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Utilizing OncomiR and TSmiR as Biomarkers for Screening Breast Cancer

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Abstract

Breast cancer stands out as a significant threat to women globally, emphasizing the urgent need for reliable diagnostic and prognostic markers.Numerous studies have shown that miRNAs assist as either an oncogene or tumor suppressor. As a result, they are recognized as non-invasive biomarkers for diagnosing and predicting cancer outcomes.Through an in-depth literature review, we have identified numerous upregulated miRNAs, including miR-9, miR-10b, miR-21, miR-29a, miR-92a, miR-148a-3p, miR-155, miR-221, miR-222, and miR-373. Conversely, we have identified downregulated miRNAs, including miR-34a, miR-96, miR-99a, miR-125b, miR-145, miR-200c, miR-203, miR-214, miR-411, and miR-486.We used the miRWalk database to predict target genes associated with each miRNA and constructed a comprehensive network. Additionally, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted to delve deeper into the functional significance and molecular pathways associated with the identified miRNAs target genes. Additionally, we constructed a Protein-Protein interaction networkbased on the miRNA-target genes.Further analysis was directed towards the target genes of OncomiRs (EGFR, MYC, CTNNBI, TP53, CCDNDI, BCL2) and TS-miRs (TP53, CTNNBI, AKT1), exploring their involvement in signaling pathways.This study delves into the utilization of miRNA for the early screening and monitoring of breast cancer.

Keywords: Breast Cancer, Functional Enrichment, Oncomir, TS Mir, PPI Network

Introduction

Despite the existence of various diagnostic modalities, breast cancer continues to exhibit a high prevalence among women. The primary reason lies in the constraints of current screening methods. These include the prevalence of false positives and false negatives, challenges in detecting abnormalities within dense breast tissue, and the restricted frequency of screening intervals (1). As a result, there is a need for alternative methods to identify early-stage breast cancer(BC). This has led to a heightened focus on biomarkers, given their remarkable ability to identify specific molecules or genetic irregularities associated with cancer cells. Conventional biomarkers such as HER2, ER, andPR status are widely utilized in breast cancer diagnosis and classification. While these biomarkers target specific molecular pathways, they may exhibit limited sensitivity and fail to fully capture tumor heterogeneity. Moreover, blood-based markers like CA 15-3 and CA 27.29 may also demonstrate lower sensitivity and specificity compared to tissue-based counterparts. miRNA biomarkers have demonstrated notable sensitivity and specificity across multiple studies, effectively distinguishing between breast cancer patients and healthy individuals, as well as discerning different subtypes and stages of breast cancer.In recent years, there has been a growing emphasis on small non-coding miRNAs as biomarkers. Their variability in tissues and biological fluids compared to normal samples has garnered attention. Their stability renders them ideal for screening and precise prognostication purposes (2). They exert a crucial influence on gene expression by either impeding mRNA translation or

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promoting mRNA degradation. Furthermore, miRNAs impact cellular proliferation, migration, invasion, and differentiation, thereby contributing to tumorigenesis through the regulation of oncogenes and tumor suppressor genes.

The dysregulation of Oncomirs and TSmiRs in breast cancer and their potential as biomarkers offer exciting prospects for improving breast cancer detection, diagnosis, prognosis, and treatment. OncomiRs (oncogenic microRNAs) and TS miRs (tumor suppressor microRNAs) have emerged as promising biomarkers for breast cancer screening due to their central roles in modulating essential cellular processes implicated in cancer initiation and progression. Typically, oncomiRs, which inhibit tumor suppressor genes, are upregulated in cancer cells, while TS miRs, which counteract oncogenes, are downregulated. Modulating the levels of these oncomiRs or TS miRs, whether through inhibition or augmentation, can induce significant changes in cancer cell behaviors. OncomiR dysregulation contributes to the uncontrolled growth and survival of cancer cells. In breast cancer, specific oncomiRs have been implicated in various aspects of the disease, including metastasis and resistance to therapy. Identifying and measuring the levels of these oncomiRs in biological samples, such as blood or tissue biopsies, can provide valuable information about the presence and progression of breast cancer.TSmiRs help maintain normal cellular functions by inhibiting processes like cell proliferation and invasion. In breast cancer, the loss of certain TS miRs has been associated with increased tumor aggressiveness and poor prognosis. Therefore, detecting the reduced expression of these miRs in patient samples can indicate cancer development and progression (3). By elucidating common molecular mechanisms and identifying potential therapeutic targets, these findings pave the way for interdisciplinary collaborations and innovative approaches to cancer diagnosis and treatment. Understanding miRNA function in breast cancer offers insights into tumorigenesis, metastasis, and treatment resistance, guiding the development of therapeutic strategies. Exploring miRNA expression across cancers may reveal universal biomarkers and shared tumorigenic pathways.

