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Integrated in Silico Analysis Reveals miR-551b-3p as a Potential Tumor Suppressor and Prognostic Biomarker in Breast Cancer

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Abstract

Background: Tumor progression in breast cancer is largely driven by microRNA-mediated post-transcriptional regulatory networks; however, the biological and clinical roles of miR-551b-3p remain insufficiently defined. The present study aimed to characterize the expression profile, molecular interactions, and prognostic relevance of miR-551b-3p in breast cancer via in silico analyses.

Method: Designed as an in silico bioinformatic analysis, weassessed miR-551b-3p expression and clinical correlations using the University of Alabama at Birmingham Cancer data analysis portal (UALCAN) and OncomiR. Associated transcription factors were retrieved from TransmiR v3.0, functional enrichment was performed using the Protein Analysis Through Evolutionary Relationships suite, and survival outcomes were evaluated using Kaplan–Meier Plotter. Statistical significance was evaluated via UALCAN, OncomiR, and KM-Plotter using t-tests, ANOVA, and log-rank tests, with the threshold set at $P < 0.05$.

Results: Our analyses revealed a significant downregulation of miR-551b-3p in breast tumor tissues (log-fold change [log-FC] = -1.1, $P < 0.0001$). *TEAD1*, protein tyrosine kinase 2, *SMG1*, *TIMELESS*, *SLC5A3*, and *NUP93* emerged as strong target genes, implicating key pathways involved in proliferation, DNA damage response, and metastatic progression. Furthermore, central transcription factors such as *E2F*1, *ESR1*, *FOXA1*, *CBX3*, and *RAD21* were associated with reduced miR-551b-3p expression. In silico screening identified lncRNAs *AC069281.2*/*AL359258.1* and *USP9X*/*TMEM2*-derived circRNAs as potential target-directed microRNA degradation (TDMD) candidates. Clinically, miR-551b-3p downregulation associated with metastasis and unfavorable survival, serving as a supplementary molecular indicator.

Conclusion: Collectively, these findings support miR-551b-3p as a potential tumor suppressor and prognostic biomarker in breast cancer, warranting further experimental validation.

Keywords: MicroRNAs, Breast neoplasms, Computational biology, Gene expression regulation, Survival analysis

Introduction

Breast cancer is the most common malignancy among women worldwide and remains a major global health challenge due to its high incidence, mortality, and biological heterogeneity.[1, 2] Although current treatment strategies include surgery, radiotherapy, and systemic therapies, clinical outcomes vary markedly across molecular subtypes. This heterogeneity arises from genomic alterations, diverse signaling pathways within the tumor microenvironment, and subtype-specific biological characteristics. In particular, triple-negative breast cancer exhibits aggressive behavior with limited targeted treatment options and a higher likelihood of recurrence and metastasis compared with other subtypes.[3]

In recent years, non-coding RNAs—especially microRNAs (miRNAs)—have emerged as key regulators of post-transcriptional gene expression and play essential roles in breast cancer biology.[4] miRNAs are short endogenous RNA molecules that fine-tune gene expression by targeting the untranslated regions of mRNAs, thereby inducing degradation or translational repression.[5, 2] Through these mechanisms, miRNAs regulate proliferation, apoptosis, epithelial–mesenchymal transition, invasion, metastatic dissemination, and therapy resistance. Notably, subtype-specific miRNA expression signatures have demonstrated strong potential as diagnostic, prognostic, and treatment-response biomarkers in breast cancer.[6, 7]

Among these regulatory molecules, microRNA-551b-3p (miR-551b-3p) has gained attention due to its context-dependent functions. In breast cancer, elevated nuclear transport of miR-551b-3p via importin-8 (*IPO8*) activates signal transducer and activator of transcription 3 (*STAT3*) signaling and correlates with poor clinical outcomes.[8] Conversely, reduced expression of miR-551b-3p promotes oncogenic activity in other tumor types, such as lung cancer, by enabling long non-coding RNA (lncRNA) PVT1-mediated increases in cellular proliferation and invasion.[9] Additional studies highlight diverse regulatory axes involving miR-551b-3p, including *Erb-B2* receptor tyrosine kinase 4 (*ERBB4*) suppression in breast cancer[10] and its participation in lncRNA- or autophagy-related pathways in gastric cancer and head and neck carcinoma.[11, 12] Collectively, these findings indicate that miR-551b-3p exerts tumor-suppressive or oncogenic effects depending on the molecular context.

Despite these specific reports, a system-wide view of the miR-551b-3p regulatory landscape and its target interactome in breast cancer remains elusive. In contrast to previous studies that focused on isolated signaling axes, our work integrates high-stringency CLIP-seq binding frequency data, evolutionary conservation metrics, and novel target-directed microRNA degradation (TDMD) scoring. This multi-layered approach aims to systematically identify the miR-551b-3p target network and reconcile its context-dependent roles through large-scale pan-cancer clinical data. By elucidating these biological pathways, we demonstrate that miR-551b-3p acts as a central regulatory node, offering new insights into breast cancer progression and its potential as a supplementary prognostic indicator.

