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Review

The Role of miR-21 in HER2-Positive Breast Cancer: A Comprehensive Review of Molecular Mechanisms, Biomarker Potential, and Therapeutic Implications

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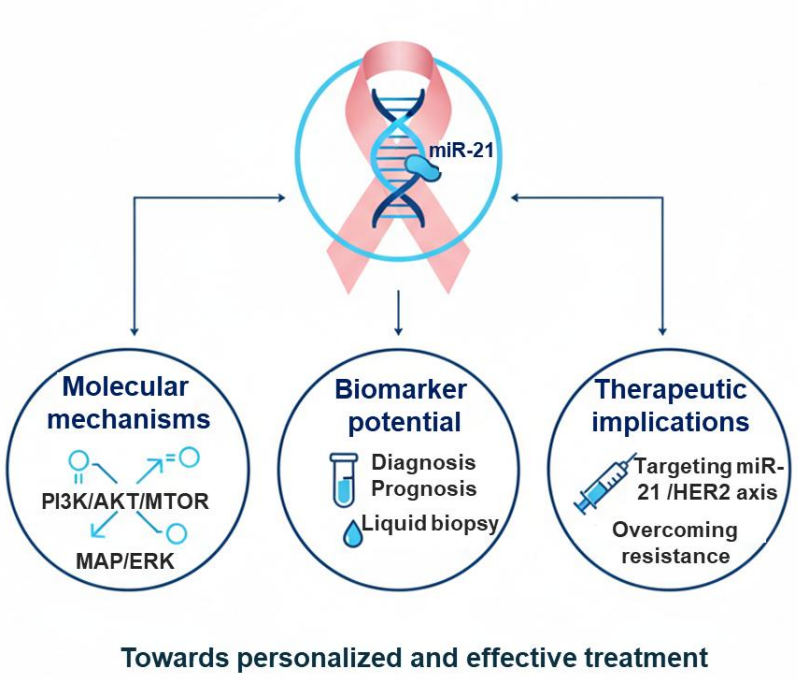
Abstract

Breast cancer continues to be the most prevalent cancer affecting women, with Human Epidermal Growth Factor Receptor 2-positive (HER2-positive) breast cancer presenting particular difficulties because of its aggressive nature and resistance to treatments aimed at specific targets. MicroRNA-21 (miR-21) has been identified as an important factor in controlling HER2-related cancer development, significantly contributing to tumor growth, spread, and resistance to therapy. miR-21 is upregulated by approximately 2-5-fold in HER2-positive tumors compared to HER2-negative tumors, as reported in several clinical studies, correlating with advanced disease stage, lymph node metastasis, and resistance to HER2-targeted therapies. This study explores the multifaceted contributions of miR-21 to HER2-positive breast cancer biology, emphasizing its regulatory role in key oncogenic pathways, including PI3K/AKT/mTOR and MAPK/ERK. Here, discuss the possibility of miR-21 as a biomarker for diagnosis, prognosis, and monitoring therapeutic responses, particularly in liquid biopsy formats such as serum and extracellular vesicles (EVs). Furthermore, this review examines promising therapeutic strategies targeting the miR-21/HER2 axis, highlighting ongoing preclinical developments and future clinical applications. Despite the challenges in standardizing miR-21-based diagnostics and therapies, this review highlights the increasing potential of miR-21 as a therapeutic target, particularly in overcoming resistance to HER2-targeted treatments. This offers a promising pathway toward more personalized and effective options for HER2-positive breast cancer. However, while miR-21 shows significant promise, no therapies targeting miR-21 have been approved for clinical use, emphasizing the need for further research and clinical trials.

Graphical Abstract



1. Introduction

Breast cancer is the most prevalent cancer diagnosed in women globally, with several molecular subtypes influencing prognosis and treatment responses [1]. One of the most aggressive forms is HER2-positive breast cancer, characterized by HER2 gene amplification or protein overexpression [2]. These subtypes are classified based on the presence of hormone receptors and HER2 overexpression, and include estrogen receptor-positive (ER^+), progesterone receptor-positive (PR^+), and HER2-positive breast cancer. Each subtype presents unique challenges for treatment, and advancements in precision medicine have highlighted the importance of subtype-specific therapies [3]. HER2-positive breast cancer is associated with poor clinical outcomes, including increased metastatic potential, higher rates of recurrence, and resistance to HER2-targeted therapies such as trastuzumab. These therapies, while initially effective, often encounter resistance due to molecular adaptations in the tumor cells, making it imperative to identify new biomarkers and therapeutic targets to improve patient outcomes. The key pathways involved in HER2-driven oncogenesis include the PI3K/AKT/mTOR and MAPK/ERK signaling cascades, both of which are pivotal in mediating tumor progression, metastasis, and resistance to apoptosis [4,5].

MicroRNAs (miRNAs) are small, non-coding RNAs that act as essential post-transcriptional regulators of gene expression, influencing a variety of cellular processes such as proliferation, differentiation, apoptosis, and cell cycle regulation. Their dysregulation is frequently observed in cancer, where they can function as either oncogenes or tumor suppressors. Among the numerous miRNAs implicated in breast cancer, miR-21 has garnered significant attention due to its pivotal role in HER2-positive breast cancer [6]. In this subtype, miR-21 is consistently upregulated and functions as a critical downstream effector of HER2 signaling. This upregulation is not only associated with tumor progression but also with increased therapeutic resistance and enhanced metastatic potential. miR-21 mediates these effects by targeting a range of key tumor suppressor genes (TSGs), including Phosphatase and Tensin Homolog (PTEN) and Programmed Cell Death 4 (PDCD4). These targets are involved in critical cellular pathways such as apoptosis, cell survival, and immune evasion. Through its repression of these tumor suppressors, miR-21 activates pro-survival and pro-metastatic pathways, including the PI3K/AKT/mTOR and MAPK/ERK cascades, both of which contribute to uncontrolled cell growth, resistance to cell death, and enhanced invasive capabilities. Furthermore, emerging evidence suggests that miR-21 also influences the tumor microenvironment (TME) by modulating immune cell functions and extracellular matrix remodeling, thus facilitating metastasis and resistance to HER2-targeted therapies. This comprehensive understanding of miR-21's role underscores its potential as both a biomarker and a therapeutic target in HER2-positive breast cancer [7-9].

This review provides a comprehensive analysis of the multifaceted roles of miR-21 in HER2-positive breast cancer. It explores the regulatory functions of miR-21 within key oncogenic pathways, with a particular focus on those driven by HER2 signaling. Additionally, the review examines the potential of miR-21 as both a diagnostic and prognostic biomarker, as well as its promise as a therapeutic target for overcoming resistance to HER2-targeted therapies. In the following sections, we will first discuss the molecular mechanisms underlying HER2 signaling in breast cancer, followed by an in-depth exploration of how miR-21 modulates these pathways. The review then delves into the clinical implications of miR-21, particularly its role in therapeutic resistance and its potential as a target for innovative treatment strategies. Finally, the review considers future research directions, highlighting the importance of standardized miRNA detection methods and discussing the clinical potential of miR-21-based therapies in advancing breast cancer treatment.

