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ORIGINAL RESEARCH

Expression and Clinical Significance of Origin Recognition Complex Subunit 6 in Breast Cancer – A Comprehensive Bioinformatics Analysis

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Objective: We aimed to investigate the expression, diagnostic and prognostic values, and potential molecular mechanisms of the origin recognition complex (ORC) in breast cancer (BC).

Methods: Kaplan–Meier estimation was used to assess the prognostic value of *ORC* genes, and Oncomine, TCGA, GEO and ULCAN databases were used to analyze their expression in BC. Wilcoxon rank-sum tests were used to evaluate the relationship between *ORC* gene expression levels and BC clinicopathological features. Receiver operating characteristic (ROC) curves were used to assess the diagnostic value of *ORC* genes in BC. Survival analysis was performed using Kaplan–Meier estimation and Cox regression. A nomogram was constructed to predict 1-, 3-, and 5-year survival probabilities in BC. Gene set enrichment analysis (GSEA) and immune infiltration were used to investigate potential molecular mechanisms of the ORC.

Results: *ORC1L* and *ORC6L* were highly expressed in BC compared with healthy tissue, while *ORC5L* expression patterns were inconsistent; no significant differences in *ORC2L, ORC3L* or *ORC4L* expression were observed between BC and healthy tissues. *ORC1L* and *ORC6L* expression levels were significantly correlated with age, tumor (T) stage and molecular subtype; *ORC5L* expression was significantly correlated with age and number of nearby lymph nodes with cancer (N stage). *ORC6L* expression had the highest diagnostic value in BC and was an independent prognostic factor for poor overall survival (OS). ORC6L may be involved in cell cycle progression and may regulate cancer signaling pathways, including NF-κB, P53, and WNT, in BC. *ORC6L* expression was also associated with immune infiltration.

Conclusion: *ORC1L* and *ORC6L* are highly expressed in BC; *ORC6L* has a high diagnostic value and is an independent prognostic factor for poor OS. ORC6L may be involved in the initiation and progression of BC by regulating cell cycle progression, promoting cancer signaling pathway activation, and influencing tumor immune cell infiltration.

Keywords: origin recognition complex, breast cancer, prognostic biomarker, nomogram, gene set enrichment analysis, immune infiltration

Introduction

Breast cancer (BC) is the most common malignancy worldwide. It is the most common cause of cancer-related deaths in women and the fifth leading cause of cancer-related deaths globally.[1] Despite rapid developments in diagnostic methods and treatment strategies for this disease in recent decades, the 5-year stage IV BC survival rate is only 20%, and 5–10% of newly diagnosed BC patients have distant

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metastases.[2] Therefore, novel diagnostic and prognostic biomarkers of BC and a better understanding of BC pathogenesis are critical to improving prognosis and survival for people with this cancer type.

Recently, bioinformatics analyses identified two of the six subunits of the origin recognition complex (ORC), ORC1L and ORC6L, are related to the maintenance of the fundamental stem cell properties of triple negative breast cancer ('stemness') and cognitive dysfunction in breast cancer survivors, respectively.[3,4] The ORC binds to the origin of DNA replication and regulates the initiation of replication.[5] In recent decades, substantial evidence has shown that the ORC is involved in other biological processes and conditions, such as heterochromatin formation, cytokinesis, viral replication, DNA damage repair, cognitive impairment, and radiation inflammatory response.[6–11] ORC genes are also known to be differentially expressed in a number of tumors, such as gliomas,[12] endometrial cancer,[13] colon cancer,[14–16] gastric cancer,[17,18] liver cancer,[19] esophageal cancer,[20] and lung cancer.[21] They are implicated in diverse biological processes, including cell proliferation, migration, invasion, and chemotherapy resistance.[13–15,21–23] ORC1L, ORC5L and ORC6L have known prognostic value in gastric cancer, liver cancer, and colorectal cancer;[16–19] however, the expression levels and roles of ORC subunits in BC tumorigenesis remain to be elucidated.

In the current study, we aimed to assess the expression levels, clinical values, and potential molecular mechanisms of ORC subunits in BC, providing a new theoretical basis for the diagnosis and treatment of BC.

Materials and Methods

Kaplan–Meier Analysis

We assessed the association between ORC genes and overall survival (OS) in BC patients using the Kaplan–Meier Plotter online tool (http://kmplot.com/analysis/, accessed on: July 16, 2021). This tool is capable of assessing the effects of more than 54,000 genes (mRNA, miRNA, protein) on survival in 21 cancer types including BC (n = 7830). The sources of data in this tool include the GEO, EGA, and TCGA databases.[24] P-values of < 0.05 were considered statistically significant in this study.

ORC Gene Expression and Clinical Features of BC

mRNA levels were used as a proxy to assess ORC gene expression in BC and healthy tissues, based on information in the TCGA database (https://portal.gdc.cancer.gov/, accessed on: July 16, 2021), the GEO database (accession numbers GSE54002 and GSE37751; https://www.ncbi.nlm.nih.gov/geo/, accessed on: July 16, 2021), and the Oncomine platform (www.oncomine.org, accessed on: July 16, 2021). We obtained gene expression data and clinical information from 1109 BC and 113 healthy breast tissue samples from the TCGA database. RNAseq data in fragments per kilobase per million mapped reads (FPKM) were converted to transcripts per million mapped reads (TPM) and log2-converted for subsequent analysis. The clinical characteristics of BC are presented in Table 1.

ORC Protein Expression Analysis

We explored the protein expression of the ORCs in BC in the UALCAN web resource for cancer-omics data analysis (http://ualcan.path.uab.edu/, accessed on: July 16, 2021) using the Clinical Proteomic Tumor Analysis Consortium (CPTAC) confirmatory/discovery dataset.[25]

Construction and Verification of Nomogram

We used the independent factors found to be associated with BC prognosis in this study to construct a nomogram to predict 1, 3, and 5-year OS. The nomogram was generated in R, using the rms and survival packages.[26] Harrell's concordance index (C-index) was used to quantify the predictive accuracy for each factor, ranging from 0.5 (no predictive power) to 1 (perfect prediction). In addition, calibration plots were generated to examine the performance characteristics of the predictive nomogram.

