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REVIEW ARTICLE

miR-143-3p: A Critical Regulator of KRAS and HRAS with Potential for Targeted Therapies in Breast Cancer

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Abstract

Breast cancer is a heterogeneous disease with distinct subtypes, including the highly aggressive triple-negative breast cancer (TNBC), which lacks estrogen, progesterone, and HER2 receptors. The rapid recurrence, high metastatic potential, and poor clinical outcomes of breast cancer are driven by dysregulated signaling pathways, such as PI3K/AKT/mTOR, MAPK/ERK, and JAK/STAT, which contribute to breast cancer progression, therapy resistance, and metastasis. Among these, the Ras family of proteins, particularly KRAS and HRAS, is pivotal in promoting tumor survival, epithelial-to-mesenchymal transition (EMT), and chemotherapy resistance. Recent research highlights the regulatory role of microRNAs (miRNAs) in these pathways, with miR-143-3p emerging as a critical modulator of the RAS signaling cascade. miR-143-3p acts as a tumor suppressor by targeting key components of the RAS pathway, including KRAS, thereby influencing downstream effectors. This regulation impacts cellular processes such as proliferation, apoptosis, and EMT, crucial in breast cancer progression and therapeutic resistance. This review focuses on the role of miR-143-3p in breast cancer, particularly its regulation of the RAS pathway. It discusses its potential as a therapeutic target for improving outcomes in aggressive breast cancer subtypes.

Keywords: Breast cancer, Gene regulation, HRAS, KRAS, MicroRNA, miR-143-3p, RAS mutation

Introduction

Breast cancer is a pathological condition that occurs in breast tissue, where most cases originate from the milk ducts, while a smaller portion arises from the lobules. Cancer that develops in the milk ducts is called ductal carcinoma, whereas cancer that develops in the lobules is referred to as lobular carcinoma [1,2]. TNBC is a highly aggressive subtype of breast cancer, defined by the absence of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2). This lack of receptor expression makes TNBC unresponsive to traditional treatments such as hormone therapy and HER2-targeted therapies, which are effective in other breast cancer subtypes. As a result, chemotherapy, particularly anthracyclines and taxanes - remains the primary treatment option. However, despite initial responses to chemotherapy, TNBC is known for its rapid recurrence, high metastatic potential, and poor prognosis. The median survival for patients with metastatic TNBC is approximately 13.3 months, with a 5-year survival rate dropping to just 11 % when the cancer has spread to distant organs [3].

These challenges highlight the need for novel therapeutic strategies to improve treatment outcomes.

TNBC comprises about 15 – 20 % of all breast cancer cases globally, with approximately 2.08 million cases diagnosed worldwide in 2018 [4]. Studies in Asia, such as in Indonesia, show that TNBC constitutes around 15 - 25 % of the 50,000 breast cancer cases diagnosed annually. In addition, TNBC disproportionately affects African American women, who have a higher incidence and worse survival outcomes compared to Caucasian women. This racial disparity underscores the urgency of developing more effective treatments tailored to high-risk populations.

The poor prognosis associated with TNBC is largely due to its molecular complexity and the lack of targeted therapies. Unlike hormone receptor-positive and HER2-positive breast cancers, TNBC lacks specific molecular targets, making treatment more difficult. TNBC tumors exhibit significant molecular heterogeneity, further complicating diagnosis and therapy selection [3,5,6]. A major contributor to the aggressiveness of TNBC is the dysregulation of key signaling pathways, such as the PI3K/AKT/mTOR, MAPK/ERK, and JAK/STAT pathways, which drive tumor proliferation, survival, invasion, and metastasis. These pathways often become hyperactivated in TNBC, promoting tumor progression and contributing to resistance against conventional therapies.

One of the central factors in TNBC resistance to therapy is the dysregulation of the RAS family of proteins, particularly KRAS and HRAS. Mutations in these genes are frequently observed in various cancers, including TNBC, and play a crucial role in promoting tumorigenesis and chemotherapy resistance [7]. Oncogenic KRAS mutations, for instance, drive chemotherapy resistance by activating downstream signaling pathways such as PI3K/AKT and MAPK/ERK, which are involved in cell survival and proliferation [8]. KRAS mutations also enhance the expression of anti-apoptotic proteins like Bcl-2, Bcl-xL, and survivin, making cancer cells more resistant to cell death [9]. In addition, KRAS mutations contribute to the epithelial-to-mesenchymal transition (EMT), a process that enables tumor cells to become more invasive and metastatic. The KRAS mutations further complicate treatment, as EMT enhances tumor spread and immune evasion, leading to poor clinical outcomes.

The tumor microenvironment (TME) also plays a significant role in the progression and treatment resistance of TNBC. Tumor-associated macrophages (TAMs) and tumor-infiltrating lymphocytes (TILs) are key TME components that influence tumor growth and immune evasion. TAMs have been shown to promote a pro-tumorigenic environment by secreting cytokines that support tumor growth and metastasis [10]. Meanwhile, TILs, although part of the immune response, often fail to mount an effective attack against TNBC cells due to the immunosuppressive nature of the TME. These immune-related factors, combined with the aggressive behavior of TNBC, highlight the need for new therapeutic approaches to overcome immune evasion and resistance mechanisms.

Given the complexity of TNBC, one promising area of research is the role of microRNAs (miRNAs) in regulating key signaling pathways, including the Ras pathway. miR-143 has emerged as a significant regulator of tumor progression in various cancers, including breast cancer. miR-143 targets key components of the Ras signaling pathway, potentially modulating tumor cell growth, invasion, and resistance to chemotherapy [11]. Recent studies suggest that miR-143 can function as a tumor suppressor in TNBC by inhibiting Ras-related signaling and affecting downstream targets involved in cell survival and metastasis [12,13]. miR-143 forms two isoforms: miR-143-3p (the guide strand) and miR-143-5p (the passenger strand), with miR-143-3p being more prevalent in tissues like fibroblasts and smooth muscle cells [13,14]. miR-143-5p is often downregulated in breast cancer, esophageal squamous cell carcinoma, colorectal cancer, and gastric cancer. Its overexpression can suppress breast cancer progression through HIF-1α-related GLUT1, inhibit metastasis, reduce cell proliferation, and promote apoptosis [15,16].