In this study, we have curated upregulated and downregulated miRNAs through literature mining and constructed a comprehensive miRNA-target regulatory network. The process began with a comprehensive literature review focused on studies investigating miRNA dysregulation in breast cancer. This involved searching relevant databases such as Google Scholar, PubMed, and ScienceDirect conducted on articles over the past five years this involved reviewing articles that contained computational analyses, and experimental validations. Within the literature, miRNAs known to play roles as either oncogenes (OncomiRs) or tumor suppressors (TS miRs) in breast cancer were identified based on reported dysregulation patterns and functional studies. Identified OncomiRs and TS miRs were compiled into lists based on their reported upregulation or downregulation in breast cancer samples compared to normal controls. Further network analysis elucidates how miRNAs intricately regulate the expression of their target genes, thereby exerting an effecton critical processes such as proliferation, differentiation, apoptosis, and development.To delve deeper into the functional roles of miRNA-target interactions within the network, we conducted GO and KEGG pathway analyses. GO analysis elucidates the molecular mechanisms driving biological processes, identifying candidate biomarkers, and facilitating the prioritization of genes for further experimental validation. On the other hand, KEGG pathway analysis involves mapping genes or gene products to their respective pathways and uncovering significantly enriched pathways within the gene list. Furthermore, we conducted a PPI network analysis utilizing the miRNA-target network. Subsequent analysis focused on identifying target genes among OncomiR and

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TS-miR, aiding in the exploration of pivotal pathways.This computational approach facilitates the understanding of miRNAs and their associated genes within breast cancer pathways, rendering them viable biomarkers for screening purposes.

Materials and Methods

Literature Mining

A comprehensive literature review was conducted to identify miRNAs associated with breast tumors and their presence in circulating blood. Search engines such as Google Scholar, PubMed, and ScienceDirect were conducted on articles over the past five years.

Mirna-Target Interaction Network

We utilized miRwalk 2.0 (https://mirwalk.umm.uni-heidelberg.de)to forecast miRNA targets. miRWalk is an extensive repository, offering the most extensive collection of predicted and experimentally validated microRNA-target interactions (4). A regulatory network was created to depict the complex interplay among identified miRNAs and their corresponding target genes using Cytoscape (V3.10.1.) (https://cytoscape.org).

Functional Enrichment Analysis

We employed the ClueGO plugin (Version 2.5.9) within Cytoscape (Version 3.10.1) for investigating GOand KEGG pathways. Statistical validation was conducted, with a significance threshold set at $P < 0.05$, and a kappa score of 0.4 was utilized to ensure the reliability of the enriched terms (5).

Protein-Protein Interaction (PPI) Network

To investigate the interaction among miRNA targets, we utilized the STRING database (Version 12.0) (https://string-db.org/) to construct a PPI network, with a criteria score threshold of ≥ 0.4. The network was visualized using Cytoscape software (Version 3.10.1). Laterhub genes were acquired utilizing the CytoHubba plugin (V 0.1) within Cytoscape.Scoring methods such as Bottleneck and Degree identify and emphasize the central and densely interconnected nodes within thePPI network (6).

Result and Discussion

Literature Mining

Through an exhaustive literature mining approach, we identified elevated expression of miRNAs found in breast tumors, as well as their presence in circulating blood which includeshsa-miR-9, hsa-miR-10b, miR-21, miR-29a, miR-92a, miR-148a-3p, miR-155, miR-221, miR-222, and miR-373(7-11). Conversely, the selected downregulated miRNAs encompassed miR-34a, miR-96, miR-99a, miR-125b, miR-145, miR-200c, miR-203, miR-214, miR-411, andmiR-486(12-16).

Construction of A Mirna-Target Gene Interaction Network

To explore the relationship between miRNAs and their targets and gain insights into regulatory connections and their roles in various breast cancer (BC) processes and pathways, we utilized miRwalk. This tool employs the TarPmiR algorithm, which is a random-forest-based approach for miRNA target site prediction. Our selection criteria focused on identifying targets with a high score of 1 and miRNA binding sites specifically located in the 3'-UTR of the gene, as the 3'-UTR is known for its abundance of miRNA binding sites. This process resulted in the prediction of 215 target genes for oncomiRs and 118 target genes for tumor suppressor (TS) miRNAs, each associated with specific miRNAs. To visualize this intricate miRNA-target network, we utilized Cytoscape software (Figures 1 and 2).

Functional Enrichment Analysis

We utilized the ClueGO plugin within Cytoscape to import the target genes of all oncomiRs and TS miRs, aiming to explore potential GO categories and KEGG pathways. miRNAs are expected to target genes crucial for various biological

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processes, molecular functions, and KEGG pathways, some of which are discussed below.

