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Tumor Discovery

ORIGINAL RESEARCH ARTICLE

RIBC2 expression as a potential diagnostic and prognostic biomarker associated with tumor progression and immune modulation in breast cancer

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Abstract

Breast cancer remains one of the deadliest diseases around the world. This study systematically investigates the expression profile and clinical significance of *RIBC2* in breast cancer. By integrating multi-omics data from public databases, such as The Cancer Genome Atlas and The Genotype-Tissue Expression project, combined with bioinformatic analyses and molecular docking simulations, we demonstrated that *RIBC2* is significantly overexpressed in breast cancer tissues. Elevated *RIBC2* expression was positively correlated with higher T stage, advanced pathological stage, and poorer overall survival. Functional enrichment analyses indicated that *RIBC2* may facilitate tumor invasion by regulating extracellular matrix remodeling and activating the mitogen-activated protein kinase signaling pathway. Multivariate Cox regression analysis confirmed high *RIBC2* expression as an independent poor prognostic factor, with its inclusion slightly increasing the area under the curve of the prognostic model from 0.762 to 0.764. Moreover, immune infiltration analysis revealed that high *RIBC2* expression was associated with increased infiltration of regulatory T cells and M2-polarized macrophages, indicating a potential role in shaping an immune suppressive tumor microenvironment. Spearman correlation analysis between *RIBC2* and immune checkpoints showed significant positive associations only for lymphocyte activation gene 3 protein ($p<0.001$, absolute correlation ≈ 0.1) and human leukocyte antigen-C ($p=0.045$, absolute correlation ≈ 0.08), indicating *RIBC2* may selectively modulate specific immunosuppressive pathways. Drug sensitivity analysis further suggested that *RIBC2* overexpression may contribute to chemotherapy resistance. The methylation level of *RIBC2* was found to be significantly negatively correlated with its expression, suggesting that hypermethylation in the promoter or regulatory regions may suppress gene expression through epigenetic silencing mechanisms. Furthermore, quantitative polymerase chain reaction revealed a 4.2-fold *RIBC2* upregulation in triple-negative breast cancer cells (MDA-MB-231) compared to normal mammary cells (Hs578bst), exceeding levels in luminal subtypes (MCF-7). Collectively, these findings highlight *RIBC2* as a promising prognostic biomarker and provide a theoretical foundation for developing combination therapeutic strategies targeting *RIBC2*.

Keywords: Breast cancer; *RIBC2*; Prognostic marker; Metastasis; Immunity

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1. Introduction

Breast cancer remains one of the most prevalent malignancies among women worldwide and a leading cause of cancer-related mortality. Its clinical course is shaped by receptor-defined subtypes with distinct molecular programs and therapeutic strategies.[1] Among subtypes, triple-negative breast cancer (TNBC), which lacks expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2), is notably aggressive and therapeutically challenging, with limited targeted treatment options and a dismal prognosis.[2,3] Hormone-receptor-positive, HER2-negative tumors follow luminal programs driven by estrogen-receptor signaling, show frequent phosphoinositide 3-kinase (PI3K) pathway activation, and often acquire endocrine resistance through *ESR1* alterations.[4] Standard care combines endocrine therapy with cyclin-dependent kinase 4/6 inhibition and, when appropriate, PI3K inhibition.[5] HER2-positive cancers are driven by *ERBB2* amplification or overexpression and now achieve durable control with HER2-targeted antibodies and antibody-drug conjugates, with benefits that extend to some HER2-low disease under contemporary testing criteria.[6] The complex pathophysiology of breast cancer requires deeper molecular insights to support the development of personalized treatment approaches. TNBC exhibits aggressive clinical behavior, high rates of early recurrence, and a strong propensity for distant metastases, including to the brain and lymph nodes, resulting in poor patient prognosis. Due to the lack of specific molecular targets, systemic chemotherapy remains the mainstay of treatment; however, the survival benefit is limited.[7] Therefore, identifying novel biomarkers and elucidating the underlying molecular mechanisms are urgent for improving TNBC patient stratification and developing targeted therapies. Clinically, hormone receptor-positive disease carries a persistent risk of late recurrence, HER2-positive disease often attains prolonged control under targeted therapy, and TNBC tends to relapse earlier and behave more aggressively despite recent advances.[8] Across subtypes, converging evidence implicates cytoskeletal dynamics and membrane-trafficking pathways governed by small guanosine triphosphatases in invasion, plasticity, receptor recycling, immune engagement, and drug uptake.[9-11] This biological mechanism motivates investigation of *RIBC2*-related mechanisms as potential modulators of progression and treatment response, with particular relevance to TNBC and possible implications across the broader spectrum of breast cancer.

In recent years, advances in single-cell and multi-omics technologies have provided unprecedented opportunities to dissect the molecular heterogeneity and tumor microenvironment of breast cancer. For instance, single-cell RNA sequencing of breast cancer brain metastases has revealed distinct malignant subclusters and identified RNA-binding protein interleukin enhancer-binding factor 2 as a driver of metastatic programs, validated both *in vitro* and *in vivo*.[12] Similarly, integrative multi-omics profiling has characterized disulfidptosis, a novel form of cell death triggered by disulfide stress, and identified glycogen synthase 1 as a TNBC-specific therapeutic target.[13] These studies underscore the power of multi-omics approaches in uncovering molecular vulnerabilities in aggressive breast cancer subtypes. Recent multi-omics integrative analyses, including transcriptomic, genomic, and epigenomic datasets, have identified novel biomarkers and therapeutic targets.[14-16] For example, prognostic gene signatures derived from weighted gene co-expression network analysis and least absolute shrinkage and selection operator regression have shown clinical utility in cervical cancer,[17] while obesity-associated transcriptomic profiles have shed light on immune microenvironmental heterogeneity in hepatocellular carcinoma.[18,19] These findings highlight the potential of combining high-throughput data with clinical parameters to uncover key regulators of tumor progression and therapy response.

Among the emerging candidates is the RIB43A domain with the coiled-coils 2 (*RIBC2*) gene, which encodes a scaffolding protein implicated in cytoskeletal reorganization and cell migration.[20] Although the oncological role of *RIBC2* remains largely underexplored, recent studies have uncovered its broader biological functions. Katsuma *et al.*[21] revealed that RIBC1 and RIBC2 function as microtubule inner proteins essential for maintaining sperm motility in mice; single knockouts had mild effects, double knockout mice showed significantly reduced sperm velocity and fertility despite no overt structural defects in axonemes, indicating that *RIBC2* contributes to microtubule function in a cooperative manner.[2,21] Similarly, Kwon *et al.*[22] demonstrated that RIBC2 interacts with creatine kinase B and Y-box-binding protein 2 proteins and plays a critical role in ciliary beating in multiciliated cells. Its depletion disrupted fluid flow and defective cilia function in *Xenopus laevis*, reinforcing its role in motility-related cytoskeletal dynamics.[22] Furthermore, *RIBC2* has been identified as a hub gene in a six-gene prognostic model for cervical cancer, suggesting that it may have oncogenic potential across multiple tumor types.[23] In particular, RIBC2 is part of the dynein-decorated doublet microtubules within cilia, and its presence is required for proper motile cilia beating.[22] This implies that RIBC2 helps maintain the structural integrity and oscillatory movement of cilia, a fundamental aspect of cellular development and tissue physiology. Disruption of ciliary structure or function often leads to

developmental disorders and aberrant cell regulation.[21] As a ciliary structural protein, RIBC2 contributes to this regulatory landscape by maintaining functional cilia. Across proper cilia beating and structure, RIBC2 indirectly influences how cells sense extracellular cues and regulate downstream gene expression during development.

In the context of breast cancer, emerging bioinformatic and functional evidence suggests that *RIBC2* expression is altered in tumors, with implications in regulating pathways relevant to metastasis and immune evasion. Notably, the single-nucleotide polymorphism rs2272804, located in the 5' untranslated region of *RIBC2*, introduces an upstream open reading frame, which may interfere with ribosomal scanning and initiation of translation at the canonical start codon.[20] This regulatory alteration is predicted to attenuate translational efficiency and reduce RIBC2 protein synthesis, which may impair ciliary function and indirectly contribute to dysregulated signaling and gene expression during tumorigenesis. In addition, overexpression of *RIBC2* has been linked to the activation of *TRIM37*, a modulator of the Wnt/β-catenin pathway implicated in tumor metastasis.[24] High *RIBC2* expression also impresses the expression of *TRAF2*, a key player in inflammatory and apoptotic signaling.[20] These regulatory interactions indicate a potential oncogenic axis involving *RIBC2* and key signaling networks in breast cancer pathogenesis. *RIBC2* is a significantly differentially expressed gene in breast cancer tissue; the reduced expression of *RIBC2* in TNBC could diminish the ciliary signaling of cancer cells.[20] This loss may allow tumor cells to escape the usual developmental restraints imposed by cilia-mediated pathways, contributing to unchecked proliferation or loss of differentiation. Dysregulation of *RIBC2* in TNBC may disrupt the balance of signaling that regulates cell growth, highlighting its potential role as a tumor suppressive factor through maintenance of ciliary regulation.

Despite these intriguing findings, the precise roles of *RIBC2* in breast cancer subtype specificity, tumor progression, and the tumor microenvironment remain inadequately characterized. Whether *RIBC2* contributes to therapy resistance, immune escape, or metastasis in aggressive subtypes, such as TNBC is yet to be determined. In this study, we investigate the role of *RIBC2* in breast cancer through a combination of bioinformatic analysis, *in vitro* validation, and clinical correlation studies (Figure 1). By characterizing its expression, molecular function, and pathway associations, we seek to define whether *RIBC2* may serve as a novel biomarker or therapeutic target for breast cancer in the era of precision oncology.

