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ORIGINAL RESEARCH

SIRT3 Expression Predicts Overall Survival and Neoadjuvant Chemosensitivity in Triple-Negative Breast Cancer

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Background: The Sirtuin (SIRT) family consists of seven evolutionary conserved NAD-dependent deacetylases that play important roles in various cancers, including breast cancer (BC). SIRTs expression has been reported to have prognostic value in BC, but these studies used limited sample size and yielded inconsistent conclusions. This study evaluated the association of SIRT3 and other SIRT family members with survival and neoadjuvant chemotherapy outcomes.

Methods: BC patients' data was obtained from the TCGA-BRCA, METABRIC and GEO databases, comprising 4336 samples. SIRTs expression and overall survival (OS) were analyzed using Kaplan-Meier analysis and Cox proportional hazards regression. SIRT3 expression levels were compared between pathologic complete response (pCR) and non-pCR groups after neoadjuvant chemotherapy in triple-negative breast cancer (TNBC). Protein-protein interaction networks were constructed using the STRING database. Gene set enrichment analysis (GSEA) was performed to explore potential functions of SIRT3.

Results: Through systematic analysis of SIRTs expression and OS of BC using three independent cohorts: TCGA-BRCA, METABRIC and GSE16446, we found that high SIRT3 expression was significantly associated with worse OS in TNBC in the TCGA-BRCA cohort, which was validated in the METABRIC and GSE16446 cohorts. SIRT3 expression was correlated with BC subtypes and American Joint Committee on Cancer (AJCC) T stage, but not with age-at-diagnosis, race, or tumor stage. Moreover, TNBC patients with higher SIRT3 expression had lower pCR rates after neoadjuvant chemotherapy ($p = 6.40e-03$) and SIRT3 expression was significantly lower in the pCR group than in the non-pCR group in TNBC ($p = 4.2e-03$). GSEA indicated that SIRT3 was involved in drug-related pathways such as oxidative phosphorylation, metabolism of xenobiotics by cytochrome P450, and drug metabolism.

Conclusion: Our study suggests that SIRT3 is a potential biomarker for both OS and neoadjuvant chemosensitivity in TNBC. It may also assist in selecting suitable candidates and treatment options for TNBC patients.

Keywords: SIRT3, biomarker, breast cancer, neoadjuvant chemotherapy, prognosis

Introduction

Breast cancer (BC) is the most prevalent cancer in women worldwide.[1] It is a complex and heterogeneous disease that requires tailored treatment strategies depending on the stage and subtype.[2] However, some patients, especially those with triple-negative breast cancer (TNBC), still have a poor prognosis despite the advances in understanding the disease mechanisms and discovering novel biomarkers.[3] TNBC was the subset of breast cancers lacking expression of molecular markers estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). More than 50% of TNBC patients relapsed in the first 3–5 years, and the median overall survival is significantly shorter than other subtypes.[4] Therefore, finding biomarkers to guide patient stratification and treatment selection is crucial for improving BC outcomes.

Sirtuins (SIRTs) are an evolutionary conserved family of NAD-dependent deacetylases (SIRT1-7) that regulate various cellular processes in cancer, such as oxidative stress, angiogenesis, the Warburg effect, genome stability, autophagy.[5–12] SIRTs expression has been implicated in BC progression and prognosis. We previously reported that SIRT7 inhibits metastasis[13] and predicts prognosis in BC.[14] High expression of SIRT3 predicts overall survival (OS) and shorter disease-free survival (DFS) in BC.[15] A meta-analysis suggests SIRT1 and SIRT6 are associated with poor OS

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in BC.[16] Besides, SIRT7 has been shown to regulate chemotherapy response in hepatocellular carcinoma.[17] Thus, SIRTs are potential biomarkers and therapeutic targets for BC treatment.

However, the clinical significance of SIRTs in BC has not been systematically explored. For example, though high SIRT3 expression was reported to predict worse OS in BC,[15] it was analyzed in a limited sample size and did not stratify samples into different BC subtypes. Considering the high heterogeneity of BC, some important information may be missed. Besides, the results were not consistent in different studies, for example high SIRT3 expression was also reported to be linked to better OS in BC.[18] Thus, we aimed to evaluate the association of SIRTs expression with survival and neoadjuvant chemosensitivity (NAC) in BC using multiple large public data cohorts from the TCGA-BRCA, METABRIC, and GEO databases. Our results indicate that SIRT3 may be a promising predictive biomarker of long-term overall survival and response to neoadjuvant chemotherapy in TNBC.

Materials and Methods

Datasets and Flow Design

The datasets and the overall design are demonstrated in Figure 1.

