 Family: This family, which includes miR-200a, miR-200b, miR-200c, miR-141, and miR-429, is a master regulator of epithelial phenotype. By specifically targeting ZEB1 and ZEB2, transcriptional repressors of E-cadherin, they preserve epithelial integrity. By forming a double-negative feedback loop with ZEB factors, the miR-200 family produces bistable switches that regulate the epithelial-mesenchymal transition (EMT). Beyond controlling EMT, they affect several facets of the development of breast cancer by targeting BMI1 (polycomb protein), SEC23A (vesicle trafficking), and CXCL1 (chemokine) (28).

The let-7 Family: Members of the let-7 family, which were among the first miRNAs to be identified, target several oncogenes, such as RAS, HMGA2, MYC, and LIN28, to act as powerful tumor suppressors. In breast cancer stem cells, let-7 expression is often lost, which increases the cells' capacity for self-renewal and resistance to treatment. Remarkably, another regulatory circuit that is dysregulated in aggressive breast cancers is created by the reciprocal inhibition of let-7 biogenesis by the RNA-binding protein LIN28 (29).

miR-125 Family: Targeting HER2/ERBB2 and HER3/ERBB3, miR-125a and miR-125b are especially important in HER2-positive breast cancer. A poor prognosis and trastuzumab resistance are associated with decreased miR-125b expression. In addition to HER signaling, they exhibit pleiotropic tumor suppressor activities by controlling ETS1 (transcription factor), ENPEP (angiogenesis), and Bak1 (apoptosis) (30).

Additional Key Tumour Suppressor miRNAs: miR-145 effectively suppresses the characteristics of cancer stem cells by targeting SOX2, OCT4, and MUC1. miR-205 targets ZEB1 and HER3 to preserve epithelial integrity. miR-31 targets integrin-α5, WAVE3, and RhoA to prevent metastasis. miR-22 targets CD44 and SNAI1 to control EMT and stemness. By specifically targeting ESR1, miR-206 modifies estrogen receptor signaling (31).

4.3 Mechanisms of Tumour Suppression

Tumor suppressor miRNAs prevent the spread of cancer by coordinating the regulation of several key features:

Cell Cycle Control: By targeting cyclins (CCND1, CCNE1), CDKs (CDK4, CDK6), and CDK activators (CDC25A), miRNAs such as miR-15/16, miR-34, and miR-449 enforce cell cycle arrest. Additionally, miR-15/16 targets WNT3A and BCL2, connecting the regulation of apoptosis and proliferation (32).

Apoptosis Induction: By focusing on anti-apoptotic proteins, several tumor suppressor miRNAs encourage programmed cell death. miR-205 sensitizes cells to apoptotic stimuli by targeting HER3 and LYN, miR-15/16 targets BCL2, and miR-34 targets SIRT1 and BCL2.

Metastasis Suppression: Several stages of the metastatic cascade are inhibited by tumor suppressor miRNAs. EMT initiation is prevented by the miR-200 family and miR-205, invasion and angiogenesis are inhibited by miR-31 and

miR-126, and colonization at distant sites is suppressed by miR-335 and miR-206 (33).

Stemness Inhibition: Stemness factors like BMI1, SOX2, and KLF4 are targeted by miRNAs like miR-200c, miR-203, and miR-600. By specifically targeting SOX2 and OCT4, miR-140 and miR-145 control the populations of breast cancer stem cells.

DNA Repair Regulation MiR-96 controls RAD51, miR-182 targets BRCA1, and miR-373 affects DNA damage response by targeting RAD23B. These rules have the potential to establish artificially lethal connections with agents that damage DNA (34).

4.4 Subtype-Specific Tumour Suppressor miRNAs

Tumor suppressor miRNA expression and functional significance differ significantly among molecular subtypes of breast cancer:

Luminal Subtypes: miR-342 and miR-26a/b, which typically limit estrogen signaling and cell cycle progression, are specifically lost in ER-positive cancers. Tamoxifen sensitivity is increased and proliferation is suppressed when these miRNAs are restored. Targeting metadherin (MTDH) and YAP1, miR-375 is often downregulated in luminal cancers and affects the response to endocrine therapy (35).

HER2-Positive Breast Cancer: These tumors show significant suppression of miR-125a/b, which increases trastuzumab resistance and HER2/3 signaling. Restoring miR-205, which targets HER3 and ZEB1 and is often lost in HER2-positive cancers, makes cells more susceptible to HER2-targeted treatments.