OncomiRs, linked to numerous targets, significantly influence various biological processes in BC. For example, in TNBC, CTNNB1 impacts basal-like and immune subtypes, regulating critical cellular functions like proliferation, migration, and angiogenesis (17). Additionally, miRNAs miR-221/222, miR-21, and miR-29a, along with their associated genes PTEN and TIMP3, activate the AKT pathway, contributing to TRAIL-Activated Apoptotic Signaling (18). Moreover, AKT1 regulates the import of long-chain fatty acids across the plasma membrane, promoting tumor growth in TNBC by disrupting the cell cycle or enhancing apoptosis. Furthermore, heightened fatty acid metabolism is linked to therapy resistance in HER2-positive breast cancer, suggesting potential avenues for treatment (Figure 3 a). Further analysis of molecular functions underscores the importance of several key processes in breast cancer. For instance, the positive regulation of MAP kinase activity, particularly through the MEK1/MAPK1/2 signaling axis and pro-survival autophagy, is critical in overcoming resistance to antiestrogen treatment and enhancing the effectiveness of hormone therapies. Additionally, EGFR activity inhibits the pro- apoptotic function of BimEL,

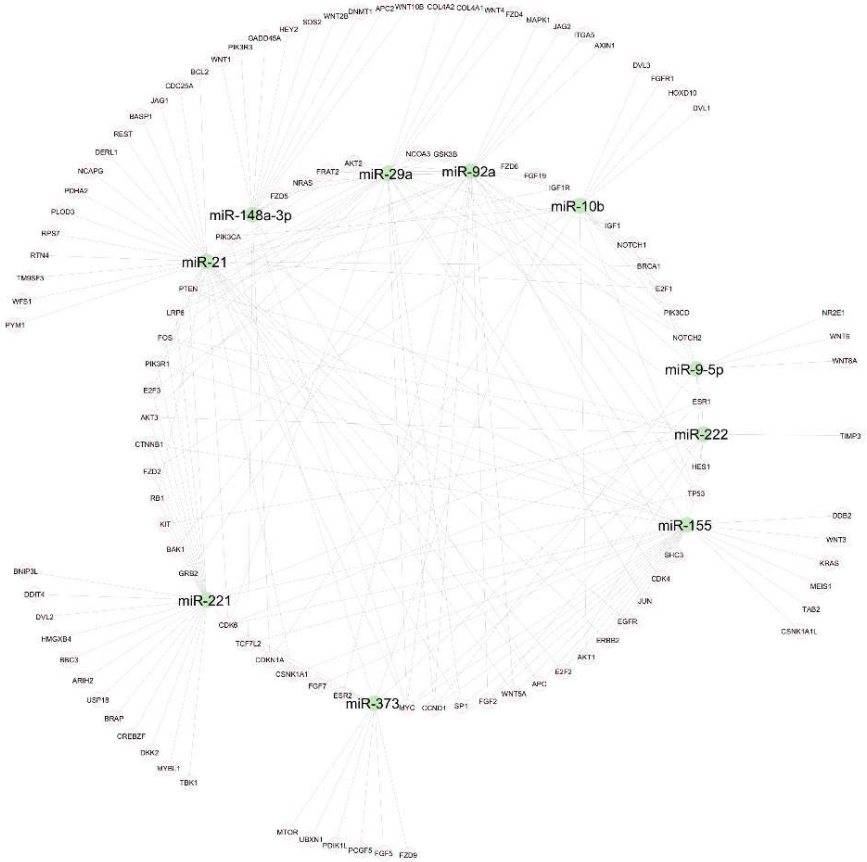

Figure 1: The regulatory network of OncomiRs their target genes: Green nodes represent Upregulated miRNA, where's white nodes represent its target

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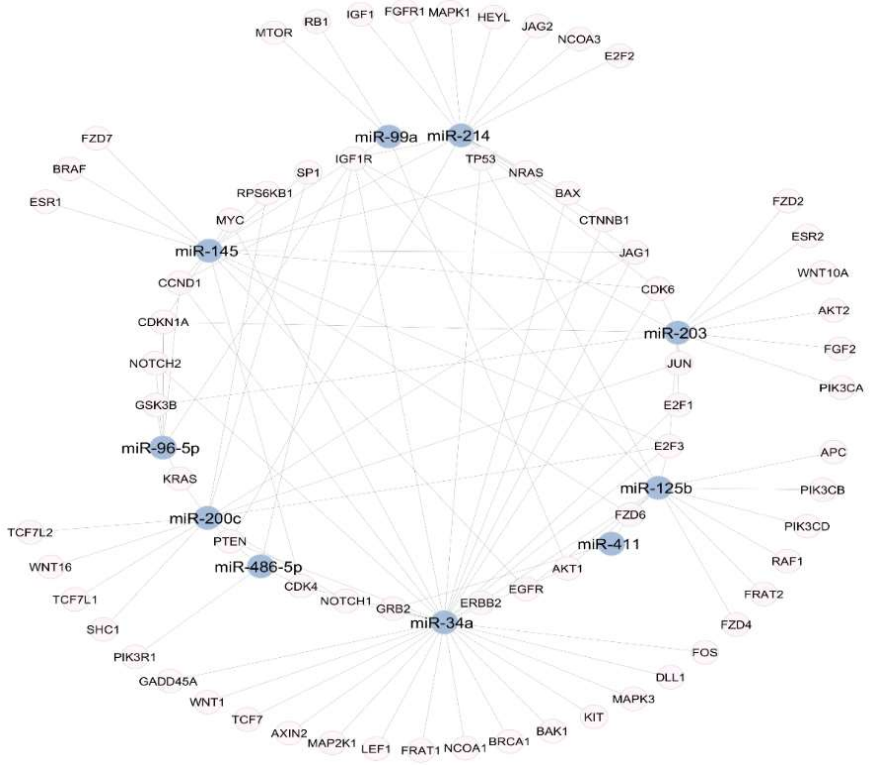