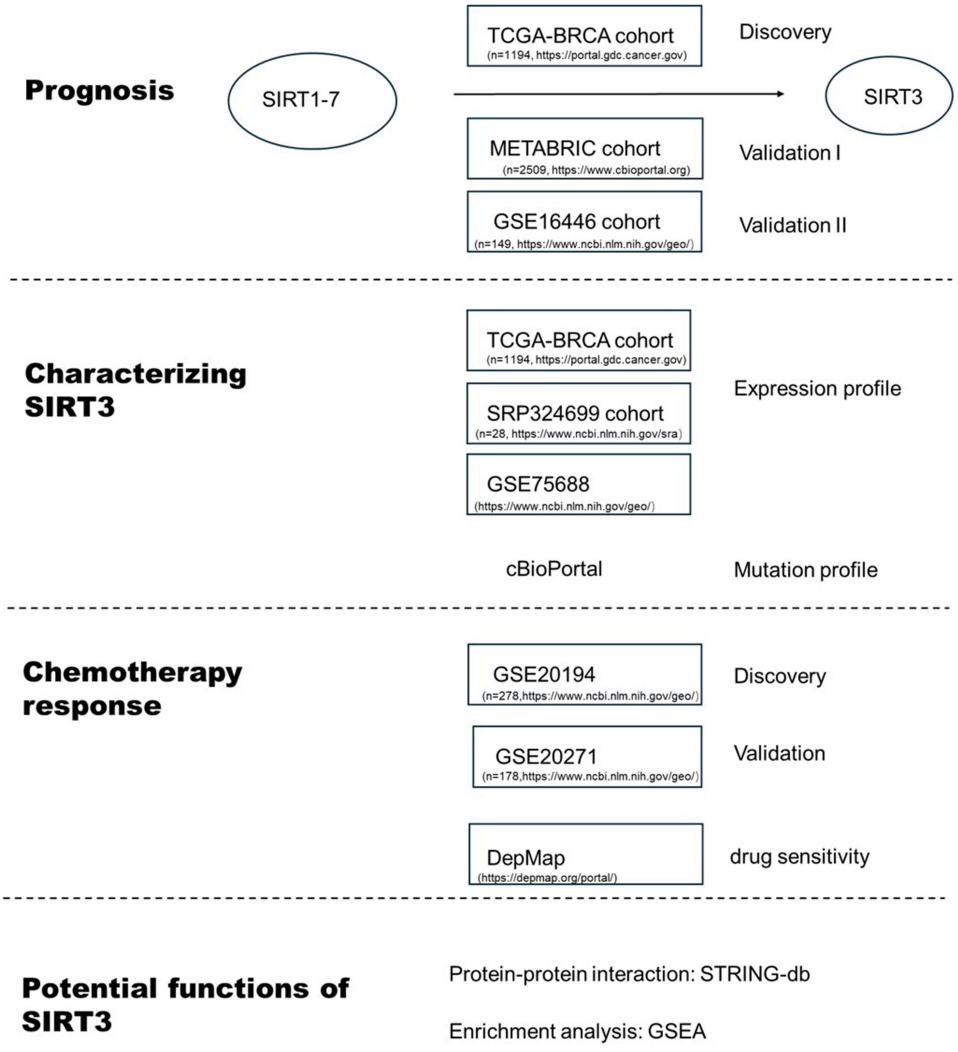

Figure 1 The datasets used in this study and the overall design.

Different Expression Analysis

We aimed to identify the differentially expressed SIRTs genes between normal and tumor samples in BC. We downloaded the TCGA-BRCA cohort[19] (n=1194) from the Genomic Data Commons portal (https://portal.gdc.cancer.gov) and an RNA-seq dataset cohort (n=28, SRP324699) from the NCBI Sequence Read Archive (SRA) database (https://www.ncbi.nlm.nih.gov/sra). The TCGA-BRCA consisted of 1075 tumors and 112 adjacent normal samples, while SRP324699 had 14 normal and 14 tumors. We normalized the gene read counts using quantile normalization and then log-transformed. We performed differential expression analysis using two-tailed t-tests and set p=0.05 as a significant cut-off. For more details on the data processing the RNA-seq data, refer to our previous publication.[20]

Single-Cell RNA-Seq Dataset Analysis

We aimed to explore the expression levels of SIRT3 among different cell types in BC. The single-cell RNA-seq dataset (GSE75688[21]) was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). The cells were annotated into five cell types: tumor, T, B, stromal, and myeloid cells.[21] A one-way ANOVA test was used to compare the expression levels of SIRT3 among cell types.

Survival Analysis

We aimed to evaluate the prognostic value of SIRTs genes in BC. Three cohorts (TCGA-BRCA, METABRIC,[22] GSE16446[23]) with overall survival information were used. We obtained the METABRIC cohort (n=2509) from the cBioPortal database (https://www.cbioportal.org)[24] and GSE165446 cohort (n=149) from the GEO database. We used the Python package lifelines (https://lifelines.readthedocs.io/en/latest/, version 0.26.4)[25] to perform Kaplan–Meier curve, Log rank test, univariate and multivariate Cox proportional hazards regression analyses. We considered genes with $p < 0.05$ to be significant.

Association Between Gene Expression and Treatment Response

We aimed to assess the association of SIRT3 expression with NAC response in BC. Two cohorts (GSE20194,[26] GSE20271[27]) with NAC responses were downloaded from the GEO database. GSE20194 had 278 samples and GSE20271 had 178 samples. The median expression of SIRT3 was used to split samples into high and low SIRT3 groups in each cohort. Single and multiple variable logistic regression analysis were used to test the association of SIRT3 expression and clinical features with NAC response. A logistic regression predictive model was built and 10-fold cross-validation was used to estimate the area under the curve (AUC).

To test the drug sensitivity in vitro, we obtained the AUC of drugs from the Dependency Map (DepMap) portal (https://depmap.org/portal/). The SIRT3 expression in cell lines was obtained from The Cancer Cell Line Encyclopedia (CCLE) database.[28] Eleven estrogen receptor (ER)- and human epidermal growth factor receptor 2 (HER2)-negative cell lines (HCC1599, AU565, HCC1419, UACC-812, HDQ-P1, JIMT-1, MDA-MB-175-VII, MDA-MB-231, CAMA-1, BT-483, and CAL-51) were included.

Protein Interaction, Gene Ontology, and Kyoto Encyclopedia of Genes and Genomes Enrichment Analysis

We aimed to explore the potential functions and pathways of SIRT3 in BC. The STRING database (https://string-db.org/)[29] was used to identify SIRT3-related protein-protein interactions. Gene set enrichment analyses (GSEA) were performed using GSEApy (https://github.com/zqfang/GSEApy)[30] with gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) gene sets. Three GO terms biological processes (BPs), cellular components (CCs), and molecular functions (MFs) were included. We used a false discovery rate (FDR) of 0.25 as the significance criterion.

Other

We used Python software (version 3.10) for all analyses. A one-way analysis of variance (ANOVA) and Fisher's exact test, chi-square test was used for group comparison as appropriate. Boxplots were used to show median and interquartile values. The significance level was set at 0.05 unless otherwise specified.

Results

SIRT3 from SIRT Family Associated with Prognosis in Triple-Negative Breast Cancer

We aimed to investigate the clinical relevance of SIRTs expression in BC by performing survival analysis in different BC subtypes ER+/PR+, HER2+, TNBC using two large independent cohorts, TCGA-BRCA and METABRIC. For each SIRT gene, samples were divided into low and high expression groups based on the tercile cut-off that yielded the lowest p-value in the Log rank test. We set p=0.05 as a significant cut-off.

Although most SIRTs were somewhat associated with BC OS in the TCGA-BRCA cohort, they were not validated in the METABRIC except SIRT3. The Kaplan–Meier curves for SIRT1-7 were showed in Supplemental Figures 1 and 2 for TCGA-BRCA and METABRIC cohorts, respectively. In the TCGA-BRCA cohort, high expression of SIRT3 was associated with better OS in ER+/PR+ samples (p = 2.25e-02) but with worse OS in TNBC samples (p = 3.94e-02) (Figure 2A). In the METABRIC cohort, high expression of SIRT3 was associated with worse OS in TNBC samples (p = 5.38e-03), but not in ER+/PR+ samples (p >0.05) (Figure 2B).To further confirm this result, we used a third dataset GSE16446, which also showed that high expression of SIRT3 was associated with worse OS in TNBC samples (p = 2.74e-02, Figure 2C). Thus, we focused on SIRT3 in the following analysis.

