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ORIGINAL RESEARCH

Identification of *CISD1* as a Prognostic Biomarker for Breast Cancer

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Background: Although *CISD1* (CDGSH iron sulfur domain 1) is upregulated in many cancer types, the potential role of *CISD1* in breast cancer is still unclear. The purpose of this study was to investigate its clinical significance in breast cancer.

Methods: We obtained 1109 breast cancer samples and 113 normal samples from The Cancer Genome Atlas (TCGA) and GTEx databases to demonstrate the relationship between *CISD1* expression and pancancer characteristics. We analysed the relationship between *CISD1* and breast cancer using the *t*-test and the chi-square test to evaluate the expression level of *CISD1* and its clinical significance in breast cancer. The prognostic value of *CISD1* in breast cancer was determined by Kaplan–Meier and Cox regression analyses. The biological pathways were screened by gene set analysis and Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and single-sample gene set enrichment analysis (ssGSEA), of which the correlation between the level of immune infiltration and the expression of *CISD1* in breast cancer was then analysed. Finally, we verified the conclusion by qPCR, immunohistochemistry, and CCK8.

Results: *CISD1* is highly expressed in breast cancer patients. In addition, we verified a higher expression of *CISD1* expressed in the BRCA (breast cancer) cell line, whereas *CISD1* has a high diagnostic value, with an AUC of 0.718. Kaplan–Meier survival and Cox regression analyses showed that high expression of *CISD1* was independently associated with adverse clinical outcomes. In turn, GO and KEGG analyses showed that most genes were related to rRNA metabolic process, rRNA processing. Moreover, PCR and immunohistochemistry showed that *CISD1* in breast cancer tissues was upregulated significantly, with CCK8 results showing that the proliferation of breast cancer cells decreased after *CISD1* knockout.

Conclusion: A high level of *CISD1* is associated with poor prognosis and immune infiltration in breast cancer.

Keywords: breast cancer, cancer prognosis, immunotherapy, bioinformatics, *CISD1*

Introduction

Bearing in mind that breast cancer is the most common cancer in women and the leading cause of cancer death, its burden is increasing worldwide.[1,2] Although an early diagnosis and a comprehensive treatment strategy have shown an improvement in the prognosis of breast cancer patients, the overall survival rate of 5 years is less than 20% once metastasis occurs.[3,4] It is therefore imminent to find biomarkers related to the prognosis of breast cancer.

Ferroptosis describes a type of programmed cell death different from apoptosis that is iron-dependent and characterized by lipid peroxidation and the production of reactive oxygen species.[5] Studies have shown that some ferroptosis-related genes have been identified as suppressor genes in the process of BRCA.[6,7]

CISD1 is a protein containing the CDGSH iron-sulfur domain, which is located in the outer membrane of mitochondria and is known to negatively regulate ferroptosis.[8] While studies have found that *CISD1* plays an important role in promoting tumorigenesis and tumor progression in many cancer types,[9] recent studies also suggest that *CISD1* can be used as a biomarker and target for breast cancer.[10] At present, although there is not much research on *CISD1* in breast cancer, we were able to determine the role of *CISD1* in breast cancer through TCGA-BRCA database analysis.

With the maturity of high-throughput sequencing technology, the generation of large-scale omics data has become possible,[11,12] and the characteristics of these TCGA-BRCA genes can explain the etiology of cancer and have significant

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diagnostic and prognostic value. In this study, the transcriptional level and prognostic significance of *CISD1* were analysed by reviewing the data obtained by TCGA-BRCA, and we explored its biological mechanism through GO and KEGG analysis and further evaluated the association between *CISD1* and immune infiltration levels, with q-PCR, immunohistochemistry, and cell proliferation experiments also confirming our conclusion.

Materials and Methods

Data Processing

Gene expression data of 1109 BRCA tissues and 113 adjacent tissues were downloaded from the TCGA database, which we then screened for clinicopathological features and prognostic data. Unified processing of RNA-seq data in TPM format of TCGA. The expression of *CISD1* was analysed by the TCGA database, and we extracted *CISD1* from UCSC Xena to assess *CISD1* expression levels in pancancer (https://xenabrowser.net/datapages/).

Patients and Tissues

Bearing in mind, all participants were informed by written consent. A total of 12 breast cancer samples and matched nontumor tissues were obtained from Liaoning Cancer Hospital. The study was also approved by the Ethics Committee of Liaoning Cancer Hospital. Breast cancer tissue was frozen rapidly in liquid nitrogen and stored at −80°C after the operation for q-PCR detection.

Gene Enrichment Analysis

In this study, gene expression data were divided into high expression and low expression *CISD1* groups (R package cluster profile). According to the transcriptional sequence of TCGA, we used GO and KEGG to identify the genome and pathway related to *CISD1*.