In contrast, miR-143-3p is more widely implicated in various cancers compared to miR-143-5p. It is frequently downregulated in cancers such as lung adenocarcinoma, breast cancer, hepatocellular carcinoma, colorectal cancer, gallbladder carcinoma, endometrial cancer, and melanoma [14,17,18]. In TNBC, Nanostring analysis shows miR-143-3p downregulation correlates with poor prognosis and reduced survival rates [19]. However, in brain metastases, miR-143-3p is upregulated, potentially

enhancing cancer cell invasion and angiogenesis, facilitating their penetration through the blood-brain barrier (BBB) [20]. Given that miR-143-3p is more widely associated with various cancers than miR-143-5p, understanding its interaction with the Ras pathway in TNBC could offer valuable insights for developing novel therapeutic strategies to overcome treatment resistance and improve outcomes.

This review will summarize the role of miR-143-3p in regulating the Ras signaling pathway in breast cancer. We will discuss how miR-143-3p modulates key molecular mechanisms, including apoptosis, EMT, and metastasis. We will discuss how targeting this miRNA could offer a promising therapeutic approach for combating breast cancer's aggressive nature and resistance to conventional therapies. By providing a deeper understanding of miR-143-3p and Ras signaling, we aim to highlight potential therapeutic opportunities that could lead to more effective treatments for breast cancer patients.

Materials and methods

The aim of this review was to summarize the existing data and information. This study utilized narrative approaches to conduct comprehensive review analysis, following 3 key steps: literature screening, data extraction, and analysis. Relevant literature was searched using the Scopus database with the keywords “miR-143-3p; AND breast; AND cancer” and “miR-143-3p; AND RAS; AND signaling” within the section "article title, abstract, and keyword." The inclusion criteria for the literature search encompassed research articles and review articles published in English between 2015 and 2024. In addition to Scopus, we conducted a search for supporting relevant literature on Google Scholar using the same keywords.

The extracted data were categorized into 3 main areas:

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1) The role of RAS mutations in breast cancer cell lines.

2) The regulatory role of miR-143-3p in the RAS signalling pathway.

3) The impact of miR-143-3p on cancer cell proliferation, metastasis, and apoptosis.

To guide the analysis, several research questions were addressed, including how RAS mutations influence breast cancer cell lines and their implications for tumour progression and therapy resistance. We also investigated how miR-143-3p regulates the RAS signalling pathway and its downstream targets. Lastly, we discussed the effects of miR-143-3p on critical cellular processes such as proliferation, metastasis, and apoptosis in breast cancer, as well as its potential therapeutic value in reducing tumour aggressiveness and enhancing treatment outcomes.

The RAS family proteins

In general, the RAS protein regulates proliferation, differentiation, cell survival, and protein localization on the cell membrane, which plays a role in various signaling processes, including oncoprotein signaling [21]. In addition to increasing oncoprotein signaling, RAS also plays a role in regulating cell proliferation and apoptosis [22]. RAS protein activation occurs when epidermal growth factor (EGF) binds to tyrosine kinase receptors such as epidermal growth factor receptor (EGFR). When RAS is activated, many signals are initiated. The major signaling pathways that have been well characterized are the mitogen-activated protein kinase (MAPK) pathway via the Raf/MEK/ERK (MEK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase) and PI3K/AKT/mTOR (PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B; mTOR, mechanistic target of rapamycin) phosphorylation cascades [23,24]. RAS activation and signaling are shown in **Figure 1**.

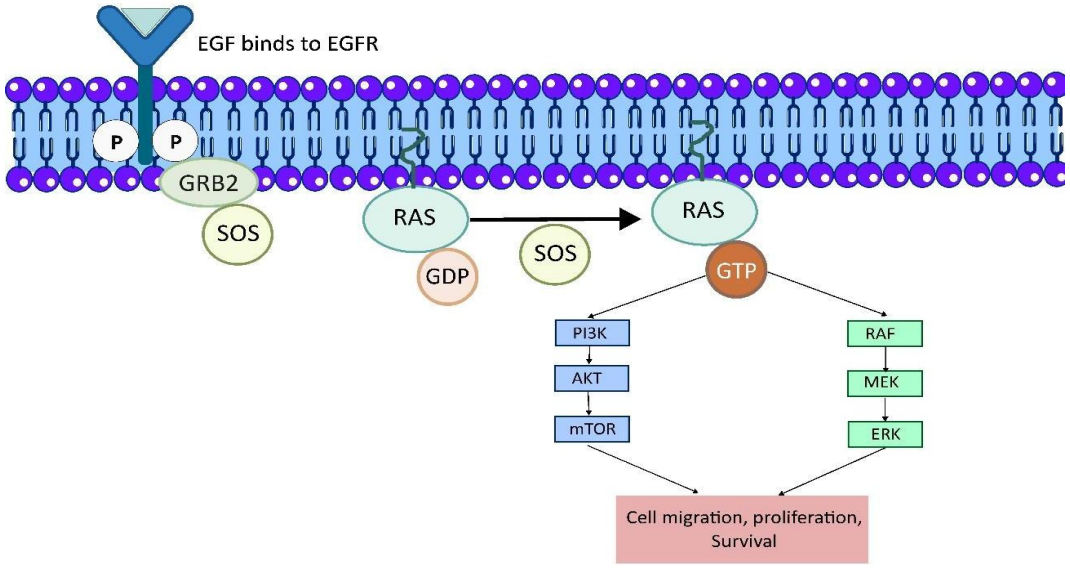

Figure 1 RAS Activation and Signalling.

Ras protein has 3 isoforms: NRas, Hras, and KRas. The functions of KRas, HRas, and NRas are specific depending on the context and mutations [24]. Several theories explain the specific functions of Ras isoforms. First, the specificity of a particular Ras isoform is related to the level of expression in a particular cancer. Second, the specific function of Ras is related to the potential of Ras isoforms to trigger cancer in specific tissues. From a genetic perspective, Ras isoforms have different DNA repair efficiencies due to slight sequence differences in Ras isoforms at codons 12, 13, and 14. The mutation status of the RAS gene influences the level of RAS gene expression. Genetic expression and mutation status of the RAS gene are related to chromatin accessibility in certain regions because chromatin accessibility determines the binding ability of transcription factors [25-27].