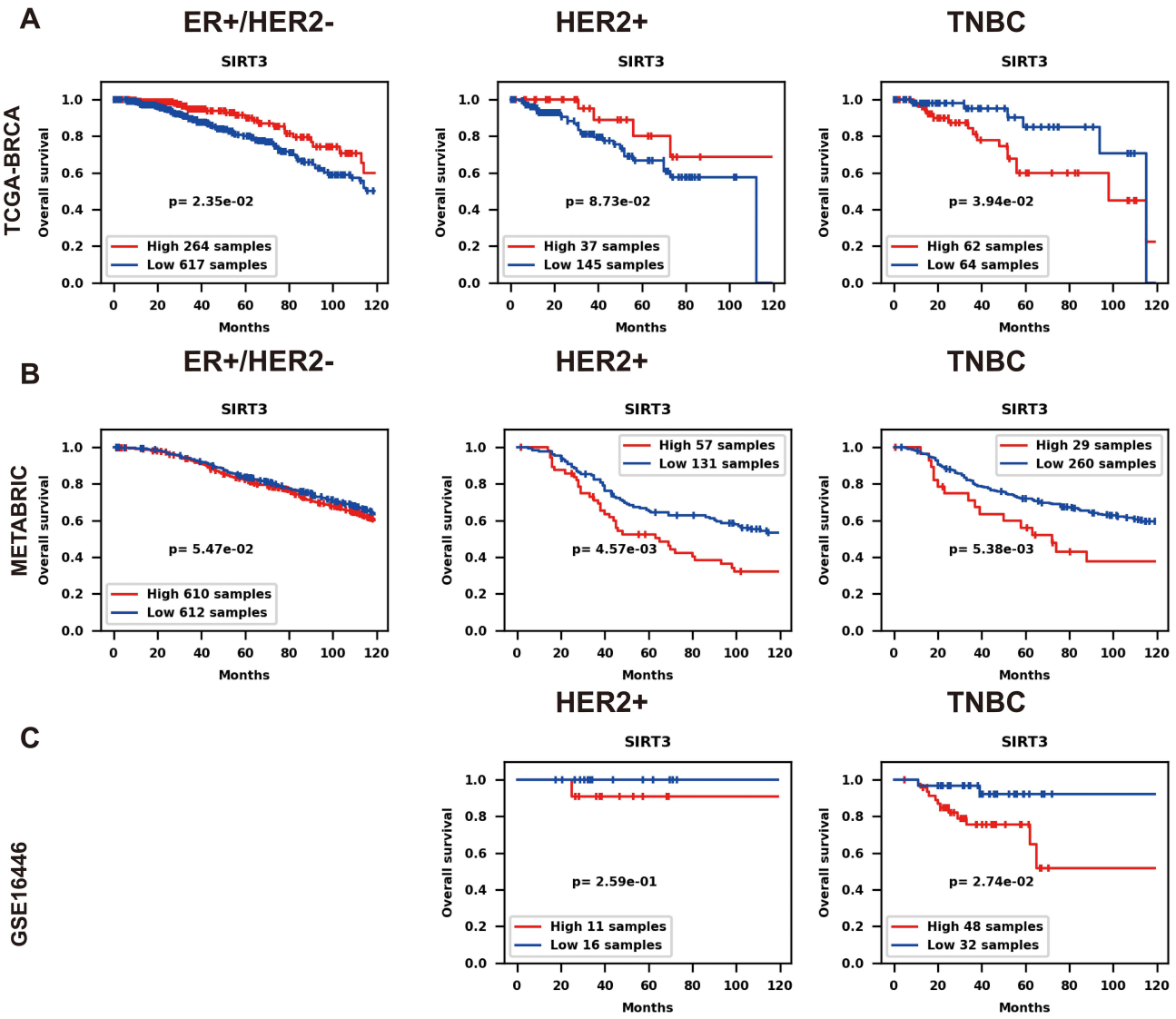

Figure 2 SIRT3 and overall survival of patients in ER+/HER2-, HER2+, TNBC from the TCGA-BRCA, METABRIC and GSE16446 cohorts. The Kaplan-Meier curves for the TCGA-BRCA (**A**), METABRIC (**B**) and GSE16446 (**C**) cohorts.

Characterizing SIRT3 Expression and Mutation Profile in Breast Cancer

We then analyzed SIRT3 expression profile in BC. SIRT3 was significantly downregulated in BC compared to the normal samples in both the TCGA-BRCA cohort (p=1.37e-05, two-tailed *t*-test) and the SRP324699 cohort (p=4.59e-06, two-tailed *t*-test). Figure 3A shows the boxplot of the expression of SIRT3 in these two cohorts. We also compared the expression of SIRT3 in different clinical stages (Figure 3B), molecular subtypes (Figure 3C), and age-at-diagnosis groups (Figure 3D). SIRT3 was significantly lower in basal than other molecular subtypes (p<0.05, one-way ANOVA), and slightly higher in patients with age≥50 group than in patients with age<50 group (p=3.5e-03, two-tail *t*-test). We did not find significant differences in SIRT3 expression among clinical stages (p>0.05, one-way ANOVA).

Based on the single-cell RNA-seq dataset (GSE75688) that annotated cells into five types (tumor cells, T cells, B cells, stromal cells, and myeloid cells), we found that SIRT3 was mainly expressed in the tumor cell type (p=9e-04, one-way ANOVA), indicating the high cell type specificity of SIRT3 (Figure 3E). We further explored the DNA mutations using cBioPortal and found only 2 non-driver mutations in SIRT3 of 996 samples from the Breast Invasive Carcinoma cohort (Figure 3F), suggesting that gene mutations may not be the cause of tumor genesis.