2. Overview of HER2 Signaling Pathways in Breast Cancer

HER2-positive, also known as ERBB2-positive subtype, which comprises around 15–20% of all breast cancer cases [1]. This variant is marked by an overexpression of the HER2 protein or amplification of the HER2 gene, and is typically linked with unfavorable outcomes, including a greater likelihood of metastasis, disease recurrence, and resistance to HER2-targeted treatments like trastuzumab and lapatinib [2]. Despite considerable improvements in clinical outcomes following the introduction of HER2-targeted agents, intrinsic and acquired resistance continue to represent a major therapeutic challenge, necessitating the identification of complementary molecular biomarkers and novel therapeutic targets [10]. Among all miRNAs implicated in breast cancer pathogenesis, miR-21 is robustly upregulated in HER2-positive tumors and demonstrates a bidirectional relationship with HER2 signaling, functioning both as a downstream effector and regulator of oncogenic pathways [11]. The activation of HER2 is initiated by the binding of a Growth Factor to its extracellular domain, which triggers a signaling cascade across the cell membrane. This activation branches into two main downstream pathways: the PI3K/AKT Pathway and the RAS/RAF/MEK/ERK Pathway (MAPK Pathway). The PI3K/AKT pathway, activated by HER2, leads to the phosphorylation of AKT and its target mTOR, fundamentally driving Cell Survival, Proliferation, Cell Growth, Differentiation, and contributing to Anti-apoptosis. Concurrently, the RAS/RAF/MEK/ERK pathway is activated through the sequential signaling of RAS, RAF, MEK, and ERK, which is essential for Cell Growth and Differentiation. Activation of the PI3K/AKT/mTOR axis promotes cell survival, proliferation, metabolic reprogramming, and resistance to apoptosis, and is one of the most frequently dysregulated pathways in HER2-positive breast cancer. Concurrently, the RAS/RAF/MEK/ERK pathway regulates cell cycle progression, differentiation, migration, and invasion, contributing to tumor growth

and metastatic dissemination [12] (Figure 1). Growing evidence from extensive clinical and molecular investigations indicates that miR-21 overexpression is closely intertwined with HER2 amplification, advanced disease stage, lymph node metastasis, and therapy resistance, positioning it as a critical node in the molecular landscape of HER2-driven tumor progression [12,13]. MiR-21 is frequently upregulated across multiple subtypes of breast cancer, although its roles and mechanisms of action can differ depending on the tumor context. In HER2-positive breast cancer, miR-21 is a key effector of HER2-driven oncogenic signaling and therapeutic resistance. In contrast, in triple-negative breast cancer (TNBC), miR-21 is often associated with aggressive tumor behavior and poor prognosis, largely through its impact on TSGs and metastasis-related pathways. Similarly, in ER^+ breast cancer, miR-21's role is more nuanced, potentially being modulated by estrogen signaling, but still contributing to tumor progression and resistance to endocrine therapies [7,14,15]. This comprehensive review synthesizes findings to delineate the complex molecular interplay between miR-21 and HER2 signaling, explore its mechanistic roles in tumor progression and therapeutic resistance, evaluate its clinical utility as both a tissue-based and non-invasive biomarker, and discuss emerging therapeutic strategies targeting this axis to overcome resistance in HER2-positive breast cancer.

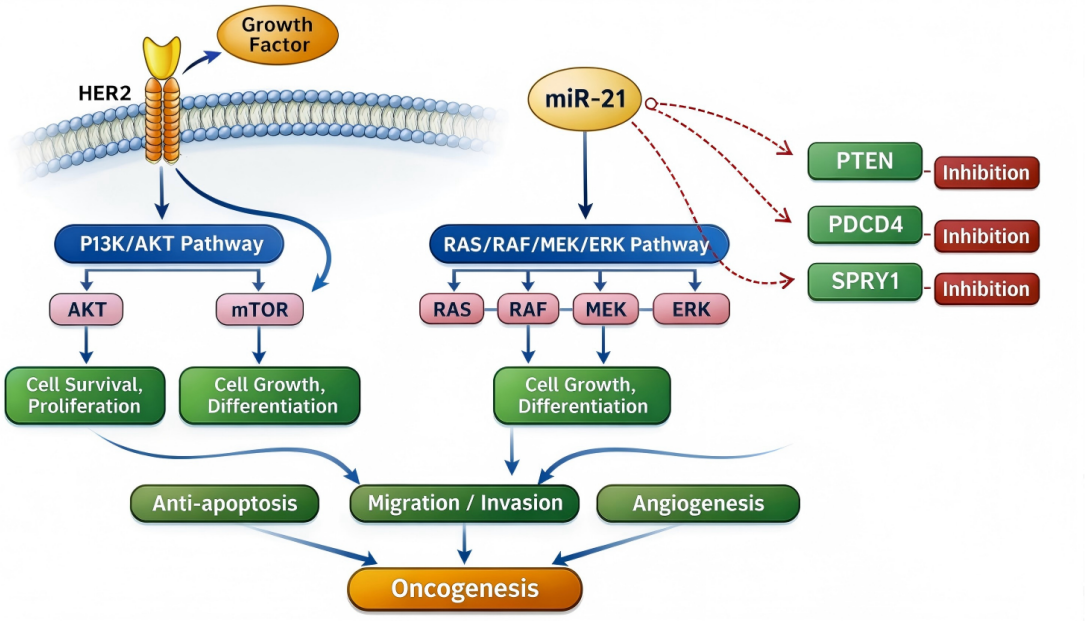


Figure 1. HER2 signaling pathways and regulation by miR-21 in oncogenesis. This figure illustrates the principal downstream signaling cascades activated by the HER2 receptor tyrosine kinase and the subsequent regulatory role of miR-21 in promoting cancer development.

3. miR-21 as a Downstream Effector and Amplifier of HER2 Signaling

Multiple lines of evidence demonstrate that HER2 overexpression directly induces miR-21 expression, establishing a feed-forward oncogenic loop. HER2 activates canonical downstream pathways, including MAPK/ERK and PI3K/AKT, which transcriptionally upregulate miR-21 via Activator Protein 1 (AP-1) and Signal Transducer and Activator of Transcription 3 (STAT3) binding sites in its promoter region [16,17]. Furthermore, a novel mechanism has been identified wherein the HER2 locus itself, through co-amplification of the intronic miRNA miR-4728-3p, stabilizes mature miR-21-5p by inhibiting its degradation via the poly(A) polymerase (PAPD5), thereby amplifying its oncogenic output independently of canonical receptor kinase activity. This dual-mode regulation-transcriptional activation and post-transcriptional stabilization-positions miR-21 as a key molecular amplifier of HER2-driven malignancy [18]. Crucially, miR-21 executes its oncogenic function by targeting key TSGs, most prominently PTEN. Repression of PTEN by miR-21 removes the primary inhibitory brake on the PI3K/AKT pathway, which is itself responsible for miR-21's transcriptional activation, thereby creating a highly potent and self-sustaining positive feedback loop that continually reinforces the HER2-driven signaling cascade This amplification loop results in sustained cell proliferation, survival, and resistance to apoptosis. In alignment with these findings, miR-21 expression is markedly elevated in HER2-positive tumors relative to HER2-negative tumors or normal breast tissue, with expression levels showing a direct correlation with HER2 immunohistochemistry (IHC) scores ranging from 1+ to 3+ [9,10]. Importantly, this relationship remains statistically significant after controlling for estrogen receptor (ER) and progesterone receptor (PR) status, indicating a regulatory effect that is specifically attributable to HER2 [11]. While multiple studies have demonstrated that HER2 signaling induces miR-21 expression through the PI3K/AKT and MAPK/ERK pathways, the reproducibility of these findings across different cohorts and datasets is not always consistent. For example, variations in HER2 expression levels, tumor heterogeneity, and sample types may influence miR-21 induction. Additionally, some studies have reported only modest correlations between HER2