Gene Set Enrichment Analysis (GSEA)

GSEA (http://www.broadinstitute.org/gsea/index.jsp, accessed on: July 16, 2021) was used to assess the correlation between gene expression signatures based on low versus high *ORC6L* expression. GSEA is generally used to determine whether a set of genes show statistically significant, concordant differences between two biological states, in this case, BC versus healthy tissue.[27] Publicly available microarray expression data for 1109 BC samples were downloaded from TCGA; the significance threshold in our GSEA was set at $P < 0.05$ and the false discovery rate (FDR) at <0.25.

Assessment of Correlation Between BC Risk and Immune Cell Infiltration

The relative tumor infiltration levels of 24 immune cell types were quantified using ssGSEA of GSVA package.[28] The

Table 1 The Clinical Characteristics of BC Patients (TCGA)

Characteristic	Low Expression of ORC6L, n (%)	High Expression of ORC6L, n (%)	P value
n	541	542	
T stage			< 0.001
T1	162 (15%)	115 (10.6%)	
T2	281 (26%)	348 (32.2%)	
T3	78 (7.2%)	61 (5.6%)	
T4	20 (1.9%)	15 (1.4%)	
N stage			0.016
N0	261 (24.5%)	253 (23.8%)	
N1	173 (16.3%)	185 (17.4%)	
N2	50 (4.7%)	66 (6.2%)	
N3	50 (4.7%)	26 (2.4%)	
M stage			1
M0	434 (47.1%)	468 (50.8%)	
M1	10 (1.1%)	10 (1.1%)	
Pathologic stage			0.036
Stage I	105 (9.9%)	76 (7.2%)	
Stage II	287 (27.1%)	332 (31.3%)	
Stage III	126 (11.9%)	116 (10.9%)	
Stage IV	10 (0.9%)	8 (0.8%)	
Age, n (%)			< 0.001
≤60	272 (25.1%)	329 (30.4%)	
>60	269 (24.8%)	213 (19.7%)	
Histological type			< 0.001
Infiltrating Ductal Carcinoma	315 (32.2%)	457 (46.8%)	
Infiltrating Lobular Carcinoma	172 (17.6%)	33 (3.4%)	
PAM50			< 0.001
Normal	28 (2.6%)	12 (1.1%)	
LumA	437 (40.4%)	125 (11.5%)	
LumB	52 (4.8%)	152 (14%)	
Her2	16 (1.5%)	66 (6.1%)	
Basal	8 (0.7%)	187 (17.3%)	
OS event			0.194
Alive	473 (43.7%)	458 (42.3%)	
Dead	68 (6.3%)	84 (7.8%)	

correlation between risk scores and immune infiltration was calculated using Spearman's rank correlation coefficient analysis. Results with correlation coefficient $|r| > 0.3$ and $P < 0.05$ were considered statistically significant.

Statistical Analysis

Statistical analysis and mapping were performed in R (v3.6.3). Wilcoxon rank sum tests were used to compare gene expression between BC and normal breast tissues and to assess the correlation between gene expression and clinicopathological features. Receiver operating characteristic (ROC) curves were plotted using the pROC package. Kaplan–Meier and Cox proportional risk regression models were used to evaluate the prognostic value of ORC genes. P values <0.05 were considered statistically significant.

Results

ORC Gene Expression Levels in BC

We found that the expression of *ORC1L* and *ORC6L* were significantly increased and *ORC5L* significantly decreased in BC compared with healthy tissue (Figure 1A). Furthermore, patients with high levels of *ORC5L*