Figure 2: The regulatory network of TS miRs their target genes: Blue nodes represent downregulated miRNA, where's white nodes represent its target

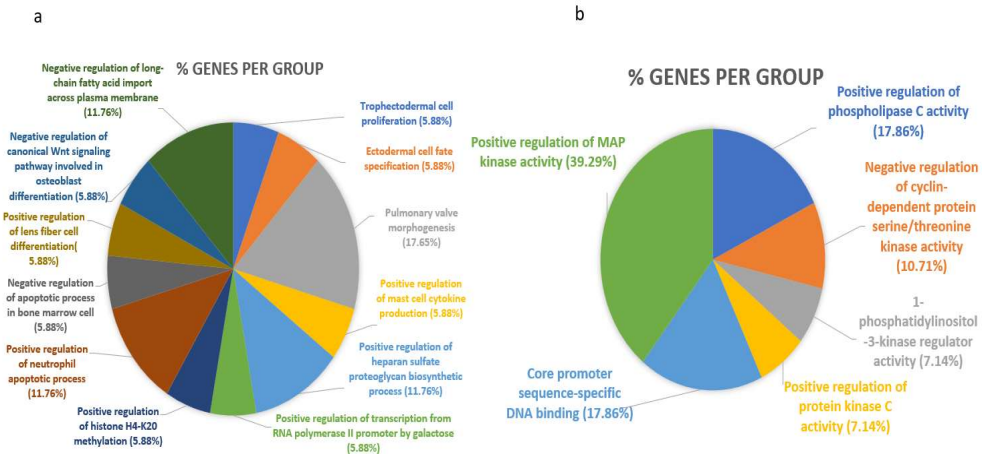

Figure 3: The pie chart depicts the functional connections between (a) biological processes and (b) molecular functions, which are enriched by upregulated miRNAs target genes

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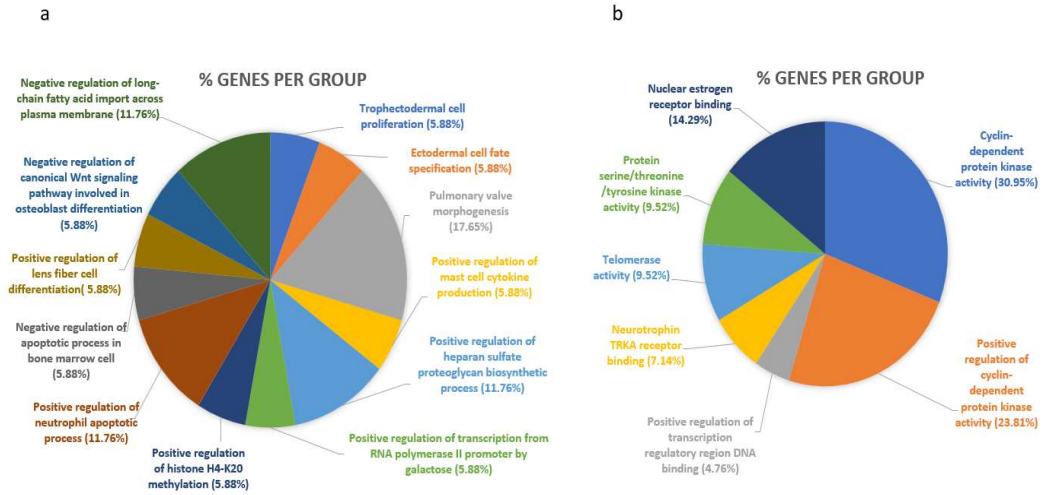

Figure 4: The pie chart depicts the functional connections between (a) biological processes and (b) molecular functions, which are enriched by downregulated miRNAs target genes

a vital regulator of apoptosis in ERα-positive breast cancer cells. Furthermore, the positive regulation of phospholipase C activity, influenced by EGFR, impacts the expression of PLC-γ1, an enzyme involved in EGFR-associated pathways that affect cell migration (19). Lastly, core promoter sequence-specific DNA binding, particularly involving the MYC gene, regulates the transcriptional activity of genes essential for tumor progression, including those involved in cell cycle regulation, apoptosis modulation, and angiogenesis promotion (20) (Figure 3 b).