Methylation Analysis of the *CISD1* Gene

We used the MethSurv (https://biit.cs.ut.ee/methsurv/, accessed on October 17, 2022) database to conduct multivariate survival analysis on the DNA methylation of breast cancer patients,[13] which was used to analyse the impact of *CISD1* methylation on the survival and prognosis of breast cancer patients.

Immune Cell Infiltration

While we performed ssGSEA (single sample gene set enrichment analysis) to assess the relative abundance of infiltrating immune cells in tumor tissues, we also analysed the infiltration level of immune cells in BRCA expression profile data by using "GSVA" (R package) and the immune data set, which included 24 immune cells.

We also used TIMER (http://timer.cistrome.org/, accessed on October 18, 2022) to examine the relationships among immune cells and breast cancer.[14]

Connections Between Small Molecules and Genes via a Connectivity Map

The differentially expressed genes between the CMap (connectivity map) database and *CISD1* in breast cancer were used to reveal the interactions among drugs, compounds and diseases.[15]

Survival and Prognosis Analysis

We used the R package "survival" to obtain the overall survival (OS) survival map of *CISD1*, of which a critical value of 50% was selected as the division threshold, and the cohort was divided into high expression and low expression groups. Additionally, we used the R package "ROC" to analyse and visualize it using "ggplot2" to evaluate the value of *CISD1* in predicting the prognosis of breast cancer patients.

Cell Culture and Transfection

The MCF7 cell line belonged to the Chinese Academy of Sciences and was cultured in MEM supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin–streptomycin in an incubator humidified with 5% CO2 at 37 °C. Moreover, MCF7 cells were seeded in 50–60% confluent six-well plates 24 hours before transfection, and siRNA was then transfected with Lipofectamine 2000 according to the manufacturer's instructions.

RNA Isolation and qPCR Analysis

RNA extraction from tissues was performed using TRIzol reagent. RNA was reverse-transcribed into cDNA using the QuantiTect Reverse Transcription Kit. qPCR analyses were quantified by SYBR-Green, and levels were normalized to GAPDH levels. The primers used were as follows: *CISD1* forwards primer, 5′-GCTCTCGGTTACCTGGCTTA-3′; reverse, 5′-TTGTCTCCAGTCTCCTCATTGT-3′.

Immunohistochemistry

BRCA samples were fixed in 10% formalin, embedded in paraffin and processed into 5-μm sequential sections. Samples were dewaxed with ethanol and blocked to inhibit endogenous peroxidase activity. Samples were incubated overnight at 4 °C with rabbit anti-*CISD1* (Thermo Fisher Scientific PA5-106281), followed by incubation with horseradish peroxidase-coupled goat anti-rabbit secondary antibody at 37 °C for 30 min and then stained using 3,3′-diaminobenzidine. Cell nuclei were stained blue with hematoxylin. The sections were then dehydrated, cleared with xylene, and mounted. *CISD1* expression was determined