2. Materials and methods

2.1. Data sources and gene information retrieval

To investigate *RIBC2*'s basic genetic characteristics, the chromosomal location and subcellular localization were retrieved from the GeneCards database. Protein topology was analyzed using the Protter tool, and tissue-specific expression patterns were obtained from the Human Protein Atlas (HPA). Disease associations were identified using the Open Targets platform to screen tumor types and pathological mechanisms significantly related to *RIBC2*. All uniform resource locators are listed in Table 1.

2.2. Clinical data acquisition and differential expression analysis

To further evaluate differential *RIBC2* expression between tumor and normal tissues, RNA-sequencing data for breast cancer and normal breast tissues were downloaded from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) databases. TCGA data are mainly derived from cancer patient samples in the United States, whereas the GTEx data are primarily obtained from multiple tissue samples of healthy individuals in the United States. Data were integrated and pre-processed through the University of California, Santa Cruz Xena platform. Differential expression analysis was conducted using the DESeq2 R package (v1.26.0). Differentially expressed genes were identified using thresholds of $|\log_2(\text{fold change})| > 1.5$ with a false discovery rate <0.05. The Wilcoxon signed-rank test was applied for paired samples and the Mann–Whitney U test for unpaired samples.

2.3. Prognostic and clinicopathological correlation analyses

To explore the prognostic significance and clinicopathological relevance of *RIBC2* expression, patients from the TCGA cohort were stratified into high- and low-expression groups based on median expression levels. Kaplan–Meier survival curves were generated for overall survival (OS) and progression-free survival, with differences assessed using the log-rank test. Cox proportional hazards regression models were used to examine the association between *RIBC2* expression and TNM stage, histological grade, and survival outcomes, with results expressed as hazard ratios (HRs) and 95% confidence intervals (CIs). Gene set variation analysis (GSVA) was performed to assess pathway activity alterations related to *RIBC2* expression, referencing hallmark and Kyoto Encyclopedia of Genes and Genomes (KEGG) gene sets. Univariate and multivariate Cox proportional hazards regression models were used to examine the association between *RIBC2* expression and clinical outcomes. For the multivariate

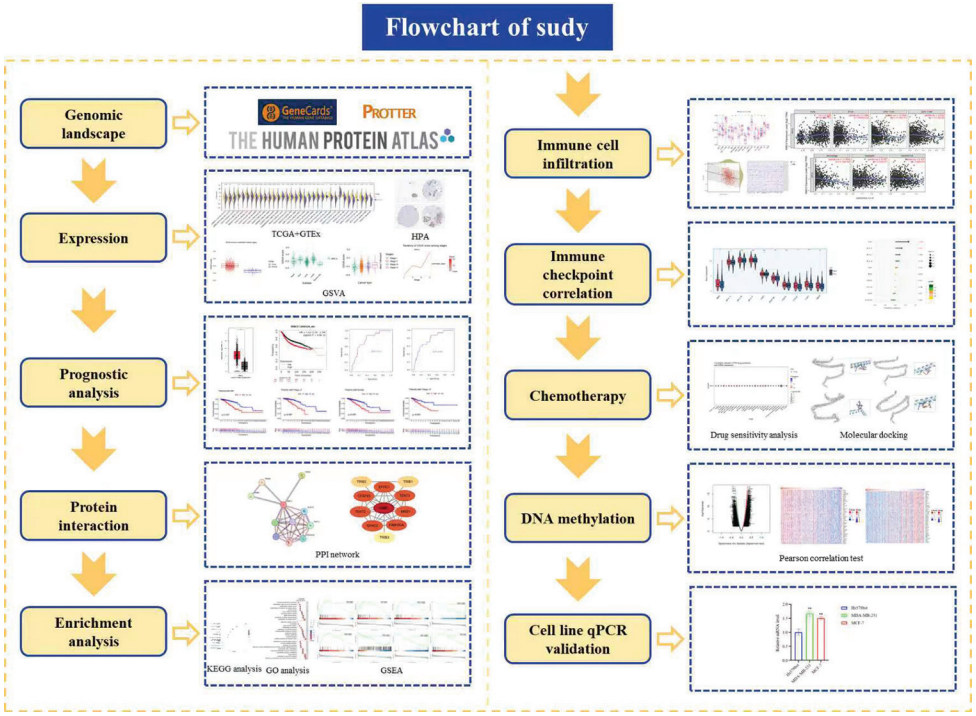


Figure 1. Flowchart of the study. This study systematically explored the role of *RIBC2* in breast cancer through multiple analytical steps. First, the genomic landscape of *RIBC2* was investigated using GeneCards, Protter, and the Human Protein Atlas (HPA) databases. Then, expression analysis was conducted based on The Cancer Genome Atlas and Genotype-Tissue Expression datasets, supplemented by HPA immunohistochemistry and Gene set variation analysis scores. Third, prognostic significance was assessed through Kaplan–Meier survival analyses. Fourth, a protein-protein interaction network was constructed. Subsequently, functional enrichment analyses, including Kyoto Encyclopedia of Genes and Genomes, Gene Ontology, and gene set enrichment analysis, were performed to explore potential biological pathways. Then, immune infiltration analysis was conducted to evaluate associations with the tumor microenvironment, followed by an immune checkpoint correlation conducted using public datasets. Subsequently, chemotherapy response and potential drug interactions were assessed through drug sensitivity profiling and molecular docking. DNA methylation analysis was performed to investigate the epigenetic regulation of *RIBC2.* Finally, quantitative polymerase chain reaction validation of *RIBC2* expression was conducted.

Table 1. Data sources and platforms used in the study

Platform	Accessible website	Function
GeneCards	https://www.genecards.org/	Gene information
Protter	https://wlab.ethz.ch/protter/start/	Protein structure prediction
Human Protein Atlas	http://www.proteinatlas.org/	Protein expression in various tissues, cells, and disease states
Open Targets	https://www.targetvalidation.org/	Systematic drug target validation and prioritization
UCSC Xena	https://xenabrowser.net/	Integrated analysis of cancer genomics data (e.g., TCGA, GTEx)
Kaplan-Meier Plotter	https://kmplot.com/analysis/	Survival analysis based on gene expression profiles
STRING	https://cn.string-db.org/	Protein-protein interaction network construction and functional enrichment
Cancer Therapeutics Response Portal database	https://portals.broadinstitute.org/ctrp/	Exploration of cancer cell line drug sensitivity profiles
AlphaFold	https://alphafold.com/	High-accuracy protein structure prediction
PubChem	https://pubchem.ncbi.nlm.nih.gov/	Chemical compound database for drug discovery and pharmacology
LinkedOmics	http://www.linkedomics.org	Multi-omics data analysis in cancer research

Abbreviations: GTEx: Genotype-Tissue Expression; STRING: Search Tool for the Retrieval of Interacting Genes/Proteins; TCGA: The Cancer Genome Atlas; UCSC: University of California, Santa Cruz.

model, covariates included age, tumor size, lymph node metastasis status, TNM stage, histological grade, hormone receptor status, HER2 status, *BRCA1/2* mutation status, and expression levels of established prognostic markers

(antigen Kiel 67, cancer embryonic antigen, cancer antigen 125 [mucin 16], cancer antigen 15-3 [mucin 1], and cancer antigen 19-9 [fucosyltransferase 3/ST3 beta-galactoside alpha-2,3-sialyltransferase 3]). The proportional hazards assumption was verified using the cox.zph function, and multicollinearity was excluded by calculating variance inflation factors with a threshold of <10 using the "car" package in R (version R-4.3.1). HRs and 95% CIs were computed, and Wald *p*-values were reported. In addition, receiver operating characteristic (ROC) curves were constructed to evaluate the predictive performance of models with and without *RIBC2*, with the area under the curve (AUC) used as the primary metric.

2.4. Functional enrichment and protein-protein interaction network analysis

This study utilized the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database for online analysis to predict the potential interacting proteins of RIBC2 and its functional network. The specific steps were as follows. First, the STRING database was accessed, and "RIBC2" (*Homo sapiens*) was entered in the search bar. Next, the correct protein entry was selected, and the interaction score was set to "medium confidence" to ensure the reliability of the prediction results. Subsequently, the "Search" button was clicked, and the list of interacting genes was extracted. Gene ontology (GO) and KEGG functional enrichment analyses were performed using the clusterProfiler package (v4.6.2) in the R platform. The GO analysis comprises three categories: Biological process, molecular function, and cellular component. The KEGG analysis was used to identify potential signaling pathways associated with the interacting genes. The enrichment analysis employed the hypergeometric test method, with a screening criterion of adjusted *p* (*p*.adjust) <0.05, and combined visualization results to systematically predict the biological functions of RIBC2.