Materials and Methods

This study was designed as *in silico* bioinformatic analysis based exclusively on publicly available datasets (The Cancer Genome Atlas (TCGA) and Molecular Taxonomy of Breast Cancer International Consortium (METABRIC)); therefore, no pharmacological agents, placebos, or pharmaceutical products were used.

Expression level of miR-551b-3p in breast cancer

The expression levels of miR-551b-3p in breast cancer tissues and normal controls were assessed using the University of Alabama at Birmingham Cancer data analysis portal (University of Alabama at Birmingham Cancer data analysis portal (UALCAN), https://ualcan.path.uab.edu), which provides miRNA-seq and clinical metadata derived from TCGA cohort.[13] Additional expression data, including statistical metrics such as T-test *P*-value, T-test false discovery rate (FDR), tumor Log_2 mean expression, and normal Log_2 mean expression, were retrieved from the OncomiR database (https://oncomir.org), a TCGA-based resource for miRNA dysregulation in cancer.[14]

Potential target genes of miR-551b-3p and enrichment analysis in breast cancer

Potential mRNA targets of miR-551b-3p were obtained from the Encyclopedia of RNA Interactomes (ENCORI) database.[15] High-confidence interactions were selected based on CLIP-supported binding (CLIP-Data ≥ 5), AGO-paralog evidence, and concordance across multiple prediction algorithms (PITA, miRanda, miRmap, TargetScan, PicTar, microT). For each gene, ENCORI-provided metrics—including TMDB score, TCGA-derived Pearson correlation coefficients, *P*-values, FDR, and prediction-tool support—were collected. Genes passing FDR significance thresholds were retained for downstream functional and pathway enrichment analyses.

The identified target genes were analyzed for gene ontology (GO) and pathway enrichment using the Protein Analysis Through Evolutionary Relationships (PANTHER) classification system.[16] Enrichment analyses included GO biological process (BP), cellular component (CC), and molecular function (MF) categories. Pathway enrichment was assessed using the PANTHER Pathway module, with statistical significance set at $P < 0.05$.

Potential lncRNAs and circRNAs of miR-551b-3p in breast cancer

Potential lncRNA and circular RNA (circRNA) interactors of miR-551b-3p were retrieved from the ENCORI database using AGO-CLIP–supported interactions (AGOExpNumber ≥ 5) as the primary filtering criterion. Transcription factors (TFs) regulating miR-551b-3p expression were identified through the TransmiR v3.0 database.[17] These datasets were used to construct the extended miRNA-centered regulatory network, including non-coding RNA interactors and upstream transcriptional regulators.

Survival analysis of miR-551b-3p in breast cancer

Survival analysis of miR-551b-3p in breast cancer was performed using the Kaplan–Meier Plotter (http://www.kmplot.com).[18] Overall survival (OS) curves were generated using TCGA breast cancer datasets with the default 'auto–best cutoff' option. Hazard ratios (HRs) with 95% confidence intervals and log-rank p-values were computed automatically by the KM Plotter statistical module. The maximum follow-up time reached approximately 300 months, based on the TCGA breast cancer survival data used in the Kaplan–Meier analysis. All available cases from the TCGA and METABRIC cohorts were included to maximize statistical power; therefore, a formal sample size calculation was not performed.

Analysis of miR-551b-3p expression in relation to breast cancer clinical parameters

The association between miR-551b-3p expression and clinical parameters in invasive breast carcinoma was assessed using the OncomiR database.[14] miR-551b-3p expression levels were analyzed in relation to tumor stage and grade by selecting the corresponding miRNA and cancer type. Only associations with statistical significance at $P < 0.05$ were considered. These analyses provided insights into the potential clinical relevance of miR-551b-3p in breast cancer.

Results

Expression of miR-551b-3p in breast cancer tissues

In this study, the expression profile of miR-551b-3p in breast cancer was systematically characterized. The analyses demonstrated a significant downregulation of miR-551b-3p in primary breast tumors compared with normal breast tissues (Figure 1a; $^{****}P < 0.0001$), indicating a robust differential expression pattern. A similar trend was observed across breast cancer molecular subtypes (Figure 1b; $^{***}P < 0.001$), reinforcing the notion that miR-551b-3p loss may be functionally relevant in breast tumorigenesis.

OncomiR analyses further validated this decrease, revealing a statistically significant reduction in tumor samples (two-tailed t-test, $P = 2.90 \times 10^{-8}$; FDR = 1.10×10^{-7}). The mean $\log_2$ expression values (1.96 in normal tissues vs. 0.86 in tumor tissues; Table 1) highlight a pronounced downregulation of miR-551b-3p in breast cancer.

