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Identifying miR-616–Regulated Molecular Mechanisms and Novel Interacting Genes in Triple-Negative Breast Cancer

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ABSTRACT

Background: Among the major subtypes of breast cancer, triple-negative breast cancer (TNBC) is recognized as the most aggressive form of invasive breast cancer, associated with a poor prognosis and high mortality rate. Consequently, gaining insights into the underlying mechanisms of TNBC is of paramount importance. We focused on investigating the molecular mechanism of miR-616, a confirmed metastasis-related microRNA, in the pathogenesis and metastatic behavior of TNBC.

Methods: We obtained the mRNA dataset (GSE38959) from the Gene Expression Omnibus (GEO) to identify differentially expressed genes (DEGs). The target genes of miR-616 were predicted using the miRWalk and TargetScan databases. Subsequently, the genes that overlapped between these predictions were used to construct a protein-protein interaction network. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses were performed. Module discovery was conducted using Molecular Complex Detection, visualized through Cytoscape, and further annotated using ClueGO. Finally, a literature review followed by a survival analysis was carried out.

Results: We identified 1725 DEGs (1109 upregulated and 616 downregulated), 116 of which overlapped with miR-616 targets. Among these, 31 downregulated genes were selected due to their reciprocal regulation with miR-616 expression. These genes were enriched in several cancer-associated pathways, specifically the estrogen, neurotrophin, JAK-STAT, and PI3K-Akt signaling pathways. We identified 16 novel candidate genes involved in miR-616–related TNBC pathogenesis, with *KCNE1* showing a significant correlation with overall patient survival (hazard ratio = 0.72; 95% CI, 0.52–0.99).

Conclusion: These findings shed light on how miR-616 exerts its regulatory effect, underscoring its pivotal role in metastasis development in patients with TNBC.

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INTRODUCTION

Breast cancer is the most prevalent form of cancer in women and represents a significant global public health concern. Its incidence is projected to rise over the next decades.[1] Advances in breast cancer awareness and imaging techniques have contributed

to enhanced diagnosis and screening.[2] However, despite progress in screening, diagnosis, and treatment, approximately 12% of individuals diagnosed with breast cancer progress to metastatic stages. Currently, there is no cure for metastatic breast cancer. The prognosis remains poor, with a 5-year survival rate of approximately 26%. Breast cancer is categorized into 3 main subtypes based on molecular markers for estrogen and progesterone receptors, as well as human epidermal growth factor 2 (ERBB2 or HER2). Among these, triple-negative breast cancer (TNBC), characterized by the absence of these markers, is particularly aggressive and associated with a higher likelihood of disease progression and lower survival rates compared with other types of breast cancer.[3] TNBC is the most challenging form of invasive breast cancer to treat and carries a higher risk of metastasis, particularly to the brain and bones, potentially due to its propensity for hematogenous spread rather than lymphatic.[4] patients with TNBC do not respond to hormonal or HER2-targeted therapies, necessitating specific treatment approaches.[5]

MicroRNAs (miRNAs) are a class of noncoding RNA molecules that play a crucial role in regulating gene expression. They have garnered significant attention for their potential applications from diagnosis to prognosis in various diseases, including TNBC.[6,7] Dysregulation of miRNA expression is believed to impact processes such as tumorigenesis, cancer progression, and disease pathogenesis. Additionally, miRNAs influence a wide array of cellular activities, including proliferation, metabolism, apoptosis, invasion, and migration.[7,8] One such miRNA, miR-616, has been implicated as an oncogene in multiple cancers, including breast, hepatocellular, and gastric cancers, where it promotes metastasis by targeting genes like *TIMP2* and modulating MMP signaling.[9,10] Studies suggest that miR-616 may act as an oncogene and could potentially serve as a biomarker for breast cancer. Elevated levels of miR-616 have been observed in TNBC tissues and cell lines, indicating its potential role in TNBC cell metastasis and invasion. However, the precise mechanism remains unclear. Recent research has revealed a link between miR-616 and TNBC, showing that it promotes progression and metastasis by directly targeting the *TIMP2* gene. Given the multifunctional nature of miRNAs and their ability to target multiple genes, it is likely that miR-616 may also impact other crucial genes involved in TNBC.[10]

The advent of high-throughput technologies and the integration and analysis of corresponding data have revolutionized our approach to understanding various aspects of medicine, from diagnosis to treatment.[11] Our investigation used a systems biology approach to uncover the underlying molecular mechanism of miR-616 in TNBC to provide deeper insights into the metastasis and overall pathogenesis of the disease.