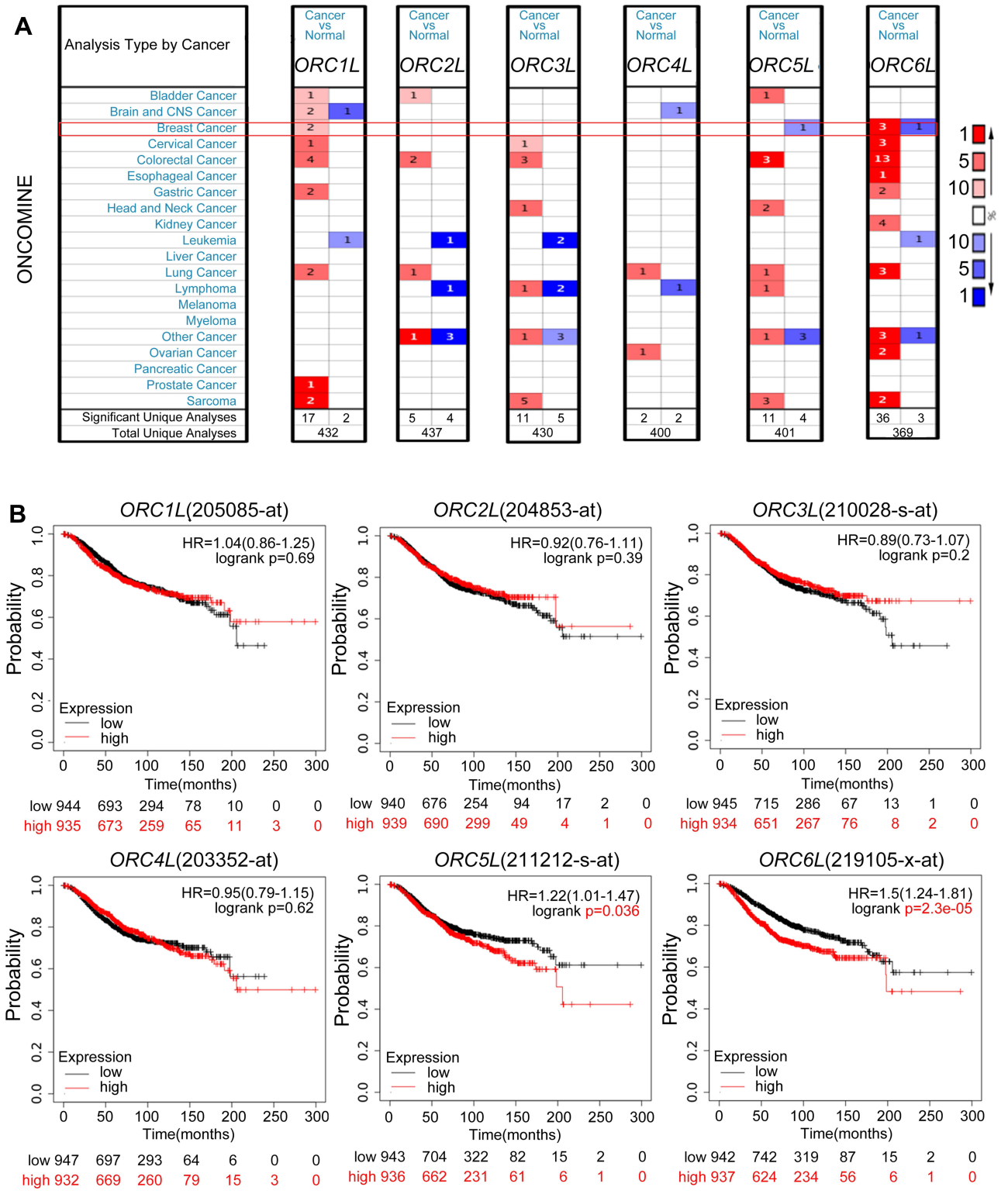

Figure 1 Expression of ORCs in various tumors and their prognostic value in BC. (**A**) Comprehensive analysis of ORCs mRNA expression in various tumor tissues compared with normal tissues using Oncomine database. The left box in red indicated the number of datasets with high expression and the right box in blue indicated the number of datasets with low expression after comparing cancerous and normal tissues. (**B**) The Kaplan–Meier curve for OS of BC patients with high and low ORCs mRNA expression from Kaplan–Meier Plotter.

expression were found to have significantly worse OS (hazard ratio [HR] = 1.22, 95% confidence interval [CI] = 1.01–1.47, Log rank P = 0.036). Similarly, BC patients with high levels of *ORC6L* expression also had significantly worse OS [HR = 1.5, 95% CI = 1.24–1.81, Log rank P < 0.001], ie, the expression of *ORC5L* and *ORC6L* were negatively correlated with OS in people with BC (Figure 1B). In contrast, *ORC1L, ORC2L, ORC3L*, and *ORC4L* expression levels were not associated with OS in those with BC. These results suggest that *ORC5L* and *ORC6L* may be useful prognostic factors in BC.

To account for tissue-specific expression of ORC genes, we studied their expression in BC a combined multiple database. *ORC1L, ORC5L*, and *ORC6L* expression levels were significantly higher in BC than in healthy tissues, while *ORC2L, ORC3L*, and *ORC4L* expression levels were not significantly different (Figure 2A). Results of analyses using the GEO datasets GSE54002 and GSE37751 had concordant results (Figure 2B and C). Our comprehensive analysis using the Oncomine, TCGA and GEO databases therefore showed that *ORC1L* and *ORC6L* expression was significantly increased in BC compared with healthy tissue, while the results for *ORC5L* were inconsistent between databases, and there were no significant differences in the expression of *ORC2L, ORC3L* or *ORC4L* in BC versus healthy tissue.

UALCAN cancer-omics data were further used to reveal ORC protein levels in 125 BC and 18 normal tissue samples. Consistent with the gene expression results, ORC1L and ORC6L protein levels were significantly increased in BC compared with healthy tissues. However, ORC5L levels were low in BC compared with healthy tissues, which was inconsistent with the gene expression levels (Figure 2D). Interestingly, ORC2L and ORC3L levels were different between BC and healthy tissues despite no significant difference in gene expression in this study, indicating post-transcriptional regulation of these factors in BC. In conclusion, ORC1L, ORC5L and ORC6L were found to be differentially expressed in BC tissues; these proteins may therefore affect incidence and development of BC.

Relationship Between ORC Subunits and Clinicopathological Characteristics in BC

We further explored the relationship between the expression of *ORC1L, ORC5L*, and *ORC6L* and the clinicopathological characteristics of BC patients using clinical data from TCGA, including tumor patient age, molecular subtype, tumor volume, lymph node metastasis, distant metastasis, and pathological stage. Our results suggested that high *ORC1L* and *ORC6L* expression occurred mainly in the HER2-enriched subtype of BC; their expression was negatively correlated with patient age and positively correlated with tumor size but not with lymph node metastasis, distant metastasis, or tumor stage (Figure 3A and C). *ORC5L* expression was also negatively correlated with age and positively correlated with lymph node metastasis but not with BC molecular subtype, tumor size, or any other characteristics (Figure 3B).

Diagnostic and Prognostic Values of *ORC1L, ORC5L* and *ORC6L* in BC

ROC curves were used to evaluate the diagnostic value of *ORC1L, ORC5L*, and *ORC6L* in BC. *ORC6L* had the highest diagnostic value, with an AUC of 0.907 (95% CI = 0.882–0.932) (Figure 4A). The AUC for *ORC1L* was 0.885 (95% CI = 0.858–0.913), and the AUC for ORC5L was 0.739 (95% CI = 0.694–0.785).

We used the Kaplan–Meier method to assess the impacts of *ORC1L, ORC5L*, and *ORC6L* on patient survival. Interestingly, *ORC1L, ORC5L*, and *ORC6L* expression were not significantly correlated with OS in BC patients (Figure 4B). This is inconsistent with previous results that *ORC5L* and *ORC6L* have prognostic value in the context of survival based on KM-plot online analysis. Since the expressions of *ORC1L, ORC5L*, and *ORC6L* were related to clinicopathological factors, such as age and molecular subtype, univariate and multivariate Cox proportional risk regression models were used to eliminate any confounding effects of these factors. Univariate analysis showed that T stage (size and extent of the tumor), N stage (number of nearby lymph nodes with cancer), M stage (metastatic status), pathological stage, molecular subtype, and age were found to be significantly associated with OS. Further multivariate regression analyses were performed, in which *ORC6L* level, N stage, M stage, pathological stage, molecular subtype, and age were determined to be independent risk factors in OS (Table 2, Figure 5A). The risk of death in people with high *ORC6L* expression was found to be 1.538-fold higher than in those with low *ORC6L* expression.