Triple-Negative Breast Cancer: The most significant changes in tumor suppressor miRNA expression are seen in TNBC. This subtype's mesenchymal phenotype, stemness characteristics, and resistance to treatment are all influenced by the global downregulation of the miR-200, miR-34, let-7, and miR-205 families. Restoring these miRNAs specifically reverses aggressive characteristics and makes TNBC more responsive to traditional treatments (36).

4.5 Clinical Implications and Therapeutic Potential

These miRNAs' tumor suppressor properties have significant clinical ramifications. Prognosis, treatment response, and metastatic propensity are all correlated with their expression patterns. Restoring tumor suppressor functions through miRNA-based therapies is an emerging field. A number of strategies are being developed:

Synthetic double-stranded RNA molecules created to resemble natural tumor suppressor miRNAs are known as miRNA mimics. A liposomal miR-34a mimic called MRX34 showed preclinical efficacy but encountered difficulties in clinical trials because of immune-related side effects. Next-generation mimics with enhanced delivery mechanisms and chemical alterations are being developed (37).

Gene Therapy Methods: Tumor suppressor miRNA-expressing viral vectors may provide long-term expression. In preclinical breast cancer models, adeno-associated viruses (AAVs) that expressed miR-26a demonstrated effectiveness.

Small Molecule Activators: Substances that target the transcriptional regulators or epigenetic modifiers of tumor suppressor miRNAs to increase their endogenous expression. For example, HDAC inhibitors can reactivate the expression of miR-34 and miR-15/16.

Combination Strategies: Tumor suppressor miRNA restoration exhibits synergistic effects when paired with targeted agents or traditional therapies. While miR-200 restoration improves chemotherapy response in TNBC, miR-34 mimics increase the effectiveness of PARP inhibitors in BRCA-mutant models (38).

5. The Dual Role: miRNAs with Context-Dependent Functions

5.1 Paradigms of miRNA Context-Dependency

Since many miRNAs exhibit context-dependent functions, the functional categorization of miRNAs as exclusively oncogenic or tumor suppressive is oversimplified. The intricacy of gene regulatory networks and the impact of the cellular environment on miRNA activity are reflected in this plasticity.

This duality is best illustrated by miR-205, which targets ZEB1, HER3, and E2F1 and primarily acts as a tumor suppressor in the majority of breast cancer contexts. However, by suppressing PTEN and activating AKT, miR-205 can increase survival and therapy resistance in particular microenvironments or molecular subtypes. The cellular context, which includes the relative expression of target genes and cooperating signaling pathways, determines how these opposing functions are balanced (39).

Similar complex behavior is shown by miR-7, which inhibits proliferation and survival signaling in many breast cancer models by acting as a tumor suppressor by targeting EGFR, RAF1, and AKT. On the other hand, miR-7 can encourage invasion and metastasis in advanced metastatic settings or in particular genetic backgrounds by inhibiting genes like FAK and PXN that are involved in cell adhesion and cytoskeletal organization.

By targeting ERBB2 and ERBB3 in HER2-positive breast cancer, miR-125b exhibits subtype-specific functionality. In other contexts, however, it may promote stemness and therapy resistance through alternative target regulation.

5.2 Molecular Determinants of Dual Functionality

Multiple factors contribute to the context-dependent behavior of miRNAs:

Cellular Compartmentalization: Functional outcomes are strongly influenced by the subcellular localization of miRNAs and their targets. Context-specific effects include target mRNA localization, nuclear-cytoplasmic shuttling of miRNAs, and the presence of RNA-binding proteins that alter miRNA accessibility (40).

Target Abundance and Affinity: Competitive interactions are produced by the relative expression levels of various target mRNAs and their binding affinities for a particular miRNA. The miRNA may have net tumor suppressive effects in situations where tumor suppressor targets are predominant, but it may also promote malignancy in settings where oncogenic targets are abundant.

Network Topology: Functional outcomes are influenced by the location of miRNA targets within regulatory networks. Depending on network state and feedback regulation, miRNAs that target hub genes with multiple interactions may have more significant and possibly contradictory effects.

Cooperative Interactions: RNA-binding proteins or cooperating miRNAs can significantly change the function of miRNAs. For example, miR-145 is often co-transcribed with miR-143, and its activities vary based on the co-expression of miR-143 (41).