Target genes of miR-551b-3p in breast cancer

Potential target genes of miR-551b-3p were identified by integrating multiple high-stringency miRNA–mRNA prediction algorithms with expression correlation analyses (Table 2). The combined analysis revealed a set of candidate genes exhibiting supportive prediction evidence together with statistically significant positive correlations in TCGA-BRCA (The Cancer Genome Atlas Breast Invasive Carcinoma) expression data. To ensure biological relevance, candidates were prioritized based on a stringent intersection of multiple high-stringency parameters.

Among them, *TEAD1*, *SMG1*, and *SLC5A3* emerged as the most prominent potential targets, characterized by a combination of integrative metrics, including high AGO-binding frequency (CLIP-seq), evolutionary conservation, and predictive scores ($P < 0.01$, FDR < 0.05) (Figure 2).

GO and pathway enrichment analysis of target genes

GO and pathway analyses revealed that the target genes of miR-551b-3p are predominantly associated with cellular processes (31%) and biological regulation (26%). At the MF level, most genes participate in binding (37%) and catalytic activity (32%), while CC analysis indicated that the majority of genes localize to nuclear and cytoplasmic structures (Figure 3).

Pathway analysis highlighted protein tyrosine kinase 2 (*PTK2*) as a central component of Integrin, vascular endothelial growth factor (*VEGF*), and Angiogenesis signaling pathways, suggesting that miR-551b-3p may modulate cell adhesion, migration, and angiogenic processes (Table 3).

Prediction of potential lncRNA targets of miR-551b-3p

miRNA–lncRNA interactions were identified by intersecting AGO-binding regions derived from CLIP-seq data with predicted target sites (Table 4). Based on correlation coefficients and supportive predictive metrics, *AC069281.2* and A*L359258.1* emerged as the most prominent candidate lncRNAs, characterized by consistent negative correlations ($R = -0.128$, $P < 0.001$) and high significance levels (FDR < 0.001).

DLX6-AS1 was identified as a secondary candidate due to its moderate correlation, whereas *LINC00599* exhibited lack of statistical significance ($P > 0.05$). Expression analyses using the UALCAN platform further supported these findings; TCGA data demonstrated a marked upregulation of *AC069281.2* in primary tumor tissues, while *AL359258.1*—nearly undetectable in normal breast tissue—showed a statistically significant increase in tumor samples (Figure 4).

Prediction of potential circRNA targets of miR-551b-3p

CLIP-seq–based integration identified a set of circRNAs with potential binding interactions with miR-551b-3p (Table 5). Since the ENCORI database

reports circRNA candidates using parent gene symbols rather than specific isoform IDs, the names listed in the table (e.g., *TMEM2*, *USP9X*, and *UBC*) represent circRNAs originating from these genomic loci. A joint evaluation using multiple parameters—including AGO-binding frequency, evolutionary conservation, and predictive degradation scores—highlighted *TMEM2*-, *USP9X*-, and *UBC*-derived circRNAs as the most prominent candidates for further analysis.

The relationship between miR-551b-3p and TFs in breast cancer

The potential TFs regulated by miR-551b-3p were identified using the TransmiR v3.0 database, revealing TFs involved in proliferation, differentiation, and cancer-related pathways. GEPIA3 analysis indicated that many of these TFs—particularly *E2F*1, *FOXA1*, *ESR1*, *KDM5B*, *CBX3*, and *RAD21*—are markedly upregulated in breast cancer tissues (Table 6).

Prognostic value of miR-551b-3p in breast cancer patients

The prognostic significance of miR-551b-3p was evaluated using the Molecular Taxonomy of Breast Cancer International Consortium. OS analysis revealed that patients with low miR-551b-3p expression had significantly poorer survival outcomes compared with those exhibiting high expression levels (HR = 0.64; 95% confidence interval (CI): 0.52–0.79; log-rank $P = 3.5\times10^{-5}$; FDR = 1%) (Figure 5).

Clinical and pathological correlates of miR-551b-3p expression

Bioinformatic and statistical analyses showed that miR-551b-3p expression is significantly associated with metastasis status (Pathologic M) in breast cancer (analysis of variance (ANOVA) $P = 1.41 \times 10^{-4}$; FDR = 1.65×10^{-3}; Table 7). In the multivariate survival analysis, however, the association with OS reached a near-significant level ($P = 0.055$) but did not retain statistical significance after FDR correction (FDR = 0.559). Similarly, analyses stratified by tumor size (Pathologic T) revealed no statistically significant associations following FDR adjustment.

Discussion

In this study, we systematically characterized the expression profile, molecular interactions, and clinical relevance of miR-551b-3p in breast cancer. Our large-scale analysis using TCGA and METABRIC cohorts revealed a significant downregulation of miR-551b-3p across various breast cancer tissues and molecular subtypes, pointing toward a predominant tumor-suppressive loss. Furthermore, we identified key downstream oncogenic target genes and potential upstream non-coding RNA regulators (lncRNAs and circRNAs) associated with this miRNA's depletion. Clinically, we demonstrated that the global loss of miR-551b-3p is robustly associated with disease progression and metastatic status.