Correlation Between SIRT3 Expression and Clinicopathological Characteristics

Next, we analyzed the association between the expression level of SIRT3 and clinicopathological characteristics. Samples were divided into SIRT3-high and SIRT3-low groups based on the median expression of SIRT3 in the TCGA-BRCA dataset. We compared the distribution of clinical features such as age-at-diagnosis groups (≥50 vs <50), race (black, Asian, white), tumor stages (I, II, III, IV), American Joint Committee on Cancer (AJCC) T stage, AJCC N stage, AJCC M stage, and subtypes (ER+/HER2-, HER2+, TNBC) between the two groups. No significant difference was

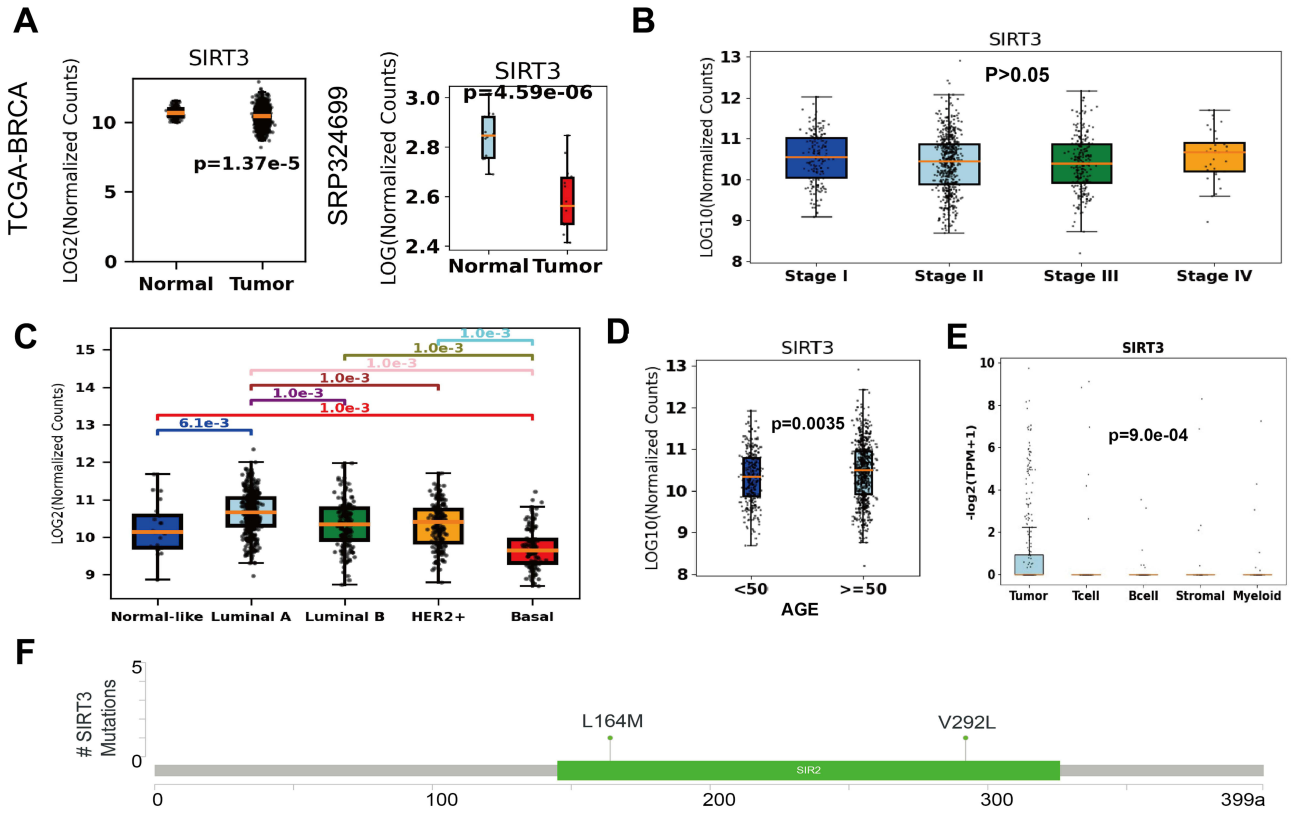

Figure 3 Expression and mutations profile of SIRT3 in breast cancer. (**A**) Boxplot of SIRT3 expression in TCGA-BRCA and SRP324699 cohorts. (**B**) Boxplot of SIRT3 expression in clinical stage I, II, III, and IV. No significant difference was found among stages. (**C**) Boxplot of SIRT3 expression in molecular subtype normal-like, Luminal A, Luminal B, HER2 and Basal. (**D**) Boxplot of SIRT3 expression in age groups, <50 and ≥50 years. (**E**) Expression of SIRT3 according to tumor, T, B, stromal, and myeloid cells in the single-cell RNA-seq dataset (GSE75688). (**F**) The lollipop plot of mutations of SIRT3 in the TCGA-BRCA cohort from cBioPortal.

found in clinical features such as age-at-diagnosis groups, race, tumor stages, AJCC N stage, or AJCC M stage between the SIRT3-low and -high groups (Table 1). However, we found a significant difference in AJCC T stage between the two groups (p=0.0036), with more T2 cases in the SIRT3-low group than in the SIRT3-high group. The most pronounced difference was found in subtypes between the SIRT3-low and -high groups (p=1.12e-17). The SIRT3-high group had more ER+/HER2- cases than the SIRT3-low group (74.3% vs 46.6%), while the SIRT3-low group had more HER2+ and TNBC cases than the SIRT3-high group (27.5% vs 19.8% and 25.8% vs 5.8%, respectively). These results suggest that SIRT3 expression is correlated with clinicopathological characteristics in BC, especially with molecular subtypes.

Table 1 Associations Between SIRT3 Expression Level and Baseline Clinical Characteristics in Breast Cancer

Clinical Characteristics	SIRT3-Low (n=597)	SIRT3-High (n=597)	p*
Age at diagnosis (years)			0.074
≥50	415 (69.5%)	443 (74.2%)	
<50	182 (30.5%)	153 (25.6%)	
Median	56.0	60.0	
IQR	48–66	49–69	
RACE			0.14
Black	93 (15.6%)	94 (15.7%)	
Asia	39 (6.5%)	23 (3.9%)	
White	413 (69.2%)	436 (73.0%)	
Other	52 (8.7%)	44 (7.3%)	
AJCC T-category			**0.0036**
T1	136 (22.8%)	171 (28.6%)	
T2	376 (63.0%)	316 (52.9%)	
T3	60 (10.0%)	85 (14.2%)	
T4	24 (4.0%)	23 (3.9%)	
AJCC N-category			0.73
N-	279 (46.7%)	272 (45.6%)	
N+	318 (53.3%)	325 (54.4%)	
AJCC M-category			0.67
M-	525 (97.9%)	478 (97.4%)	
M+	11 (2.1%)	13 (2.6%)	
Stage at diagnosis			0.19
I	87 (14.9%)	112 (19.1%)	
II	346 (59.3%)	335 (57.1%)	
III	141 (24.2%)	127 (21.6%)	
IV	9 (1.5%)	13 (2.2%)	

(*Continued*)

Table 1 (Continued).