5.3 The Tumour Microenvironment as a Functional Modifier

A key factor in determining miRNA function is the tumor microenvironment, which has several elements affecting miRNA activity:

Hypoxia: By altering target availability and RNA-binding protein expression, oxygen deprivation not only modifies the patterns of miRNA expression but also their functional outcomes. Certain miRNAs, such as miR-210, which exhibits context-dependent roles in angiogenesis and metastasis, can have their activity modulated by hypoxia-inducible factors (HIFs) (42).

Extracellular Matrix Composition: Through mechanotransduction pathways, the extracellular matrix's stiffness and composition affect miRNA function. In soft versus stiff microenvironments, miRNAs involved in cytoskeletal regulation, such as members of the miR-200 family, exhibit distinct activities.

Immune Cell Interactions: Immune cells and cancer cells interact to form dynamic circuits that regulate miRNA. Depending on the immune context, miRNAs that control immune responses, like miR-146a and miR-155, can have conflicting effects on the development of tumors (43).

Therapeutic Stress: By altering the cellular transcriptome and proteome, radiation, chemotherapeutic agents, and targeted therapies can significantly change miRNA function. Depending on the therapeutic context, miRNAs that control DNA damage response pathways may change from protective to harmful roles.

5.4 Systems Biology Approaches to Context-Dependency

Systems-level methods that incorporate various data types and computational modeling are necessary to comprehend miRNA context-dependency:

Network Analysis: Context-specific interactions and functional modules are revealed when miRNA-mRNA regulatory networks are constructed from multi-omics data. These analyses show that distinct regulatory modules in various subtypes or stages of breast cancer can involve the same miRNA (44).

Dynamic Modeling: Critical transition points and functional switches can be predicted by mathematical models that take into account feedback loops, cellular context, and miRNA-target interactions.

Single-Cell Analysis: Single-cell high-resolution profiling identifies uncommon cell states where miRNAs may perform non-canonical functions and reveals cell-to-cell variability in miRNA function.

Machine Learning Approaches: Therapeutic development can be guided by predictive models trained on multi-dimensional datasets, which can identify contexts in which particular miRNAs are likely to perform oncogenic versus tumor suppressive functions (45).

6. Exosomal miRNAs in Breast Cancer Progression

6.1 Biogenesis and Secretion of Exosomal miRNAs

The endosomal system produces exosomes, which are tiny extracellular vesicles (30–150 nm) that form intraluminal vesicles inside multivesicular bodies (MVBs). Both selective and non-selective mechanisms, including ceramide-dependent processes and RNA-binding proteins like hnRNPA2B1 and YBX1, are used to load miRNA into exosomes. RAB GTPases and SNARE complexes control exosome secretion, and cancer cells frequently produce more exosomes. Oncogenic transformation in breast cancer modifies the amount and composition of secreted exosomes, resulting in communication networks that promote tumor growth (46).

6.2 Role in Metastasis (Brain, Bone, Lung tropism)

By creating pre-metastatic niches, exosomal miRNAs are essential for organotropic metastasis. Exosomes enriched in miR-181c, which targets PDPK1 and COX2 to disrupt the blood-brain barrier, are secreted by brain-tropic breast cancer cells. Exosomal miR-21, which stimulates osteoclast differentiation by targeting PDCD4, and miR-19a, which increases osteolytic activity by inhibiting SOCS1, both aid in bone metastasis. Exosomal miR-200 family members, which increase endothelial permeability and inflammatory responses in pulmonary tissues, are responsible for lung metastasis (47). The significance of exosomal miRNAs in identifying metastatic patterns is emphasized by these organ-specific mechanisms.

6.3 Exosomal miRNAs as Non-Invasive Biomarkers

Promising non-invasive biomarkers for the identification and tracking of breast cancer are circulating exosomal miRNAs. Certain signatures have been found for various clinical applications: miR-21, miR-155, and miR-10b are useful for early detection; miR-210 and miR-373 are associated with hypoxia and aggressive behavior; miR-34a and miR-122 are predictive of treatment response; and members of the miR-200 family are indicative of EMT status and metastatic potential (48). Exosomal miRNAs are especially appealing for liquid biopsy applications due to their exceptional stability in circulation, defense against RNase degradation, and reflection of tumor heterogeneity.