Our initial analyses revealed a consistent downregulation of miR-551b-3p across both bulk breast tumor tissues and various molecular subtypes, suggesting a potential tumor-suppressive role in the general breast cancer landscape. While previous literature has indicated that miR-551b-3p might exhibit oncogenic properties in specific contexts—such as through STAT3 activation in triple-negative breast cancer[8]—our large-scale findings point toward a broader trend of depletion. This discrepancy may align with the context-dependent behavior observed in other microRNAs, such as miR-206 and miR-205, which can function either as suppressors or oncogenic drivers depending on the molecular environment.[19, 20] Indeed, the complex regulatory landscape of breast cancer involves both tumor-suppressive miRNAs (e.g., let-7 and miR-139-5p)[21, 22] that are frequently diminished, and oncomiRs (e.g., miR-21 and miR-221/222)[23, 24] that promote invasiveness. Therefore, despite potential subtype-specific oncogenic functions, the widespread reduction of miR-551b-3p

observed in our cohorts suggests that its predominant functional impact may be closely tied to tumor suppression.

Our integrative bioinformatic approach identified several high-confidence downstream targets of miR-551b-3p, including *TEAD1*, *PTK2*, and *NUP93*. Consistent with existing literature, these genes are recognized as central players in cell proliferation and metastatic dissemination.[25, 26, 27,28, 29, 30] Within this context, it is plausible that the escape of *TEAD1*—a key effector of the Hippo–*YAP* pathway—from miR-551b-3p-mediated repression might potentially drive epithelial–mesenchymal transition and invasion.[26] Interestingly, our analysis also revealed positive correlations between miR-551b-3p and some of these targets, a finding that appears to challenge the classical paradigm of unidirectional miRNA-mediated silencing. This non-canonical pattern could potentially reflect shared upstream regulatory mechanisms or sophisticated feedback loops, wherein target mRNA abundance might influence miRNA stability to maintain cellular homeostasis. Ultimately, these non-canonical relationships underscore the intricate complexity of the miR-551b-3p regulatory network, suggesting that its functions may extend well beyond a simple 'one-way' repression model.

Another layer of regulation identified in our analysis involves potential upstream sponges and degraders, including *TMEM2*- and *USP9X*-derived circRNAs, alongside lncRNAs such as *AC069281.2*. Consistent with emerging literature on competing endogenous RNAs,[31, 32] the high AGO-binding frequency and elevated target-directed microRNA degradation scores of these candidates suggest that they might actively contribute to miR-551b-3p depletion through sequestration or degradation.[15, 33, 34] Furthermore, the evolutionary conservation of these interaction sites supports the potential existence of a preserved regulatory module. Consequently, these findings suggest that the depletion of miR-551b-3p may not merely be a bystander effect, but could potentially be driven by a highly orchestrated non-coding RNA interactome in malignant tissues.

From a clinical perspective, our findings established a robust association between diminished miR-551b-3p expression and pathologic metastasis status. While miR-551b-3p did not emerge as an independent prognostic factor for overall survival in our multivariate models, its strong correlation with metastatic progression highlights its potential clinical utility. This profile suggests that the prognostic value of miR-551b-3p might partially overlap with established clinical variables, yet it continues to provide valuable biological insights specifically relevant to the metastatic behavior of the tumor. Therefore, rather than serving as a standalone survival predictor, miR-551b-3p could potentially function as an important supplementary biomarker for evaluating metastatic risk.

A major strength of the present study lies in its comprehensive, multi-layered in silico approach, which integrates large-scale clinical cohorts (TCGA and METABRIC), high-stringency RNA-binding data, and evolutionary conservation metrics to systematically characterize the miR-551b-3p network. Despite these methodological strengths, it is important to acknowledge certain limitations. The molecular interactions and prognostic significance identified here are predominantly based on genomic datasets and predictive algorithms. Notably, while the identified Integrin and *VEGF* signaling pathways are influenced by a complex network of competing regulators, our results represent only a specific regulatory facet of this intricate landscape. Furthermore, while high TDMD scores suggest a potential mechanism for miRNA depletion, TDMD remains an emerging concept in RNA biology that requires careful interpretation. Therefore, these computational predictions should be viewed

as robust candidates for further investigation rather than definitively established biochemical pathways. Ultimately, extensive experimental validation through in-vitro assays and in-vivo models will be essential to definitively map the precise biochemical mechanisms and evaluate the therapeutic potential of these identified regulatory nodes in breast cancer.

Conclusion

The present multi-layered bioinformatic investigation characterizes miR-551b-3p as a significant tumor-suppressive regulator in breast cancer. Our findings demonstrate that the marked downregulation of miR-551b-3p across molecular subtypes is associated with the dysregulation of key oncogenic nodes, including *TEAD1*, *PTK2*, and *NUP93*. The identification of potential upstream inhibitors—specifically lncRNAs *AC069281.2* and *AL359258.1*, alongside high-TDMD-score circRNAs—provides a novel mechanistic framework for the observed miRNA depletion in malignant tissues.