TS miRNA target genes exhibit significant enrichment in biological processes, particularly in signaling pathways like Ectodermal cell fate specification involving Wnt, Notch, and BMP. These pathways, crucial in regulating ectodermal cell fate and implicated in cancer progression, include the canonical Wntsignaling pathway, notably involving FZD7, which inhibits tumor growth by reducing cell proliferation in TNBC (21). Additionally, LEF1, a downstream component of Wnt/β-catenin signaling, influences stem cell maintenance, organ development, and EMT. Dysregulation of LEF1 promotes tumorigenesis by enhancing cancer cell proliferation, migration, and invasion, extending its influence to bone marrow cells in breast cancer, where its activation suppresses apoptosis, driving disease progression. Moreover, PIK3CB and PIK3CD, encoding different PI3K isoforms, positively regulate neutrophil apoptosis, prolonging neutrophil survival via PI3K signaling and fostering inflammation, angiogenesis, and tumor cell survival, contributing to tumor progression (22) (Figure 4 a). TS miRNAs are anticipated to target genes essential for a variety of molecular functions. For instance, they may regulate cyclin-dependent protein kinase activity, where AKT1 triggers CDKs by suppressing CDK inhibitors like CDKN1A (p21), thereby facilitating cell cycle advancement(23). Additionally, they might influence positive nuclear estrogen receptor binding, such as the role of Ets1 in mediating interactions between nuclear hormone receptors and coactivators like NCOA1, which intensifies estrogen sensitivity and fosters tumor growth. Furthermore, TS miRNAs could modulate protein serine/threonine/tyrosine kinase

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activity, exemplified by RPS6KB1's function in breast cancer, where dysregulation contributes to tumor progression by affecting cellular processes like growth, proliferation, and survival (24) (Figure 4b).

In the KEGG pathway, there are overlapping pathways present in both oncomirs and tumor-suppressive microRNAs (ts-miRNAs). Common microRNA-associated genes are found in both types of pathways. The same gene can demonstrate oncogenic and tumor-suppressive roles, which vary depending on factors such as the cellular environment, specific mutations, interactions with other signaling pathways, and the microenvironment. These factors collectively influence the gene's function, determining whether it promotes tumorigenesis or inhibits tumor growth. Increased expression of E2F1 can enhance mitophagy in breast cancer cells by transcriptionally regulating genes involved in mitochondrial dynamics and quality control, facilitating the removal of damaged mitochondria and maintaining cellular balance. Conversely, decreased E2F1 expression may hinder mitophagy, leading to the accumulation of damaged mitochondria and heightened oxidative stress, factors associated with breast cancer progression. Also, upregulation of TP53 positively influences mitophagy by activating genes involved in mitochondrial quality control, whereas downregulation or loss of TP53 function can impair mitophagy, resulting in mitochondrial dysfunction and increased genomic instability, characteristic of breast cancer development (Figure 5 a). Therefore, while elevated levels of TP53 and E2F1 promote mitophagy and cellular equilibrium, their downregulation or functional loss may disrupt mitophagy, exacerbating breast cancer progression (25).CTNNB1 serves as a pivotal element within adherens junctions, facilitating cell-cell adhesion and signaling processes.Dysregulation of CTNNB1 leads to abnormal initiation of the Wntsignaling pathway, consequently disrupting adherens junctions.This disruption fosters tumor cell invasion and metastasis in breast cancer. Similarly, EGFR signaling modulates the dynamics of adherens junctions and cellular adhesion (26). When EGFR is overexpressed or activated, it disrupts the regulation of adherens junction proteins, contributing to heightened tumor cell motility, invasion, and metastasis in breast cancer (Figure 5 b). As a result, targeting EGFR signalingis avaluable therapeutic method to hinder tumor progression and metastasis in breast cancer. EGFR upregulation enhances hepatocellular carcinoma (HCC) metastasis to breast tissue by promoting cell motility and survival, while downregulation inhibits metastatic potential.