6.4 Therapeutic Delivery of miRNA Mimics via Exosomes

Exosomes are a natural way to deliver miRNA-based treatments. Tumor suppressor miRNA mimics (like miR-34a and let-7a) or antagomiRs against oncomiRs (like anti-miR-21 and anti-miR-155) can be loaded into engineered exosomes. Tumor-specific delivery is made possible by surface modification with targeting ligands (such as iRGD peptides and HER2 antibodies), and effective cellular uptake is ensured by endogenous trafficking mechanisms (49). Although there are still issues with manufacturing scalability and quality control, preclinical research shows that exosome-mediated miRNA delivery can successfully

inhibit tumor growth and metastasis with fewer off-target effects when compared to synthetic nanoparticle systems.

7. Clinical Utility of miRNAs in Breast Cancer

7.1 Diagnostic Applications

There is a lot of promise for diagnosing breast cancer using circulating miRNA signatures. When separating breast cancer patients from healthy controls, panels containing miR-145, miR-155, and miR-382 achieve high sensitivity (85–92%) and specificity (88–95%) (50). Certain combinations have been found to identify interval cancers that appear between screening mammograms (miR-18a, miR-181a, miR-222), distinguish breast cancer from benign breast conditions (miR-21, miR-106a, miR-155), and detect early-stage disease (miR-148b, miR-376c, miR-409-3p). These circulating biomarkers may serve as supplements or substitutes for current screening techniques, especially in cases of dense breast tissue where mammography sensitivity is diminished.

7.2 Prognostic and Predictive Biomarkers

Beyond typical clinicopathological parameters, miRNA expression patterns offer useful prognostic information. Shortened metastasis-free and overall survival across subtypes is predicted by high-risk signatures such as miR-210, miR-21, and miR-10b. Certain miRNAs are linked to specific metastatic patterns: lung metastasis is associated with the miR-200 family, brain metastasis with miR-181c, and bone metastasis with miR-122 (51). MiR-125b for anthracycline-based chemotherapy resistance, miR-221/222 for tamoxifen resistance, and miR-340 for trastuzumab response are examples of predictive biomarkers. Risk stratification and treatment selection may be improved by integrating miRNA signatures with proven prognostic tools.

7.3 miRNA-Based Therapeutics

MiRNA mimics to restore tumor suppressor function and antagomiRs (anti-miRs) to inhibit oncomiR activity are the two main therapeutic approaches that target miRNAs. The liposomal miR-34a mimic MRX34 showed encouraging preclinical activity but encountered difficulties in clinical trials because of immune-related side effects. In preclinical models of TNBC and inflammatory breast cancer, anti-miR-21 and anti-miR-155 compounds are effective (52). Stability and binding affinity are improved by chemical modifications such as locked nucleic acid (LNA) backbones, 2'-O-methyl, and 2'-fluoro. Delivery systems are still developing, with conjugate technologies, polymer-based carriers, and new lipid nanoparticles enhancing cellular uptake and tissue targeting.

7.4 Challenges in Clinical Translation

The clinical application of miRNA-based treatments and diagnostics is hampered by a number of issues. Context-dependent functions, off-target effects, and miRNA redundancy are examples of biological obstacles. Assuring circulation stability, attaining effective delivery to tumor sites, and reducing immune activation are technical challenges. Tumor heterogeneity, inter-individual variability, and integration with current treatment paradigms are examples of clinical challenges (53). Clear pathways are necessary for regulatory considerations of miRNA-based products as medications or diagnostic tools. Despite these obstacles, the field is still progressing thanks to better delivery methods, large-scale cohort biomarker validation, and a deeper comprehension of miRNA biology in various breast cancer contexts.

8. Cutting-Edge Technologies for miRNA Profiling in Breast Cancer

8.1 Single-Cell miRNA Sequencing

Our knowledge of cellular heterogeneity in breast cancer has completely changed since the development of single-cell RNA sequencing (scRNA-seq) technologies. Unprecedented insights have been uncovered by recent modifications for single-cell resolution miRNA profiling:

High-Throughput sc-miRNA-seq: Parallel profiling of miRNAs and mRNAs in thousands of individual cells is made possible by new protocols such as scGET-seq (single-cell genome and epigenome by transposase sequencing) and scTRAP (single-cell transcriptome and regulome analysis). These methods have revealed unique patterns of miRNA expression in immune cell subsets within the tumor microenvironment, therapy-resistant subpopulations, and breast cancer stem cells (54).

Multimodal Single-Cell Analysis: miRNA detection is now combined with protein abundance measurements in technologies like REAP-seq (RNA expression and protein sequencing assay) and CITE-seq (cellular indexing of transcriptomes and epitopes by sequencing), which provide integrated views of regulatory networks at single-cell resolution.