KRAS and HRAS in breast cancers

Cell proliferation

KRAS signaling promotes TNBC cell growth. The inhibition of the PI3K and MEK pathways by PI3K inhibitor effectively suppressed proliferation in MDA-MB-231 cells in a dose-dependent manner. TNBC has higher KRAS expression than other breast cancer subtypes. So, a RAS downstream effector inhibitor could inhibit the TNBC cells proliferation [13]. Besides KRAS signaling, HRAS signaling also plays an important role in driving cancer cell proliferation. The HRAS G12D mutation in TNBC cell line Hs578T plays a pivotal role in driving tumor progression by activating critical signaling pathways that regulate proliferation. This mutation leads to constitutive activation of HRAS promoting oncogenic signaling cascades that enhance the malignant phenotype [28]. Elevated HRAS expression, although relatively rare in breast cancer, is frequently observed in aggressive subtypes like TNBC, making it a potential biomarker for malignancy. Additionally, the H-Ras G12D mutation makes Hs578T cells particularly susceptible to the effects of FTI-277, a farnesyltransferase inhibitor. FTI-277 effectively inhibits the proliferation and invasive phenotypes of Hs578T cells in a dose-dependent manner, with an IC50 value of 14.87 μM for 48 h [28]. By interfering with the localization and activation of H-Ras at the cell membrane, FTI-277 suppresses H-Ras-driven oncogenic traits, mitigating the aggressive behaviour of these cancer cells.

Cell migration and metastasis

KRAS activation also contributes to increasing cancer cell migration and metastasis in TNBC, basal, also luminal breast cancer. In MDA-MB-231 cells, KRAS activation induces the expression of SLUG, a key transcription factor involved in epithelial-mesenchymal transition (EMT), while having little to no effect on other EMT-related factors like SNAIL, TWIST, or ZEB1. The elevated expression of SLUG underscores its critical role in maintaining the mesenchymal and malignant characteristics of MDA-MB-231 cells.

Moreover, research demonstrates that siRNA-mediated downregulation of KRAS significantly reduces the migratory and invasive properties of these cells, highlighting KRAS's pivotal role in driving tumor progression [29].

Notably, KRAS activation is both necessary and sufficient to induce mesenchymal features in breast cancer cells [13,29,30]. For instance, ectopic expression of KRAS in luminal-type breast cancer cells was sufficient to induce mesenchymal phenotypes, emphasizing KRAS's unique role in promoting metastatic potential. Despite the relatively low overall frequency of KRAS mutations in breast cancer, the functional significance of KRAS activation, as seen in MDA-MB-231 cells, highlights its critical impact on basal-type TNBC. These findings suggest that KRAS, through its regulation of SLUG and downstream signaling pathways, represents a key oncogenic driver in basal-type TNBC. Targeting KRAS and its regulatory mechanisms offers a promising therapeutic strategy for addressing the aggressive behavior of KRAS-driven TNBC, particularly in cases like MDA-MB-231 [29].

An increase in HRAS expression also increases breast cancer cells metastasis ability. In another TNBC cell line, Hs578T cells, HRAS activity is closely linked to lipid rafts, where the protein flotillin-1 acts as a crucial regulator [31]. Flotillin-1 facilitates HRAS activation by supporting pathways such as Rac1-p38 signaling, which drives invasive and migratory behaviors. Its depletion disrupts H-Ras micro localization and signaling, significantly reducing the invasive capacity of Hs578T cells *in vitro* and tumor growth *in vivo*. Research findings further reveal that flotillin-1 is overexpressed in breast cancer tissues, correlating with elevated H-Ras levels, particularly in TNBC. Clinical data link flotillin-1 expressions to poor survival outcomes and higher metastatic potential, emphasizing its role as a prognostic marker and therapeutic target in aggressive breast cancers [31].

EMT, marked by loss of E-cadherin and altered β-catenin signaling, facilitates tumor invasion and metastasis, exacerbating the aggressive nature of HRAS-mutated SUM159PT cells, another TNBC cell line. The SUM159PT has the loss of E-cadherin expression resulting from promoter hypermethylation [32]. This epigenetic inactivation is characteristic of the spindle-shaped basal morphology of SUM159PT and contributes to epithelial-to-mesenchymal transition (EMT) that continues to cell metastasis [32].

Cell cycle regulations and apoptosis

The MDA-MB-231 cell line, a widely studied TNBC model, harbours the KRAS G13D mutation, significantly contributing to its aggressive and invasive phenotype [30]. In MDA-MB-231, KRAS expression was upregulated compared to the normal mammary cell line MCF10A [13]. Notably, MCF7, BT474, and MDA-MB-231 exhibited particularly high levels of KRAS expression. Among these, TNBC displayed significantly higher KRAS signaling compared to estrogen receptor (ER)-positive breast cancers. KRAS mutations, including those at hotspots G12 and G13, are frequently implicated in TNBC, associated with poor prognosis and limited therapeutic options [30]. In MDA-MB-231 cells, the mutation influences the MAPK/ERK pathway, promoting the expression of anti-apoptotic proteins like BCL-x and Cathepsin D, further enhancing cell survival and invasiveness. These findings underscore the critical role of KRAS mutations in TNBC biology and therapeutic resistance [30].

Another TNBC cell model, SUM159PT cell line, harbors a G12D mutation in the HRAS oncogene alongside mutations in PIK3CA (H1047L) and the tumor suppressor TP53 [32]. While HRAS mutations are rare in TNBC, they can activate oncogenic signaling pathways critical for tumor progression. The G12D mutation in HRAS leads to constitutive activation of HRAS, driving abnormal cell malignant phenotypes [33]. In SUM159PT cells, this oncogenic signaling is compounded by additional genetic alterations. PIK3CA, frequently mutated in breast cancer, is constitutively active, enhancing downstream PI3K/AKT pathway signaling. TP53 mutations further disrupt cell cycle regulation and apoptosis, collectively contributing to the aggressive behavior of these cells [32,34].