METHODS

Identification of differentially expressed genes

In this study, we utilized the mRNA expression dataset GSE38959 obtained from the Gene Expression Omnibus (GEO) database.[12] This dataset encompasses profiling information from 30 samples of TNBC, 13 samples of normal mammary ductal cells, and 4 samples from other healthy tissues. For our analysis, we focused exclusively on the TNBC and normal mammary ductal cell samples. The overall study process is illustrated in Figure 1. The initial step involved using GEO2R (R version 4.2.1), an openly accessible online tool within GEO, for detecting differentially expressed genes (DEGs). We selected a fold-change threshold of $|\log FC| > 1.5$ to prioritize genes exhibiting biologically meaningful differential expression, as this cutoff has been widely adopted in transcriptomic analyses of TNBC to focus on robust changes associated with tumor aggressiveness.[13] This methodology was employed to compile the list of DEGs. Quality control was performed using boxplots and principal component analysis to assess sample distribution and detect outliers (Figure S1).

Predicting miR-616 targets

The prediction of miR-616 target genes was conducted using 2 distinct tools: miRWalk (version 3.0; http://mirwalk.umm.uni-heidelberg.de/) server and TargetScan (version 7.2; http://www.targetscan.org/vert_72/). From the results obtained from miRWalk, we refined the selection based on the following criterion: a binding probability exceeding 0.9, and the 3′ UTR as the position of miR-616 on the mRNA. These thresholds balance sensitivity and specificity, allowing for the identification of key regulatory networks without excessive noise.[14] TargetScan predictions were filtered by context++ score and conserved seed matches. miRWalk targets were filtered by binding probability > 0.9 and 3′ UTR localization. Subsequently, we employed Venny 2.1 to identify the genes that overlapped across our 3 candidate gene lists. This process was undertaken to ensure a comprehensive and accurate set of potential miR-616 target genes.

Protein-protein interaction construction

Protein-protein interaction (PPI) network analysis has recently become a crucial concept for systems biologists due to its various applications. In our study,

we performed the construction of a PPI network of 116 overlapping genes with the help of the STRINGdb R package (version 11.5). We focused on downregulated genes due to their reciprocal regulation with upregulated miR-616, as miRNAs typically suppress target mRNAs. Gene Ontology (GO) enrichment analysis of the gene set was performed using the Enrichr web tool to identify significantly overrepresented terms in the biological process, molecular function, and cellular component ontologies. Pathway enrichment was also carried out using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database in Enrichr. An adjusted *P* value less than 0.05 was set as statistical significance. Subsequently, Jensen disease, a data mining–based tool, was utilized to check the validity of the constructed PPI network based on gene-disease association from the literature.