expression and miR-21 levels, suggesting that other regulatory factors or co-mutations might contribute to miR-21 upregulation in HER2-positive breast cancer [19,20]. Several studies have reported a 2-5-fold increase in miR-21 expression in HER2-positive tumors compared to HER2-negative tumors. For instance, a study by Huang et al. (2009) demonstrated a significant correlation between HER2 overexpression and miR-21 upregulation (correlation coefficient = 0.87, $p < 0.01$) in a cohort of breast cancer samples. Similarly, miR-21 expression in HER2-enriched tumors, as classified by the PAM50 signature, showed a marked elevation compared to other breast cancer subtypes, reinforcing its role as a key player in HER2-driven oncogenesis [16].

The Prediction Analysis of Microarray 50 (PAM50) gene expression classifier further substantiates this concept by identifying miR-21 as a pivotal regulatory hub within HER2-enriched tumors, orchestrating the expression of nearly all genes within the PAM50 signature-a phenomenon not evident in normal breast tissue or other breast cancer subtypes [12]. These findings imply that miR-21 functions as a master transcriptional regulator in HER2-positive breast cancer, driving transcriptomic reprogramming that promotes cellular proliferation, survival, and metastatic potential (Figure 2).

This schematic illustrates the complex dual mechanism by which HER2 signaling synergistically amplifies the expression of miR-21, resulting in a robust oncogenic drive in HER2-positive breast cancer. The primary regulatory axis involves transcriptional upregulation: activation of the HER2 receptor initiates canonical downstream pathways, specifically the Phosphatidylinositol 3-Kinase/Protein Kinase B (PI3K/AKT) and Mitogen-Activated Protein Kinase/Extracellular-signal-regulated Kinase (MAPK/ERK) cascades, which subsequently lead to the nuclear translocation and activation of transcription factors such as AP-1 and STAT3, which bind to the promoter of the primary miR-21 transcript (pri-miR-21). Concurrently, a post-transcriptional mechanism may also contribute, where the co-amplification of the intronic miRNA miR-4728-3p or the action of other factors may stabilize the mature miR-21-5p molecule, preventing its degradation and further increasing its functional concentration within the cell. The resultant high levels of mature miR-21 then exert their oncogenic function by binding to and repressing target TSGs, including PDCD4, Reversion-inducing-cysteine-rich protein kinase (RECK), and critically, PTEN; the repression of PTEN is key, as it removes the negative regulation on the PI3K/AKT pathway, thereby establishing a powerful and self-reinforcing positive feedback loop that sustains aggressive cellular phenotypes such as enhanced proliferation, survival, invasion, and resistance to therapy.

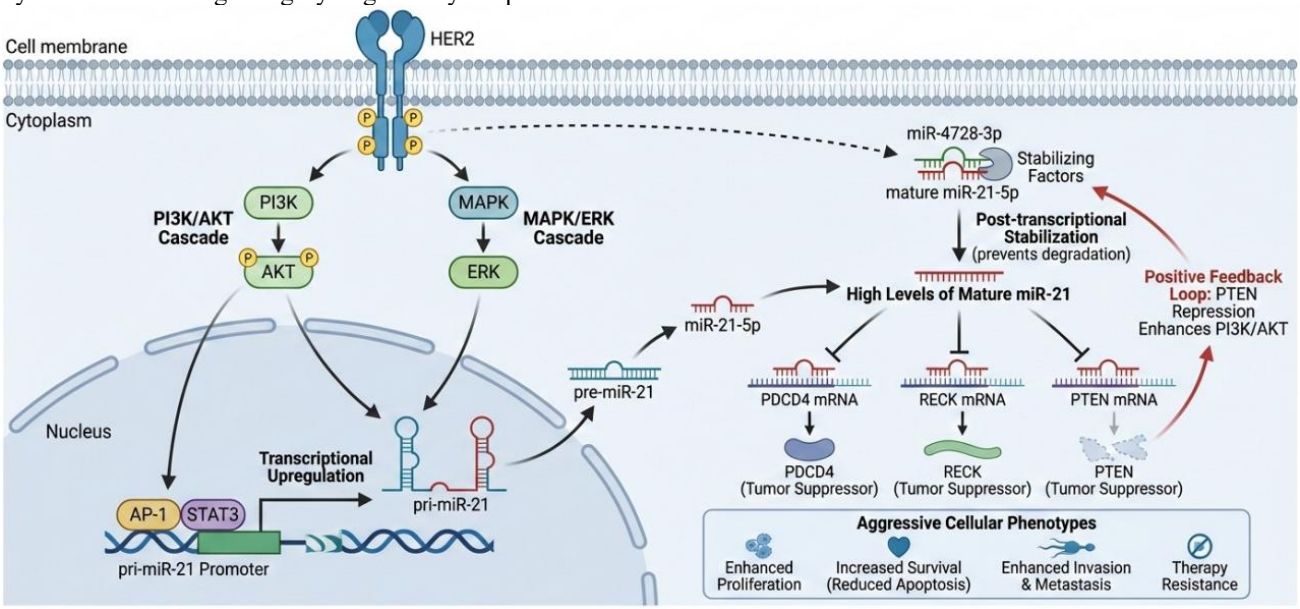


Figure 2. Dual-Mode regulation and oncogenic amplification of miR-21 by HER2 signaling in breast cancer. Abbreviations: HER2: Human Epidermal Growth Factor Receptor 2; miR-21: MicroRNA-21; PI3K: Phosphatidylinositol 3-Kinase; AKT: Protein Kinase B; MAPK: Mitogen-Activated Protein Kinase; ERK: Extracellular-signal-regulated Kinase; AP-1: Activator Protein 1; STAT3: Signal Transducer and Activator of Transcription 3; PTEN: Phosphatase and Tensin Homolog; PDCD4: Programmed Cell Death 4.

4. miR-21 as a Mediator of Tumor Progression and Metastasis via Tumor Suppressor Repression

miR-21 has emerged as a central oncomiR in breast cancer and other malignancies, functioning as a potent mediator of tumor progression, metastasis, and therapy resistance through its capacity to repress multiple TSGs. Consistent overexpression of miR-21 has been documented in breast tumors compared with matched normal tissue, supporting its oncogenic role [21,22]. In the context of HER2-positive breast cancer, several validated miR-21 targets have been identified, including PTEN, PDCD4, STARD13, ZNF132, TIMP3, and MSH2 [16,23-27].