Immune response regulation

KRAS signaling is known to be associated with progression in several cancers. Upregulation of KRAS signaling usually occurs in cancers with a high KRAS mutation rate, such as pancreatic cancer. KRAS mutations create an immunosuppressive tumor microenvironment, while the efficiency of chemotherapy is influenced by immune cell infiltration.

Thus, cancers with high KRAS mutations do not respond well to chemotherapy. In breast cancer, KRAS mutations are infrequent. Thus, only 2 % of breast cancers have KRAS mutations [13].

High KRAS expression is associated with a worse prognosis of breast cancer. KRAS expression was associated with TNBC progression since MEK and PI3K, both downstream effectors of KRAS inhibitors, suppressed tumor cell proliferation. The change of tumor microenvironment due to KRAS activation may be worsening tumor progression. CD8+ T cell infiltration decreases in high KRAS expression groups in breast cancers. RAS/MAPK activity inhibits MHC-I and MHC-II expression in breast cancer cells. The inhibition causes failed immune recognition of tumor cells and inflammation. Meanwhile, the infiltration level of T cells enhanced after KRAS activation. The immune response changes also happen if the target of drugs is downstream effectors of KRAS. Inhibition of PI3K and ERK1/2 reduced PD-L1 expression. Reduction of PD-L1 in tumor cells can reduce the probability of tumor cells escaping immune system recognition [35].

Interestingly, while KRAS activation generally drives breast cancer progression, it is paradoxically associated with better survival outcomes in TNBC patients. This may be attributed to its influence on the tumor immune microenvironment (TIME), which shows increased infiltration of anti-tumor immune cells, including CD8+ T cells, B cells, and M1 macrophages, in KRAS-high TNBC. At the same time, pro-tumor immune cells, such as regulatory T cells (Tregs), M2 macrophages, and central memory CD4+ T cells (Tcm), were less prevalent in KRAS-high TNBC [13]. These findings suggest that although KRAS promotes tumor progression, its role in enhancing anti-tumor immunity may improve survival in some instances.

Chemotherapy resistance

HRAS mutations are common in the salivary gland, urinary tract, gastrointestinal and upper respiratory tract, and cervical cancers. HRAS mutations in breast cancer are rare because they are only detected in < 1 % of all breast cancer subtypes [36]. So, information about the impact of HRAS mutations on breast cancer progression is very lacking.

However, Increased HRAS expression is most common in HER-2 positive, ER-negative breast cancer in an advanced stage. Increased HRAS expression occurs due to crosstalk between HER and epidermal growth factor receptor (EGFR). Therefore, increased HRAS expression causes resistance to EGFR inhibitor therapy. Interestingly, when only HER2 status was considered without considering the hormone receptor status, no affiliation with H-RAS mRNA levels was found in breast cancer patients. This perception underlines the need to investigate the organic part of H-RAS in advance, as it was connected to HER2 and to hormone receptor-dependent pathways [37]. The relationship between mutations, increased KRAS, and HRAS expression has been studied in breast cancer. Thus, several alternative breast cancer treatments have been developed that target HRAS and KRAS. However, the prognostic and predictive value of RAS overexpression still needs to be studied in breast cancer [36].

miRNAs: Expression, function, and mechanism of action

miRNAs are non-coding RNAs approximately 22 – 24 nucleotides long that play a crucial role in regulating gene expression at both transcriptional and post-transcriptional level. regulating gene expression at both transcriptional and post-transcriptional levels. By binding to the 3' or 5' untranslated regions (UTRs) of target mRNAs, miRNAs can mediate mRNA degradation or translational inhibition, thus influencing various cellular processes such as differentiation, identity, and tissue homeostasis. In cancer, miRNAs can act as oncogenes or tumor suppressors, influencing tumor progression, metastasis, and invasion. [28,38,39].

miRNAs are pivotal regulators of gene expression, influencing both transcriptional and post-transcriptional processes. They primarily function by silencing mRNA through binding to the 3' or 5' untranslated regions (UTRs), resulting in mRNA degradation or translational repression. Conversely, miRNAs can also activate transcription by interacting with promoters. The miRNA-mediated gene silencing process relies on the miRISC complex, where complementary binding between miRNA and its target mRNA at miRNA response elements (MREs) recruit proteins like AGO2 and GW182. This interaction leads to mRNA degradation or translational repression. Most MREs exhibit partial complementarity to miRNAs, which

prevents AGO2 endonuclease activity and allows GW182 to recruit deadenylase complexes for mRNA silencing [39].

In certain physiological conditions, such as nutrient starvation, miRNAs may enhance translation instead of silencing it. For instance, they can interact with AGO2, FXR1, and AREs or bind to the 5' UTR of ribosomal protein mRNAs, facilitating increased translation during amino acid starvation [39]. Dysregulation of miRNAs is a hallmark of many diseases, including cancer, where they can act as either tumor suppressors or oncogenes (oncomiRs). For example, the loss of miR-15 and miR-16 in chronic lymphocytic leukemia promotes cell proliferation, while overexpression of oncomiRs like miR-21 and miR-10b is associated with metastasis and poor prognosis in breast cancer [40].

Therapeutic strategies to modulate miRNA activity involve inhibiting oncomiRs using anti-miRNA oligonucleotides, sponges, or locked nucleic acids, and restoring tumor-suppressive miRNAs with miRNA mimics. For instance, miR-34 mimics have shown promise inducing apoptosis and suppressing tumor growth in lung, colon, and pancreatic cancers [40,41]. Although miRNAs hold great potential in cancer treatment, challenges remain in characterizing their diverse origins, which include not only their genes but also transcripts from 5p or 3p arms, long non-coding RNAs, small nucleolar RNAs, and spliced introns [28,42,43].

miR-143-3p regulation of KRAS and HRAS in breast cancer

One of the anti-oncogenic miRNAs is miR-143. miR-143 is categorized as a tumor suppressor because its expression is downregulated in most cancer cell lines. it is also downregulated in primary tumor samples [44]. miR-143-3p expression is downregulated in several cancers, mainly colorectal, prostate, cervical, ovarian, and B-cell lymphoma [38]. miR-143-3p is also downregulated in breast cancer. Expression level, gene target, and role of miR-143-3p in various cancers are summarized in **Table 1**. The knockdown of miR-143-3p in breast cancer cells showed a higher tumorigenesis potential in breast tumor cells through induction of RAS signaling, which increased sensitivity to MEK inhibition [28].