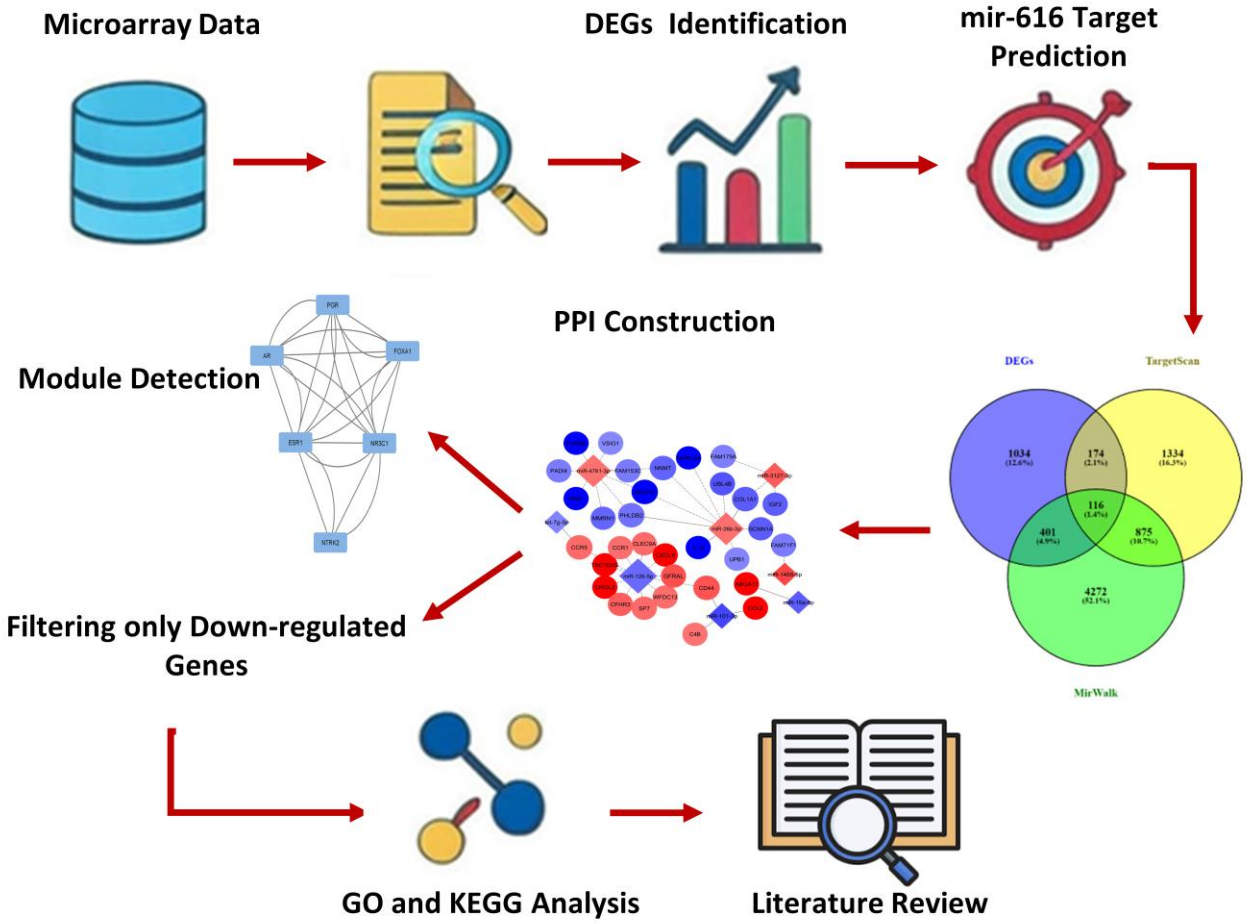

Figure 1. Workflow of the study design. This flowchart outlines the key steps: DEG identification from GSE38959, miR-616 target prediction via miRWalk and TargetScan, overlap extraction, PPI network construction in STRING, enrichment analyses (GO/KEGG), module detection with MCODE, and survival validation in GEPIA.

Module detection and enrichment analysis

In addition, the Molecular Complex Detection (MCODE) plugin (version 2.0.0) of Cytoscape (version 3.9.1) was used to identify the most important modules in the network. Modules are highly connected subnetworks that have practical applications as they share common pathways or encode special protein complexes. Degree Cutoff = 2 and K-Core = 2 were selected as standard parameters in MCODE to identify highly interconnected subnetworks while minimizing false positives. Subsequently, we performed GO analysis for each module of the PPI network with ClueGO (version 2.5.8). Finally, a current literature review was performed to determine the number of genes in our set with established direct links to breast cancer and other malignancies. Afterward, survival analysis was conducted using Gene Expression Profiling Interactive Analysis (GEPIA) on The Cancer Genome Atlas (TCGA)/Genotype-Tissue Expression (GTEx) data for invasive breast carcinoma (BRCA), encompassing TNBC subtypes.

RESULTS

Identification of miR-616-related DEGs

In our analysis, we identified 1725 DEGs (1109 were upregulated and 616 downregulated). Figure 2 illustrates the overall distribution and clustering of

DEGs between TNBC and normal samples. The volcano plot (Figure 2A) shows upregulated and downregulated genes according to logFC and adjusted *P* values.