Among these targets, PTEN repression appears particularly critical from a clinical standpoint. MiR-21 binds directly to the 3′ untranslated region (3′UTR) of PTEN mRNA, leading to decreased PTEN protein expression, which in turn results in activation of the

PI3K/AKT/mTOR signaling cascade [21,23]. This activation is a well-known driver of cell survival, proliferation, and notably resistance to therapies such as Trastuzumab in HER2-positive breast cancer [23,25]. Experimental inhibition of miR-21, for instance, via antisense oligonucleotides in HER2-positive, trastuzumab-resistant cell lines and xenograft models restored PTEN expression and resensitized tumors to trastuzumab, underscoring the potential of miR-21 antagonism as a therapeutic strategy [19].

In addition to PTEN, miR-21–mediated suppression of PDCD4 contributes to enhanced invasiveness and metastatic behavior. PDCD4 is a translational inhibitor of pro-metastatic factors and functions as a tumor suppressor by restraining cellular migration and invasion. In HER2 overexpressing cells, HER2 signaling via the MAPK/ERK pathway induces miR-21 expression, which then downregulates PDCD4, thereby promoting epithelial-to-mesenchymal transition (EMT), invasion, and metastasis [16,17,24].

The role of miR-21 in regulating extracellular matrix remodeling and metastatic dissemination is further supported by its direct targeting of TIMP3. TIMP3 normally inhibits matrix metalloproteinases (MMPs), thereby constraining extracellular matrix degradation, invasion, and metastasis [28]. *In vitro* studies demonstrated that miR-21 suppression increases TIMP3 mRNA and protein levels, and luciferase reporter assays confirmed that the TIMP3 3′UTR contains functional miR-21 binding sites, linking miR-21 expression to increased invasive potential in breast cancer cells [26].

More recently, investigations have expanded the spectrum of miR-21 targets in breast cancer. For instance, an analysis focusing on the minor arm miR-21-3p - transcribed from the lagging strand of the MIR21 locus - found that elevated miR-21-3p levels are associated with poorer survival, lymph node positivity, larger tumor size, higher grade, and HER2 positivity [3]. Importantly, among the most significantly downregulated genes correlated with high miR-21-3p were STARD13 and Zinc Finger Protein 132 (ZNF132), both recognized as tumor suppressors implicated in cell migration, invasion, and broader transcriptional control of cancer-associated gene networks [9]. MutS Homolog 2 (MSH2), a key DNA mismatch repair protein, undergoes post-transcriptional repression by miR-21 in HER2-transformed cells via Transforming Growth Factor Beta co-signaling, which contributes to increased genomic instability and chemoresistance [26].

Collectively, the cumulative evidence positions miR-21 as a multi-target oncomiR that orchestrates a coordinated program of proliferation, survival, invasion, metastasis, and genomic instability hallmarks characteristic of aggressive HER2-positive disease. In addition, miR-21 expression has been correlated with poor prognosis in breast cancer overall, including worse overall survival (OS) and recurrence rates [11]. Given its widespread role across oncogenic processes and therapeutic resistance, miR-21 represents a promising candidate for diagnostic, prognostic, and therapeutic applications, including the potential for miRNA-based interventions in breast cancer [29-32]. The temporal regulation of miR-21 in HER2-positive breast cancer is not fully understood. Some studies suggest that miR-21 induction occurs early in the tumorigenic process, preceding HER2 overexpression, while others propose that miR-21 upregulation follows HER2 signaling as a secondary response. miR-21 expression was found to increase significantly following HER2 activation, suggesting that HER2 signaling may be a key driver of miR-21 upregulation during tumor progression [12,33]. MiR-21 exists in two major isoforms: miR-21-5p and miR-21-3p, each with distinct target preferences and roles in cancer biology. While miR-21-5p is the more widely studied isoform and is primarily involved in tumor progression and metastasis, recent research has highlighted the importance of miR-21-3p in certain contexts. For example, miR-21-3p has been shown to preferentially target different tumor suppressors, such as STARD13 and ZNF132, in HER2-positive breast cancer, suggesting a potential isoform-specific role in regulating tumor metastasis and resistance to therapy [32,34].

While these findings collectively position miR-21 as a robust amplifier of HER2 signaling, some discrepancies remain. For example, several clinical cohorts reported modest correlations between HER2 IHC score and miR-21 levels, suggesting that additional regulatory factors (e.g., stromal miR-21, hormonal crosstalk, intratumoral heterogeneity) may modulate this relationship. Furthermore, most mechanistic studies rely on cell lines with extreme HER2 amplification, raising questions about generalizability to lower-expressing tumors. These unresolved issues highlight the need for integrative analyses that combine genomic, transcriptomic, and EV-based miRNA profiling.

Key validated targets of miR-21, including PTEN, PDCD4, STARD13, ZNF132, TIMP-3, and MSH2, contribute to HER2-driven progression through coordinated tumor-suppressor repression (Table 1).

Table 1. Validated miR-21 targets and functional consequences in HER2-positive breast cancer.

Target Gene / Protein	Physiological Function	Mechanism of miR-21–Mediated Regulation	Evidence in HER2-positive Context	Oncogenic Consequence in HER2-positive Breast Cancer	Ref
PTEN	Negative regulator of PI3K/AKT/mTOR signaling; promotes apoptosis	Direct binding of miR-21 to 3′UTR reduces mRNA and protein levels	Elevated miR-21 expression correlates with reduced PTEN in HER2-positive tumors and trastuzumab-resistant cell lines; restoration of PTEN observed upon miR-21 inhibition	Constitutive activation of PI3K/AKT signaling, cell survival, proliferation, and resistance to HER2-targeted therapy	[35]
PDCD4	Translation inhibitor; suppresses invasion and EMT	miR-21 represses PDCD4 through post-transcriptional silencing	Reduced PDCD4 expression detected in HER2-positive breast tissue and cell lines with high miR-21; linked to enhanced MAPK/ERK activity	Promotion of EMT, migration, and metastatic dissemination	[36]
STARD13	Regulator of Rho GTPases and cytoskeletal stability	miR-21 binding to 3′UTR decreases transcript stability	Downregulation observed in HER2-positive invasive ductal carcinoma compared with HER2-negative subtypes	Enhanced motility, invasion, and cytoskeletal remodeling	[37]
ZNF132	Transcriptional repressor of migration-related genes	Direct 3′UTR targeting by miR-21	Reduced ZNF132 mRNA levels correlate with high miR-21 in HER2-positive tumors	Increased cell migration and invasiveness	[9]
TIMP-3	Inhibitor of matrix metalloproteinases (MMPs)	miR-21-induced mRNA degradation and translational repression	Inverse correlation between miR-21 and TIMP-3 expression reported in HER2-positive invasive carcinomas	Matrix degradation and facilitation of metastasis	[26]
MSH2	DNA mismatch-repair protein maintains genomic stability	Post-transcriptional repression via TGF-β/miR-21 co-signaling	Suppressed MSH2 expression noted in HER2-transformed breast cells with high miR-21 activity	Genomic instability, chemoresistance, and enhanced survival under stress	[27]

Taken together, these observations indicate that miR-21 does not act through a single dominant pathway but instead exerts oncogenic pressure by simultaneously weakening multiple tumor suppressive networks. This multi-node repression produces a systems-level shift toward PI3K/AKT hyperactivation, cytoskeletal plasticity, extracellular matrix remodeling, and genomic instability. Importantly, this combinatorial mechanism may explain why miR-21 overexpression is consistently associated with aggressive HER2-positive phenotypes and why its inhibition often yields disproportionately large therapeutic effects relative to the restoration of any single target.