Table 1 Role of miR-143-3p in various cancers.

Cancer type	Expression level	Targets	Function	Ref.
Lung Adenocarcinoma (LUAD)	Downregulation	HOXA10	Promote cell proliferation, migration, invasion, and metastasis	[45]
Non-small cell lung cancer (NSCLC)	Downregulation	SOX5, RRM2	Promote cancer cell proliferation, migration, and inhibit apoptosis	[46,47]
Lung Cancer (LC)	Downregulation	CDK1	Promote cancer cell proliferation, survival, and reduce apoptosis	[48]
Breast Cancer (BC)	Downregulation	SMAD3, PCMT1, HK2, MSI2, COX-2, PCAT6, MYBL2, MAPK7, LIMK1, COL1A1	Promote proliferation, migration, invasion, metastasis, survival, tumorigenicity, inhibit apoptosis	[28,49-57]
Hepatocellular Carcinoma (HCC)	Downregulation	FOSL2, MSI2, ZEB1, FGF1	Promote cell proliferation, invasion, EMT, and cancer progression	[58-61]
Gastric Cancer (GC)	Downregulation	MAP3K7, IGF1R	Promote cell proliferation and metastasis, and inhibit apoptosis	[62,63]
Colorectal Cancer (CRC)	Downregulation	KLF5, CTNND1, ASAP3	Promote proliferation, invasion, and migration.	[64-66]

Cancer type	Expression level	Targets	Function	Ref.
Colon Cancer (CC)	Downregulation	BCL2, SMAD3, CDK1, VDAC1	Promote cancer cell proliferation, migration, invasion, metastasis, and inhibit apoptosis	[67-69]

Cell viability and proliferation

KRAS is known to be directly targeted by miR-143 in breast cancer. Direct targeting of KRAS in breast cancer has been studied in two cell lines, namely MDA-MB-231 and MCF-7. The qPCR results showed that KRAS mRNA expression decreased after transfection of synthetic miR-143-3p. Western blot also showed the same results for the KRAS protein. KRAS downstream effectors such as AKT and MAPK7 also decreased. There is also a decrease in the viability of both cell lines. Those findings mean that miR-143-3p inhibits the growth of breast cancer cells by directly targeting KRAS [13]. miR-143-3p can also indirectly regulate RAS mRNA by regulating downstream effectors regulated by RAS. Previous studies in osteosarcoma cell line U2OS showed that overexpression of MAPK7 with pcDNA-MAPK7 overexpression vector and co-transfection of miR-143-3p mimics reversed the inhibitory effects of miR-143-3p on the cell proliferation because miR-143-3p expression decreased. Those findings show that miR-143-3p also target KRAS downstream effectors such as MAPK7 [52].

Similar results were also found in breast cancer. The expression of miR-143 was inversely proportional to the expression of MAPK7 or ERK5 mRNA. Both are KRAS downstream effectors. MCF-7 cell viability decreased when MAPK7 or ERK5 expression increased. The level of miR-143 expression will decrease when MAPK7 or ERK5 expression and phosphorylation increase in breast cancer tissue compared with normal tissue [70]. Meanwhile, no study has specifically discussed the interaction of HRAS and miR-143 in breast cancer.

Cell cycle regulation and apoptosis

miR-143 plays a role in inhibiting apoptosis in TNBC. *In vivo* studies involving synthetic miR-143 transfection into nude mice infected with MDA-MB-231 TNBC cells and MCF-7 luminal breast cancer cells demonstrate that miR-143 has antitumor effects and induces apoptosis, particularly in MDA-MB-231 cells. Overexpression of miR-143 reduces AKT activity by downregulating AKT, a downstream effector of RAS. However, the effect of miR-143 is more pronounced in MCF-7 cells, which lack a KRAS mutation. Conversely, MDA-MB-231 cells exhibit resistance by upregulating alternative survival pathways, such as ERK1/2, another downstream effector of RAS [13].

The efficacy of miR-143 in inducing apoptosis is enhanced when combined with other miRNAs. Recent studies show that transfecting miR-99a and miR-143 into MCF-7 and TNBC T47D cells synergistically induces apoptosis more effectively than individual miRNAs. miR-99a targets mTOR, a downstream effector of RAS in the PI3K pathway, reducing breast cancer cell survival and inhibiting tumorigenesis by inducing G1-phase cell cycle arrest and apoptosis. miR-143 directly targets KRAS, inhibiting the Akt/mTOR pathway and KRAS downstream effectors, including AKT and ERK. Both miRNAs also regulate overlapping target genes, such as Akt1 and CDK6, which are critical for cell cycle progression. Additionally, they affect mitogenic signalling pathways, including receptor tyrosine kinase (RTK) and PI3K/AKT/mTOR, influencing the expression of cyclin D and CDK4/6. Together, miR-143 and miR-99a effectively reduce cancer cell survival rates by targeting key regulators such as CDC25A, KRAS, CDK6, and Akt1 [71].

Cell migration and metastasis

Direct and indirect targeting of RAS by miR-143-3p can suppress the migration of breast cancer cells. However, there has been no study that explains explicitly that miR-143-3p inhibits metastasis in breast cancer. There is a study that targeting the RAS pathway can reduce the level of metastasis in breast cancer as in the study [72]. In this study, black rice was used to determine the mechanism of metastasis inhibition in MDA-MB-453 (HER2+) cells. The anthocyanin content in black rice inhibits the phosphorylation of mitogen-activated protein kinase kinase (RAF) and MAPK by reducing the activity of KRAS, the upstream kinase of

RAF1. RAF kinase in HER2+ breast cancer plays a vital role in the RAS/RAF/MEK/JNK survival signaling pathway. According to this research, we hypothesized that if miR-143-3p targets RAS, then miR-143-3p can potentially reduce the metastatic ability of HER2+ breast cancer cells [72].