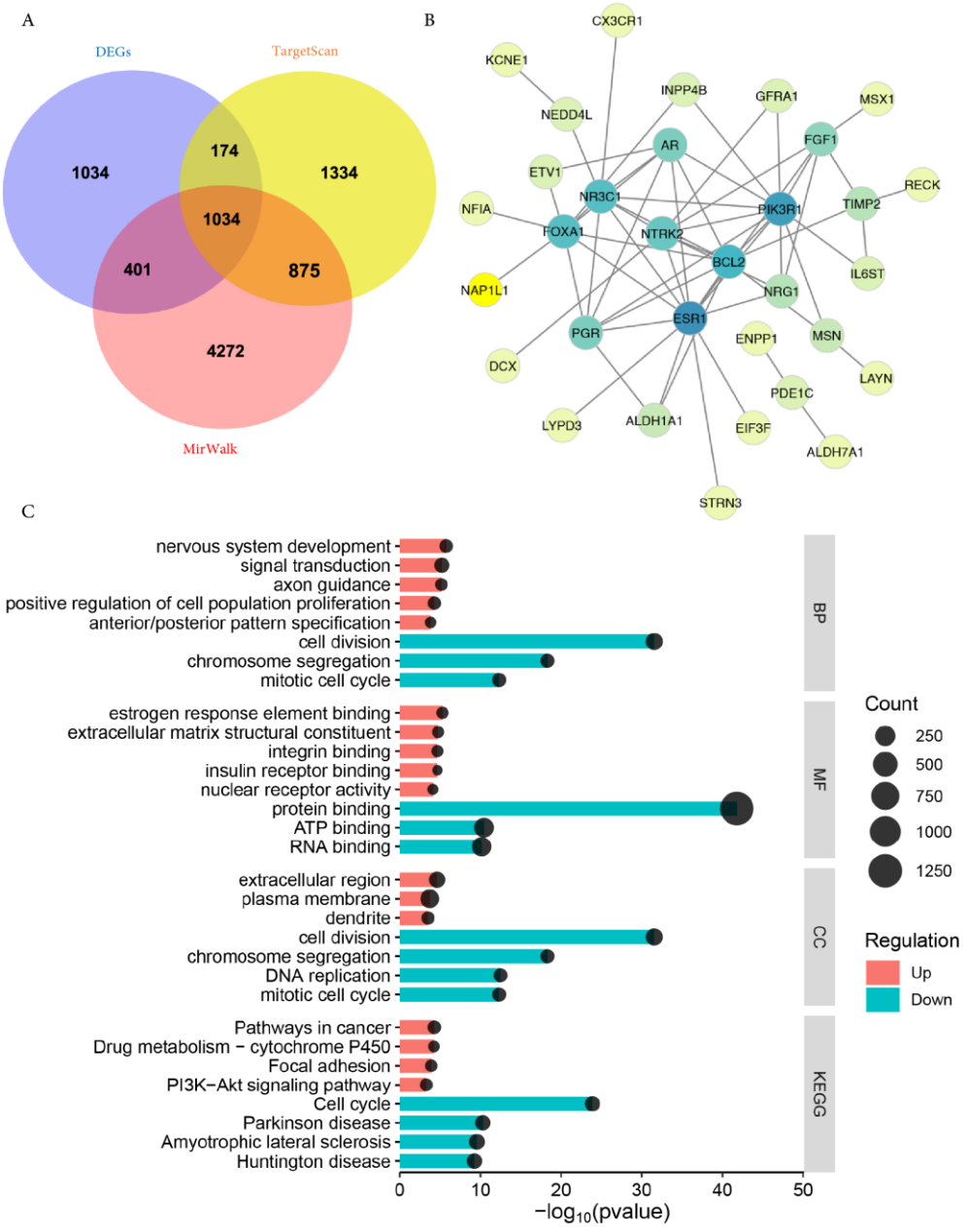

Figure 2. Identification and functional enrichment analysis of differentially expressed genes (DEGs) in TNBC. (A) Venn diagram of overlaps (116 genes) between DEGs (GSE38959), miRWalk targets, and TargetScan. (B) Network analysis. (C) Biological processes (BP); Molecular function (MF); Cellular components (CC) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

Subsequently, we conducted miR-616 target gene prediction using miRWalk, resulting in 5664 entries after filtering. From TargetScan, we selected the top 2500 target genes. Through this process, we uncovered 116 genes (1.4%) that overlapped between our predicted target genes and the identified DEGs.

These 116 genes were enriched in cancer pathways (PI3K-Akt) and steroid signaling, suggesting roles in metastasis. Using this overlapping list, we proceeded to construct a PPI network. We removed 61 nodes in the network that lacked interactions (from 116 to 55), focusing on biologically relevant hubs; isolated nodes may represent indirect effects. This network represents the miR-616–related DEGs, comprised of 55 nodes and 159 edges (Figure 2B). The PPI network was visualized using a force-directed layout in Cytoscape, with nodes grouped by enriched functions (signaling pathways). This network provides a visual representation of the relationships among these genes, shedding light on their potential interactions and functional relevance.