5. miR-21 as a Central Driver of Therapeutic Resistance in HER2-Positive Breast Cancer

Resistance to anti-HER2 therapies, particularly trastuzumab, remains a formidable clinical barrier in the management of HER2-positive breast cancer, and miR-21 has emerged as a central, multifaceted mediator of this resistance through a constellation of interconnected molecular mechanisms [25,38,39]. Elevated miR-21 expression is consistently associated with poor pathological complete response to neoadjuvant trastuzumab-based regimens and reduced overall and disease-free survival (DFS), with experimental overexpression directly conferring resistance while its inhibition restores drug sensitivity in both cell lines and xenograft models [40,41].

While miR-21 is among the most studied mediators of trastuzumab resistance, additional miRNAs - including miR-221 and miR-155 - also contribute to therapy failure in HER2-positive breast cancer. miR-221 promotes resistance through suppression of p27^Kip1 and activation of AKT signaling, whereas miR-155 enhances STAT3-dependent survival pathways and modulates immune-evasion programs [42]. Comparative analyses indicate that miR-21 exhibits the strongest and most reproducible correlation with trastuzumab resistance, supported by its multifaceted targeting of PTEN, PDCD4, and mismatch-repair proteins. In contrast, miR-221 and miR-155 show subtype-specific or context-dependent associations. These comparisons support miR-21 as the most consistently validated miRNA-based predictor of HER2-targeted therapy response [25,43].

Mechanistically, miR-21 drives resistance primarily through suppression of the tumor suppressor PTEN, leading to hyperactivation of the PI3K/AKT/mTOR pathway and inhibition of pro-apoptotic signaling [24]; this axis is further reinforced by its targeting of PDCD4, which enhances survival and EMT programs [16]. Beyond direct target repression, miR-21 cooperates with non-coding RNAs, as exemplified by the loss of the tumor-suppressive lncRNA Growth Arrest-Specific 5 (GAS5) in resistant tumors, which normally sequesters miR-21 via Competing Endogenous RNA (ceRNA) activity to preserve PTEN; GAS5 depletion thus unleashes miR-21-mediated oncogenic signaling [41]. Additionally, miR-21 fosters an immunosuppressive TME by activating the IL-6/STAT3/NF-κB axis, thereby

reducing cytotoxic T-cell infiltration and upregulating Programmed Death-Ligand 1 (PD-L1) expression, which may limit the efficacy of immunomodulatory combinations [23]. It also promotes cancer stem cell (CSC) maintenance and radioresistance in HER2-positive cells, likely through modulation of pluripotency factors such as SRY (Sex-determining Region Y)-Box 2 (SOX2) and Octamer-binding Transcription Factor 4 (OCT4), contributing to therapy persistence and relapse [44]. Notably, miR-21 is not only induced by HER2 signaling but also by extrinsic stressors: chemotherapy agents like paclitaxel upregulate miR-21 to enhance BCL2-mediated survival and suppress PDCD4/PTEN, while catecholamine-driven β2-adrenergic receptor (β2-AR) activation triggers HER2/STAT3-dependent miR-21 induction a pathway pharmacologically reversible by β-blockers such as propranolol, offering a novel repurposing strategy [45]. Furthermore, in the context of genomic co-amplification, the 17q23 locus harboring both MIR21 and WIP1 (a phosphatase that inactivates p53) creates a synergistic oncogenic unit that amplifies trastuzumab resistance and tumor progression independently of canonical HER2 kinase activity [46]. Importantly, while some studies caution that tumor miR-21 levels alone may lack predictive power in the adjuvant setting [47], its dynamic changes in circulation during therapy particularly declines correlating with improved outcomes combined with integration into multi-analyte panels (e.g., with CTCs, EVs, or other miRNAs), significantly enhance its utility as a real-time, non-invasive biomarker for monitoring resistance and guiding therapeutic adjustments [47,48]. Collectively, these findings position miR-21 not merely as a passive marker but as a master regulatory node whose multifunctional roles span genomic, transcriptional, post-transcriptional, metabolic, and immunological dimensions of therapy resistance, making it a compelling target for next-generation combinatorial interventions in HER2-positive breast cancer. Despite strong preclinical evidence implicating miR-21 in trastuzumab resistance, clinical validation has been inconsistent. Some cohorts have failed to correlate tumor miR-21 levels with trastuzumab benefit, suggesting that circulating or EV-enriched miR-21 may be more informative than tissue levels. Moreover, the majority of mechanistic studies rely on transient miR-21 inhibition, whereas stable or clinically translatable suppression remains challenging. These limitations emphasize the need to determine whether miR-21 is a true driver of resistance in patients or whether it primarily reflects downstream activation of broader stress-response pathways

6. Circulating and Extracellular Vesicle-Derived miR-21 as Non-Invasive Biomarkers

The exceptional stability of miR-21 in biofluids, particularly when protected within EVs shed by the tumor, establishes it as a prime candidate for sophisticated liquid biopsy applications in HER2-positive breast cancer [49-51]. Circulating miR-21 serves multifaceted roles, spanning early detection, prognostic stratification, and real-time therapeutic monitoring (Figure 3).

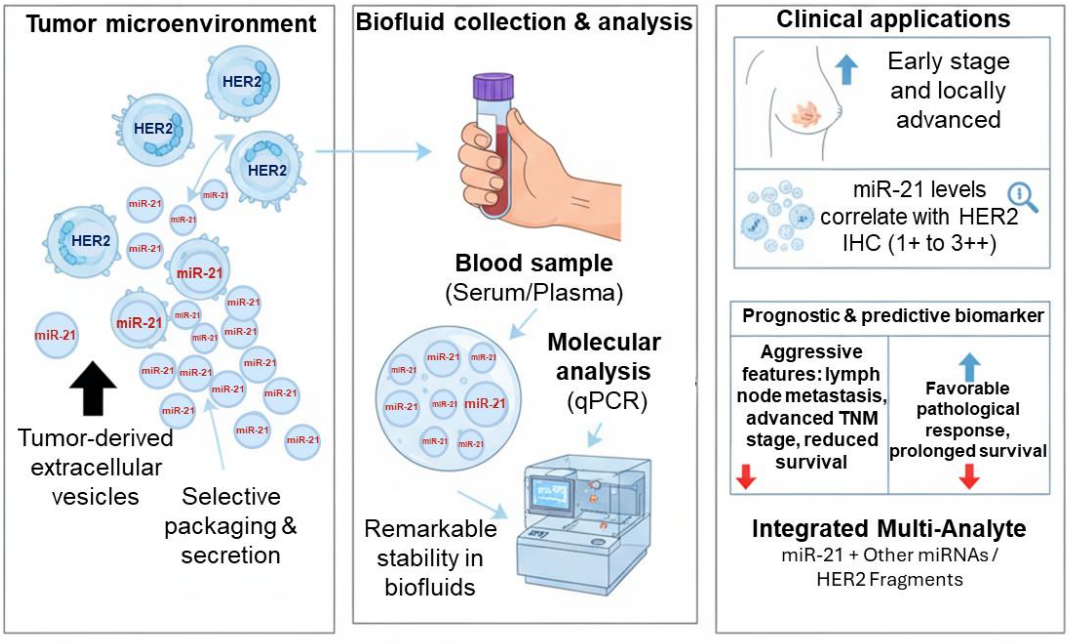


Figure 3. Circulating and extracellular vesicle-derived miR-21 as a non-invasive biomarker in HER2-positive breast cancer. This diagram details the mechanism by which miR-21 enters the circulation and its potential as a liquid biopsy biomarker, highlighting its role as both a diagnostic/prognostic tool and an intercellular messenger. (Abbreviations: miR-21: MicroRNA-21; HER2: Human Epidermal Growth Factor Receptor 2; TNM: Tumor, Node, Metastasis stage; IHC: Immunohistochemistry.)