Clinical relevance of miR-143-3p in breast cancer

MiR-143, frequently downregulated in breast cancer, is a tumor suppressor by targeting key oncogenic signaling pathways, particularly KRAS/HRAS and their downstream effectors, such as MAPK/ERK and PI3K/AKT [11,73]. These pathways are critical for cell proliferation, survival, migration, and epithelial-to-mesenchymal transition (EMT), hallmarks of cancer progression. In breast cancer, miR-143-3p is consistently downregulated, contributing to enhanced tumor growth, metastasis, and resistance to apoptosis [39]. Its reduced expression is associated with aggressive tumor phenotypes and poor clinical outcomes, making it a potential biomarker for diagnosis and prognosis. MiRNA-seq data from TCGA confirm significantly decreased miR-143-3p levels in breast cancer tissues [56]. Restoration of miR-143-3p levels has shown promise in suppressing these oncogenic pathways, reducing tumor cell proliferation and migration. Mechanistically, miR-143-3p directly binds to the 3′ untranslated region (UTR) of KRAS/HRAS mRNA, promoting its degradation or translational inhibition, thereby dampening downstream signaling cascades critical for cancer progression [73].

Overexpression of miR-143-3p in breast cancer cell lines, such as MDA-MB-231 and HCC-1937, inhibit proliferation, migration, and invasion, as evidenced by reduced clone formation, MTT assay results, and decreased wound healing and trans well migration rates [56]. MiR-143-3p targets LIMK1, reducing its expression, downregulating CFL1, a key player in cellular migration [56]. Additionally, miR-143-3p arrests cells in the G0/G1 phase by suppressing CDK4/6-Cyclin D1 and CDK2-Cyclin E1, and inhibits MMP2 and MMP9, which are crucial for cancer cell invasiveness. In CAMA-1 cells, miR-143 overexpression also inhibits cell proliferation, migration, invasion, and bone metastasis by targeting MAPK3, suppressing Jag1, and inactivating the ROCK signaling pathway [74]. miR-143 further induces apoptosis by increasing Bax and decreasing Bcl-2 expression. Bioinformatic and experimental analyses confirm MAPK3 as a direct target of miR-143, and its overexpression reverses miR-143's tumor-suppressive effects [74]. Another study found that miR-143-3p is significantly downregulated in breast cancer tissues and cell lines (MDA-MB-468, MDA-MB-231, and MCF-7) compared to normal cells (MCF-10A). miR-143-3p targets COL1A1, reducing its expression at both mRNA and protein levels. The lncRNA PSMG3-AS1 regulates COL1A1 by interacting with miR-143-3p, and silencing PSMG3-AS1 decreases COL1A1 and PCNA expression, further linking it to breast cancer progression [57].

The tumor-suppressive functions of miR-143-3p and its association with multiple oncogenic pathways underline its potential as a biomarker for breast cancer. It could serve as a diagnostic indicator of aggressive breast cancer phenotypes and provide prognostic information based on its expression levels. Besides, its expression levels offer potential utility in distinguishing tumor from normal tissues and predicting treatment responses Furthermore, its functional roles in reducing proliferation, migration, invasion, and inducing apoptosis highlight its therapeutic potential, supporting its development as a novel target for breast cancer treatment.

Therapeutic potential of targeting miR-143-3p in KRAS/HRAS-Driven breast cancer miRNA mimics and gene therapy

miR-143-3p has emerged as a promising therapeutic target in various cancers, including breast cancer, TNBC, osteosarcoma, and LSCC. In breast cancer, frequently downregulated miR-143-3p exhibits significant tumor-suppressive functions by inhibiting migration, invasion, and proliferation. Mechanistically, miR-143-3p targets the mutant KRAS gene and its downstream effectors, reducing oncogenic markers like Vimentin, CXCR4, and MMP-9 while increasing epithelial markers such as E-Cadherin, thereby suppressing metastasis and tumor progression [75].

In TNBC, restoring hsa-miRNA-143-3p expression enhances chemosensitivity, particularly to paclitaxel, by downregulating CIAPIN1 and reducing MDR1 expression while upregulating P53 levels.

Preclinical models demonstrated that targeted delivery of hsa-miRNA-143-3p using a recombinant lentivirus limited tumor growth and improved drug efficacy, emphasizing its therapeutic potential in managing chemoresistant breast cancer subtypes [75]. Similarly, miR-143-3p mimics inhibit tumor progression in osteosarcoma by targeting and downregulating FOSL2, a transcription factor linked to cell adhesion, migration, and TGF-beta-mediated pathways. Knockdown of FOSL2 enhances the inhibitory effects of miR-143-3p on tumor growth and metastasis [38].

In other cancers, such as LSCC, low miR-143-3p levels correlate with poor prognosis, including advanced tumor stage, lymph node metastasis, and reduced overall survival. Restoration of miR-143-3p expression inhibits cell proliferation, migration, and invasion while promoting apoptosis by downregulating the KRAS/Raf/MEK/ERK signaling pathway, repressing EMT, reducing MMP-9 expression, and regulating mitochondrial apoptotic proteins such as Bax, Bcl-2, and cleaved caspase-3 [76]. These findings show that miR-143-3p mimics as a potential therapeutic strategy across various cancers by targeting key oncogenic pathways.

miR-143-3p delivery challenges

The development and clinical application of miRNA-based therapeutics, such as miR-143-3p mimics, face significant delivery, stability, specificity, and scalability challenges. One primary obstacle is achieving effective and targeted delivery to tumor cells while minimizing uptake in healthy tissues [77]. miR-143-3p are prone to degradation in the bloodstream and require protective systems, such as lipid nanoparticles (LNPs) or exosomes, to ensure stability and bioavailability without compromising biological activity or eliciting immune responses [78,79]. For instance, LNPs have been successfully used in FDA-approved siRNA therapies like patisiran, showcasing their potential for miRNA delivery [80]. Exosomes, as naturally occurring vesicles, also offer biocompatibility and targeted delivery capabilities, which can be enhanced with tumor-homing peptides or antibodies to improve specificity, as demonstrated in KRAS-driven breast cancer models (**Figure 3**) [41,81,82].