2.5. Immune infiltration and tumor microenvironment analysis

To assess the relationship between *RIBC2* expression and immune infiltration, the abundance of 22 immune cell types in the tumor microenvironment was estimated using the CIBERSORT and xCell algorithms (implemented via the GSVA R package, v3.6). The association between *RIBC2* expression and immune cell markers was analyzed using Spearman's correlation. Immunohistochemistry images from the HPA database were utilized to validate differential protein expression of *RIBC2* between normal and breast cancer tissues. To investigate potential crosstalk between *RIBC2* and immune evasion, breast cancer patients from the TCGA cohort were stratified into high- and low-*RIBC2* expression groups using the median expression as a cutoff. A panel of immune checkpoints was analyzed: T cell immunoreceptor with Ig and ITIM domains (TIGIT), lymphocyte-activation gene 3 (LAG3), T-cell immunoglobulin and mucin domain 3 (TIM-3; *HAVCR2*), Cluster of Differentiation 47 (CD47), major histocompatibility complex I (human leukocyte antigen [HLA]-A/B/C), indoleamine 2,3-dioxygenase-1 (IDO1), programmed death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte associated protein 4 (CTLA4). Spearman's rank correlation (robust to non-normal distributions) was used to assess relationships, with coefficients (ρ) and *p*-values calculated through R's "cor.test" function. For visualization, the ggplot2 package generated dot plots where dot size represented |ρ| (0–0.5 scale) and color encoded *p*-values (green = low *p*, yellow = high *p*). Statistical significance was defined as $p<0.05$ (exploratory analysis, with focused validation planned).

2.6. Drug sensitivity analysis and molecular docking

To explore the potential therapeutic implications of *RIBC2*, its correlation with drug sensitivity (half-maximal inhibitory concentration values) was analyzed using data from the Cancer Therapeutics Response Portal (CTRP) through Spearman correlation ($p<0.05$). For molecular docking, the RIBC2 protein sequence was retrieved from UniProt, and a three-dimensional structure was predicted using AlphaFold (https://alphafoldserver.com) (AlphaFold 3 model). The structure was pre-processed in PyMOL (v2.5) by removing water molecules, adding hydrogens, and optimizing force fields. Small molecule structures were retrieved from PubChem, and ligand files were prepared using AutoDock Vina (v1.2.3). Docking simulations were performed using AutoDock Tools (v1.5.6) and AutoDock Vina, and binding affinities (kcal/mol) were calculated to assess ligand-receptor binding strength. Lower binding energies indicated stronger binding stability. Binding sites and hydrogen bond interactions were visualized using PyMOL.

2.7. Analysis of *RIBC2* gene methylation levels

The methylation levels of the *RIBC2* gene in breast cancer were analyzed using the Meth450 methylation platform available on the LinkedOmics database. A Pearson correlation test was performed to evaluate the relationship between *RIBC2* methylation and its expression levels in breast cancer, providing insights into the epigenetic regulation of this gene.

2.8. Quantitative polymerase chain reaction of *RIBC2* expression in breast cancer cell lines

Human normal mammary epithelial cells (Hs578bst), luminal breast cancer cells (MCF-7), and TNBC cells

(MDA-MB-231) were obtained from Hangzhou Comet Biotechnology Co., Ltd., China. All cells were cultured in Dulbecco's Modified Eagle Medium (Gibco, Thermo Fisher Scientific Inc., United States). The culture medium was supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (MacRoll Biotech Co., Ltd., China). The cells were maintained at 37°C in a humidified atmosphere containing 5% carbon dioxide in a carbon dioxide incubator until 90–100% cell confluency was achieved. Total RNA was isolated using TRIzol reagent (Invitrogen, Thermo Fisher Scientific Inc., United States), and purity was confirmed using a spectrophotometer (A260/A280 >1.8; NanoDrop 2000, Thermo Fisher Scientific Inc., United States). Complementary DNA synthesis was performed using the PrimeScript RT Reagent Kit (Takara, Japan). The SYBR Green Master Mix (Roche, Switzerland) with the primers from Table 2 was used for the quantitative polymerase chain reaction (qPCR) procedure (Thermo Fisher Scientific, China).

2.9. Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences 25.0 (SPSS Inc., United States) and GraphPad Prism 9.0 (GraphPad Software, United States). For quantitative comparisons, we used either one-way analysis of variance or a two-tailed Student's *t*-test, while qualitative data were analyzed using the non-parametric χ^2 test. Statistical significance was set at $p<0.05$, with significance levels denoted as follows: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, and $^{****}p<0.0001$. The exact *p*-values are indicated in the figures.

3. Results

3.1. Expression characteristics of *RIBC2* in breast cancer

The chromosomal location and subcellular localization data obtained from the GeneCards database showed that *RIBC2* is located in the 12q24.31 region of chromosome 12 and is distributed in both the cytoplasm (60%) and the nucleus (40%) (Figure 2A and B). Topological analysis revealed that RIBC2 protein contains multiple SH3 domains, suggesting potential roles in signal transduction and cytoskeletal remodeling (Figure 2C). Further analysis demonstrated a positive correlation between *RIBC2* expression levels and tumor grade (Figure 2D). This result suggests the potential of *RIBC2* as a diagnostic biomarker for the diagnosis of breast cancer.

Table 2. Primers used in quantitative polymerase chain reaction

Primer name	Primer sequence (5' to 3')
RIBC2-Forward	AAATCTCTGTAGGGCTAT
RIBC2-Reverse	TCCGAACATCATTATCTG
GAPDH-Forward	TGACAACAGCCTCAAGAT
GAPDH-Reverse	GAGTCCTTCCACGATACC

3.2. Correlation of *RIBC2* expression with clinical features and molecular mechanisms

To further elucidate the relationship between *RIBC2* and clinicopathological features, differential expression analyses were conducted using TCGA, GTEx, and TCGA+GTEx datasets. Results showed that *RIBC2* expression was significantly positively correlated with breast cancer T stage, and high *RIBC2* expression was associated with advanced pathological stage and reduced OS in the M0 subgroup (Figure 3A-C). Immunohistochemistry images confirmed that RIBC2 protein expression was markedly higher in tumor tissues than in normal breast tissue (Figure 3D). Scatter plot analysis further demonstrated a strong positive correlation between *RIBC2* expression and T stage, suggesting that elevated *RIBC2* expression may contribute to enhanced local invasion (Figure 3E). Survival curves (Figure 3F) revealed that patients with high *RIBC2* expression exhibited significantly poorer OS. GSVA-based pathway analysis suggested that *RIBC2* may promote tumor microenvironment remodeling and invasive capacity by regulating specific pathways. In addition, subtype-specific GSVA score comparisons (Figure 3G and H) revealed significant heterogeneity among different breast cancer subtypes (Basal, HER2, Luminal A, Luminal B, and Normal-like), with Basal and HER2 subtypes exhibiting higher pathway activities. These findings suggest a subtype-specific role of *RIBC2* in breast cancer, providing a potential molecular basis for precision therapy.

3.3. Correlation between *RIBC2* expression and clinicopathological parameters

Analysis of *RIBC2* expression and T stage demonstrated a significant positive correlation (Figure 4A). High *RIBC2* expression was also significantly associated with advanced pathological stage and poorer OS in the M0 subgroup (Figure 4B-E). GSVA score analysis indicated that RIBC2-associated pathway activities, particularly those related to extracellular matrix degradation and invasion, progressively increased with advancing tumor stage (I–IV) (Figure 4F and G), supporting the clinical associations at the molecular level. Multivariate Cox proportional hazards regression analysis further confirmed that high *RIBC2* expression was an independent prognostic factor for poor OS in breast cancer, even after adjusting for age, tumor size, lymph node metastasis, TNM stage, hormone receptor status, HER2 status, *BRCA1/2* mutation status,