We included all statistically significant factors, including N stage, M stage, pathological stage, molecular subtype, age, and *ORC6L* expression, in a multivariate Cox

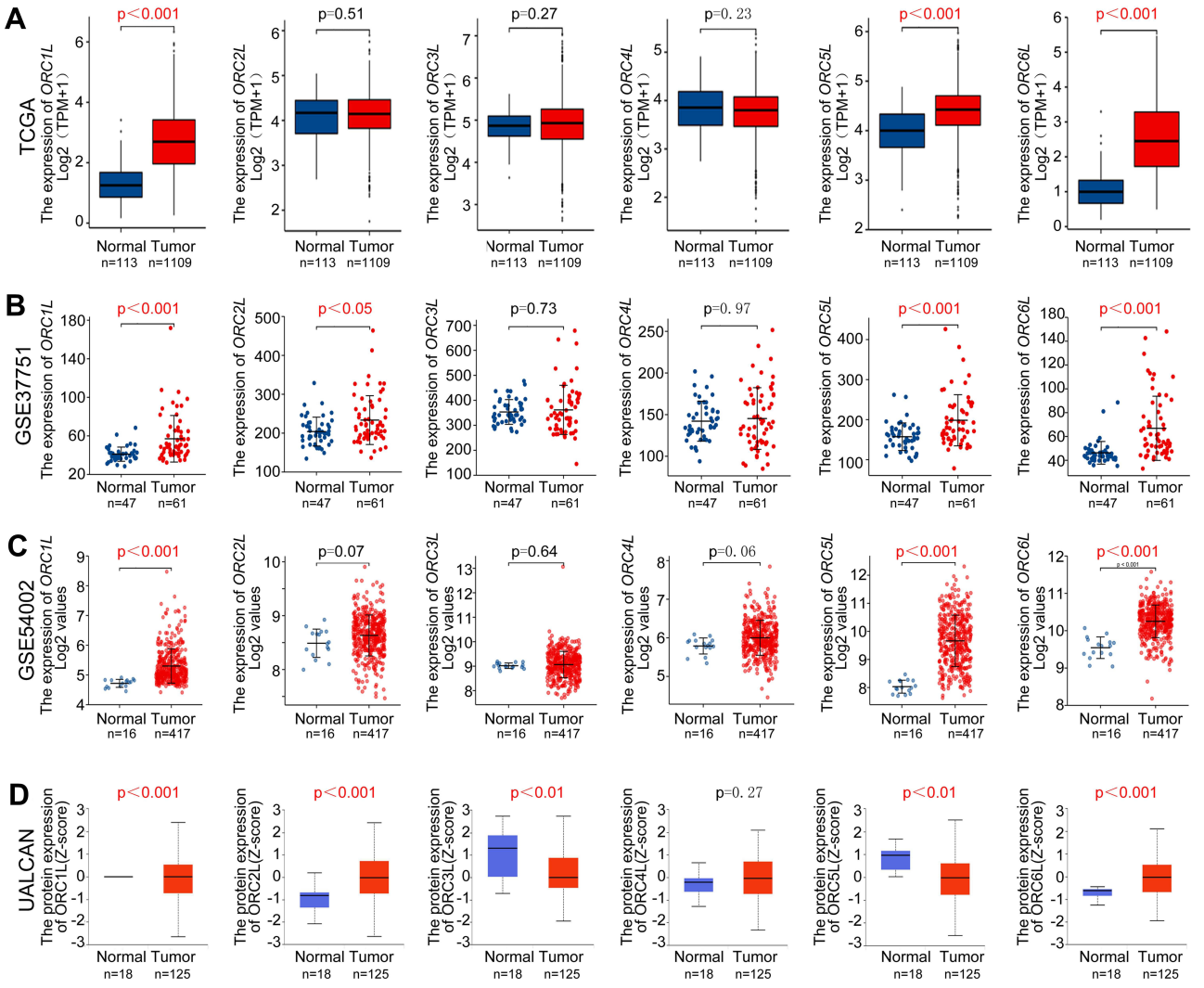

Figure 2 mRNA and protein expression of ORCs in BC. (**A**) Relative mRNA expression of ORCs in human BC and normal tissues obtained from TCGA. (**B** and **C**) Relative mRNA expression of ORCs in human BC and normal tissues obtained from GEO (GSE54002, GSE37751). (**D**) ORCs protein expression in BC and normal tissues from UALCAN cancer OMICS data. n=number of cases. P-value < 0.05 was considered statistically significant.

proportional risk regression model to construct a nomogram for predicting 1, 3, and 5-year survival in people with BC (Figure 5B). The C-index, calculated by internal re-sampling verification, was 0.716 (95% CI 0.692–0.741), indicating that the predicted results were likely in good agreement with the real results. Our calibration plot also indicated that the nomogram was well calibrated, with mean predicted probabilities close to observed probabilities (Figure 5C). ORC6L therefore has prognostic value in BC.

Enrichment Analysis of ORC6L-Related Signaling Pathways in BC

A GSEA was performed to identify the potential molecular mechanisms of ORC6L in BC. In this analysis, patients in the TCGA database were divided into "high" and "low" expression groups based on the median value of *ORC6L* expression in people with BC in this study. Overall, 14 gene sets were strongly enriched in those with high compared with low *ORC6L* expression (Figure 6A, Table 3). Some of these gene sets, including those involved in cell cycle checkpoints and cellular senescence, were markedly activated in those with high *ORC6L* expression, implying a potential cell cycle regulatory mechanism for ORC6L in BC. Pathways that modulate cancer-related functions, including transcriptional regulation by P53, FCER1-mediated nuclear factor (NF-κB) activation, and T-cell factor (TCF)-dependent WNT signaling, were positively correlated with *ORC6L* expression. In addition, GSEA revealed a relationship between *ORC6L* expression and