6.1 Diagnostic and Prognostic Utility

Serum and plasma levels of miR-21 are consistently and significantly elevated in patients with HER2-positive disease compared to healthy individuals, demonstrating utility even in early-stage, non-metastatic settings. This highlights its potential for early detection and risk stratification independent of conventional imaging or

tissue biopsy [52-54]. As a prognostic indicator, elevated circulating miR-21 strongly correlates with aggressive clinicopathological features, including lymph node metastasis, advanced Tumor, Node, Metastasis (TNM) stage, high histological grade, and significantly reduced OS and DFS [55-57]. These consistent findings across ethnically diverse cohorts underscore its biological robustness and global applicability as a powerful prognostic marker [58,59]. Several studies reporting diagnostic accuracy metrics highlight the robustness of circulating miR-21. For example, Wang et al. reported an AUC of 0.86 (sensitivity 78%, specificity 82%) for serum miR-21 in differentiating early-stage breast cancer from controls. EV-derived miR-21 demonstrated even higher performance, with an AUC of 0.92 (sensitivity 85%, specificity 88%) in early detection cohorts. Multi-miRNA panels incorporating miR-21 further improved diagnostic accuracy; Kim et al. achieved an AUC of 0.97 with a 4-miRNA exosomal panel including miR-21. These metrics underscore the clinical utility of miR-21 as part of multimodal biomarker strategies [49,50].

6.2 Monitoring Therapeutic Efficacy and Resistance

Crucially, serial monitoring of circulating miR-21 during neoadjuvant or targeted therapy provides dynamic, real-time molecular insight into treatment efficacy. A marked decline in levels correlates with favorable pathological response and prolonged survival, indicating drug sensitivity. Conversely, persistently high or rising concentrations predict poor therapeutic outcomes and the emergence of acquired resistance, allowing for timely clinical intervention and adaptive treatment strategies [38,56]. Longitudinal profiling of circulating and EV-derived miR-21 reveals that its dynamic changes during treatment carry significant predictive value. In multiple neoadjuvant trials, patients who ultimately developed trastuzumab resistance exhibited persistently elevated or rising miR-21 levels in serial plasma samples, often preceding radiologic progression by several months. Conversely, patients achieving pathologic complete response showed a marked early decline in circulating miR-21. These findings suggest that miR-21 kinetics function as an early molecular indicator of emerging resistance and support the integration of serial liquid-biopsy monitoring into precision-medicine frameworks for HER2-positive breast cancer [39,56].

6.3 Enhancing Specificity with Multi-Analyte Panels and EV Isolation

The clinical relevance of miR-21 is superior when its detection is refined. EV-associated miR-21 is considered particularly valuable compared to total circulating miR-21, as it is selectively packaged and secreted by tumor cells in response to HER2-driven oncogenic signaling. This selective encapsulation provides a more accurate reflection of active tumor biology and the intercellular crosstalk occurring within the TME [50,60]. The specificity and sensitivity are further enhanced when miR-21 is integrated into multi-analyte panels: when combined with HER2-positive Circulating Tumor Cells (CTCs), diagnostic accuracy for metastatic disease improves substantially, and when paired with other miRNAs (e.g., miR-10b, miR-210) or circulating HER2 fragments, it improves subtype classification and prediction of recurrence [48,61,62]. The specificity and sensitivity are further enhanced when miR-21 is integrated into multi-analyte panels: when combined with HER2-positive CTCs, diagnostic accuracy for metastatic disease improves substantially, and when paired with other miRNAs (e.g., miR-10b, miR-210) or circulating HER2 fragments, it improves subtype classification and prediction of recurrence. Nevertheless, reliance on circulating miR-21 alone risks overinterpretation. Numerous conditions including inflammation, metabolic stress, and non-malignant tissue injury can elevate plasma miR-21, raising concerns about specificity. Furthermore, technical variation in EV isolation, storage, and normalization creates noise that can mask biologically meaningful changes. Thus, multi-marker signatures integrating miR-21 with subtype-specific molecules may offer more reliable performance than miR-21 alone.

A variety of analytical platforms are used to quantify circulating or EV-derived miR-21, each with distinct sensitivity profiles. qRT-PCR remains the most widely used method due to its affordability and high analytical specificity, though its performance depends heavily on reference-gene normalization. Next-generation sequencing (NGS) provides unbiased discovery of miRNA signatures and superior dynamic range, yet requires larger input RNA and higher cost. Branched Rolling Circle Amplification (RCA) offers ultrasensitive detection suitable for low-abundance miRNAs in early-stage disease and has demonstrated superior performance in serum-based early detection studies. Integrating these methodologies allows comprehensive and cross-validated miRNA characterization for clinical translation [54].

This comprehensive diagram illustrates the dual role of miR-21 in the circulation, serving both as a dynamic non-invasive biomarker for liquid biopsy and as a critical mediator of intercellular communication within the TME. Driven by oncogenic signaling from the Human Epidermal Growth Factor Receptor 2 (HER2), cancer cells overexpress miR-21 and selectively package it into protective structures, predominantly EVs such as exosomes, which are then secreted into the bloodstream; a fraction also circulates bound to proteins like Argonaute 2 (Ago2). These highly stable circulating levels, particularly the EV-miR-21 fraction, are utilized in liquid biopsy for clinical applications: they serve as reliable diagnostic and prognostic indicators, with elevated concentrations correlating strongly with advanced TNM stage, poor OS, and reduced DFS. Furthermore, serial monitoring of circulating miR-21 levels provides real-time insight into therapeutic efficacy, as a decline predicts successful response, while persistent elevation forewarns of therapeutic resistance and relapse; the predictive value is often enhanced when used in conjunction with other circulating markers like CTCs. Beyond its biomarker potential, the oncogenic role of miR-21 extends to intercellular crosstalk, as tumor-derived EV-miR-21 is internalized by recipient cells in the TME, including immune cells and Cancer-Associated

Fibroblasts (CAFs), thereby transferring its function to reprogram these cells, promoting an immunosuppressive TME (e.g., inducing pro-tumorigenic M2 Macrophages) and facilitating invasion and metastasis at distant sites.