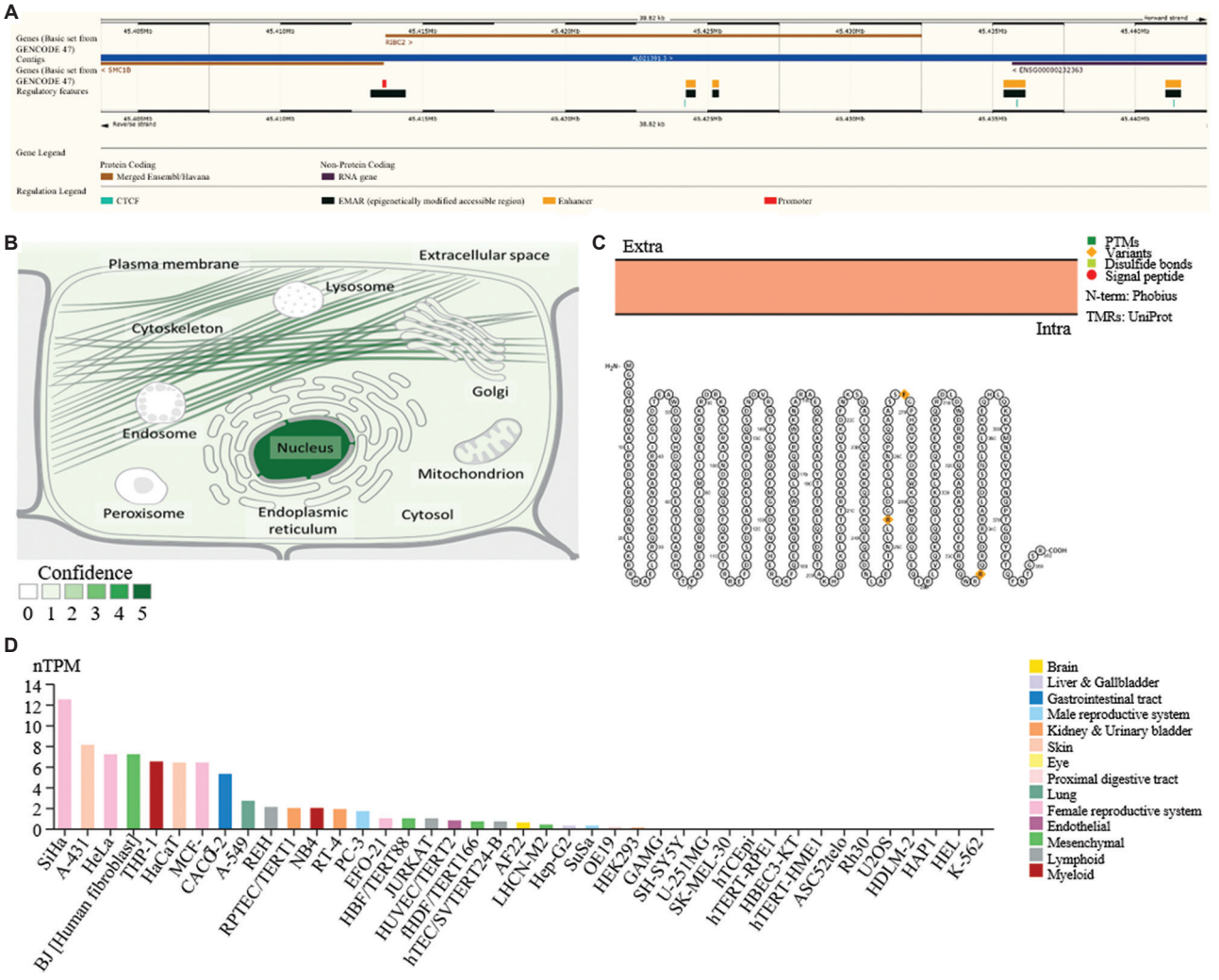


Figure 2. Genomic and cellular characteristics of the *RIBC2* gene. (A) Chromosomal localization of *RIBC2*, mapped to chromosome 12q24.31. (B) Subcellular localization of the RIBC2-encoded protein, showing dual distribution in the cytoplasm and nucleus. (C) Topological structure of the RIBC2 protein, highlighting the presence of SH3 domains. (D) Tissue-specific expression pattern of *RIBC2* across various human organs, based on proteomic profiling.

Abbreviations: nTPM: Normalized transcripts per million; PTMs: Post-translational modifications; RIBC2: RIB43A domain with coiled-coils 2.

and expression of established markers (HR = 1.32, 95% CI: 1.15–1.52, $p<0.001$). ROC curve analysis showed that the model incorporating traditional clinicopathological factors and established markers yielded an AUC of 0.762, while inclusion of *RIBC2* slightly improved the AUC to 0.764 (Figure 4H and I). These findings indicate that *RIBC2* contributes incremental prognostic value beyond conventional clinicopathological parameters and established markers.

3.4. *RIBC2*-associated protein interactions and functional enrichment analysis

Prediction results from the STRING database suggest that RIBC2 potentially interacts with multiple proteins. RIBC2 likely regulates cytoskeleton reorganization and cell migration through the Ras homolog family member A/Rho-associated coiled-coil kinase signaling pathway, with potential interactions to proteins, such as cilia- and flagella-associated protein 45 and tektin 2, implicating roles in ciliary assembly or cell motility. Its association with telomere repeats binding family proteins may also indirectly modulate the mitogen-activated protein kinase pathway, influencing cell proliferation or stress responses (Figure 5A and B). Based on the KEGG results, *RIBC2* plays a role in multiple biological processes and signaling pathways. The significantly enriched pathways include neuroactive ligand-receptor interaction, cytokine-cytokine receptor interaction, calcium signaling pathway,

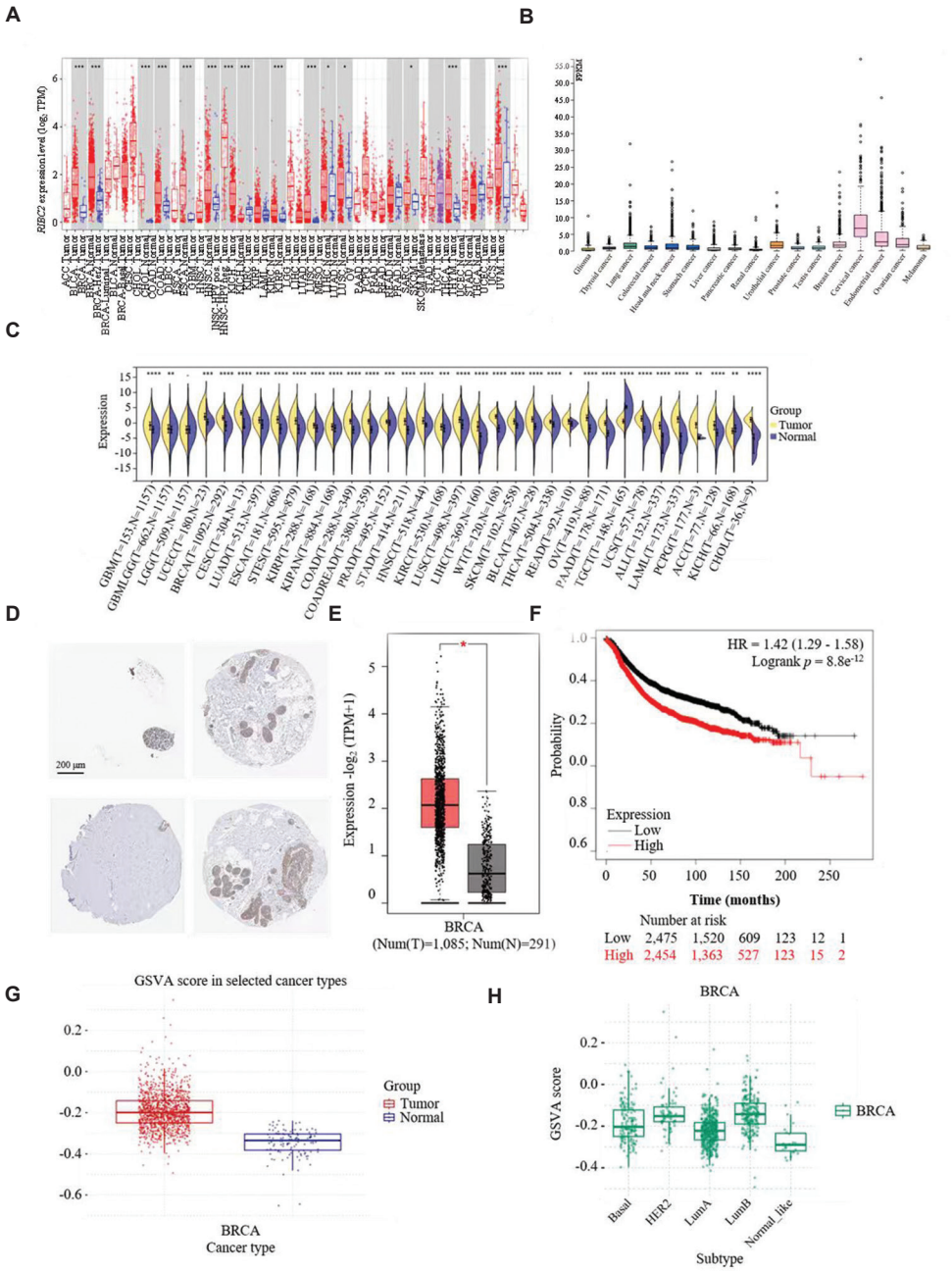


Figure 3. Disease relevance and expression profile of *RIBC2* in breast cancer. (A-C) *RIBC2* expression levels in breast cancer compared to normal tissues based on The Cancer Genome Atlas (TCGA), Genotype-Tissue Expression (GTEx), and combined TCGA + GTEx datasets. (D) Elevated expression of *RIBC2* in breast cancer samples. Scale bar: 200 μm, magnification: 50×. (E) High *RIBC2* expression is associated with poorer overall survival. (F) Predicted protein expression of RIBC2 in normal and cancerous breast tissues based on the Human Protein Atlas database. (G) Gene set variation analysis (GSVA) score comparison between tumor and normal samples. (H) GSVA score distribution across breast cancer subtypes, highlighting subtype-specific pathway activity.

Abbreviations: BRCA: Breast cancer; HER2: Human epidermal growth factor receptor 2; HR: Hazard ratio; Lum: Luminal; RIBC2: RIB43A domain with coiled-coils 2; TPM: Transcripts per million.

protein digestion and absorption, extracellular matrix-receptor interaction, and complement and coagulation cascades. These results suggest that *RIBC2* may regulate immune responses, signal transduction, and inflammatory responses, among other biological processes (Figure 5C). The GO analysis results indicate that *RIBC2* and its