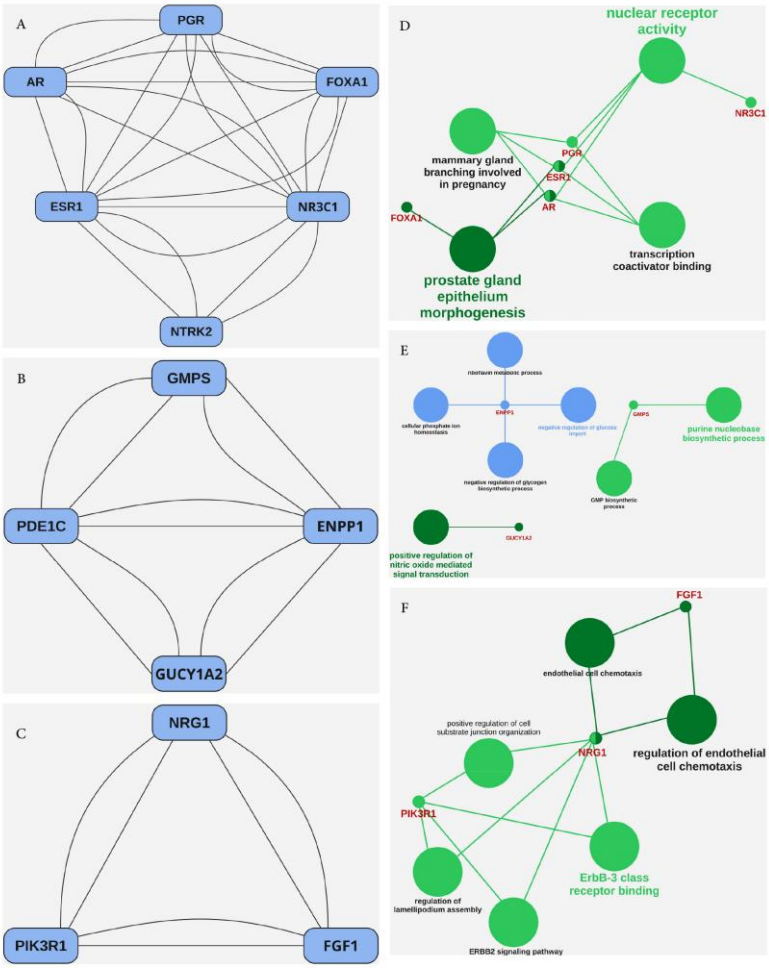

Figure 3. (A-C) Detected modules within the PPI network via the Cytoscape software. (D-F) biological representation of detected modules, A, D: The first module, B, E: second module, and C, F: third module.

Enrichment analysis

As illustrated in Figure 2C, the GO analysis unveiled significant enrichments in several biological processes. Notably, these pathways comprised specific mechanisms critical to cancer biology, including steroid hormone–mediated signaling, intracellular steroid hormone receptor signaling, and the regulation of protein kinase B (Akt) signaling. Furthermore, in terms of molecular functions, the most enriched activities of the genes were associated with DNA-binding transcription activator activity, RNA polymerase II–specific and RNA polymerase II general transcription initiation factor binding.

Regarding cellular components, the phosphatidylinositol 3-kinase complex, class I, and the integral component of the plasma membrane emerged as crucial locations where these genes predominantly carry out their functions. In addition to GO analysis, the KEGG pathway analysis demonstrated that alterations in genes significantly impacted pathways related to cancer, chemical carcinogenesis, and the estrogen signaling pathway. Lastly, Jensen disease results validated the robust construction of our network. It indicated that breast fibroadenoma and breast disease were the 2 most enriched diseases, further reinforcing the relevance and accuracy of our network in relation to breast cancer–related conditions.

PPI network analysis and module annotation

Using the MCODE plugin, we identified 3 distinct modules based on the criteria of Degree Cutoff = 2,

K-Core = 2, Node Score Cutoff = 0.2, and Max Depth = 100 (Figure 3A-F). The structural relationships within each module are depicted in Figure 3A-C. For the first module (Figure 3D), comprising 6 nodes and 24 edges, GO analysis revealed enrichment in processes related to transcription coactivator binding, nuclear receptor activity, mammary gland branching involved in pregnancy, and prostate gland epithelium morphogenesis. The second module (Figure 3E) encompassed 4 nodes and 10 edges, and it was primarily enriched for functions related to the nucleotide biosynthetic process and the nitric oxide signaling pathway. The third module (Figure 3F) included 3 nodes and 6 edges. It showed involvement in pathways such as endothelial cell chemotaxis, ErbB2 signaling pathway, ErbB-3 class receptor binding, regulation of lamellipodium assembly, and positive regulation of cell-substrate junction organization. These findings provide valuable insights into the functional roles and interactions within each of these modules.

Following this, we refined our selection by focusing solely on the downregulated genes, aiming to narrow down our targets to those with higher confidence. As a result, we identified only 31 downregulated genes within the PPI network (Table 1).

Table 1. List of 31 Downregulated miR-616 Target Genes in the Triple-Negative Breast Cancer Protein-Protein Interaction Network