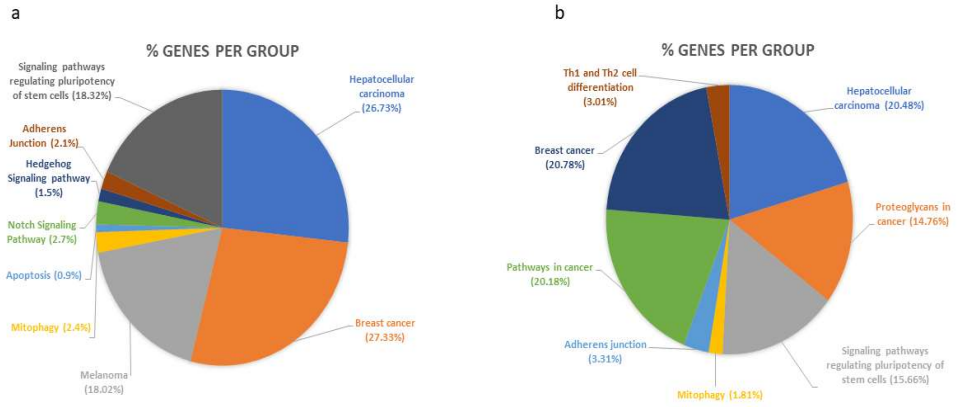

Figure 5: This figure depicts the KEGG pathways associated with target genes regulated by oncomiRs and TS miRNAs.

Utilizing OncomiR and TSmiR as Biomarkers

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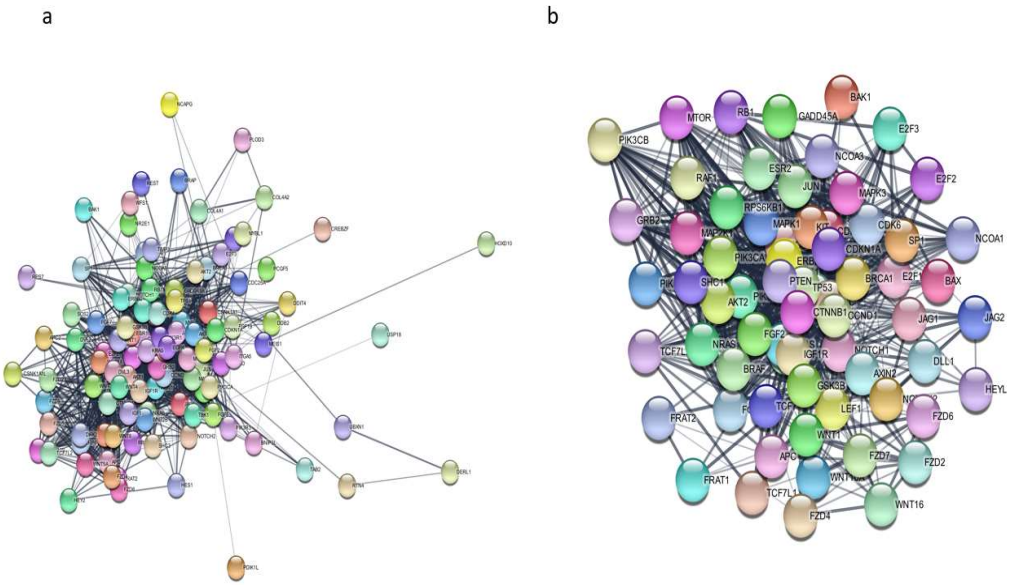

Figure 6: Protein-Protein interaction of miRNA target genes- a) Upregulated miRNA-targets. (b) Downregulated miRNA-targets.

Similarly, AKT1 upregulation aids metastasis via enhanced proliferation and invasion, while its downregulation impedes metastatic spread. EGFR and AKT1 levels significantly influence HCC metastasis to breast tissue, although further research is required to elucidate underlying mechanisms (27).

Protein-Protein Interaction (PPI) Network

The miRNA-target network was employed to build a PPI network, comprising 113 nodes and 140 edges for OncomiR targets, and 69 nodes with 1050 edges for TSmiR targets (Figure 6). The top 10 hub genes ranked by bottleneck and degree for OncomiR and Ts miR-target genes are seen in (Figures 7 and 8). The bottleneck node serves as a critical bridge linking distinct sections of the network. Meanwhile, the degree of a node represents the count of connections it maintains with other nodes within the network. Target genes that were common to both parameters(Bottleneck and degree)for OncomiR (EGFR, MYC, CTNNBI, TP53, CCDNDI, BCL2) and TS-miR (TP53, CTNNBI, AKT1)were chosen.The expression of miRNA along with its corresponding genes and its function areseen in (Tables 1 and 2).

Thus, miRNA emerges as a promising biomarker, functioning as both oncogenes and tumor suppressors, offering a potential breakthrough in early detection methods. Abnormally elevated levels of OncomiRs in cancer promote the proliferation, migration, and metastasis of cancer cells by downregulating the expression of several tumor suppressor genes through direct binding to their corresponding mRNA molecules. Consequently, inhibiting OncomiR function emerges as a promising strategy for cancer treatment. The synthetic anti-miRs, which are complementary to OncomiRs, into cancer cells inhibits the interaction between OncomiRs and their target RNAs.This intervention effectively suppresses cancer cell growth and metastasis. In contrast to OncomiRs, decreased expression of TSmiRs in cancer contributes to the initiation and advancement of malignancy by avoiding the suppression of several cancer-promoting genes targeted for downregulation.