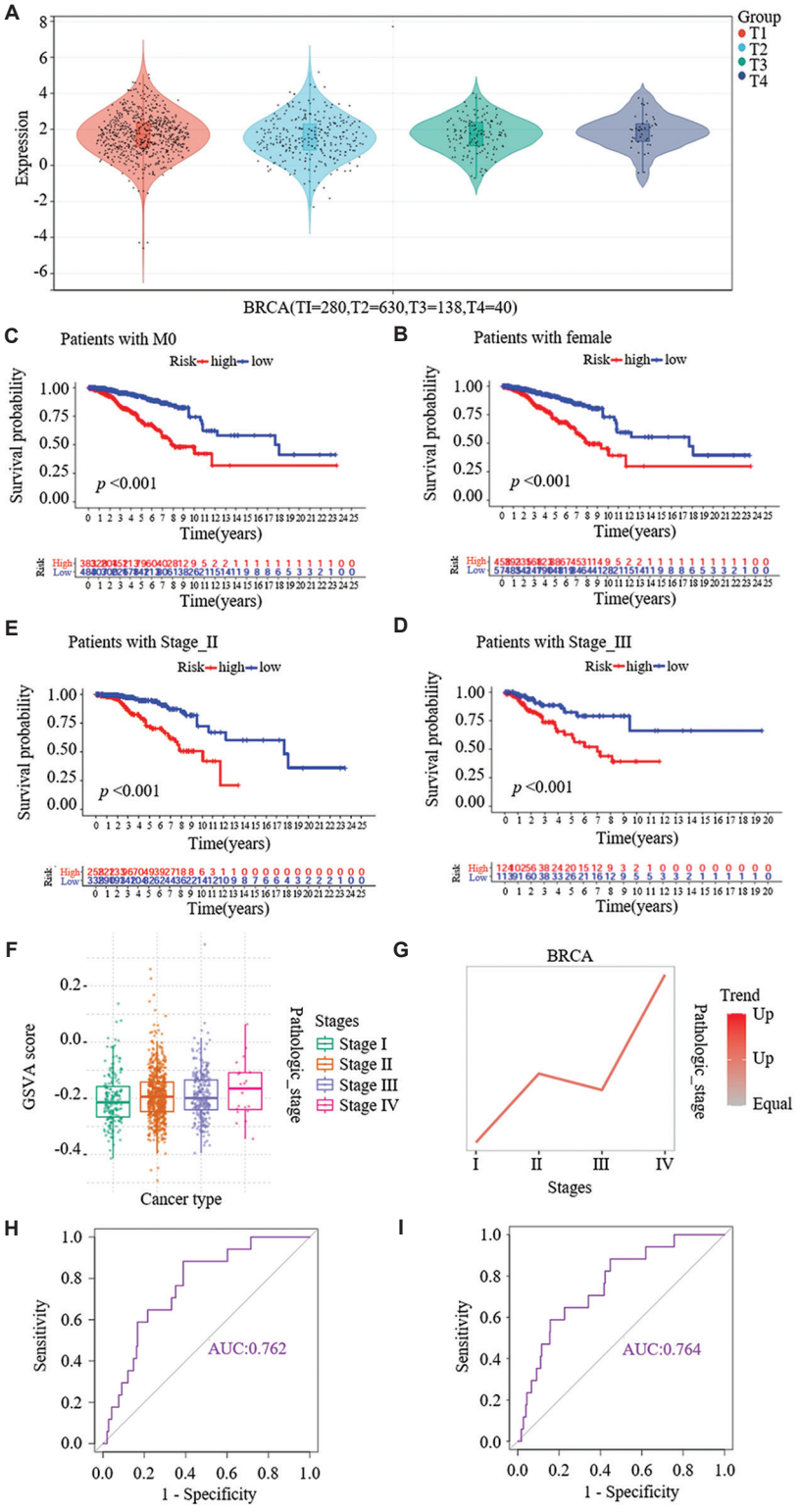


Figure 4. Correlation between *RIBC2* expression and clinicopathological parameters in breast cancer. (A) Association between *RIBC2* expression and tumor T stage. (B-E) Higher *RIBC2* expression is associated with poorer prognosis in patients with advanced pathological stage, M0 subgroup, stage III, and stage II breast cancer. (F) GSVA scores across cancer stages I–IV. (G) Trend analysis of GSVA scores across progressive cancer stages. (H) ROC curve of the prognostic model without *RIBC2* in breast cancer. (I) ROC curve of the prognostic model with *RIBC2* in breast cancer.
Abbreviations: AUC: Area under the curve; BRCA: Breast cancer; GSVA: Gene set variation analysis; ROC: Receiver operating characteristic.

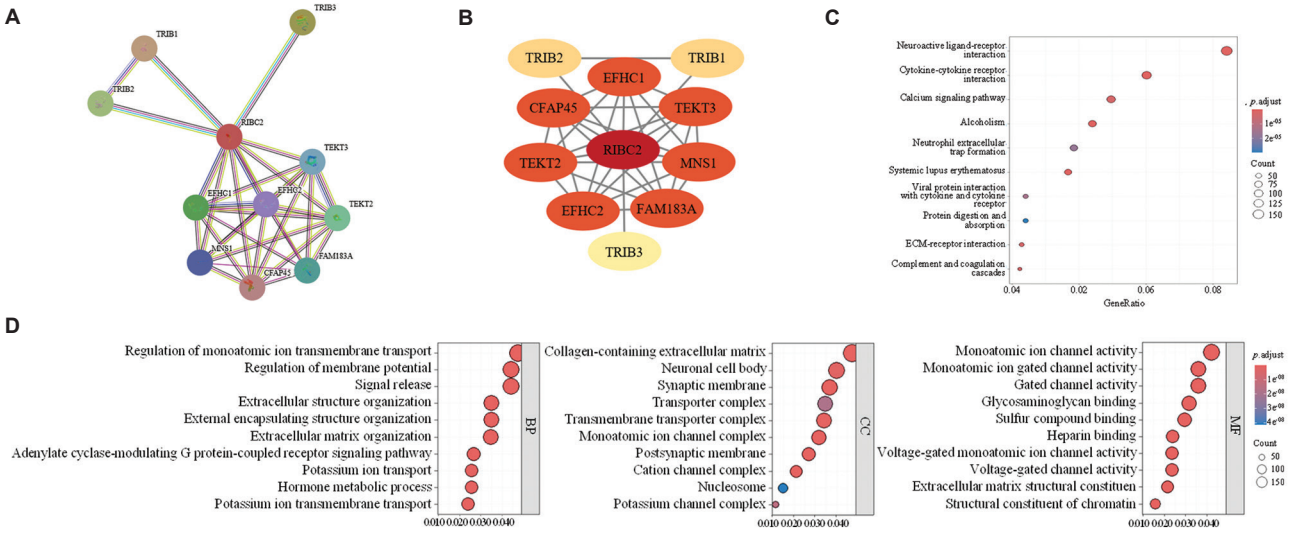


Figure 5. RIBC2-associated protein interactions and functional enrichment analyses. (A and B) Predicted protein-protein interaction network for RIBC2 based on STRING database analysis. (C) KEGG enrichment of the top 100 genes positively correlated with *RIBC2*. (D) GO enrichment (biological process, cellular component, molecular function) of *RIBC2*-associated genes.

Abbreviations: GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; RIBC2: RIB43A domain with coiled-coils 2; STRING: Search Tool for the Retrieval of Interacting Genes/Proteins.

interacting proteins may play a central role in neural conduction, tissue homeostasis, and hormone response by regulating ion channel activity (particularly potassium channels) and the dynamics of collagen-containing extracellular matrix organization. These mechanisms are implicated in epigenetic regulation and hormone signaling pathways in breast cancer (Figure 5D). These findings provide essential theoretical support for further investigation into the specific functional mechanisms of *RIBC2* and its interacting proteins in breast cancer.

3.5. Gene set enrichment analysis (GSEA) of *RIBC2* expression

This study utilized the GSEA to uncover the core biological pathways regulated by *RIBC2*. The enrichment results were divided into two categories: Highly significantly enriched pathways (p.adjust < 10^{-5}) and moderately significantly enriched pathways ($10^{-5} < p$.adjust $< 10^{-3}$). Systemic lupus erythematosus, neutrophil extracellular trap formation, and alcoholism pathways showed extremely significant enrichment (p.adjust = 8.2×10^{-9}), suggesting that *RIBC2* may be involved in the progression of complex diseases through immune-inflammation regulation or metabolic reprogramming (Figure 6A-C). For example, the formation of neutrophil extracellular traps is closely related to autoimmune diseases, and the enrichment of alcoholism may be associated with the metabolic stress response mediated by *RIBC2*. Cell cycle (p.adjust = 1.057×10^{-8}) enrichment indicates that *RIBC2* may directly regulate tumor cell proliferation or DNA repair, which is closely related to the occurrence and development of cancer (Figure 6D). Viral carcinogenesis (p.adjust = 6.33×10^{-7}) and metabolic pathways (p.adjust = 1.832×10^{-6}) results suggest that *RIBC2* may be involved in virus-induced tumorigenesis or energy metabolism remodeling, especially in virus-related cancers, such as liver cancer (Figure 6E and F). The enrichment of two metabolism-related pathways, the adenosine monophosphate-activated protein kinase signaling pathway (p.adjust = 9.746×10^{-6}) and the peroxisome proliferator-activated receptor signaling pathway (p.adjust = 1.883×10^{-5}), further supports the role of *RIBC2* in energy homeostasis regulation, which may involve physiological processes, such as lipid metabolism and insulin sensitivity (Figure 6G and H). These results indicate that the cell cycle pathway has the highest enrichment degree, while the p.adjust of the peroxisome proliferator-activated receptor signaling pathway is relatively high. These findings suggest that *RIBC2* may be a multifunctional oncogene, and its targeted intervention may be a potential strategy to inhibit breast cancer progression and enhance the sensitivity of chemotherapy.