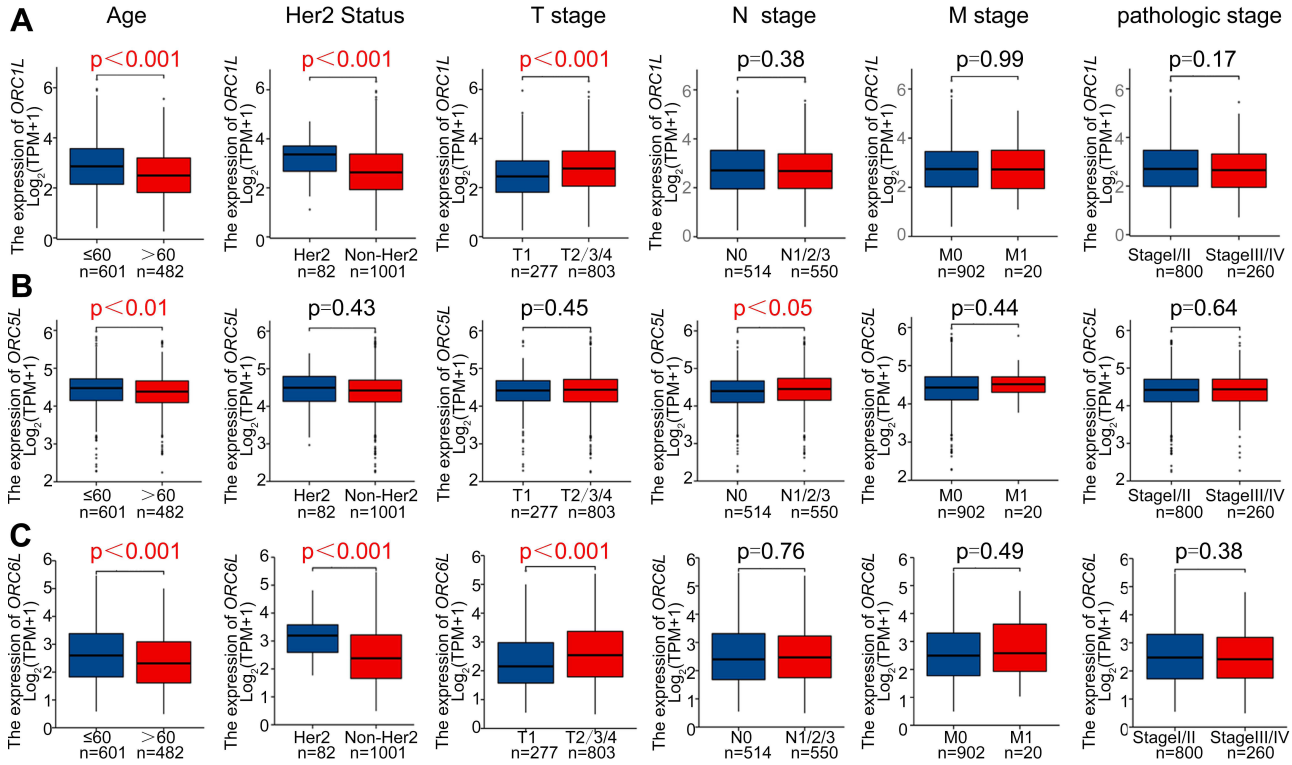

Figure 3 Relationship between the ORCs and clinicopathological features in BC. (**A**) The relationship between *ORC1L* and age, molecular subtype, T stage, N stage, M stage, and pathologic stage. (**B**) The relationship between *ORC5L* and age, molecular subtype, T stage, N stage, M stage, and pathologic stage. (**C**) The relationship between *ORC6L* and age, molecular subtype, T stage, N stage, M stage, and pathologic stage. n=number of cases. P-value < 0.05 was considered statistically significant.

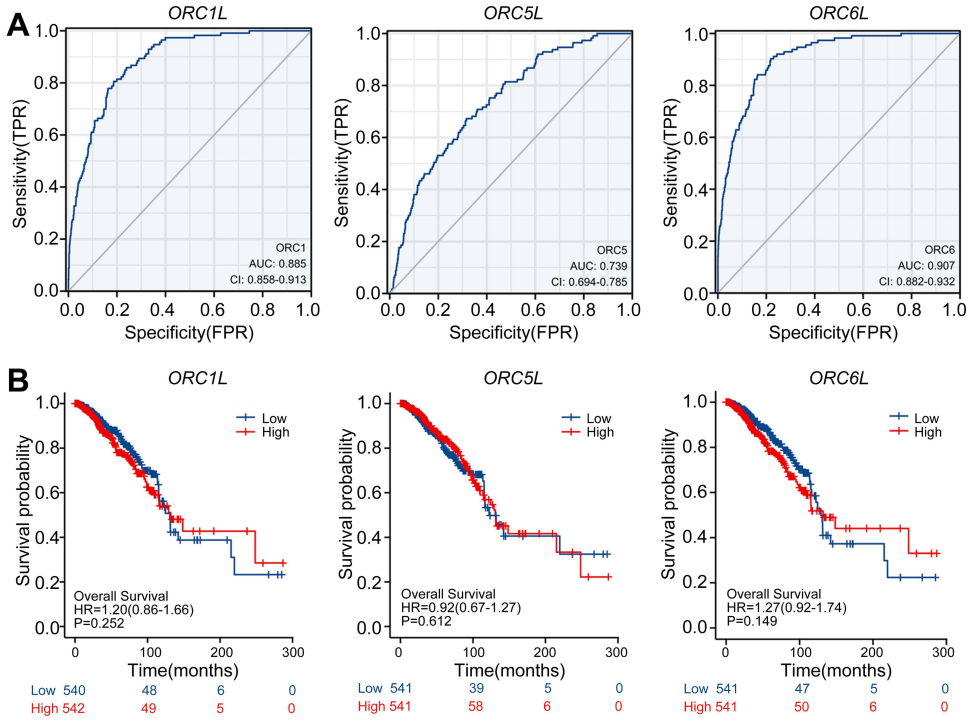

Figure 4 Diagnostic and prognostic values of *ORC1L*, *ORC5L* and *ORC6L* in BC. (**A**) The ROC was plotted to estimate the diagnostic value of *ORC1L*, *ORC5L* and *ORC6L* expression in BC. (**B**) Kaplan–Meier method illustrated the impacts of ORC1L, *ORC5L*, and *ORC6L* on OS.