7. Interplay with Hormonal and Subtype-Specific Pathways

Although miR-21 is best known for its strong association with HER2-positive breast cancer, its expression and oncogenic impact are modulated by a complex network of hormone and subtype-specific signaling pathways - underscoring that miR-21 functions as a pan-subtype oncomiR whose roles are shaped by cellular context rather than subtype identity alone. Recent reviews emphasize miR-21 as a "classic example" of a microRNA whose frequent upregulation across breast cancer subtypes contributes to tumor growth and invasion via suppression of TSGs such as PTEN and PDCD4, leading to activation of PI3K/AKT and related downstream oncogenic pathways [14,63].

In hormone receptor-positive (ER^+)/HER2-positive contexts, estrogen signaling appears to modulate miR-21 expression [64]. Although earlier work demonstrated that 17β-estradiol (E2) reduces miR-21 levels in ERα-positive breast cancer cells, leading to increased expression of miR-21 targets including PTEN, PDCD4, and BCL2, which could have pro-apoptotic and anti-proliferative effects, this hormone-miRNA axis suggests that ER signalling may counteract miR-21-driven oncogenic pathways in certain luminal settings [63,65]. Nonetheless, the hormonal regulation of miR-21 is likely dynamic and influenced by additional variables such as receptor co-factors, receptor subtype (ERα vs ERβ), and cross-talk with other pathways, indicating that the effect observed *in vitro* may differ *in vivo*, especially in the context of co-expression of HER2 [17].

Emerging evidence also underscores the capacity of miR-21 to integrate signals from diverse oncogenic pathways beyond hormone receptors and HER2. For example, some research highlighted that miR-21 participates in multiple breast cancer-relevant signaling cascades (e.g., PI3K/AKT, TGF-β, NF-κB), and can interact with non-coding RNA networks (including long non-coding RNAs) to modulate tumorigenesis, metastasis, and therapy resistance [66-68]. Therefore, these observations support the notion that miR-21 may function as a universal biomarker of tumor aggressiveness across breast cancer subtypes, even though its upstream regulation and downstream functional consequences are modulated by subtype-specific and hormone-dependent signaling [69].

Given its ubiquity and functional versatility, miR-21 remains a prime candidate for therapeutic targeting - but designing such interventions will likely require subtype- and context-specific strategies (for example combining miR-21 inhibition with endocrine therapy in hormone-positive disease, or with immune-modulatory / PI3K-pathway inhibitors in TNBC). As recently reviewed in the context of miRNA-based therapeutics, miR-21 is among the most studied miRNAs proposed for intervention, particularly in TNBC models [70].

8. Therapeutic Targeting of the miR-21/HER2 Axis: Emerging Strategies

Given its central role in HER2-driven oncogenesis and therapy resistance, miR-21 represents a compelling therapeutic target, with preclinical studies demonstrating multiple promising strategies to disrupt its oncogenic activity. Antisense oligonucleotides (ASOs) targeting miR-21 effectively restore expression of tumor suppressors PTEN and PDCD4, suppress tumor growth, and re-sensitize HER2-positive xenografts to trastuzumab, highlighting a direct path to overcoming resistance [25,38]. To enhance delivery and efficacy, lipid- and polymer-based nanoparticles carrying anti-miR-21 oligonucleotides have shown superior tumor targeting and therapeutic outcomes in murine models, while peptide-conjugated anti-miR-21 constructs demonstrate significant tumor burden reduction in both TNBC and HER2-positive contexts, suggesting broad applicability across subtypes. Critically, combinatorial approaches co-targeting miR-21 and HER2, such as trastuzumab with anti-miR-21, exhibit synergistic effects in resistant models, with dual inhibition of miR-21 and WIP1 overcoming trastuzumab resistance in tumors with 17q23 amplification [41,46]. Indirect targeting strategies also show promise, including pharmacologic β-blockade (e.g., propranolol) to inhibit β2-AR signaling, which reduces miR-21 expression and restores trastuzumab sensitivity by disrupting the HER2/STAT3/miR-21/PTEN axis [46]. Emerging epigenetic approaches further expand the therapeutic arsenal, targeting HER2 locus-driven transcription or PAPD5-mediated stabilization of miR-21-5p to dismantle the HER2-miR-21 oncogenic axis at the transcriptional level [18]. Despite these robust preclinical advances, no miR-21-targeted therapy has yet advanced to clinical approval, underscoring the urgent need for biomarker-guided trials that integrate dynamic miR-21 monitoring (e.g., via circulating EVs) to identify patient subgroups most likely to benefit from these precision interventions and translate mechanistic insights into tangible clinical outcomes for HER2-positive breast cancer. Although no miR-21-specific inhibitor has yet entered clinical trials, miRNA-based therapeutic platforms targeting other oncogenic miRNAs (such as anti-miR-155 and miR-34 mimics) have advanced to Phase I testing, demonstrating the feasibility and safety of systemic miRNA modulation in humans. Lipid-nanoparticle and peptide-conjugated antisense scaffolds used for miR-21 inhibition in preclinical HER2-positive models are based on the same chemistries already evaluated in Phase I trials, indicating that the translational barrier is technological rather than conceptual. Furthermore, PAPD5 inhibitors-currently in early-stage development for hematologic malignancies-represent an emerging class of agents with potential repurposing for destabilizing miR-21 in HER2-positive breast cancer [71]. However, translation of anti-miR-21 approaches to the clinic faces substantial challenges, including off-target suppression of miR-21 in normal tissues, delivery barriers for nucleic acid therapeutics,

and the context-dependent nature of miR-21 signaling. Thus, early-phase clinical trials must incorporate biomarker-guided patient selection and longitudinal monitoring to determine whether miR-21 inhibition provides consistent therapeutic benefit in HER2-positive disease (Figure 4).

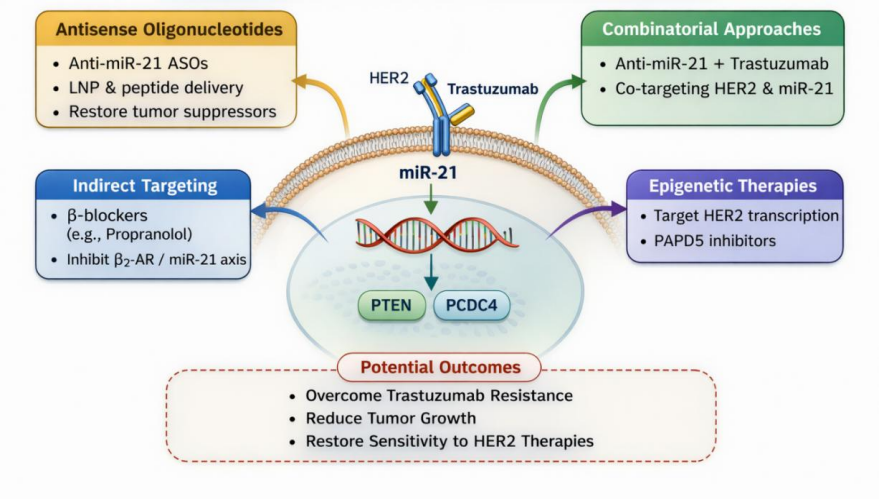


Figure 4. Therapeutic strategies targeting the HER2 signaling cascade and the regulatory miR-21 Axis.