Clinical Characteristics	SIRT3-Low (n=597)	SIRT3-High (n=597)	p*
Subtypes			**1.12e-17**
ER+/HER2-	188 (46.6%)	281 (74.3%)	
HER2+	111 (27.5%)	75 (19.8%)	
TNBC	104 (25.8%)	22 (5.8%)	

Notes: *p: the P-value was calculated using the chi-square test or Fisher's exact test. The significant p-values were marked as bold.
Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple negative breast cancer; AJCC: American Joint Committee on Cancer.

SIRT3 Expression Independently Associated with Worse Overall Survival in Triple-Negative Breast Cancer

To investigate the independence of the association between SIRT3 expression and OS in TNBC, we performed univariate and multivariate Cox proportional hazards analyses using the TNBC samples from TCGA-BRCA dataset. There is no significant association between SIRT3 expression and any of these clinicopathological characteristics (Supplemental Table 1). In addition, the univariant analysis found that that only SIRT3 expression and tumor stage were significantly associated with OS in TNBC (Table 2). The multivariate analysis confirmed that SIRT3 expression was an independent prognostic factor for OS in TNBC after adjusting for tumor stages (Table 2). High expression of SIRT3 was significantly associated with worse OS in TNBC (hazard ratio = 17.98, 95% confidence interval = 1.66–194.09, p = 0.02). These results suggest that SIRT3 expression is independently associated with worse OS in TNBC.

SIRT3 Expression Level Associated with Pathological Complete Response to Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer

Considering SIRT3 involved mitochondrial metabolism and oxidative stress, we hypothesized that they are associated with NAC response thus affecting the patient's prognosis in BC. We compared SIRT3 expression level between pathological complete response (pCR) and residual disease (RD) groups using two GEO datasets (GSE20194 and GSE20271). We also checked the cellular response to drugs in TNBC cell lines using data from the DEPMAP portal.

We found that TNBC patients who achieved pCR after NAC had significantly lower SIRT3 expression than those who did not, but this was not the case for ER+/HER2- and HER2+ subtypes (Figure 4A; p =0.0042, 0.59, 0.82, respectively). In addition, TNBCs with low SIRT3 expression before NAC were significantly more likely to achieve pCR than those with high SIRT3 expression, but not in ER+/HER2- and HER2+ groups (Figure 4B, p = 0.0064, 1, 0.79, respectively). To

Table 2 Univariate and Multivariate Cox Proportional Hazards Analyses for Overall Survival in Triple-Negative Breast Cancer

Characteristics	Univariate Analysis		Multivariate Analysis	
	Hazard Ratio (95% CI)	**p**	**Hazard Ratio (95% CI)**	**p**
Age at diagnosis (<50 vs ≥50 years)	0.63 (0.19–2.09)	0.45	–	–
AJCC TUMOR PATHOLOGIC T stage (T1, T2 vs T3, T4)	3.36 (0.87–12.97)	0.08	–	–
AJCC NODES PATHOLOGIC PN (N0 vs N1)	1.23 (0.29–5.15)	0.78	–	–
Stages (Stage I, II vs stage III, IV)	5.06 (1.53–16.75)	**0.01**	6.16 (1.72–21.99)	**0.01**
SIRT3	15.30 (1.35–173.06)	**0.03**	17.98 (1.66–194.09)	**0.02**

Notes: P values were calculated using univariate and multivariate Cox proportional hazards analyses, significant p values were marked as bold.
Abbreviation: AJCC, American Joint Committee on Cancer.