Spatial Single-Cell miRNA Profiling: New techniques allow for the mapping of miRNA expression within tissue architecture while preserving cellular resolution by combining single-cell resolution with spatial context. These methods are illuminating the ways in which local expression patterns and miRNA gradients affect cellular behavior in different tumor regions (55).

8.2 Advanced CRISPR-based miRNA Engineering

CRISPR technology has advanced beyond basic gene editing to allow for complex miRNA function manipulation:

CRISPR Activation and Interference (CRISPRa/i): Certain miRNA genes can be precisely upregulated or downregulated without changing genomic sequences when catalytically dead Cas9 (dCas9) is fused to transcriptional activators or repressors. While CRISPRi screens have shown context-specific essential oncomiRs, CRISPRa screens have discovered new tumor suppressor miRNAs in breast cancer (56).

Base Editing and Prime Editing: Single-nucleotide changes in miRNA genes, seed regions, and regulatory elements are made possible by these accurate genome editing techniques. Certain point mutations in miR-21 seed regions have been produced using base editors, changing the target spectrum and functional results.

Epigenome Editing: By utilizing dCas9 in combination with epigenetic modifying proteins, it is possible to use targeted DNA methylation and/or histone modification to alter the levels of expression of miR-200c and miR-34a, which are two examples of silenced tumor suppressor

microRNAs (miRNAs) that have been reactivated in preclinical breast cancer models using this method (57).

Multiplexed miRNA Targeting: Dissection of combinatorial miRNA functions and identification of synthetic lethal interactions in breast cancer cells are made possible by CRISPR systems designed to target multiple miRNA genes at once.

8.3 Spatial Transcriptomics and miRNA Mapping

For miRNA profiling, spatial transcriptomics technologies have been modified to provide tissue context for miRNA expression patterns:

High-Resolution Spatial miRNA Detection: MiRNA distribution in formalin-fixed paraffin-embedded tissues can be seen at nearly single-cell resolution using techniques like miRNAscope, which combine sophisticated in situ hybridization with amplification techniques. These methods have demonstrated compartment-specific miRNA expression in metastatic sites, invasive fronts, and tumor nests (58).

Integrated Spatial Multi-omics: The simultaneous detection of proteins, mRNAs, and miRNAs in tissue sections is made possible by new platforms. The Visium HD platform from 10x Genomics offers thorough spatial molecular profiling of breast cancer tissues when paired with improvements in miRNA detection.

Dynamic Spatial Analysis: Live imaging methods with molecular beacons, smart probes and other live imaging techniques provide the ability to observe dynamic changes over time in miRNA functional changes occurring in vivo via the ability to culture on animals and animal models. As a result, live imaging provides an ongoing visualisation of the effect of miRNAs during the development of tumours and their treatment.

Computational Spatial Analysis: Advanced spatial data analysis algorithms, such as graph neural networks and spatial covariance techniques, find miRNA expression patterns that are correlated with treatment responses, clinical outcomes, and histological characteristics (59).

8.4 Artificial Intelligence and Machine Learning in miRNA Research

Functional analysis and miRNA biomarker discovery are being revolutionized by AI and ML techniques:

Deep Learning for miRNA Target Prediction: Predicting functional miRNA-target interactions with previously unheard-of accuracy is made possible by neural network models trained on CLIP-seq data and functional validation datasets. To predict targets related to breast cancer, models such as miRTar2.0 and TargetNet integrate structural accessibility, expression context, and sequence features (60).

Predictive Biomarker Development: From complicated datasets, ensemble learning techniques that combine several algorithm types (random forests, gradient boosting, neural networks) find reliable miRNA signatures for breast cancer diagnosis, subtyping, and prognosis.

Network Medicine Approaches: To find dysregulated modules and forecast treatment outcomes, graph neural networks and knowledge graphs combine miRNA data with protein-protein interactions, pathway details, and clinical data.

Generative AI for Therapeutic Design: Optimized miRNA mimics and antagomiRs with enhanced stability, specificity, and delivery characteristics are being created using transformer models and generative adversarial networks (GANs) (61).

8.5 Emerging Technologies and Future Directions

MiRNA research in breast cancer is set to advance thanks to a number of promising technologies:

Nanopore-based Direct miRNA Sequencing: Direct RNA sequencing without amplification biases is made possible by third-generation sequencing technologies, which offer more precise quantification of miRNA isoforms and modifications.