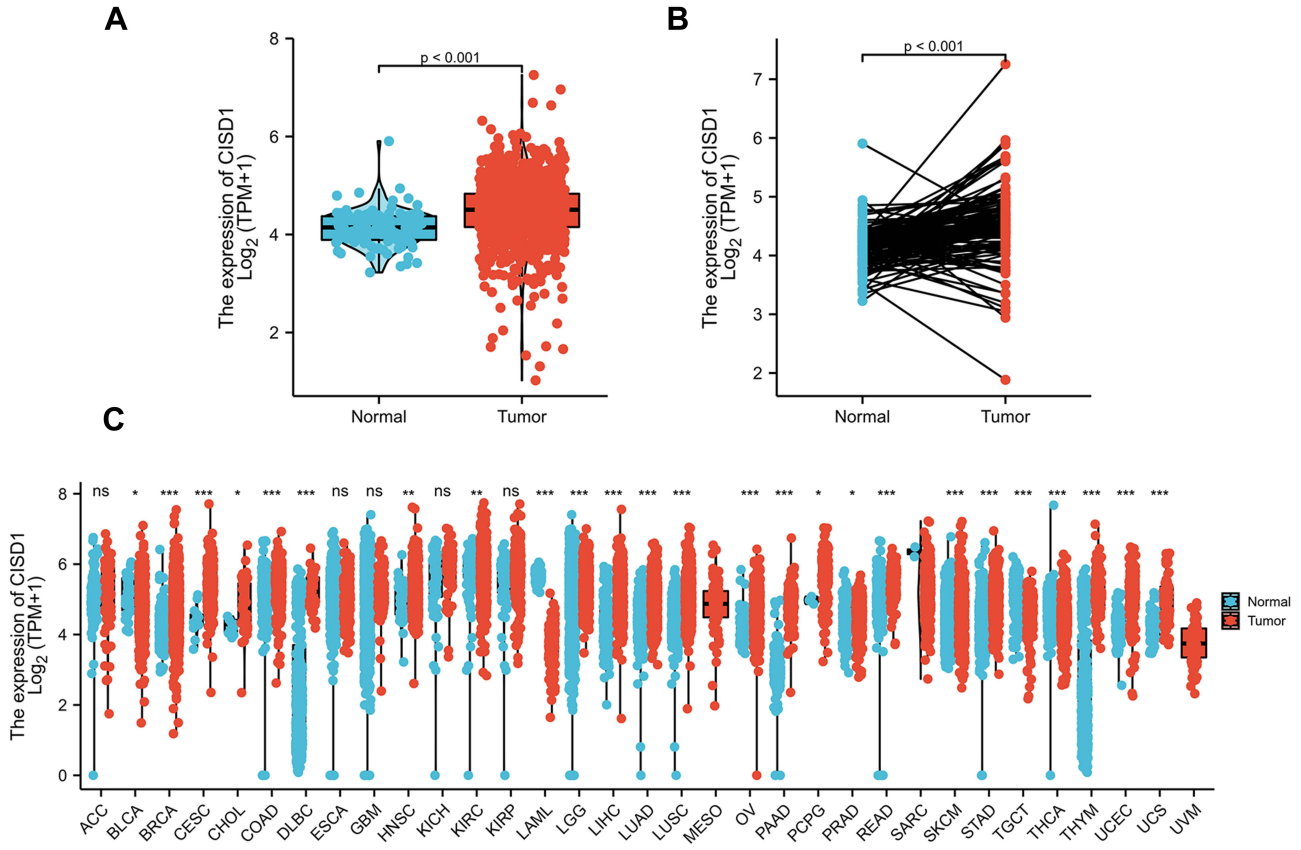

Figure 1 (**A**) Comparison of *CISD1* expression levels between BRCA and normal tissues. (**B**) *CISD1* expression levels in matched BRCA tissues and adjacent normal tissues. (**C**) Expression level of *CISD1* in different cancer types. P<0.05 indicates that the data are statistically significant; NS P>0.05, *P < 0.05, **P < 0.01, ***P < 0.001.

Abbreviations: BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; GBM, glioblastoma; HNSC, head and neck squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PRAD, prostate adenocarcinoma; READ, rectal adenocarcinoma; SARC, sarcoma; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma.

with IHC (immunohistochemistry) using a streptavidin peroxidase method, with adjacent tissues as controls. The experimental procedure was performed according to the manufacturers' instructions. Image-Pro Plus 6.0 Software (MediaCybernetics, USA) was used to analyse protein expression and perform statistics on the results obtained from immunohistochemistry.

Statistical Analysis

The statistical analysis of *CISD1* expression in normal and BRCA groups was calculated using the Wilcoxon rank-sum test, and the patients were divided into two categories according to the *CISD1*'s "median" expression. The clinicopathological features of CISD1 were analysed by the Wilcoxon rank sum test or Kruskal–Wallis test and logistic regression, whereas Kaplan–Meier survival analysis and Cox univariate and multivariate analysis were used for the prognostic analysis. The receiver operating characteristic (ROC) curve was generated using the "pROC" package to evaluate the diagnostic significance of differentially expressed genes.

Results

Analysis of *CISD1* Expression Across Cancers and Breast Cancers

While data downloaded from UCSC Xena were used to analyse the expression of *CISD1* in 33 cancers, we evaluated the expression of *CISD1* in breast cancer in TCGA database and were then able to confirm that *CISD1* was overexpressed in breast cancer. (Figure 1A and B). The results showed that *CISD1* was overexpressed in most cancers, including BLCA, BRCA, CESC, CHOL, COAD, DLBC, HNSC, KIRC, LIHC, LUAD, LUSC, OV, PAAD, PCPG, SKCM, STAD, THCA, THYM, UCEC, and UCS, but the expression of *CISD1* was low in LAML, LGG, PRAD, PEAD, and TGCT (Figure 1C).