Table 2 Univariate and Multivariate Cox Regression for OS in BC Patients (TCGA)

Characteristics	Total (N)	Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	P val	HR (95% CI)	P val
T stage	1079				
T1	277	Reference			
T2&T3&T4	803	1.482 (1.007–2.182)	0.046	1.097 (0.695–1.730)	0.691
N stage	1063				
N0	514	Reference			
N1&N2&N3	550	2.239 (1.567–3.199)	<0.001	1.688 (1.080–2.638)	0.022
M stage	922				
M0	902	Reference			
M1	20	4.254 (2.468–7.334)	<0.001	2.469 (1.213–5.028)	0.013
Pathologic stage	1059				
Stage I&Stage II	800	Reference			
Stage III&Stage IV	260	2.391 (1.703–3.355)	<0.001	1.785 (1.127–2.828)	0.013
Age	1082				
≤60	601	Reference			
>60	482	2.020 (1.465–2.784)	<0.001	2.303 (1.591–3.335)	<0.001
Histological type	977				
Infiltrating Ductal Carcinoma	772	Reference			
Infiltrating Lobular Carcinoma	205	0.827 (0.526–1.299)	0.41		
PAM50	1082				
Her2	82	Reference			
LumA&LumB&Basal&Normal	1001	0.528 (0.318–0.875)	0.013	0.555 (0.314–0.981)	0.043
ORC1	1082				
Low	542	Reference			
High	541	1.206 (0.877–1.659)	0.249		
ORC5	1082				
Low	542	Reference			
High	541	0.921 (0.670–1.268)	0.615		
ORC6	1082				
Low	542	Reference			
High	541	1.270 (0.922–1.749)	0.143	1.538 (1.060–2.231)	0.023

Note: HR (95% CI): hazard ratio (95% CI).

activation of Rho GTPase effectors, B cell receptor (BCR) signaling, and epigenetic regulation of gene expression. Together, these results indicate that ORC6L may play an oncogenic role in BC by regulating the cell cycle and activating tumor-related signaling pathways.

Correlation Between *ORC6L* and Immune Cell Infiltration in BC

Tumor growth and development are determined by both cancer cell-autonomous and microenvironmental mechanisms, including infiltration of the tumor by immune cells. Evidence has shown that tumor-infiltrating lymphocytes play an important role in the development and progression of BC.[29,30] Therefore, the ssGSEA of the GSVA package[28] was used to determine whether *ORC6L* modulates the tumor microenvironment. We found that *ORC6L* was significantly related to the infiltration of various immune cells in BC (Figure 6B). In particular, it was strongly positively correlated with T helper 1 and 2 cell (Th1 and Th2), T regulatory cell (Treg), and dendritic cell (DC) infiltration in BC. *ORC6L* expression was also closely negatively correlated with mast cell, natural killer (NK) cell,

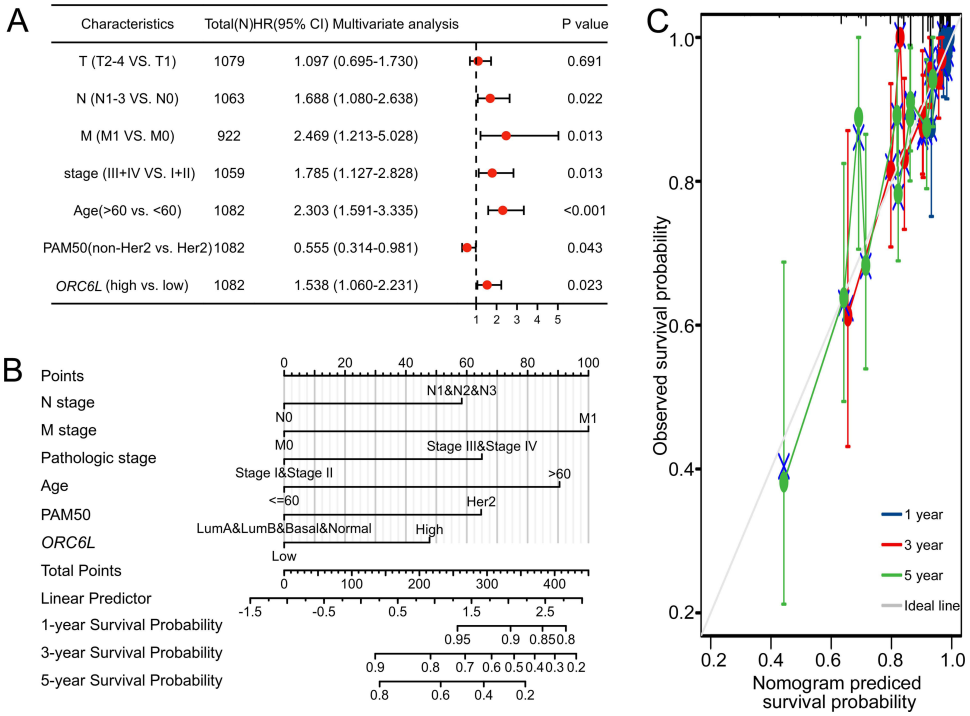

Figure 5 Multivariate Cox regression analysis and Nomogram. (**A**) *ORC6L*, age, molecular subtype, T stage, N stage, M stage, and pathologic stage were included to multivariate cox regression analysis. (**B**) According to the nomogram, the total score was obtained after adding up the scores of each factor. The probability corresponding to the total score on the scale was the 1-year, 3-year and 5-year survival probability. (**C**) The calibration plot showed the mean predicted probabilities close to observed probabilities.

eosinophil, and Th17 cell infiltration in BC. These results suggest that ORC6L may be involved in the occurrence and progression of BC through the regulation of immune cell infiltration.

Discussion

In this study, we demonstrated for the first time that ORC subunits are differentially expressed in people with BC compared with healthy individuals, and that some ORC subunits have diagnostic and prognostic values in this disease. The ORC was previously known to have a role in regulating the initiation of DNA replication, and its role in various tumors has attracted increasing attention in recent years. A previous study showed that ORC1L is upregulated in glioma and promotes the proliferation, migration and invasion of glioma cells by activating the extracellular signal-related kinase (ERK)/c-Jun N-terminal kinase (JNK) signaling pathway.[12] In lung cancer and triple-negative BC, *ORC1L* is considered a pivotal gene in the maintenance of tumor stem cells, and its expression in lung cancer is positively correlated with tumor stage.[3,21] ORC5L and ORC6L are known to have prognostic value in hepatocellular carcinoma.[19] It was reported that ORC subunits are also associated with chemotherapy resistance in various tumor types: for example, upregulation of ORC2L and ORC5L has been detected in cisplatin-resistant bladder cancer cell lines;[23] in pancreatic cancer, ORC4L is involved in phosphoinositide 4-kinase (PIK1)-mediated resistance to gemcitabine;[22] and in colorectal cancer cells, high expression of ORC6L has been shown to promote cell proliferation and induce resistance to the chemotherapeutic agents fluorouracil and cisplatin.[15] Therefore, our study adds to the evidence that ORCs have potential clinical applications in a variety of tumors, including BC.