Gene symbol	Function/pathway association[c]	Adjusted *P* value[a]	Log FC[b]
NTRK2	Neurotrophin receptor; neuronal survival; PI3K–Akt signaling	6.20×10^{-5}	−4.06
ESR1	Estrogen receptor; hormone signaling; breast cancer driver gene	2.85×10^{-6}	−3.86
HCAR1	Lactate receptor; GPCR signaling; metabolic regulation	1.88×10^{-5}	−3.80
CX3CR1	Chemokine receptor; immune cell migration; CX3CL1/CX3CR1 axis	1.40×10^{-6}	−3.59
CLSTN2	Adhesion molecule; synaptic and cell–cell interactions	1.40×10^{-4}	−3.49
FGF1	Growth factor; angiogenesis; endothelial signaling	1.03×10^{-6}	−3.07
DCX	Microtubule binding; neuronal migration pathways	5.11×10^{-3}	−2.94
ETV1	ETS family transcription factor; oncogenic driver in several cancers	2.31×10^{-4}	−2.89
PGR	Progesterone receptor; hormone signaling; breast cancer progression	1.08×10^{-4}	−2.87
INPP4B	Tumor suppressor; PI3K pathway regulation	1.69×10^{-4}	−2.86
TMTC1	ER stress response; protein modification	5.04×10^{-4}	−2.80
AR	Androgen receptor; hormone signaling and proliferation	1.47×10^{-2}	−2.66
ALDH7A1	Aldehyde detoxification; cellular metabolism	9.16×10^{-5}	−2.64
BCL2	Antiapoptotic gene; survival signaling	8.46×10^{-4}	−2.57
FOXA1	Pioneer transcription factor; hormone-dependent cancers	1.11×10^{-2}	−2.55
NR3C1	Glucocorticoid receptor; stress response; cytokine regulation	2.51×10^{-3}	−1.54
NRG1	Ligand for ErbB3/4; ErbB signaling; cell growth and migration	1.69×10^{-6}	−2.41
PDE1C	cAMP/cGMP regulation; signal transduction	2.60×10^{-4}	−2.29
NEDD4L	E3 ubiquitin ligase; PI3K–Akt regulation; tumor suppressor	6.11×10^{-6}	−2.24
RUNX1T1	Transcriptional repressor; chromatin regulation	7.57×10^{-3}	−2.15
IL6ST	gp130; JAK–STAT signaling; cytokine receptor complex	1.08×10^{-4}	−2.15
KCNE1	Potassium channel regulatory subunit; found prognostic	3.95×10^{-3}	−2.04
SEMA6D	Axon guidance; cell migration and adhesion	1.31×10^{-5}	−1.91
DIXDC1	Wnt signaling regulator; cytoskeleton remodeling	7.08×10^{-5}	−1.85
PIK3R1	PI3K regulatory subunit; cancer-associated pathway	6.66×10^{-3}	−1.83
LAYN	Immune suppressive receptor; T-cell regulation	4.29×10^{-3}	−1.78
EIF3F	Translation initiation factor; growth and proliferation	8.49×10^{-4}	−1.75
GFRA1	Neurotrophic signaling; GDNF receptor	7.60×10^{-3}	−1.73
MOB3B	Hippo pathway regulator; cell growth control	1.03×10^{-2}	−1.62
NFIA	Nuclear factor I family; transcription regulation	1.23×10^{-2}	−1.62
ENPP1	Purinergic metabolism; extracellular signaling	5.56×10^{-3}	−1.61

FDR, false discovery rate; GO, Gene Ontology; GPCR, G protein–coupled receptor; KEGG, Kyoto Encyclopedia of Genes and Genomes; Log FC, log fold change; TNBC, triple-negative breast cancer.
[a]Adjusted *P* value calculated using Benjamini-Hochberg FDR correction.
[b]Negative log FC indicates downregulation in TNBC compared with normal samples.
[c]Functions based on KEGG, GO, and published literature.

Subsequent KEGG pathway analysis for these 31 genes revealed enrichments in critical pathways, including pathways in cancer, the estrogen signaling pathway, breast cancer, the neurotrophin signaling pathway, the JAK-STAT signaling pathway, and the PI3K-Akt signaling pathway. These findings further underscore the significance of these downregulated genes in the context of breast cancer.

We took an additional step by conducting an extensive literature review on the 31 downregulated

genes and discovered 16 genes that have not been previously associated with breast cancer. To assess the prognostic significance of these newly identified genes in breast cancer, we utilized the GEPIA web server. Among these 16 genes, only potassium voltage-gated channel subfamily E regulatory subunit 1 (*KCNE1*) demonstrated a substantial impact on the overall survival of patients with invasive breast carcinoma. Figure 4 illustrates the survival curve of *KCNE1* expression in patients with invasive breast carcinoma. The hazard ratio of 0.72 (95% CI, 0.52–0.99; $P = 0.04$) indicates that higher *KCNE1* expression is protective against poor survival.

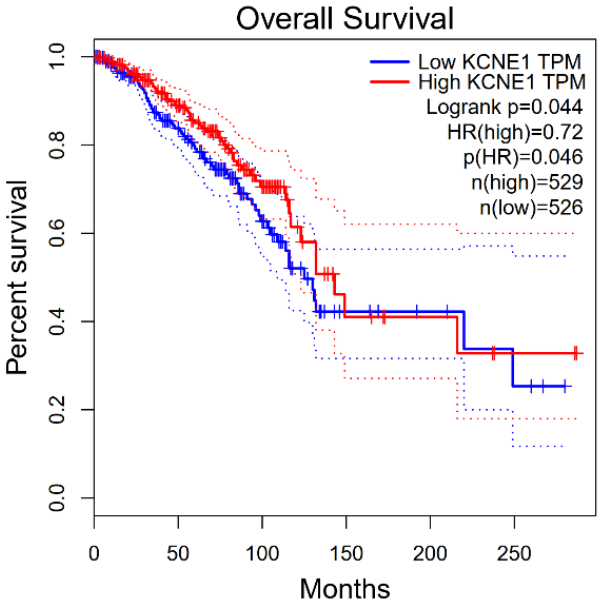

Figure 4. Survival analysis of *KCNE1* in breast invasive carcinoma patients.