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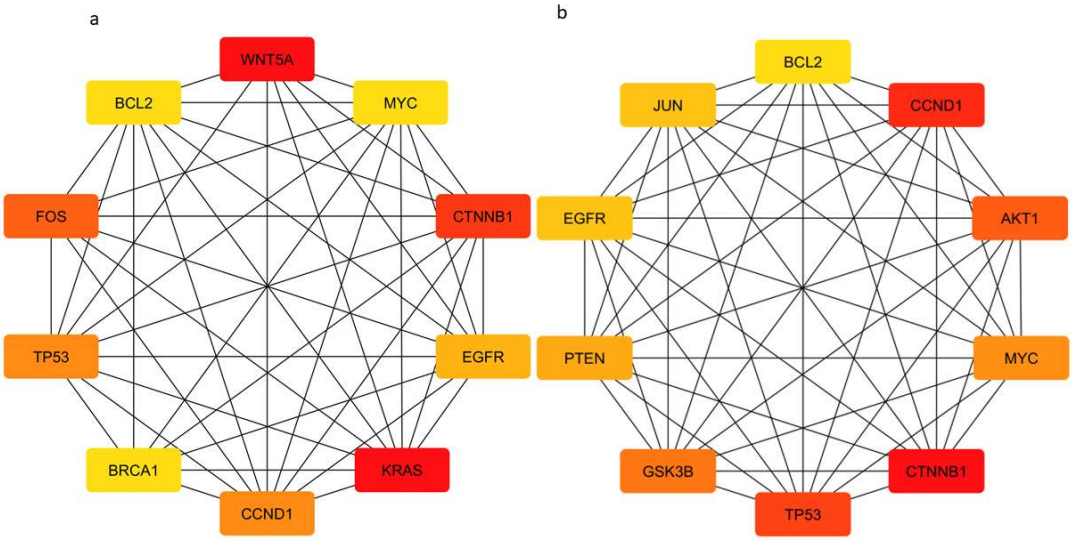

Figure 7: Top 10 hub genes among the upregulated miRNA-targets. (a) Bottleneck centrality. (b) Degree centrality

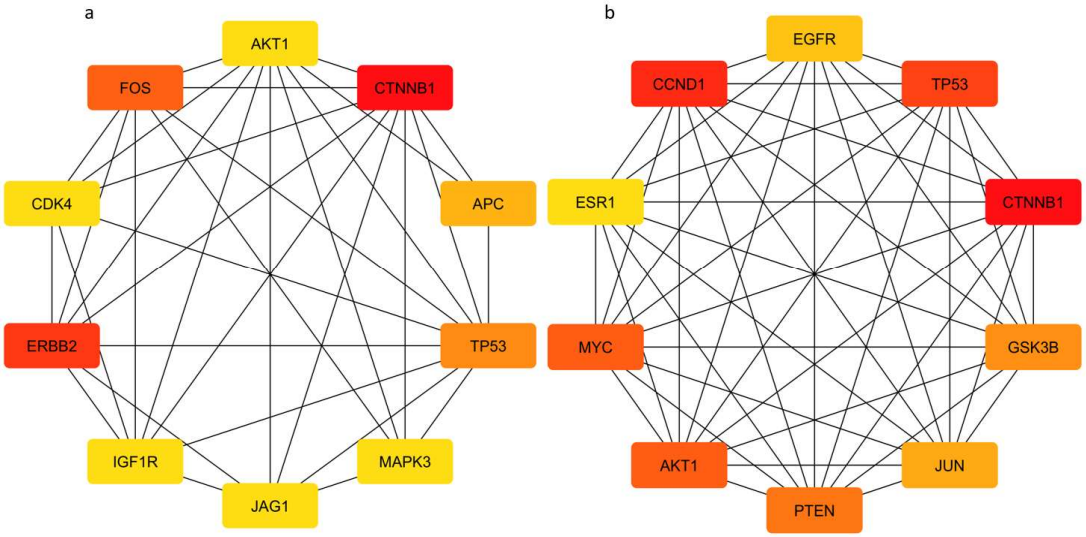

Figure 8: Top 10 hub genes among the downregulated miRNA-targets. (a) Bottleneck centrality. (b) Degree centrality

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Table 1: A list of OncomiR targets that involved breast cancer