We demonstrated that ORC1L and ORC6L mRNA and protein levels were significantly increased in BC compared with healthy tissues in an analysis of data from the Oncomine, TCGA, GEO and ULCAN databases. Interestingly, we found that the expression of ORC1L and ORC6L in BC is consistent with levels observed in lung adenocarcinoma, glioma and gastrointestinal malignancies, such as gastric and colorectal cancer.[12,15,17,21] We also found that both *ORC1L* and *ORC6L* are useful diagnostic indicators of BC. Our results suggest that *ORC1L* and *ORC6L* are potential oncogenes and may be clinically useful in BC diagnosis.

Our Kaplan–Meier analysis demonstrated that ORC expression levels were not associated with OS in BC patients, which was inconsistent with Kaplan–Meier

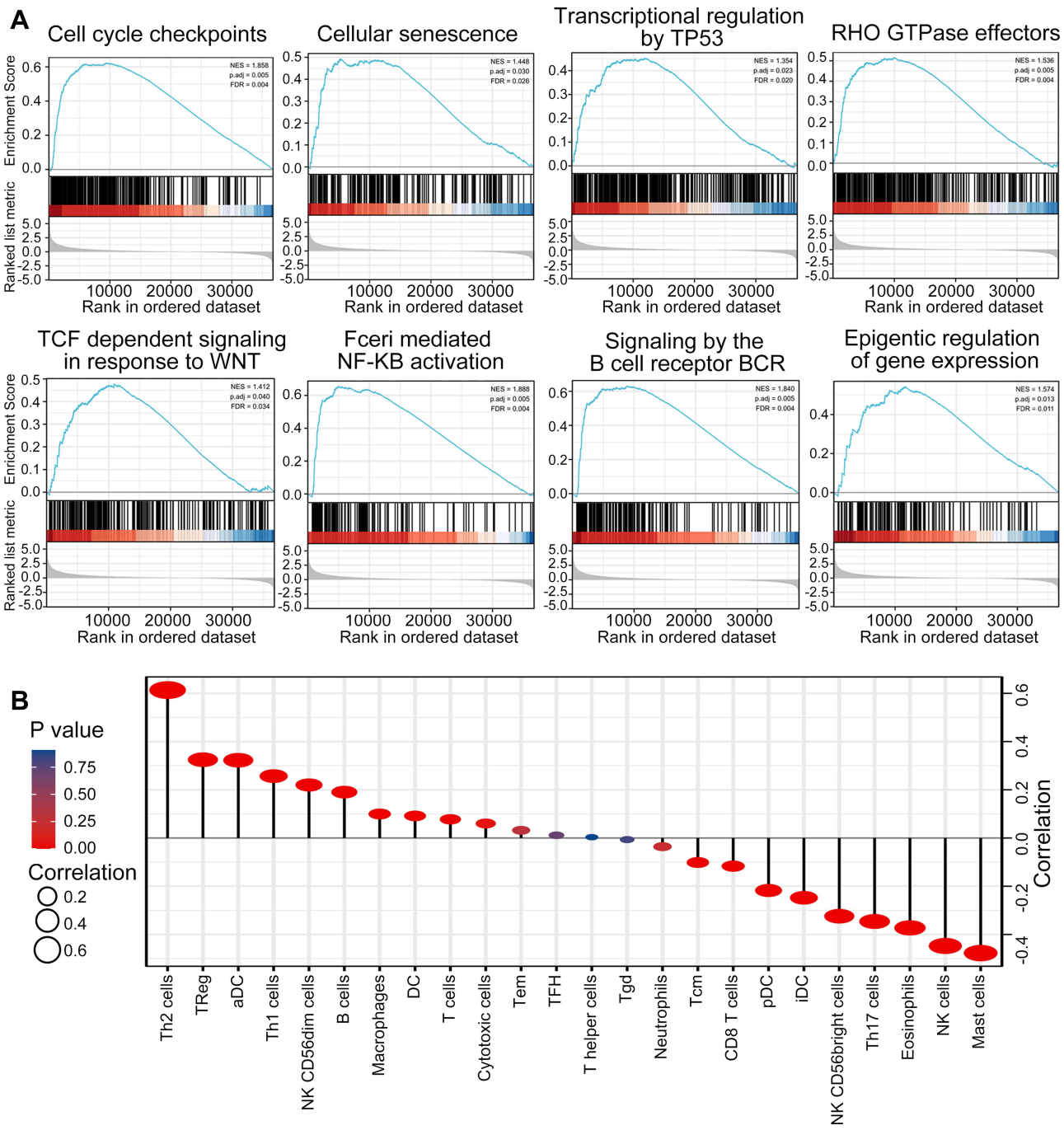

Figure 6 GSEA and Immune Cells Infiltration. (**A**) Gene set differences between ORC6L high and ORC6L low human BC specimens revealed by GSEA. 14 biological pathways were found obviously enriched in ORC6L high BC specimens. A gene set with P ≤ 0.05 was considered to be significantly enriched. (**B**) Correlation between ORC6L expression and immune cells infiltration in BC. Coefficient of correlation |r|>0.3, P-value < 0.05 was considered statistically significant.

Plotter's prediction. However, using a Cox regression analysis, which accounted for potentially confounding clinicopathological factors including age and molecular subtype of BC, we confirmed that *ORC6L* is an independent prognostic factor in this disease. *ORC1L* and *ORC5L* had no significant effect on OS, despite their differential expression in BC versus healthy tissue.