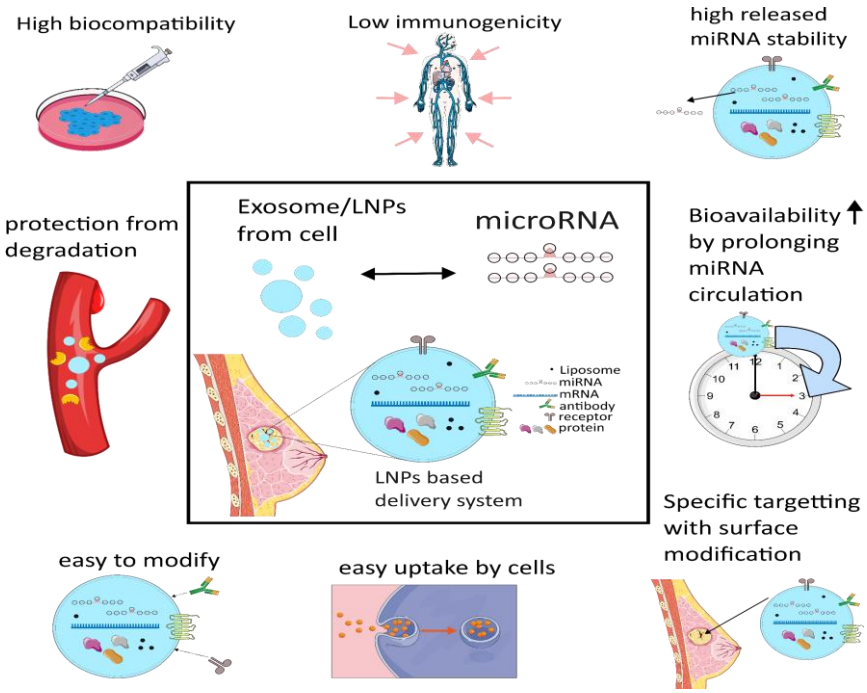

Figure 3 Exosome as miRNA delivery cargo have several advantages : high biocompatibility, low immunogenicity due to its identical structure to cell membrane, increase miRNA stability to the target, increase bioavailability of miRNA in the circulatory system, possibility to achieve specific target with exosome surface modification by adding antibody or receptor, easier for cell uptake, easy to modify, and exosome can provide protection of miRNA from enzymatic degradation in the bloodstream [83].

Maintaining the bioactivity of therapeutic miRNAs after systemic administration presents several challenges. Circulating miRNAs are often bound to proteins like Argonaute 2 (AGO2) or high-density lipoproteins (HDLs), which protect them from degradation but can hinder their cellular uptake. Interestingly, miRNAs transported through HDLs can deliver these molecules to various tissues, including the brain [84]. Advanced formulations, such as polyethylene glycol (PEG)-coated nanoparticles, have been developed to prolong circulation time and enhance cellular uptake. However, these formulations face the "PEG dilemma." The hydrophilic PEG coating improves nanoparticle stability and reduces immune clearance but simultaneously inhibits cellular uptake, endosomal escape, and membrane fusion, leading to lysosomal degradation of the therapeutic cargo [85,86]. Besides, off-target effects remain a significant concern in miRNA-based therapies, as miRNAs can bind to multiple mRNA targets due to their partial complementarity. This can result in unintended gene silencing in non-target cells. To address this issue, computational tools and experimental validation are critical for accurately predicting and minimizing these off-target interactions [78].

To enhance cellular uptake and reduce the risk of off-target effects, delivery systems like antibody-conjugated nanoparticles or tumor-specific promoters can be employed to restrict miRNA activity to target tissues, such as using HER2 antibodies to deliver miR-143-3p mimics to HER2-positive breast cancer cells, potentially improving therapeutic efficacy while minimizing systemic off-target effects. The delivery of miR-143-3p using glucose-attached reversibly ionic oligonucleotide-based nanoparticles (Glu-RION) represents a promising therapeutic tool for further clinical development [87]. The glucose moiety in Glu-RION facilitates enhanced cellular uptake, likely mediated by glucose transporters (GLUTs) or other receptors like STAB-1, achieving efficient intracellular delivery and protein signal regulation [87]. Additionally, Glu-RION demonstrated superior delivery efficiency for miR-143-3p compared to Lipofectamine, which suffers from poor nucleic acid delivery and cationic toxicity, and while effective in tumor cells, Glu-RION showed minimal apoptotic effects in normal fibroblasts and increased viability in peripheral lymphocytes, indicating its potential for selective targeting [87]. Furthermore, other delivery systems MGN-2677, such as those developed by miRagen Therapeutics, have advanced to the preclinical phase [88,89], holding great promise for future applications in treating diseases like cancer.

Cost and scalability remain significant challenges in translating miRNA-based therapeutics into clinical practice, as the production of highly pure, stable, and biologically active miRNAs at a clinical scale is still difficult. Miyamoto *et al.* highlighted the hurdles in large-scale production of RION; however, advancements in sugar-modified RNA synthesis, nanotechnology, and microfluidic production methods could make clinical applications feasible, similar to the production of mRNA vaccines [87]. Progress in synthetic biology and nanotechnology continues to address these challenges, leading to improvements in cost-effective manufacturing and the standardization of miRNA therapies [77-79].

Despite these challenges, miRNA-based therapies remain highly promising due to their unique ability to regulate multiple oncogenic pathways simultaneously. Their versatility, broad regulatory effects, and potential for personalized medicine make miRNAs, such as miR-143-3p, a powerful tool for cancer therapy. With continued advancements in delivery systems, stability, and specificity, miRNA therapeutics have the potential to revolutionize cancer treatment and other pathological conditions. miRNA delivery challenges, optimization, and opportunities are summarized in **Figure 4**.