3.6. Association between *RIBC2* expression and tumor microenvironment

We analyzed the correlation between *RIBC2* expression and immune cell infiltration in breast cancer. The results showed that the level of *RIBC2* expression was negatively correlated with the level of immune cell infiltration. The higher the expression of *RIBC2*, the lower the level of immune cell

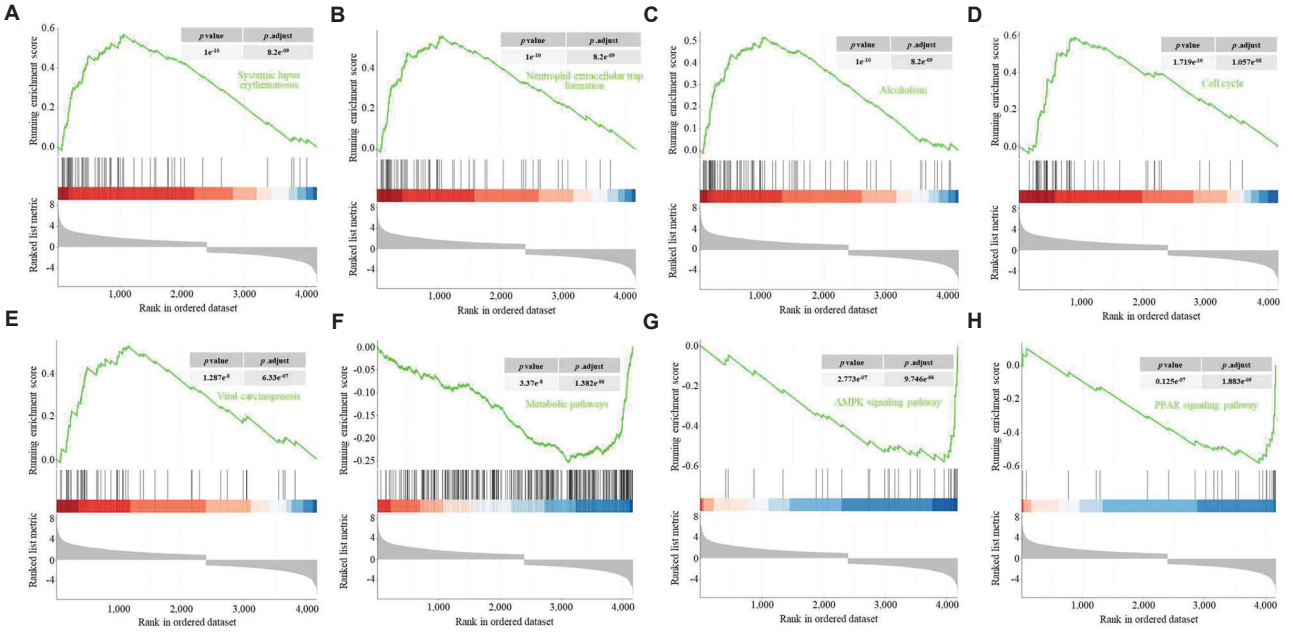


Figure 6. Gene set enrichment analysis of *RIBC2* in various biological processes. (A) Systemic lupus erythematosus. (B) Neutrophil extracellular trap formation. (C) Alcoholism. (D) Cell cycle. (E) Viral Carcinogenesis. (F) Metabolic pathways. (G) Adenosine monophosphate-activated protein kinase signaling pathway. (H) Peroxisome proliferator-activated receptor signaling pathway.

infiltration (Figure 7A). With the exception of a positive correlation with the infiltration level of neutrophils, *RIBC2* expression showed negative correlations with tumor purity, B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, and dendritic cells, several of which were statistically significant (Figure 7B). In addition, in general type breast cancer, the trend of the relationship between *RIBC2* expression level and the infiltration level of immune cells was similar to that of breast cancer, both showed negative correlation, but the specific values and significance might be different, suggesting that *RIBC2* might play a regulatory role in the tumor microenvironment of breast cancer. Its high expression may also inhibit immune cell infiltration (Figure 7C and D). These results suggest that *RIBC2* may be involved in the immune escape mechanism of breast cancer by regulating the infiltration of immune-suppressing cells, providing new targets and strategies for future breast cancer treatment. Immunoinformatics analysis revealed selective correlations (Figure 7E and F). LAG3 exhibited a significant positive correlation with *RIBC2* ($\rho = 0.10$, $p<0.001$), and HLA-C was also significant ($\rho = 0.08$, $p=0.045$). Other checkpoints, including IDO1 ($\rho = 0.04$, $p=0.936$), PD-L1 ($\rho = 0.04$, $p=0.921$), TIGIT ($\rho = 0.04$, $p=0.457$), and CTLA4 ($\rho = 0.03$, $p=0.748$), showed negligible correlation coefficients ($\rho < 0.05$) and non-significant values ($p>0.05$). The significant association with LAG3 (a marker of T cell exhaustion) suggests that *RIBC2* may promote adaptive immune suppression, while the HLA-C correlation hints at subtle alterations in antigen presentation. Notably, the lack of significant correlations with IDO1, PD-L1, or CTLA4 indicates that RIBC2's immunosuppressive role is not broadly coupled to canonical checkpoint pathways but operates through more selective mechanisms.

3.7. Correlation between *RIBC2* expression and drug sensitivity

Drug sensitivity analysis based on the CTRP database data revealed that high *RIBC2* expression was positively correlated with increased sensitivity to compounds, such as sotrastaurin D, SR8278, GSK-J4, and PD 153035 (Figure 8A). Molecular docking simulations demonstrated strong binding affinities between the RIBC2 protein and these compounds, with binding energies of −6.481 kcal/mol (sotrastaurin D), −6.239 kcal/mol (GSK-J4), −5.114 kcal/mol (PD153035), and 85.361 kcal/mol (SR8278), respectively (Figure 8B-D). The drug sensitivity experiments indicate the potential of targeting RIBC2 in breast cancer treatment through specific compounds with strong binding affinities, offering a promising therapeutic approach.

3.8. Analysis of the association between *RIBC2* methylation and gene expression

The methylation level of *RIBC2* was found to be significantly negatively correlated with its expression, suggesting that hypermethylation in the promoter or regulatory regions

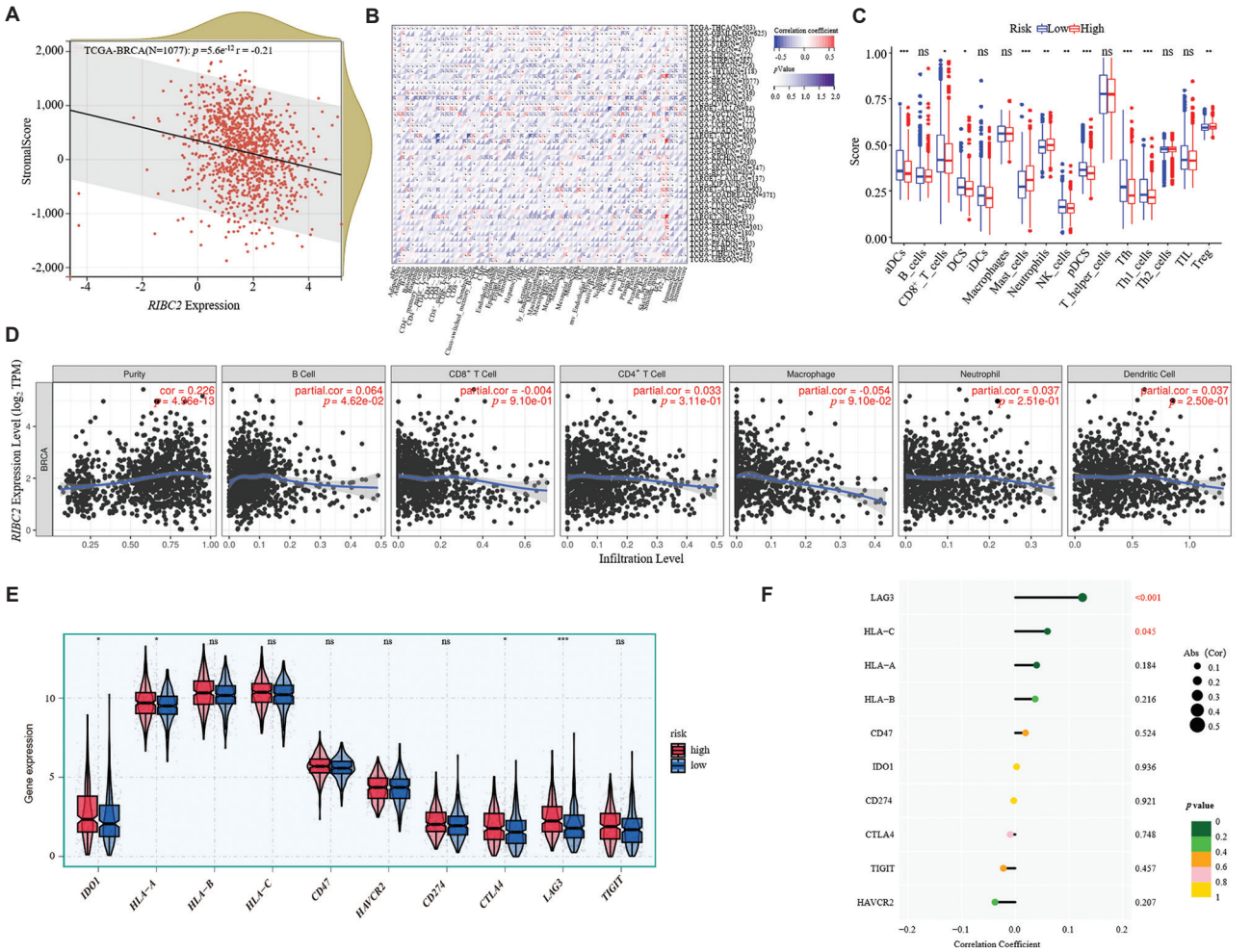


Figure 7. Association of *RIBC2* expression with immune infiltration in breast cancer. (A) Immune cell infiltration analysis of *RIBC2* expression using CIBERSORT. (B) Immune cell profiling based on the xCell algorithm. (C) Bar plot comparing immune infiltration scores between high- and low-*RIBC2* expression groups. (D) Correlation between *RIBC2* expression and seven immune cell markers based on Spearman analysis. (E) Differential expression of immune checkpoints by *RIBC2* risk groups. (F) Spearman correlation of *RIBC2* with immune checkpoints.
Abbreviations: CD: Cluster of differentiation; CTLA4: Cytotoxic T-lymphocyte associated protein 4; HAVCR2 (TIM-3): T-cell immunoglobulin and mucin domain 3; HLA: Human leukocyte antigen; IDO1: Indoleamine 2,3-dioxygenase-1; LAG3: Lymphocyte-activation gene 3; PD-L1: Programmed death-ligand 1; TIGIT: T cell immunoreceptor with Ig and ITIM domains.