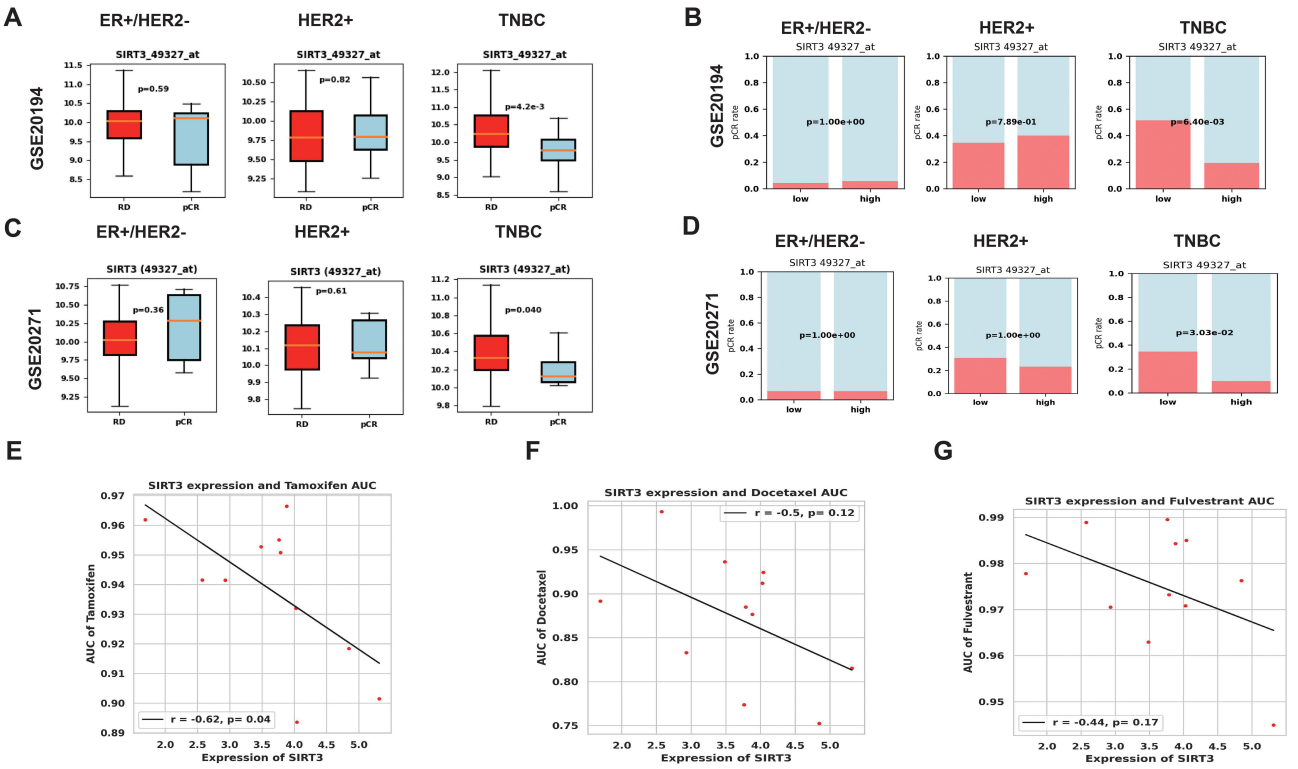

Figure 4 Association of SIRT3 expression with treatment response of human tumors and cell lines. (**A**) SIRT3 expression in pCR and RD groups in dataset GSE20194. (**B**) pCR rate in low SIRT3 and high SIRT3 groups in dataset GSE20194. (**C**) SIRT3 expression in pCR and RD groups in dataset GSE20271. (**D**) pCR rate in low SIRT3 and high SIRT3 group in dataset GSE20271. (**E**) Scatter plot of SIRT3 expression and AUC of drug sensitivity for tamoxifen in TNBC cell lines from the DEPMAP portal. The correlation coefficient and p-value are shown. (**F**) Scatter plot of SIRT3 expression and AUC of drug sensitivity for docetaxel in TNBC cell lines from the DEPMAP portal. The correlation coefficient and p-value are shown. (**G**) Scatter plot of SIRT3 expression and AUC of drug sensitivity for fulvestrant in TNBC cell lines from the DEPMAP portal. The correlation coefficient and p-value are shown. AUC: area under the curve.

validate these results, we used an independent dataset (GSE20271). We found that TNBC patients who achieved pCR after NAC still had significantly lower SIRT3 expression than those who did not, but not in ER+/HER2-, HER2+ groups (Figure 4C, p=0.04, 0.36, 0.61 respectively). In addition, TNBCs with low SIRT3 expressions before NAC still had significantly higher pCR rates than those with high SIRT3 expressions, but not in ER+/HER2- and HER2+ groups (Figure 4D, p=0.03, 1, 1, respectively).

Furthermore, we found that the level of SIRT3 expression was negatively correlated with the area under the curve (AUC) of drug sensitivity for tamoxifen (p=0.04, r=−0.62, Figure 4E), docetaxel (r=−0.5, Figure 4F), and fulvestrant (r=−0.4, Figure 4G) in TNBC cell lines from the DEPMAP portal, although the correlation with docetaxel and fulvestrant was not significant. This result suggests that high SIRT3 expression may contribute to drug resistance in TNBC cell lines.

In addition, we tested the independent effects of SIRT3 expression on NAC response in TNBCs. Univariate and multivariate logistic regression analysis were performed to assess the association between SIRT3 expression and pCR rate, adjusting for other clinicopathological variables such as T stage, N stage, Nuclear grade (BMN), and age. At the single variant level, we found that only SIRT3 expression was significantly associated with pCR rate (p=0.032) in the GSE20194 cohort, although stages T, N, and BMN were marginally significant (Table 3). When all variants were considered in the logistic model, BMN and SIRT3 expression were significantly associated with pCR rate (p=0.031 and 0.024 respectively). The odds ratio for SIRT3 expression was 0.59 (95% confidence interval: 0.37–0.93), indicating that low SIRT3 expression was associated with a higher likelihood of achieving pCR than high SIRT3 expression. We then constructed a predictive model based on logistic regression and used 10-fold cross-validation to calculate the area under the receiver operating characteristic curve (AUC) score. We found that SIRT3 expression alone had the highest predictive power for pCR rate among all parameters (AUC=0.65, Figure 5A). When combined with BMN and age, the AUC score increased to 0.72 in TNBC. Similarly, SIRT3 expression alone had an AUC score of 0.67 in GSE20271 cohort (Figure 5B).

Table 3 Univariate and Multivariate Cox Proportional Hazards Analyses for Pathological Complete Response in Triple-Negative Breast Cancer

Characteristics	Univariate Analysis		Multivariate Analysis	
	Hazard ratio (95% CI)	p	Hazard ratio (95% CI)	p
T stage	0.83 (0.69–1)	0.052	0.84 (0.45–1.58)	0.59
N stage	0.75 (0.55–1.04)	0.083	1.09 (0.56–2.13)	0.80
BMN	0.85 (0.71–1.02)	0.075	5.91 (1.18–29.65)	**0.031**
SIRT3	0.94 (0.89–0.99)	**0.032**	0.59 (0.37–0.93)	**0.024**

Abbreviation: BMN, Nuclear grade.

In conclusion, these analysis demonstrated that low SIRT3 expression was associated with higher pCR rate in TNBC patients receiving NAC treatment and may influence the cellular response to drugs in TNBC cell lines. Thus, it may be a useful independent biomarker for predicting NAC response in TNBC patients.

Potential Functions of SIRT3

To investigate the potential functions of SIRT3 in BC, we constructed a protein-protein interaction network using the STRING database (https://string-db.org/). We found that ten genes related to BC, including FOXO1, FOXO3, SOD2, NAMPT, GLUD1, ACSS1, ACSS2, NDUFA9, PPARGC1A and IDH2, can directly interact with SIRT3 (Figure 6A). These genes are involved in various biological processes such as oxidative stress response, mitochondrial metabolism, cell cycle regulation, and apoptosis, suggesting an important role of SIRT3 in breast cancer.

Then we calculated the genes correlated to SIRT3 in TNBC samples from the TCGA-BRCA cohort using Pearson's correlation coefficient. The top three correlated genes were ACBD4 (r=0.72), MOB2 (r=0.7), and LYRM9 (r=0.68) (Figure 6B). We performed gene set enrichment analysis (GSEA) on the ranked correlated genes using a false discovery rate (FDR) of 0.05 as a threshold. The enriched GO biological process terms were related to DNA replication and DNA repair. The enriched GO molecular function terms were related to DNA binding. The enriched GO cellular component terms were related to chromosomes (Figure 6C). GSEA of KEGG pathways showed that the negatively correlated genes were enriched in the Fanconi anemia pathway, DNA repair pathway, cell cycle, and mismatch repair (Figure 6D), while the positively correlated genes were enriched in the metabolism of xenobiotics by cytochrome P450, drug metabolism,