Clinically, although miR-551b-3p did not emerge as an independent prognostic factor for OS, its robust correlation with metastatic status highlights its potential as a supplementary biomarker for metastatic progression. These results suggest that miR-551b-3p primarily modulates the tumor's invasive capacity through Integrin and *VEGF*-associated signaling pathways. While our in-silico data provide strong candidates for therapeutic targeting, further experimental validation remains essential to definitively confirm the biochemical interactions and functional roles of these regulatory axes in breast cancer.

Data availability

The datasets analyzed during the present study are publicly available in the relevant repositories.

Ethics approval and consent to participate

Not applicable

Authors' Contribution

Kübra Su Eda Çelenk and Esen Çakmak contributed equally to the conception and design of the study, data collection, data analysis and interpretation, and manuscript writing and revision. Both authors read and approved the final manuscript and meet the ICMJE authorship criteria.

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Conflict of Interest

None declared.

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Table 1. Statistical comparison of miR-551b-3p expression between normal and tumor breast tissues

miRNA name	**T-Test *P*-value**	**T-Test FDR**	**Tumor log_2 mean expression**	**Normal log_2 mean expression**
miR-551b-3p	2.90×10^{-8}	1.10×10^{-7}	0.86	1.96

FDR: False discovery rate (Benjamini–Hochberg-adjusted I-value)

Table 2. Computationally predicted target genes of miR-551b-3p and their associated statistical parameters in breast cancer tissues compared with normal tissues

Genes	**TMDB score**	**Coefficient-R**	***P*-value**	**FDR**	**Prediction tools / Databases**
ADGRL1	1.80	-0.06	3.24×10^{-2}	7.27×10^{-2}	PITA, miRanda, PicTar, TargetScan
CASP2	0.75	0.07	1.37×10^{-2}	3.58×10^{-2}	PITA, miRmap, miranda
CDC42BPB	1.19	0.03	2.60×10^{-1}	3.74×10^{-1}	PITA, miRmap
CEMIP2	1.29	0.10	5.01×10^{-4}	2.35×10^{-3}	PITA, miRmap
COX10	1.56	0.08	6.37×10^{-3}	2.04×10^{-2}	PITA, miRmap
NCOA4	0.73	0.11	1.79×10^{-4}	1.08×10^{-3}	PITA, miRmap, miranda
NDEL1	0.45	0.03	3.38×10^{-1}	4.55×10^{-1}	PITA, miRmap, miranda
NDUFA9	0.71	-0.01	9.37×10^{-1}	9.55×10^{-1}	PITA, miRmap, miranda
NUP93	1.07	0.06	5.39×10^{-2}	1.11×10^{-1}	PITA, miRmap
PARD6G	0.45	0.07	1.07×10^{-2}	2.95×10^{-2}	PITA, miRmap, miranda
PDE8A	1.23	0.10	1.56×10^{-3}	5.98×10^{-3}	PITA, miRmap
PJA1	1.03	0.02	5.60×10^{-1}	6.60×10^{-1}	PITA, miRmap, microT, miRanda
PTK2	0.98	0.06	3.76×10^{-2}	8.25×10^{-2}	PITA, miRmap, miranda
SLC5A3	1.04	0.12	4.54×10^{-5}	4.74×10^{-4}	PITA, miRmap
SMG1	1.31	0.12	7.12×10^{-5}	5.78×10^{-4}	PITA, miRmap
SMYD4	-1.33	0.02	5.48×10^{-1}	6.58×10^{-1}	PITA, miRmap
SOWAHC	-0.26	0.04	1.61×10^{-1}	2.63×10^{-1}	PITA, miRmap, miranda
TEAD1	1.18	0.16	1.71×10^{-7}	7.20×10^{-6}	PITA, miRmap
TIMELESS	0.79	0.04	2.33×10^{-1}	3.50×10^{-1}	PITA, miRmap, miranda
UBALD2	0.75	-0.09	2.18×10^{-3}	8.06×10^{-3}	PITA, miRmap, miranda
ZFP36	0.52	0.02	4.07×10^{-1}	5.14×10^{-1}	PITA, miRmap, miranda, PicTar
ZNF581	1.17	-0.11	2.22×10^{-4}	1.30×10^{-3}	PITA, miRmap, miranda

TDMD: Target-directed microRNA degradation score; R: Pearson correlation coefficient; FDR: False discovery rate (Benjamini–Hochberg–corrected *P*-value)

Table 3. GO classification and pathway enrichment of computationally predicted miR-551b-3p target genes in breast cancer tissues