may suppress gene expression through epigenetic silencing mechanisms (Figure 9A). Epigenetic analyses indicate that methylation modifications in the CpG islands of the *RIBC2* promoter may inhibit its transcriptional activity by recruiting methyl-CpG-binding proteins or altering chromatin structure, thereby preventing the binding of transcription factors, such as specificity protein 1 or activator protein 1. During tumorigenesis, hypomethylation of *RIBC2* may relieve these inhibitory mechanisms, resulting in a significant upregulation of its mRNA and protein levels. This pattern of epigenetic regulation is closely associated with the malignant phenotype of breast cancer.[25] However, given the potential role of *RIBC2* in promoting cell proliferation and migration, its expression may drive tumor progression by activating canonical oncogenic pathways, such as PI3K/protein kinase B (Akt) and Wnt/β-catenin signaling. In the PI3K/Akt pathway, *RIBC2* overexpression induced by promoter hypomethylation may facilitate direct interaction with PI3K regulatory subunits, for example, *p85*, enhancing membrane localization of the PI3K complex, thereby promoting phosphatidylinositol (3,4,5)-trisphosphate production and subsequent Akt activation.[26] *RIBC2* may also suppress *PTEN* expression through epigenetic mechanisms, such as the recruitment of histone deacetylases, further relieving the negative regulation of PI3K signaling. In the Wnt/β-catenin pathway, overexpression of *RIBC2* may inhibit glycogen synthase kinase-3 beta activity by promoting

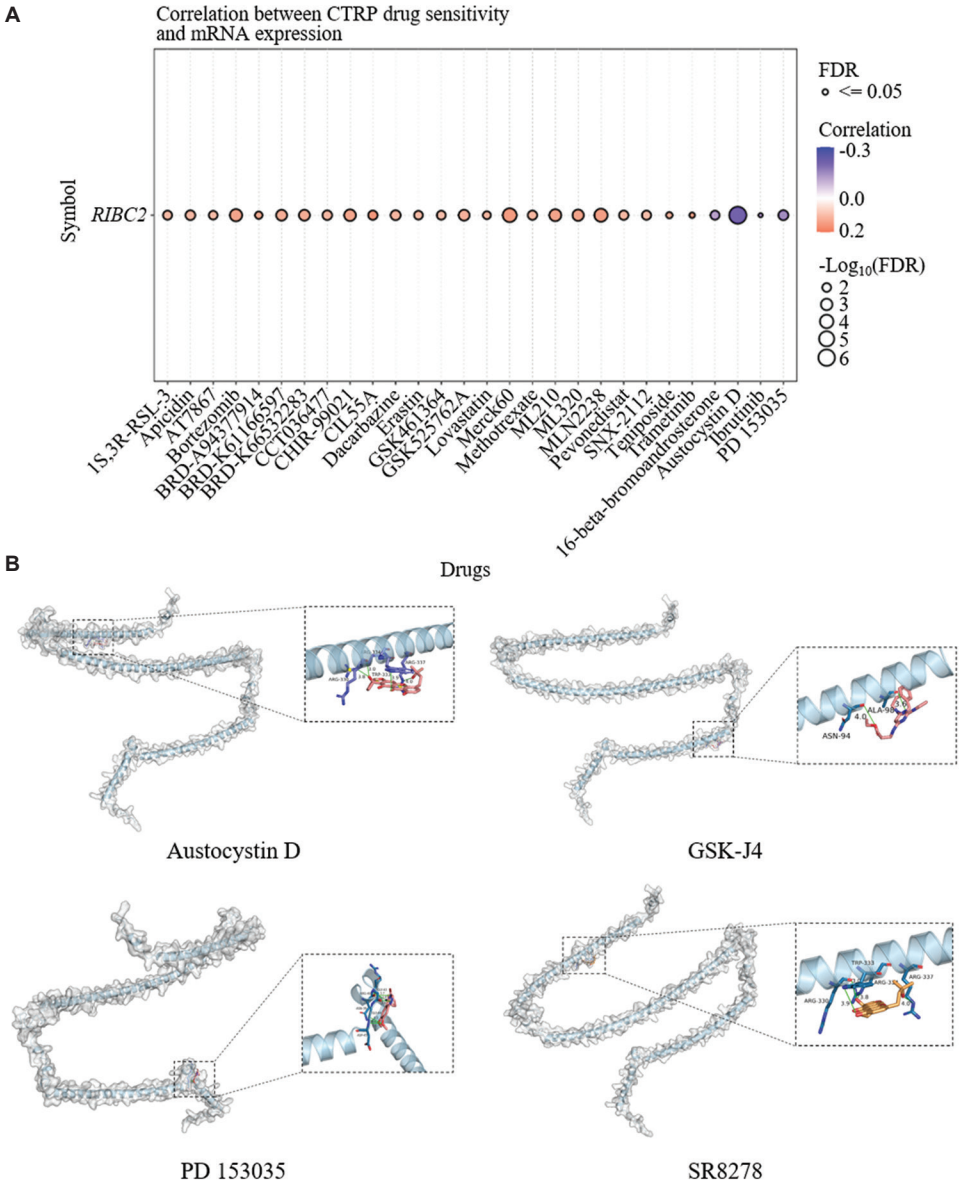


Figure 8. Association between *RIBC2* expression and drug sensitivity. (A) Correlation between *RIBC2* expression and half maximal inhibitory concentration values of selected compounds from the Cancer Therapeutics Response Portal database. (B-E) Molecular docking models showing predicted binding interactions between RIBC2 and four compounds with the highest binding affinities.
Abbreviations: FDR: False discovery rate; RIBC2: RIB43A domain with coiled-coils 2.

Akt-mediated phosphorylation at Ser9, which reduces β-catenin ubiquitination and degradation. This leads to nuclear accumulation of β-catenin and enhances Wnt signaling. Moreover, *RIBC2* may promote epithelial-mesenchymal transition (EMT) and metastatic potential by upregulating Wnt target genes, such as *SNAI1* and *TWIST1*. The statistically significant association between *RIBC2* methylation and gene expression highlights its potential as a prognostic biomarker (Figure 9B and C). If hypomethylation leads to *RIBC2* overexpression, it may promote tumor invasion and metastasis, aligning with previous findings that higher *RIBC2* expression correlates with shorter OS in patients.

3.9. Quantitative polymerase chain reaction validation of *RIBC2* expression in breast cancer cell lines

To experimentally validate the bioinformatically identified *RIBC2* overexpression patterns, we quantified its mRNA levels across breast cancer subtypes using qPCR. As shown

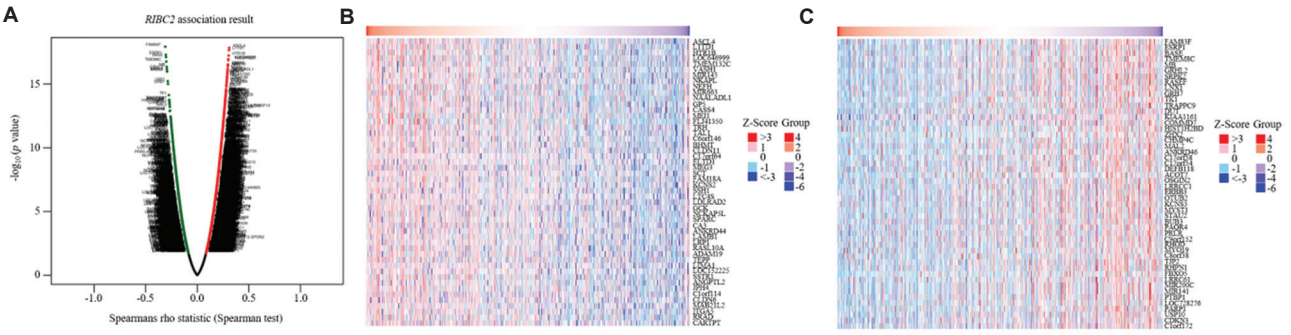


Figure 9. Analysis of *RIBC2* gene methylation levels. (A) Spearman's rho statistic based on the Spearman correlation test. (B) Significantly negatively correlated genes. (C) Significantly correlated genes.

in Figure 10, *RIBC2* expression was significantly elevated in both breast cancer cell lines compared to normal mammary epithelial cells Hs578bst (p<0.01). TNBCcells (MDA-MB-231) exhibited the most pronounced *RIBC2* upregulation (4.23 ± 0.17; 4.2-fold increase versus Hs578bst, p<0.01). Luminal-type cells (MCF-7) showed intermediate overexpression (2.85 ± 0.12; 2.8-fold increase versus Hs578bst, p<0.01). Inter-subtype differential expression was statistically significant, with MDA-MB-231 expressing *RIBC2* at 1.5-fold higher levels than MCF-7 (p<0.01). This *in vitro* validation corresponds precisely with TCGA transcriptomic data demonstrating preferential *RIBC2* overexpression in basal-like (TNBC) subtypes (Figure 10), reinforcing its subtype-specific association with aggressive tumor phenotypes.