Target genes	OncomiR	Function	References
EGFR	miR-21	Suppressed tumour growth and angiogenesis	8
MYC	miR-9	Cell migration, Invasion and metastasis	10
CTNNB1	miR-373	Tumor progression and apoptosis	13
TP53	miR-155	Cell cycle progression, anti-apoptosis, proliferation and metastasis	9
CCDNDI	miR-92a	Proliferation, migration and invasion	14
BCL2	miR-221 and miR-222	Proliferation and metastasis	12

Table 2: A list of TS-miR targets that involved breast cancer

Target genes	TS miR	Function	References
AKT1	miR-145	Inhibition of EMT	52
CTNNBI	miR-125b	Inhibiting growth and migration	52
TP53	miR-34a	Induction of cell cycle arrest and apoptosis.	20

Conclusion

The findings of this study hold significant promise in the realm of breast cancer screening and prognosis. Breast cancer remains a formidable threat to women worldwide, underscoring the critical need for reliable diagnostic and prognostic markers. Identification of OncomiR and TS miR biomarkers represents a promising avenue for overcoming limitations in current breast cancer screening methods and enhancing patient outcomes through personalized treatment strategies.These biomarkers offer the potential for detecting breast cancer at earlier stages compared to conventional screening techniques like mammography. They can identify molecular changes linked to cancer initiation and progression before tumors become visible on imaging scans, enabling timely intervention and treatment initiation, and ultimately improving survival rates. Moreover, these biomarkers provide valuable molecular insights that can augment the accuracy of breast cancer screening. Their detection can complement existing screening approaches, reducing the likelihood of misdiagnosis and unnecessary procedures. By profiling the expression levels of these biomarkers, clinicians can customize treatment plans to target specific molecular pathways driving tumor growth and advancement, thereby enhancing treatment effectiveness, minimizing adverse effects, and improving patient quality of life. Monitoring changes in miRNA expression following different treatment modalities, including chemotherapy, hormonal therapy, and targeted therapy, could provide valuable information on treatment efficacy and resistance mechanisms. Identifying treatment-specific miRNA signatures associated with favourable or adverse outcomes could facilitate personalized treatment strategies and guide to therapeutic. Additionally, real-time monitoring using these biomarkers enables clinicians to identify patients at higher risk of disease recurrence during follow-up care, allowing for the implementation of proactive management strategies such as intensified surveillance or adjuvant therapies to prevent or delay recurrence and improve long-term prognosis.

Through an extensive literature review and analysis, the study has identified

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a panel of miRNAs that exhibit dysregulation in breast cancer, both as oncogenes and tumor suppressors. The identified upregulated miRNAs, such as miR-9, miR-10b, miR-21, miR-29a, miR-92a, miR-148a-3p, miR-155, miR-221, miR-222, and miR-373, along with downregulated miRNAs like miR-34a, miR-96, miR-99a, miR-125b, miR-145, miR-200c, miR-203, miR-214, miR-411, and miR-486 present a comprehensive profile that could serve as potential non-invasive biomarkers for diagnosing breast cancer and predicting its outcomes. By utilizing the miRWalk database, the study has further elucidated the target genes associated with each miRNA, constructing a network that provides insights into the molecular mechanisms underlying breast cancer development and progression. Moreover, the gene ontology and pathway analyses conducted shed light on the functional significance of these miRNAs and their target genes, revealing their involvement in key biological processes and signalling pathways implicated in breast cancer pathogenesis. The constructed Protein-Protein interaction network adds another layer of understanding by delineating potential interactions among these target genes. Of particular significance is the exploration of target genes associated with OncomiRs and Tumor Suppressor miRNAs (TS-miRs), such as EGFR, MYC, TP53, and AKT1, elucidating their roles in crucial signalling pathways implicated in breast cancer. This deeper insight into the molecular landscape of breast cancer holds immense potential for the development of targeted therapies and personalized treatment strategies. In the context of breast cancer screening, these findings offer a promising avenue for early detection and monitoring. By leveraging the dysregulated miRNAs and their associated target genes, clinicians may be able to enhance the sensitivity and specificity of existing screening methods, enabling earlier detection of breast cancer and facilitating timely interventions. Furthermore, the non-invasive nature of miRNA biomarkers presents an attractive opportunity for routine screening and surveillance, potentially improving patient outcomes through earlier diagnosis and tailored treatment approaches. Further validation on independent cohorts of breast cancer patients and in vitro studies confirm the association between these miRNAs and breast cancer, assessing their predictive or prognostic value. Optimizing protocols for extracting miRNAs from various sample types (e.g., tissue, blood, plasma) is essential to ensure robust and reliable detection. qPCR is a widely used technique that measures the abundance of miRNAs in samples through amplification and quantification of specific nucleic acid sequences. Overall, this study represents a significant step forward in the utilization of miRNA-based biomarkers for breast cancer screening and prognosis, offering novel insights into the molecular mechanisms driving breast cancer progression and highlighting avenues for further research and clinical translation.

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