GO category	GO term	Associated genes
Biological process	Biological regulation	*PARD6G, TIMELESS, ZFP36, NDEL1, ADGRL1, PTK2, SMG1, ZNF581, TEAD1, PDE8A*
	Cellular process	*NUP93, PARD6G, TIMELESS, NDUFA9, NDEL1, ADGRL1, CDC42BPB, PTK2, SMG1, COX10, TEAD1, PDE8A*
	Developmental process	*TEAD1*
	Localization	*NUP93, NDEL1*
	Metabolic process	*NUP93, TIMELESS, NDUFA9, PJA1, CDC42BPB, SMG1, COX10*
	Multicellular organismal process	*TEAD1*
	Response to stimulus	*TIMELESS, ADGRL1, PTK2, NCOA4, PDE8A, TEAD1*
Cellular component	Cellular anatomical entity	*PARD6G, TIMELESS, ZFP36, SLC5A3, NDUFA9, NDEL1, ADGRL1, PJA1, CDC42BPB, SMYD4, PTK2, SMG1, NUP93*
	Protein-containing complex	*NUP93, TIMELESS, NDEL1, TEAD1*
Molecular function	Binding	*TIMELESS, ZFP36, NDUFA9, NDEL1, SMYD4, ZNF581, TEAD1*
	Catalytic activity	*PJA1, CDC42BPB, PTK2, SMG1, PDE8A, COX10*
	Molecular transducer activity	*ADGRL1*
	Structural molecule activity	*NUP93*
	Transcription regulator activity	*ZFP36, ZNF581, TEAD1*
	Transporter activity	*SLC5A3*
Pathway	Angiogenesis	*PTK2*
	CCKR signaling map	*PTK2*
	Gonadotropin-releasing hormone Receptor pathway	*PTK2*
	Heme biosynthesis	*COX10*
	Integrin signaling pathway	*PTK2*
	VEGF signaling pathway	*PTK2*

GO: Gene ontology; VEGF: Vascular endothelial growth factor; CCKR: Cholecystokinin receptor

Table 4. Computationally predicted lncRNA candidates regulated by miR-551b-3p and their associated statistical parameters in breast cancer tissues

Genes	TDMD score	Coefficient-R	*P*-value	FDR
AC069281.2	1.22	−0.13	2.45×10^{-5}	1.23×10^{-4}
AL359258.1	1.14	−0.13	2.45×10^{-5}	1.23×10^{-4}
DLX6-AS1	0.15	−0.09	3.74×10^{-3}	1.88×10^{-2}
LINC00599	0.74	−0.05	9.06×10^{-2}	1.84×10^{-1}

lncRNA: Long non-coding RNA; TDMD: Target-directed microRNA degradation score; R: Pearson correlation coefficient; FDR: False discovery rate (Benjamini–Hochberg–corrected *P*-value)

Table 5. Computationally predicted circRNA candidates interacting with miR-551b-3p and their associated binding and conservation parameters in breast cancer tissues

Genes	AGOExpNum	RBP	TDMD score	phyloP
RGMB	10	AGO1, AGO2	0.25	3.92
TMEM2	15	AGO1-4, AGO2	1.20	0.30
ANXA11	7	AGO1, AGO2	0.75	-0.03
RPS13	5	AGO1, AGO2	0.51	5.79
COX6A1	13	AGO1, AGO1-4, AGO2	0.46	0.65
UBC	5	AGO1, AGO2	1.18	-1.25
PAFAH1B1	6	AGO2	0.45	6.12
ATP5A1	9	AGO2	0.93	5.04
REXO1	6	AGO1-4, AGO2	0.28	-0.53
hsa_circ_0089761	10	AGO1, AGO2	0.52	2.66
USP9X	5	AGO1-4, AGO2	1.26	5.74

circRNA: Circular RNA; AGOExpNum: Argonaute binding events; RBP: RNA-binding protein; TDMD: Target-directed microRNA degradation score; phyloP: Phylogenetic conservation score across species

Table 6. Differential expression profile of transcription factors predicted to be regulated by miR-551b-3p in breast cancer tissues

TF name	Log_2 FC	*P*-value	q-value
CBX3	1.00	1.05×10^{-143}	1.70×10^{-143}
CDYL2	0.68	8.50×10^{-27}	1.91×10^{-27}
E2F1	2.01	8.83×10^{-171}	2.15×10^{-170}
ESR1	1.64	2.67×10^{-23}	5.59×10^{-24}
FOXA1	2.62	1.40×10^{-58}	5.73×10^{-59}
HDAC2	0.62	7.51×10^{-40}	2.18×10^{-40}
KDM5B	1.19	3.81×10^{-99}	3.04×10^{-99}
RAD21	1.02	2.08×10^{-59}	8.63×10^{-60}
RORC	0.87	6.92×10^{-23}	1.44×10^{-23}
SMC1A	0.83	7.54×10^{-37}	2.06×10^{-37}

TF: Transcription factor; Log_2 FC: Log_2 fold change in gene expression between tumor and normal tissues; FDR: False discovery rate (Benjamini–Hochberg-adjusted *P*-value)