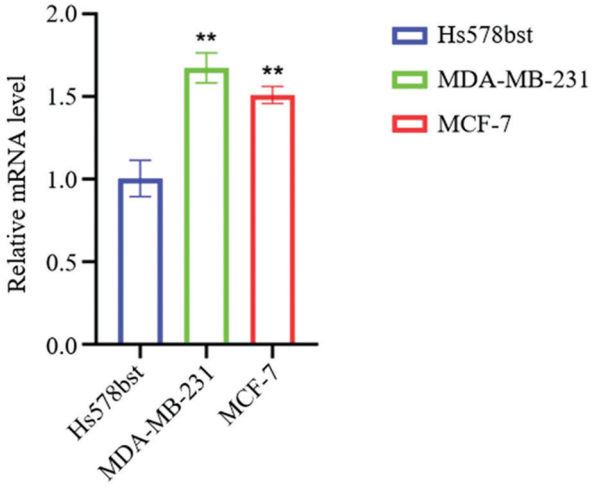


Figure 10. Triple-negative breast cancer-specific *RIBC2* overexpression revealed by quantitative polymerase chain reaction. Statistical significance at **p<0.01.

4. Discussion

In this study, we systematically characterized the expression profile and clinical significance of *RIBC2* in breast cancer through multi-omics integration and comprehensive bioinformatics analyses. We found that *RIBC2* is significantly overexpressed in breast cancer tissues, with its expression positively associated with tumor T stage, pathological stage, and reduced OS. Notably, the correlation between *RIBC2* and tumor staging was more substantial than that of traditional biomarkers, suggesting a potentially unique role for *RIBC2* in early tumor progression.

At the molecular level, functional enrichment analysis demonstrated that *RIBC2* is significantly associated with several pivotal oncogenic pathways in breast cancer, notably the PI3K/Akt signaling cascade, EMT, and the Wnt/β-catenin pathway. These pathways play a critical role in regulating cancer cell physiology and contribute to tumor progression. The PI3K/Akt pathway is a central regulator of cell proliferation, survival, metabolism, and angiogenesis. Dysregulations, such as activating mutations in *PIK3CA* or loss of *PTEN* function are among the most common genomic alterations in breast cancer.[27] These alterations lead to constitutive activation of the pathway, promoting tumor growth and resistance to therapy. Notably, the PI3K/Akt pathway is frequently activated in TNBC. EMT is a biological process wherein epithelial cells acquire mesenchymal characteristics, enhancing their migratory and invasive capabilities. In breast cancer, EMT is associated with increased metastatic potential, resistance to apoptosis, and the acquisition of stem cell-like properties. The induction of EMT involves activating transcription factors, such as snail, slug, and twist 1, which are regulated by signaling pathways, including transforming growth factor beta, Wnt, and Notch.[28,29] The Wnt/β-catenin pathway is crucial for maintaining cellular homeostasis and regulating cell fate decisions. In breast cancer, aberrant activation of this pathway contributes to tumor initiation, progression, and metastasis.[28,30] Specifically, Wnt/β-catenin signaling has been implicated in the maintenance of cancer stem cells and the promotion of EMT, thereby facilitating

tumor heterogeneity and therapeutic resistance. The association of *RIBC2* with these key pathways suggests it may function as a central modulator of breast cancer pathophysiology. By influencing the PI3K/Akt pathway, *RIBC2* could enhance tumor cell survival and proliferation. Its involvement in EMT may promote metastatic dissemination, while modulation of Wnt/β-catenin signaling could contribute to maintaining cancer stem cell populations and resistance to conventional therapies. Therefore, *RIBC2* emerges as a potential integrative node in the complex signaling networks driving breast cancer progression, representing a promising target for therapeutic intervention. In addition, GSEA confirmed the activation of the Wnt/β-catenin pathway in *RIBC2* high-expression samples, suggesting that *RIBC2* may drive tumor progression through the coordinated activation of multiple oncogenic signaling pathways. However, these mechanistic associations are primarily derived from bioinformatic predictions and warrant further validation through *in vitro* experiments.

In the tumor immune microenvironment of breast cancer, high *RIBC2* expression was associated with a distinctive immunosuppressive landscape, suggesting that *RIBC2* may play an active role in modulating immune cell infiltration and function. Our analysis revealed a significant enrichment of regulatory T cells (Tregs), identified by elevated *FOXP3* expression, in *RIBC2*-high tumors. Tregs are critical mediators of immune tolerance, suppressing the activation and cytotoxic functions of effector T cells and natural killer cells. Their accumulation within the tumor microenvironment correlates with poor prognosis, impaired anti-tumor immunity, and resistance to immunotherapeutic strategies in breast cancer. High *RIBC2* expression was also associated with increased infiltration of M2-polarized tumor-associated macrophages (M2-TAMs) and reduced cytotoxic $CD8^+$ T cell presence. M2-TAMs, marked by *CD163* expression, promote tumor progression through the secretion of immunosuppressive cytokines, angiogenic factors, and matrix remodeling enzymes.[31] Their presence is associated with enhanced tumor cell invasion, metastasis, and resistance to therapy. The convergence of these immune alterations suggests that *RIBC2* may function as a central orchestrator of immunosuppression, promoting a tumor microenvironment that favors immune evasion and progression. By facilitating the expansion of $FOXP3^+$ Tregs and $CD163^+$ M2-TAMs while simultaneously depleting $CD8^+$ effector cells, *RIBC2* may reprogram the tumor microenvironment toward an immune-tolerant state.[32] Notably, these immune alterations appear independent of PD-L1 expression, implying that *RIBC2* may act through non-canonical immune regulatory pathways distinct from traditional checkpoint axes.[33,34] Targeting *RIBC2* could therefore represent a novel therapeutic strategy to reinvigorate anti-tumor immunity and sensitize breast cancers to immunotherapies. Drug sensitivity analysis revealed that high expression of *RIBC2* in breast cancer cells exhibited increased sensitivity to GSK-J4 and other chemotherapeutic agents, potentially due to RIBC2-mediated activation of PI3K/Akt survival signaling. Molecular docking simulations further identified sotrastaurin D as a potential RIBC2 inhibitor, with a predicted binding site near the SH3 domain, suggesting a possible mechanism involving disruption of protein-protein interactions. Nonetheless, these findings are correlative, and the therapeutic efficacy of these compounds must be validated through *in vitro* drug sensitivity assays. Furthermore, qPCR analysis also experimentally validated the expression profile of *RIBC2* across breast cancer subtypes. Crucially, *RIBC2* expression in MDA-MB-231 significantly exceeded other subtypes ($p<0.01$), closely aligning with TCGA-derived basal-like subtype signatures and clinical aggressiveness phenotypes. Given the poor prognosis of TNBC, which is attributed to limited therapeutic targets and frequent metastasis, *RIBC2* is significantly overexpressed in its subtypes, suggesting that it may be a potential weak link for treatment. Mechanistically, functional enrichment supports RIBC2's coordination of PI3K/Akt and Wnt/β-catenin pathway activation, driving stromal remodeling and immune evasion in TNBC.

This research is primarily based on computational and bioinformatic analyses, relying on publicly available datasets and *in silico* predictions to infer the potential roles and molecular mechanisms of *RIBC2* in TNBC. While such approaches enable the rapid identification of gene expression patterns, putative signaling interactions, and regulatory networks, they are inherently constrained by the quality, completeness, and heterogeneity of the source datasets. Algorithmic predictions, such as those derived from co-expression analyses, network inference, or pathway enrichment, do not inherently confirm causal relationships. Building upon our findings, further experimental validation is essential to substantiate the functional role of *RIBC2* in breast cancer. *In vitro* studies involving *RIBC2* knockdown or overexpression in breast cancer cell lines, followed by proliferation, migration, apoptosis, and pathway activation assays, would confirm its oncogenic functions. In addition, bisulfite sequencing and demethylation experiments could elucidate the epigenetic regulation of *RIBC2*. Co-culture models combining tumor cells and immune cells could clarify *RIBC2*'s role in immune modulation, particularly regarding Treg and M2 macrophage recruitment, which should be further

validated in syngeneic or humanized mouse models. Progress now depends on engineering formulations that achieve consistent intratumoral distribution and on-target knockdown *in vivo*, while meeting practical constraints of manufacturability, stability, and safety for clinical translation.[35] In TNBC cell lines and *in vivo* models, evaluation should quantify effects on cilia-associated signaling and tumor immunocompetence, including interferon-pathway activation, antigen processing and presentation, and PD-L1 regulation.

5. Conclusion

In summary, this study highlights the importance of *RIBC2* as a novel biomarker and potential therapeutic target in breast cancer. Its multifaceted role in tumor progression and immune modulation offers new avenues for improving breast cancer diagnosis and treatment. Further investigation of *RIBC2* will deepen our understanding of breast cancer biology and may facilitate the development of more effective therapeutic strategies.

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Conflict of interest

The authors declare they have no competing interests.

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Writing – review & editing: All authors

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

All data analyzed have been presented in the paper.

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