9. Limitations and Future Directions

Although the cumulative evidence supporting miR-21 as a prognostic and diagnostic biomarker in breast cancer and other malignancies is compelling, several important limitations must be acknowledged, and they frame critical directions for future research. One major challenge arises from the heterogeneity in detection methods: different studies have used variable sample types (serum vs. plasma), distinct normalization controls, and divergent platforms (qRT-PCR, small RNA sequencing, exosomal RNA assays), which complicates direct comparison across datasets and undermines the robustness of meta-analytic conclusions [53,54].

Indeed, circulating miRNAs may show discordant levels between serum and plasma from the same individual, and sample processing, storage conditions, and normalization strategy (e.g., choice of internal control) all influence measured miR-21 expression [26,72].

Furthermore, despite efforts to use circulating miR-21 as a minimally invasive biomarker, there remains no consensus on optimal cutoff values or standard protocols to define “high” vs “low” expression in a clinical setting. This lack of standardization is a barrier to translating miR-21 measurements into routine diagnostic or prognostic workflows [73,74]. An additional complexity is the context-dependent roles of miR-21. While in many tumor settings miR-21 acts as an oncomiR, some data suggest that its expression and function may vary depending on the tissue microenvironment, cell type (tumor cells vs. stromal cells), and disease state. The presence of miR-21 in non-malignant cells - for example stromal fibroblasts, immune cells, or other components within the TME - may confound interpretation of bulk tissue or circulating miR-21 measurements and complicate efforts to ascribe prognostic significance solely to tumor-derived miR-21 [47,75].

Given these limitations, several priorities should guide future research. First, there is a critical need to develop standardized, validated assays for circulating miR-21, ideally “gold standard” protocols endorsed by regulatory authorities (e.g., for exosome-derived miR-21, or other circulating miR-21 fractions) that define sample collection, processing, normalization, and cutoff thresholds. Studies assessing the stability of miRNAs suggest that miRNAs, including miR-21, are relatively stable in stored serum or plasma under various conditions, supporting the feasibility of circulating miRNA assays under standardized conditions [76]. Second, recognizing the lack of disease specificity of miR-21, future efforts may be better directed toward integrating miR-21 into multi-analyte liquid biopsy panels - combining miR-21 with other miRNAs, circulating tumor DNA (ctDNA), CTCs, protein markers, or fragments of subtype-specific markers (e.g., for HER2) rather than relying on miR-21 alone. Such combinatorial approaches have already shown promise to improve diagnostic or prognostic accuracy compared with single-miRNA assays. Third, prospective, large-scale, multi-center clinical studies are needed to validate the prognostic and predictive value of circulating (or exosomal) miR-21, ideally with stratification by molecular subtype (e.g., HER2 status), treatment modalities (adjuvant, neoadjuvant, metastatic setting), and long-term follow-up for outcomes such as recurrence, metastasis (including site-specific, e.g. brain), and survival. Such studies should include rigorous methodological standardization and pre-specified cutoff definitions. For example, a 2018 prospective plasma-miRNA study in early breast cancer demonstrated that elevated pre-treatment miR-21 predicted shorter DFS and OS, but the authors noted the need for validation in independent and larger cohorts [75]. Recent advances in computational modeling and artificial intelligence have substantially accelerated biomarker discovery and validation in breast cancer, including miR-21-centered signatures. Machine-learning pipelines trained on multi-omic datasets from TCGA and METABRIC have been used to identify miRNA-mRNA interaction networks, classify HER2-positive disease with high accuracy, and predict therapeutic response based on circulating miRNA profiles. Deep-learning frameworks, including

convolutional neural networks and graph-based models, have further improved the detection of non-linear interactions among miRNAs, copy-number variants, and signaling nodes such as PI3K/AKT. These AI-based integrative platforms strengthen both the reproducibility and clinical relevance of miRNA biomarkers by reducing noise, improving feature selection, and enabling cross-cohort validation. As such computational methods mature, they are expected to play a pivotal role in standardizing and translating miR-21-based diagnostic and prognostic tools into clinical practice.

10. Conclusion

MiR-21 has solidified its position as a pivotal biomarker and therapeutic target in HER2-positive breast cancer, offering significant promise for overcoming therapeutic resistance and improving patient outcomes. Its dual role as both a downstream effector and an amplifier of HER2 signaling positions miR-21 as a central player in tumor progression, metastasis, and treatment resistance. Furthermore, the stability of miR-21 in biofluids, particularly within EVs, presents a novel avenue for non-invasive diagnostic and monitoring applications, enabling real-time insights into tumor dynamics. While the path to clinical translation of miR-21-targeted therapies remains complex, the mounting evidence supporting its multifactorial involvement in HER2-positive breast cancer biology is undeniable. Future research must focus on overcoming current limitations in detection, standardization, and clinical validation to realize the full therapeutic potential of miR-21. Ultimately, the integration of miR-21-based approaches into personalized treatment regimens holds the promise of enhancing therapeutic efficacy and improving clinical outcomes for patients with HER2-positive breast cancer. Given recent improvements in assay reproducibility and EV-based detection technologies, clinical implementation of miR-21-based diagnostics may be feasible within the next five years, pending standardized protocols and validation in prospective multi-center trials

Abbreviations

Ago2: Argonaute 2

AP-1: Activator protein 1

ASOs: Antisense oligonucleotides

CAFs: Cancer-associated fibroblasts

ceRNA: Competing endogenous RNA

CSC: Cancer stem cell

CTCs: Circulating tumor cells

ctDNA: Circulating tumor DNA

DFS: Disease-free survival

EMT: Epithelial-to-mesenchymal transition

ER: Estrogen receptor

ER^+: Estrogen receptor-positive

EVs: Extracellular vesicles

GAS5: Growth arrest-specific 5

HER2-positive: Human epidermal growth factor receptor 2-positive

IHC: Immunohistochemistry

MAPK/ERK: Mitogen-activated protein kinase/extracellular-signal-regulated Kinase

MAPK Pathway: The RAS/RAF/MEK/ERK pathway

miRNAs: MicroRNAs

miR-21: MicroRNA-21

MMPs: Matrix metalloproteinases

MSH2: MutS homolog 2

NGS: Next-generation sequencing

OCT4: Octamer-binding transcription factor 4

OS: Overall survival

PAM50: Prediction analysis of microarray 50

PDCD4: Programmed cell death 4

PD-L1: Programmed death-ligand 1

PI3K/AKT: Phosphatidylinositol 3-kinase/protein kinase B

PR: Progesterone receptor

Pri-miR-21: Primary miR-21 transcript

PR^+: Progesterone receptor-positive

PTEN: Phosphatase and tensin homolog

RCA: Rolling circle amplification

RECK: Reversion-inducing-cysteine-rich protein kinase

SOX2: SRY (sex-determining region Y)-box 2

STAT3: Signal transducer and activator of transcription 3

TME: Tumor microenvironment

TNBC: Triple-negative breast cancer

TNM: Tumor, node, metastasis

TSGs: Tumor suppressor genes

ZNF132: Zinc finger protein 132

β2-AR: β2-adrenergic receptor

Competing Interests

There is no conflict of interest.

Authors' Contributions

AZT wrote the manuscript comprehensively in all parts.

Generative AI Statement

The authors declare that no generative artificial intelligence technologies were used when preparing this manuscript.

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