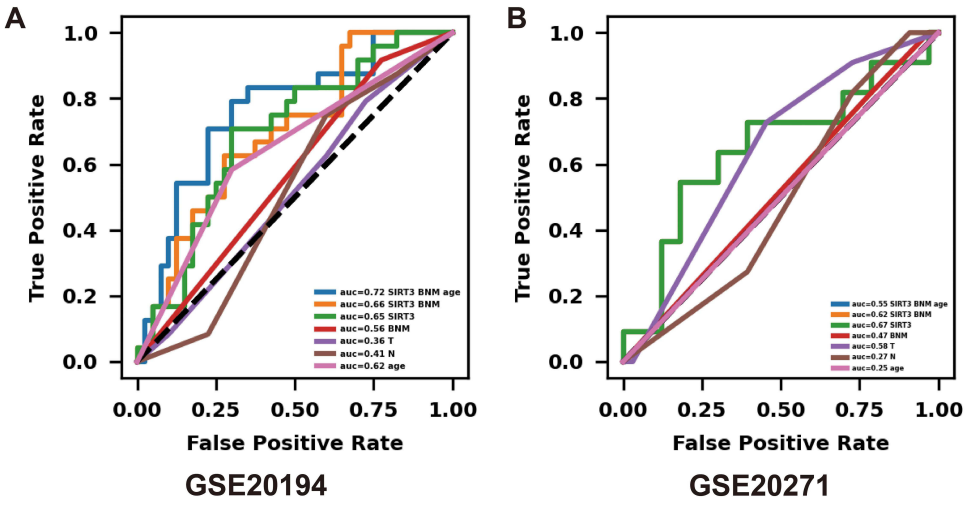

Figure 5 Receiver operating characteristic curves of different predictive models for pathological complete response. (**A**) Receiver operating characteristic curves for dataset GSE20194. (**B**) Receiver operating characteristic curves for dataset GSE20271.

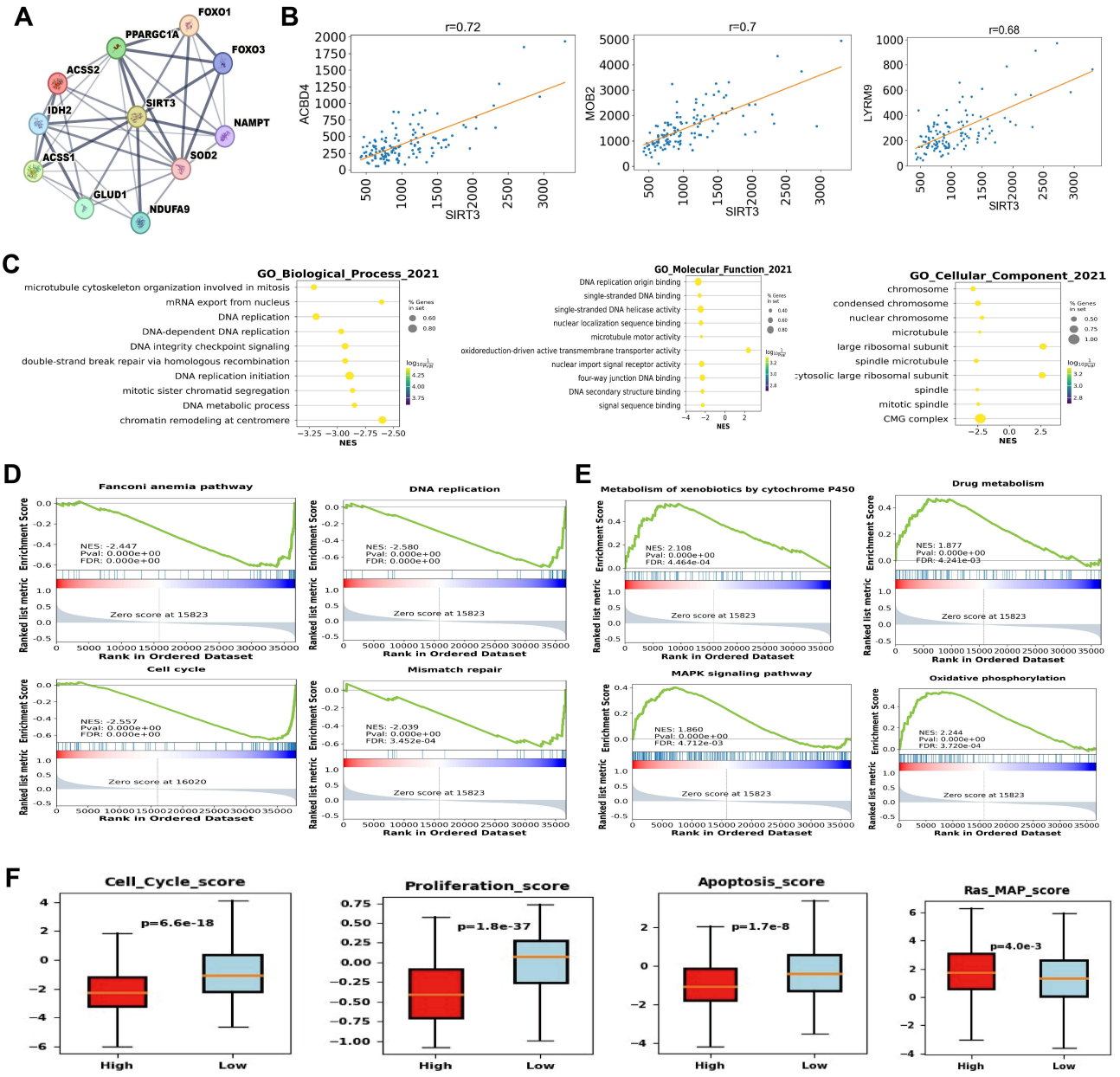

Figure 6 Pathway enrichment analysis of SIRT3. (**A**) Protein-protein interaction network from the STRING database. (**B**) Top three correlated genes with SIRT3 in TCGA-BRCA TNBC cohort using Pearson's correlation coefficient. (**C**) Dot plot of GO enriched terms using GSEA on ranked correlated genes. The dot size represents the gene ratio of each term. The color represents the normalized enrichment score (NES) of each term. (**D**) GSEA of KEGG pathways of negatively correlated genes as a threshold. The NES and FDR are shown for each pathway. (**E**) GSEA of KEGG pathways of positively correlated genes. The NES and FDR are shown for each pathway. (**F**) Boxplots of cell cycle score, proliferation score, apoptosis score, and Ras MAP score according to high or low SIRT3 expression level using the median expression value of SIRT3 as a cut-off value.

MAPK signaling pathway, oxidative phosphorylation (Figure 6E). These results indicate that SIRT3 may modulate DNA damage response and drug metabolic pathways in BC.

Using several score metrics derived from the TCGA-BRCA cohort based on gene expression profiles, we found that the samples with high SIRT3 expression had lower cell cycle scores ($p<0.001$), proliferation scores ($p<0.001$), and apoptosis scores ($p<0.001$), but higher Ras MAP scores ($p<0.001$) (Figure 6F), consistent with the KEGG enrichment analysis. These findings imply that SIRT3 may inhibit cell proliferation and apoptosis but promote Ras signaling in breast cancer. Interestingly, this is consistent with previous reports that SIRT3 overexpression represses proliferation[31] and apoptosis[32] in BC cells.