Table 7. Association between miR-551b-3p expression and clinical parameters in breast cancer patients

miRNA	Clinical parameter	ANOVA *P*-value	ANOVA FDR	Multivariate log-rank *P*-value	Multivariate log-rank FDR
miR-551b-3p	Pathologic M Status	1.41×10^{-4}	1.65×10^{-3}	5.51×10^{-2}	5.59×10^{-1}
miR-551b-3p	Pathologic T Status	4.88×10^{-2}	2.00×10^{-1}	1.29×10^{-1}	6.98×10^{-1}

Log-rank analysis was performed using a multivariate Cox proportional hazards model. ANOVA: Analysis of variance; FDR: False discovery rate (Benjamini–Hochberg-adjusted *P*-value); BRCA: Breast invasive carcinoma

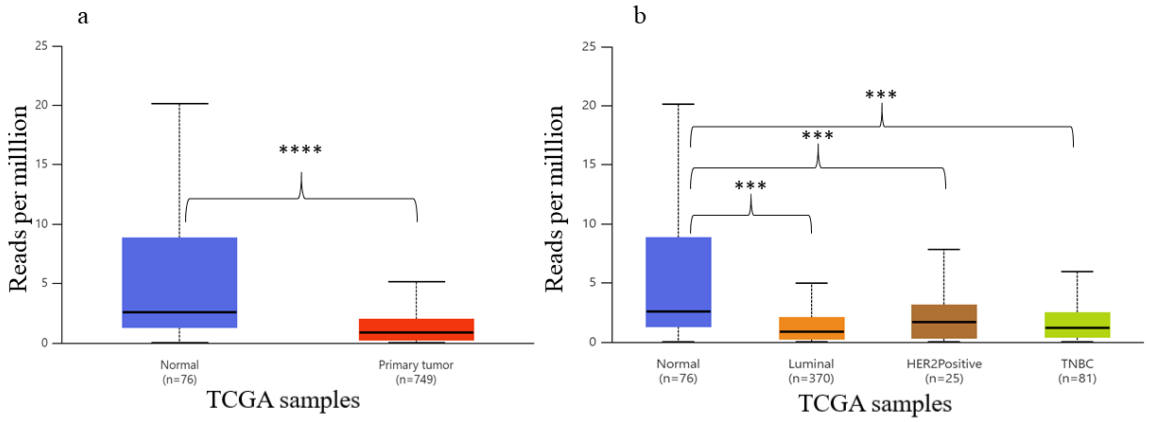

Figure 1. Differential expression of miR-551b-3p in breast cancer; (a) miR-551b-3p expression levels in normal and primary tumor tissues. (b) miR-551b-3p expression levels across different breast cancer molecular subtypes and normal tissues

$^{****}P < 0.0001$ and $^{***}P < 0.001$ indicate statistically significant differences. TCGA: The Cancer Genome Atlas; TNBC: Triple-negative breast cancer; HER2: Human epidermal growth factor receptor 2

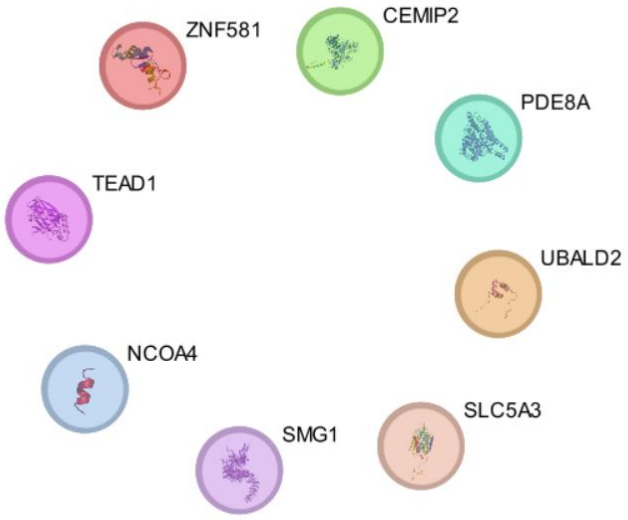

Figure 2. Significantly correlated predicted target genes of miR-551b-3p in breast cancer samples