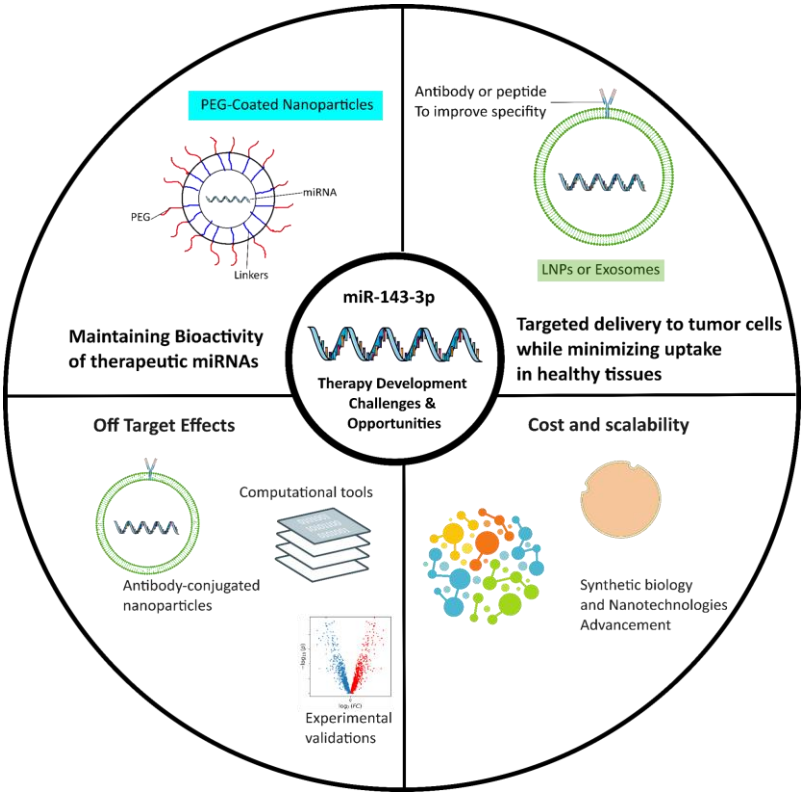

Figure 4 Summary of miRNA delivery challenges, optimization, and opportunities.

Study limitation and future directions

Based on *in-silico* and *in-vitro* studies, miRNA-based therapeutics using miR-143-3p have the potential to be new therapeutic agents for all breast cancer subtypes. However, the development of miRNA-based therapies still has many challenges, especially in the delivery efficiency and specificity of miR-143-3p. Chemical modifications to miRNA delivery, such as organic and inorganic nanotechnology, have been developed to increase the efficiency of miRNA delivery [90]. However, the development of a targeted delivery system for miR-143-3p in breast cancer is still limited. Thus, research on targeted delivery systems is needed to deliver miRNA to target cells directly. Targeted delivery systems can reduce the therapy dose to lower toxicity to normal cells. Therefore, further research is needed on tumor microenvironments in various breast cancer subtypes. Discoveries regarding the nature of tumor microenvironments in breast cancer allow the discovery of new targets more suitable for miRNA nanocarriers. Research on the miR-143-3p targeted delivery system that has been conducted *in vitro* also needs to be conducted *in vivo* on experimental animals since *in vivo* research involves more complex interaction between miRNA, its carrier, and tumor microenvironment.

In addition to miR-143-3p delivery, research on the involvement of miR-143-3p in the KRAS/HRAS pathway in breast cancer also needs to be further studied due to the limited research on regulation involving KRAS in breast cancer. Moreover, the interaction of HRAS and miR-143-3p has never been studied in any cancer. Therefore, research on the direct interaction between miR-143-3p and HRAS is needed. Research can be started *in silico* and continued *in vitro* with breast cancer cell lines expressing human estrogen receptors since HRAS mutation occurs in breast cancer cell lines with HER mutation. Clinical trials using miRNA-based therapy in breast cancer are minimal; as of December 2024, only 61 clinical trials were registered on the clinicaltrials.gov website. With the keywords "Breast cancer" AND "MicroRNA." Moreover, there have been no clinical trials using miR-143-3p in any form for breast cancer therapy. Thus, there are still many opportunities to conduct clinical trials of miR-143-3p-based therapy in any subtype of breast cancer.

While this review highlights the significant role of miR-143-3p in regulating RAS signaling and its

therapeutic potential, several limitations warrant discussion. A primary concern is the reliance on *in vitro* studies and preclinical models, which, while invaluable for understanding molecular mechanisms, may not fully capture the complexity and heterogeneity of human tumors. Tumor microenvironments *in vivo* are influenced by factors such as immune system interactions, stromal components, and metabolic conditions, all of which are challenging to replicate in laboratory models. Consequently, translating these findings into clinical applications may face significant challenges, including variability in therapeutic efficacy and potential off-target effects.

Another limitation lies in the potential biases within the reviewed literature. Many studies may preferentially report positive results, creating a publication bias that could overstate the therapeutic promise of miR-143-3p. Additionally, differences in experimental design, such as variations in cell lines, transfection methods, and miRNA delivery systems, may contribute to inconsistencies and limit the generalizability of the findings. Addressing these discrepancies will require standardization of experimental protocols and rigorous validation in diverse models. Furthermore, the review primarily focuses on miR-143-3p, which, while critical, operates within a broader network of miRNAs that modulate RAS signaling. Comparative analyses with other miRNAs, such as miR-99a, miR-34a, and let-7, could provide a more holistic view of miRNA-mediated regulation of RAS pathways. Exploring these interactions may reveal synergistic or compensatory mechanisms, highlighting the unique contributions of miR-143-3p while identifying potential combinations for enhanced therapeutic efficacy.

Finally, the lack of clinical data on miR-143-3p-based therapies underscores the need for further investigation. While preclinical studies suggest its promise, there are no robust clinical trials validating its safety, efficacy, or delivery mechanisms in humans. Future research should prioritize bridging this gap by evaluating miR-143-3p in clinically relevant models, developing effective delivery systems, and investigating potential side effects. By addressing these limitations and expanding the scope to include other miRNAs in RAS signaling, the field can move closer to realizing the therapeutic potential of miR-143-3p in cancer treatment.

Conclusions

miR-143-3p represents a promising therapeutic target in breast cancer, particularly in aggressive subtypes like TNBC, due to its tumor-suppressive effects on key oncogenic pathways such as KRAS and HRAS. By inhibiting proliferation, metastasis, and therapy resistance, miR-143-3p shows the potential to improve outcomes in cancers driven by dysregulated RAS signaling. However, challenges in efficient and targeted delivery, stability, and minimizing off-target effects must be addressed for clinical translation. Advances in nanotechnology and delivery systems, including lipid nanoparticles and exosomes, offer solutions to enhance miRNA stability and specificity. Further research into miR-143-3p's role in the tumor microenvironment and its interactions with oncogenic pathways is essential for developing effective therapies. Overall, miR-143-3p-based strategies hold significant potential to revolutionize breast cancer treatment and pave the way for personalized medicine.

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