DISCUSSION

In this study, we performed an extensive bioinformatics analysis to uncover the molecular mechanisms involving miR-616 in TNBC. Our results provide novel bioinformatics evidence for miR-616–associated regulatory networks in TNBC. Although previous evidence has suggested an oncogenic role for miR-616 in breast cancer, particularly through the suppression of *TIMP2* and subsequent activation of MMP-2/MMP-9 signaling,[10] the broader regulatory landscape of this miRNA has remained poorly characterized. By integrating differential expression analysis, miRNA target prediction, PPI network exploration, module detection, and survival analysis, our findings expand the current understanding of miR-616 and suggest several new pathways and gene candidates relevant to TNBC biology.

Our analysis, particularly the third module highlighted in Figure 3F, suggests that the ErbB signaling pathway might play a pivotal role in instigating the metastatic traits of the tumor. This finding further underscores the complexity and multifaceted nature of miR-616's influence in breast cancer progression.

Although the associations between miR-616 and some of these pathways have not been experimentally validated, our results indicate that miR-616 may influence TNBC progression by modulating genes such as *NRG1*, *PIK3R1*, and *FGF1*, which collectively participate in ErbB receptor activity, PI3K-Akt signaling, and fibroblast growth factor pathways.[15,16] Additionally, heightened expression levels of ErbB-3 have been observed in patients with breast cancer, showing a correlation with metastasis.[17] Downregulation of *NRG1* in our dataset may therefore reflect one miR-616–associated mechanism through which TNBC cells alter growth factor signaling. The enrichment of processes such as lamellipodium organization, cell-substrate junction assembly, and endothelial cell chemotaxis suggests that miR-616 might also influence pathways associated with cellular motility and the metastatic phenotype. Moreover, studies have demonstrated that the Arp2/3 complex and Scar/WAVE proteins within lamellipodia serve as key regulators of cell motility, thereby exerting a pivotal role in the metastatic process.[18] This insight underscores the potential impact of miR-616 on the metastatic behavior of breast cancer cells.

Furthermore, claudins, a prominent class of transmembrane proteins involved in forming tight junctions, play a crucial role in breast cancer development. Notably, reduced expression of claudin-6 has been demonstrated to contribute to invasiveness in breast cancer.[19] Collectively, these findings underscore that targeting intercellular junctions and modulating lamellipodium organization present a viable strategy for breast cancer treatment, particularly in the context of metastatic progression. This insight suggests potential therapeutic avenues for addressing advanced stages of breast cancer.

Solid tumors comprise a diverse array of cells, including malignant cells, fibroblasts, endothelial cells, and inflammatory cells. Within tumor tissue, certain molecules, such as vascular endothelial growth factor (VEGF) and primary fibroblast growth factor, act as chemotactic agents, attracting and influencing endothelial cells.[20,21] Based on the insights gleaned from the results in Figure 3F, these findings suggest that miR-616 may influence endothelial cell chemotaxis via FGF-1, with its involvement in cell growth, development, and potentially cancer transformation, is a noteworthy factor in this context. It has been observed that several breast cancer cell lines express FGF-1.[22] Given the pivotal role of endothelial cells within the tumor, FGF-1 may potentially contribute to the progression

toward metastatic breast cancer. This underscores the intricate interplay between various cellular components and signaling pathways in tumor development and progression.

Mammary gland morphogenesis is a tightly regulated developmental process that occurs under normal circumstances. However, any deviation from this intricate mechanism can potentially lead to the development of breast cancer. Despite its significance, the precise underlying mechanism remains elusive.[23] Within the miR-616 PPI network, the most densely connected module exhibits a strong correlation with mammary gland branching, involving key genes like *AR*, *ESR1*, and *PGR*. This suggests that miR-616 may exert influence over this process, potentially contributing to the development of cancer through a deviation from normal functioning. Notably, these genes are also believed to be involved in transcription coactivator binding, providing further evidence of miR-616's regulatory role in this context. This observation reinforces the notion that miR-616 may play a crucial role in the intricate mechanisms underlying mammary gland development and its potential disruption in the context of cancer. Our results align with and extend previous observations that miR-616 exhibits context-dependent roles in cancer biology. Several studies have reported that miR-616 can function as an oncogenic regulator by promoting proliferation, migration, invasion, and epithelial-mesenchymal transition (EMT) in diverse cancer types. For example, miR-616 is upregulated in hepatocellular carcinoma, where it enhances migration, invasion, and EMT through suppression of *PTEN*, supporting a protumorigenic role in this malignancy.[24]

We conducted a thorough review of the existing literature and identified several genes that exhibited downregulation in patients with breast cancer. These genes have been extensively investigated in prior studies and have demonstrated associations with various other types of cancers. One notable example is *FOXA1*, which belongs to the forkhead class of DNA-binding proteins. The established role of *FOXA1* in prostate cancer further highlights its significance and suggests its potential relevance in the context of breast cancer. This finding underscores the interconnectedness of genetic factors across different cancer types.[25,26] *BCL2*, which is an apoptosis-inhibiting protein, is well studied in pancreatic cancer[27-29] and may or may not have definite impacts on breast cancer. *DIXDC1* is involved in a variety of cancers, such as colon, prostate,[30] lung,[31] gastric,[28] myeloid leukemia,[32] and hepatocellular carcinoma.[33] *NEDD4L*, *NTRK2*, and *EIF3F* are each involved in many cancers.