Discussion

In this study, we evaluated the prognostic value of SIRTs in three large cohorts and found that high SIRT3 expression was associated with worse OS in the TNBC subtype. Furthermore, we showed that low SIRT3 expression was related to a higher pCR rate in TNBC after NAC, but not in ER+/HER2- and HER2+ subtypes. GSEA analysis of the genes ranked by SIRT3 correlation suggested that SIRT3 might be involved in drug metabolism pathways.

SIRT3 is an NAD+ dependent mitochondrial deacetylase that regulates the unfolded protein response, modulates energy metabolism, mitochondrial biogenesis, and oxidative stress protection.[33,34] SIRT3 also affects Wnt/β-catenin,[35] Notch[36] and mTORC1[37] signaling pathway. It can act as an oncogene or a tumor suppressor in cancers.[38] SIRT3 deficiency stabilizes HIF1α, which promotes BC growth.[31] On the other hand, SIRT3 overexpression destabilizes the oncogene MYC.[39] SIRT3 is dysregulated in many cancers and may play a vital role in BC progression.[40] It is overexpressed in colorectal cancer,[41] gastric cancer,[42] and non-small-cell lung cancer[43] but underexpressed in kidney cancer[44] and prostate cancer.[35] In BC, we confirmed that SIRT3 was significantly downregulated using two independent datasets. In addition, our analysis suggests that SIRT3 expression is heterogeneous across different BC subtypes.

Previous studies reported that SIRT3 overexpression was linked to poor prognosis in colorectal cancer[41] and non-small cell lung cancer.[43] In BC, low SIRT3 expression was related to poor locoregional relapse-free survival (RFS) in BC.[18] However, He et al report that high SIRT3 expression predicted worse RFS and OS in BC.[15] Therefore, we performed survival analysis using two large independent cohorts: TCGA-BRCA and METABRIC. We only found that high SIRT3 expression was associated with worse OS in TNBC, which we also confirmed using another dataset. This result is different from the meta-analysis[16] which reported SIRT3 was not significantly associated with the prognosis of TNBC. In that study, there are only two TNBC datasets, one has 186 samples, and the other only has 48 samples. In addition, the expression of SIRT3 were measured using different methods, IHC/QT-PCR. In ER+/HER2- samples, we only found a positive association between SIRT3 expression and OS in the TCGA-BRCA cohort, not in the METABRIC cohort. In HER2+ samples, although the associations were significant in both datasets, they were not consistent. These differences might reflect the high heterogeneity within subtypes, and further stratification of these samples might reveal the underlying causes.

Since SIRT3 is involved in several pathways such as mTORC1 and oxidative stress, it may affect BC chemotherapy response. We then examined the relationship between SIRT3 expression level and pCR response rate in two independent NAC datasets. We found that the pCR group had significantly lower SIRT3 expression than the RD group and that the low SIRT3 group had a higher pCR rate than the high SIRT3 group. In the cell line models, we found that cell lines with low SIRT3 expression are more sensitive to tamoxifen, docetaxel and fulvestrant. We also built a prediction model based on logistic regression that showed the independent predictive value of SIRT3 for pCR in NAC treatment. The AUC derived from this model (AUC=0.65, 0.67 for SIRT3 alone) is somewhat comparable to a three-gene model that achieved AUC at 0.74.[45] Combining SIRT3 with other genes or clinical information might improve the prediction accuracy. Nevertheless, these results suggest that SIRT3 might be used as a marker for predicting NAC response in BC and that it may be involved in drug metabolism.

To explore the potential function of SIRT3, we constructed a protein interaction network from the STRING database. The network indicates that SIRT3 could interact directly with FOXO1, FOXO3, SOD2, NAMPT, GLUD1, ACSS1, ACSS2, NDUFA9, PPARGC1A, IDH2. These proteins have various roles in BC. For example, FOXO3-FOXM1 axis modulates drug resistance,[46] ACSS1 and ACSS2 enhance the survival of tamoxifen-treated cells,[47] FOXO1 and SOD2 support cancer stemness,[48,49] NAMPT promotes metastasis in TNBC,[50] GLUD1 expression is associated with better patient outcomes,[51] PPARGC1A regulates mitochondrial biogenesis and oxidative phosphorylation to facilitate metastasis,[52] and SIRT3 dimerizes IDH2 to modulate cancer metabolism and tumor growth.[53] ACBD4, MOB2, and LYRM9 were the genes most correlated with SIRT3 in TNBC. These genes have not been well studied in BC, but they have been implicated in other cancers. For instance, MOB2 inhibits cell migration and invasion in glioblastoma multiforme,[54] LYRM9 might affect imatinib resistance,[55] and ACBD4 might be regulated by TP53.[56] These interactions suggest that SIRT3 is part of a complex network that might modulate drug metabolism and affect NAC response and tumor progression in BC. Consistently, GSEA analysis revealed that SIRT3 positively correlated genes were enriched in

pathways related to the metabolism of xenobiotics by cytochrome P450, drug metabolism, MAPK signaling pathway, and oxidative phosphorylation, while SIRT3 negatively correlated genes were enriched in pathways related to DNA repair, cell cycle, and mismatch repair.

Although this study shows the potential of SIRT3 as a prognostic and NAC response marker in TNBC, there are some limitations. First, though we collected many samples from public databases, the analysis of the current study may be inconclusive. Second, this study was conducted by a bioinformatics approach alone. However, our results showed consistency across multiple cohorts, in vivo and in vitro experiments to elucidate the underlying mechanism as are needed. Third, as more data may be available in the future, we expect validation of the prognostic and chemosensitivity value of SIRT3 in TNBC in future.

Conclusion

By analyzing multiple datasets, we found that high SIRT3 expression was an independent predictor of worse OS in TNBC. We also found that SIRT3 expression was associated with the pathological complete response rate after NAC treatment. Our study suggests that SIRT3 might be a useful biomarker for both prognosis and chemosensitivity in TNBC, although further biological and clinical studies are needed to confirm its role in predicting prognosis and chemosensitivity.

Data Sharing Statement

All data were downloaded from public databases and followed the data access policies.

Ethics

All data were downloaded from public databases and followed the data access policies. This study was exempt from ethical review by the ethics committee of Shenzhen Second People's Hospital.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors have no conflicts of interest to disclose for this work.

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