Evidence suggests that *ORC6L* is a potential oncogenic gene in BC and could be used as a potential prognostic factor or novel therapeutic target to improve survival in people with BC. However, the association of *ORC5L* expression with BC is unclear and its diagnostic value is relatively low. More evidence is needed to confirm whether and how this gene is differentially expressed in

Table 3 Signaling Pathways Enriched in the ORC6L High-Expression Group in BC Patients (TCGA)

Signaling Pathway	Set Size	ES	NES	p value	q value
FCERI_MEDIATED_NF_KB_ACTIVATION	136	0.654	1.888	0.005	0.004
CELL_CYCLE_CHECKPOINTS	291	0.623	1.858	0.005	0.004
SIGNALING_BY_THE_B_CELL_RECEPTOR_BCR_	166	0.630	1.840	0.005	0.004
S_PHASE	161	0.618	1.802	0.005	0.004
IMMUNOREGULATORY_INTERACTIONS_BETWEEN_A_LYMPHOID_AND_A_NON_LYMPHOID_CELL	186	0.604	1.773	0.005	0.004
M_PHASE	414	0.562	1.687	0.005	0.004
CELL_SURFACE_INTERACTIONS_AT_THE_VASCULAR_WALL	194	0.555	1.633	0.005	0.004
EPIGENETIC_REGULATION_OF_GENE_EXPRESSION	146	0.543	1.574	0.013	0.011
RHO_GTPASE_EFFECTORS	322	0.514	1.536	0.005	0.004
DNA_REPAIR	330	0.513	1.533	0.005	0.004
NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	132	0.520	1.496	0.039	0.033
CELLULAR_SENESCENCE	194	0.492	1.448	0.030	0.026
TCF_DEPENDENT_SIGNALING_IN_RESPONSE_TO_WNT	231	0.477	1.412	0.040	0.034
TRANSCRIPTIONAL_REGULATION_BY_TP53	358	0.452	1.354	0.023	0.020

Note: P value < 0.05 and FDR q value< 0.25 were considered significantly enriched; p value was adjusted.
Abbreviations: ES, enrichment score; NES, normalized enrichment score.

BC. The ORC2L and ORC3L proteins were abnormally expressed in BC, but there were no significant differences in their corresponding mRNA levels, and their expression had no effect OS in this study. ORC4L was not differentially expressed at either the protein or the mRNA level. In light of the significant differential expression and clinical significance of *ORC6L*, we constructed a nomogram to predict the probability of survival in people with BC at 1, 3, and 5 years, which was found to be highly accurate and of high clinical value.

Transcriptional profile comparisons of high versus low *ORC6L* expression using GSEA led us to identify a range of pathways (including those involved in cell cycle checkpoints and cellular senescence) that were associated with the expression of this gene. Other pathways, including such as NF-κB, P53, and WNT, were also activated with higher *ORC6L* expression. The effects of these pathways are to promote proliferation, angiogenesis, stem cell characteristics, and tumor migration. However, the exact molecular mechanism by which ORC6L acts in BC requires validation in clinical BC specimens and animal models in future to confirm these findings.

Cumulative evidence suggests that tumor-infiltrating immune cell plays essential roles in BC development and progression.[31] Th1 and Th2 cytokines have been shown to contribute to tumorigenesis in several models. Functional Th1-oriented T follicular helper tumor-infiltrating lymphocytes, which infiltrate human BC, promote effective adaptive immunity.[32] Th2 activation is associated with poor prognosis in BC.[33] Recently, Ghirelli et al described a novel granulocyte-macrophage colony-stimulating factor (GM-CSF)/pDC/Th2 axis in aggressive subtypes of BC.[34] Treg cells, which are critical for anti-tumor immunity, play a major role in the development of an immunosuppressive tumor microenvironment. Studies have shown that increased numbers of tumor-infiltrating Treg cells correlate with reduced survival, and quantification of Tregs therefore enables the identification of those with high-risk BC and those at risk of late relapse.[35] We found that the high levels of *ORC6L* expression were associated with increased Th1, Th2 and Treg cell infiltration, indicating that *ORC6L* might improve the ratio of the immune cells that infiltrate the tumor in BC through an as yet unknown mechanism, in favour of reducing tumor progression and improving clinical prognosis.

The evidence for pro- and antitumor effects of mast cells in BC progression remains conflicting.[36] Studies have showed that mast cells increase BC growth and metastasis,[37] but a study by della Rovere et al has revealed that peritumoral mast cells have a cytolytic activity against tumor cells.[38] Moreover, decreased NK-cell tumor immunosurveillance was found to enhance metastasis in BC models.[39] We found that infiltration of mast cells and NK cells was reduced in BC tissues with high *ORC6L* expression, suggesting that inhibition of tumor infiltration by ORC6L is another factor that may promote BC progression. Further studies are needed to evaluate the role of these immune cells within the BC microenvironment.

Conclusions

We demonstrated for the first time that *ORC1L, ORC5L* and *ORC6L* are differentially expressed in BC and have diagnostic value. Among them, *ORC6L* has the highest diagnostic value and is an independent risk factor that affects OS in people with BC, and could thus be used as a potential prognostic biomarker. As the expression, and diagnostic and prognostic values of ORC subunits in BC in this study were based on data from public databases, in vitro and in vivo experiments and clinical studies are required to confirm our findings.

Abbreviations

AUC, area under the curve; BC, breast cancer; CI, confidence interval; HR, hazard ratio; ORC, origin recognition complex; OS, overall survival; ROC, receiver operating characteristic.

Data Sharing Statement

The raw data used in this study were derived from TCGA (https://portal.gdc.cancer.gov/), GEO (https://www.ncbi.nlm.nih.gov/geo/), Oncomine (https://www.oncomine.org/), UALCAN (http://ualcan.path.uab.edu/) and Kaplan–Meier plotter (http://kmplot.com/analysis/) databases, which are publicly available.

Ethics Approval and Informed Consent

All data for this study were obtained from the TCGA, GEO, Oncomine, UALCAN and Kaplan–Meier plotter databases, which are publicly available. We did not obtain these data directly from patients or animals. Therefore, following ethical review, the Affiliated Hospital of Guilin Medical University Ethics Committee granted a status of exemption.

Consent for Publication

All authors gave final approval to submit the manuscript for publication.

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Disclosure

The authors declare that they have no competing interests.

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