Our literature review of the 31 downregulated miR-616–associated genes revealed 16 genes not previously linked to breast cancer. Among these, *KCNE1* emerged as the most promising candidate, demonstrating a significant association with patient survival. KCNE1 is best known as a potassium channel regulatory subunit involved in maintaining repolarization currents in excitable tissues.[34] Recent reports indicate that ion channels including KCNE family members may contribute to cancer invasion, EMT, and metabolic reprogramming.[35] Further investigation into the functions and downstream signaling pathways of KCNE1 in the context of breast cancer is strongly recommended. Additionally, exploring the potential applications of KCNE1 in both the diagnosis and treatment of breast cancer, especially in cases of metastatic breast cancer, is a prudent step forward.

Importantly, evidence from other cancer types also supports an oncogenic role for miR-616, providing broader biological context for our findings. In hepatocellular carcinoma, miR-616 has been reported to enhance proliferation, migration, and invasion by suppressing *CPEB3*, indicating its contribution to aggressive tumor behavior.[9] In non–small cell lung cancer, miR-616 activates oncogenic signaling by targeting *PTEN*, which subsequently facilitates increased proliferation and invasion.[29] Furthermore, in prostate cancer, miR-616 has been shown to function as an oncogenic microRNA by downregulating *TFPI-2*, thereby strengthening invasive and metastatic properties.[36] Collectively, these studies demonstrate that miR-616 consistently influences cancer-related signaling pathways across diverse tumor types. This cross-cancer evidence aligns with our observations in TNBC and supports the hypothesis that miR-616 may regulate multiple interconnected pathways involved in tumor progression and metastasis.

A major limitation is the lack of experimental validation; future studies could employ luciferase reporter assays for target confirmation and in vivo metastasis models for functional assessment. Additional limitations include those of the GSE38959 dataset, such as its small sample size (n = 43), cellular heterogeneity, and only 13 normal samples, potentially introducing bias.

CONCLUSION

Taken together, the aforementioned discoveries strongly advocate for a more comprehensive investigation into the role of miR-616 in metastatic breast cancer. Metastasis, being a complex and relatively enigmatic process in cancer biology, warrants closer scrutiny to enhance our comprehension of its underlying mechanisms. In

conclusion, our study not only elucidates the fundamental mechanism of miR-616 and advances our understanding of the pathways it influences, but also introduces a novel gene, *KCNE1*, which may potentially play a pivotal role in driving or impacting the progression of TNBC. However, it is important to note that further experimental studies are needed to validate and substantiate these findings in the clinical context. This research lays the groundwork for potential future advancements in the diagnosis and treatment of TNBC.

ETHICAL CONSIDERATIONS

This study utilized publicly available data from an mRNA expression dataset from the Gene Expression Omnibus (GEO) database (accession numbers: GSE38959). As a secondary bioinformatics analysis of pre-existing, publicly accessible data, no additional ethical approval or consent was required from participants.

CONFLICT OF INTEREST

All the authors declare that they have no conflict of interest.

ACKNOWLEDGMENTS

We would like to thank Negar Farhang-Doost for her help with the editing of the manuscript. This study used publicly available GEO data (GSE38959), collected under ethical approvals as per original studies.[12] No new human/animal experiments were performed.

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DATA AVAILABILITY

The datasets analyzed during the current study are publicly available in the Gene Expression Omnibus (GEO) repository under the accession number GSE38959. All bioinformatics tools and databases used (miRWalk version 3.0, TargetScan version 7.2, STRING version 11.5, Cytoscape version 3.9.1, Enrichr, GEPIA) are open access and accessible via their respective websites. Custom analysis code, including R scripts for GEO2R differential expression and Cytoscape workflows for PPI network construction, is available upon reasonable request from the corresponding author. No proprietary datasets or software were employed.

AI DISCLOSURE

This manuscript was not created using any generative AI methods for design, analysis, or writing.

AUTHOR CONTRIBUTIONS

AJ and MI: Methodology, investigation. MI and ZM: Formal analysis. AJ, MI, ZM, HA, and LS: Writing – original draft, visualization. ST: Supervision, conceptualization, writing – review and editing.

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