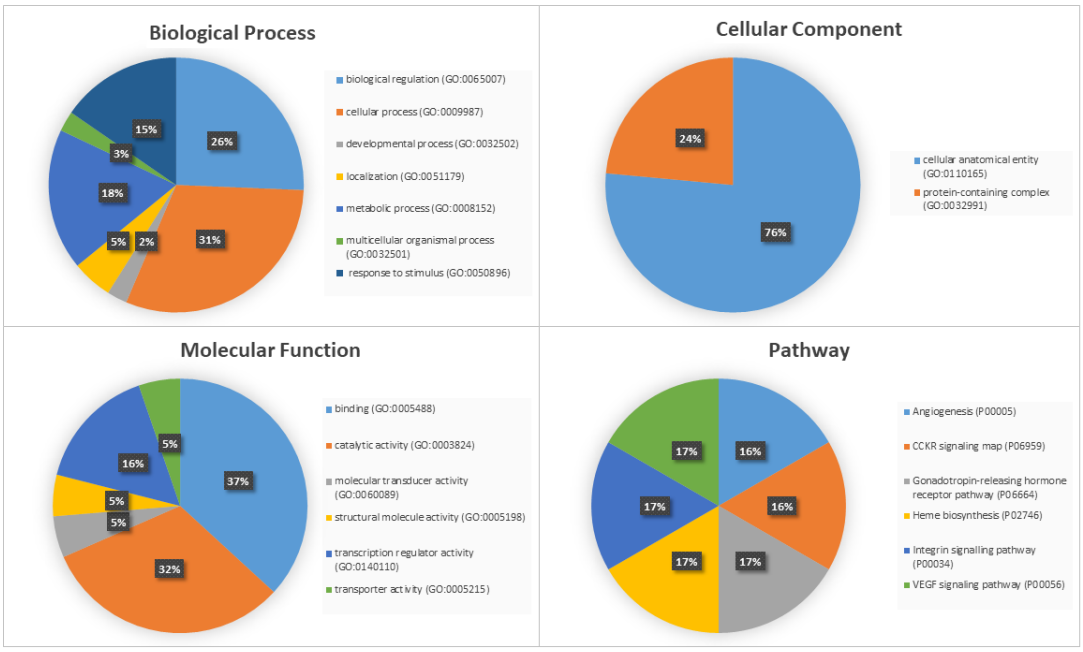

Figure 3. GO and pathway enrichment analysis of predicted miR-551b-3p target genes in breast cancer.

GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; VEGF: Vascular endothelial growth factor; CCKR: Cholecystokinin receptor

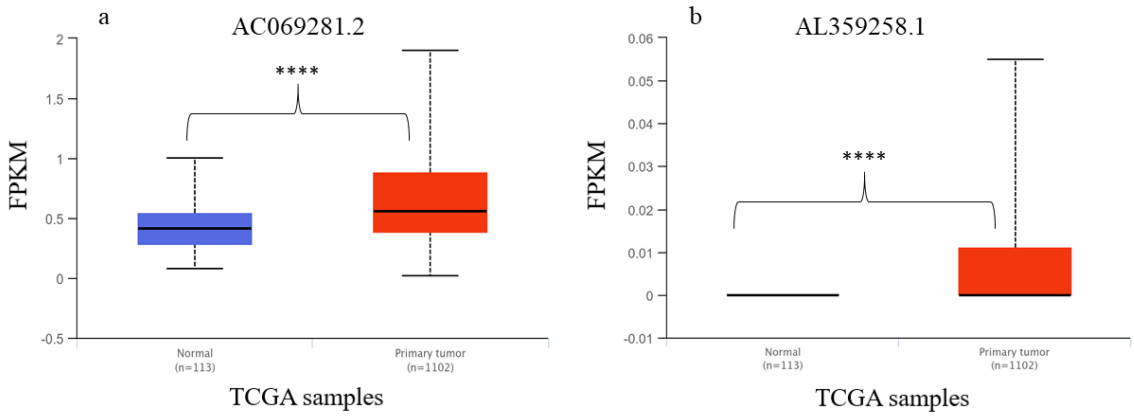

Figure 4. Differential expression of lncRNAs between normal and breast cancer tissues; (a) The expression levels of *AC069281.2*. (b) The expression levels of *AL359258.1*

$^{****}P < 0.0001$. TCGA: The Cancer Genome Atlas; FPKM: Fragments per kilobase of transcript per million mapped reads; lncRNA: Long non-coding RNA

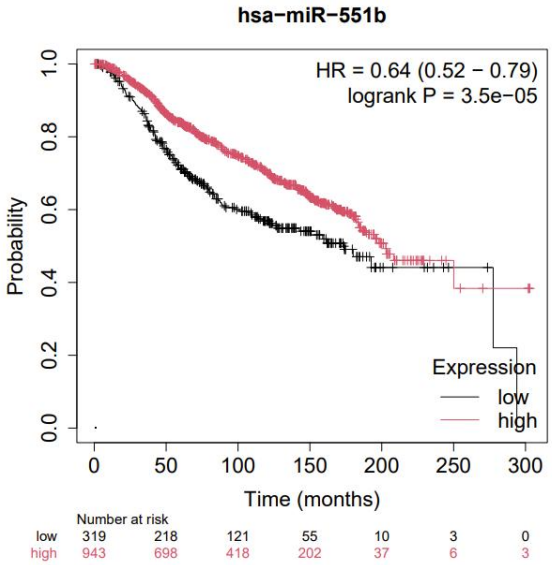

Figure 5. Overall survival analysis of breast cancer patients stratified by miR-551b-3p expression levels

HR: Hazard ratio; CI: Confidence interval