Single-Molecule Imaging: Modern microscopy methods make it possible to see individual miRNA molecules in living cells, revealing the dynamics of miRNA-mediated regulation in real time.

Microfluidic miRNA Profiling: Lab-on-a-chip devices facilitate intraoperative analysis and liquid biopsy applications by enabling ultra-sensitive miRNA detection with minimal sample input.

Quantum Dot-based Detection: For the validation of clinical biomarkers, multiplexed, extremely sensitive miRNA detection is made possible by nanocrystal technologies (57).

Integrated Multi-omics Platforms: Unprecedented insights into the regulatory hierarchy in breast cancer are being provided by comprehensive platforms that concurrently profile miRNAs, mRNAs, proteins, metabolites, and epigenetic marks from the same samples.

In addition to expanding our knowledge of the biology of miRNA in breast cancer, these technological developments are hastening the conversion of fundamental findings into practical uses, such as better diagnostics and innovative treatment approaches.

9. Future Directions and Clinical Translation

9.1 Integrating miRNA Signatures with Multi-Omics Panels

MiRNA signatures will be increasingly integrated with genomic, transcriptomic, proteomic, and metabolomic data in future biomarker development. The intricate regulatory networks influencing the course of breast cancer and the response to treatment can be captured by these multi-omics techniques. When combined with proteomic data, functional miRNA-target relationships can be identified, and integration with DNA methylation profiles may reveal miRNAs that are epigenetically regulated (62). Compared to single data types alone, these thorough profiles promise more accurate classification, prognostication, and treatment selection.

9.2 Personalized miRNA Therapy Based on Tumour Subtype

Tumor molecular subtype-matched personalized approaches are the key to the future of miRNA-based treatments. While HER2-positive tumors may respond to miR-125a/b supplementation to suppress HER2/3

signaling, luminal breast cancers may benefit from miR-342 restoration to increase endocrine therapy sensitivity. MiR-200 family restoration to inhibit EMT and miR-34a mimics to induce apoptosis could be used to target TNBC (63). For these methods to be clinically successful, patient selection based on thorough molecular profiling, including miRNA expression patterns, will be crucial.

9.3 Combining miRNA-Targeting Drugs with Immunotherapy or Chemotherapy

A promising avenue for miRNA-based treatments is rational combinations. By altering the immunosuppressive microenvironment, anti-miR-155 may improve the effectiveness of checkpoint inhibitors. In BRCA-mutant breast cancers, miR-34a mimics may make tumors more susceptible to PARP inhibitors. Resistance to taxane-based chemotherapy may be reversed by miR-21 inhibition (64). In order to improve therapeutic indices and potentially overcome resistance mechanisms, these combinations take advantage of the pathway-modulating effects of miRNAs.

9.4 Role of the Microbiome miRNA Axis in Breast Cancer Outcomes

There may be links between the gut microbiota, miRNA expression, and the prognosis of breast cancer. Short-chain fatty acids and secondary bile acids are examples of microbial metabolites that can affect miRNA expression and host epigenetic regulation. Circulating miRNA profiles linked to therapy response and survival are correlated with particular bacterial species (65). Gaining insight into these relationships could help develop microbiome-modulating techniques that maximize miRNA function and enhance treatment results.

9.5 Longitudinal Studies for Treatment Monitoring

Early indicators of response and developing resistance can be found through longitudinal monitoring of miRNA dynamics during treatment. Certain patterns linked to pathological complete response (e.g., increasing miR-125b, decreasing miR-21) are revealed by serial analysis of circulating miRNAs during neoadjuvant chemotherapy (66). Similar methods used in immunotherapy and targeted therapies may allow for early treatment strategy adaptation, potentially improving outcomes through dynamic treatment personalization.

10. CONCLUSION

As crucial regulators of oncogenic and tumor suppressor pathways, microRNAs are at the intersection of breast cancer biology. While their stability in clinical samples and subtype-specific expression patterns make them appealing biomarkers and therapeutic targets, their dualistic nature as both drivers and inhibitors of malignancy reflects the complexity of cancer regulation. Using state-of-the-art technologies to address cellular heterogeneity, creating complex delivery systems for clinical translation, and incorporating miRNA signatures into multi-omics strategies for personalized medicine are the future directions of miRNA research in breast cancer. MiRNA-based strategies have the potential to improve early detection, risk stratification, and therapeutic options for patients with breast cancer across the disease spectrum as our knowledge of miRNA biology grows and technological obstacles are removed.

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