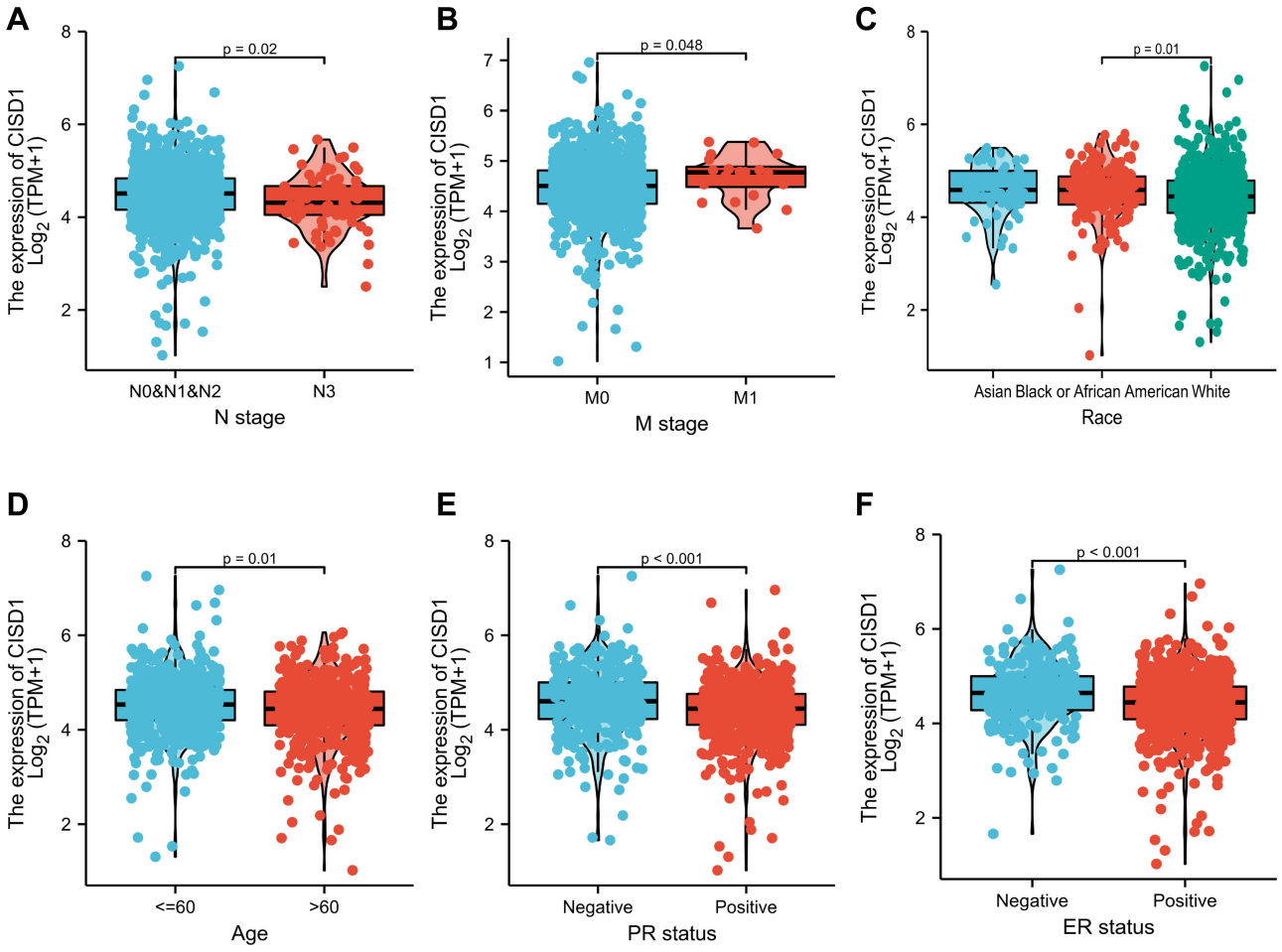

Figure 2 The mRNA expression level of *CISD1* was analysed using TCGA-BRCA data sets. (**A**) N stage (**B**) M stage (**C**) Race (**D**) Age (**E**) PR status (**F**) ER status.

In addition, we found that N stage (P = 0.02), M stage (P = 0.048), race (P = 0.01), age (P = 0.01), PR status (P < 0.001), and ER status (P < 0.001) were also significantly correlated with *CISD1* mRNA expression (Figure 2A–F).

Clinical Relevance of *CISD1* Expression in Breast Cancer Patients

The patients were divided into the *CISD1* high expression (n = 542) and *CISD1* low expression groups (n = 541), and the clinical characteristics and gene expression data of 1083 patients with primary breast cancer were downloaded from the TCGA database to investigate the correlation between *CISD1* expression level and the patients' clinicopathological features. We found that *CISD1* expression was correlated with N stage (P = 0.012), M stage (P = 0.047), age (P = 0.022), race (P < 0.001), PR status (P < 0.001), and ER status (P < 0.001) by using the chi-square test or Fisher's exact test, whereas the Wilcoxon rank-sum test showed that *CISD1* expression was associated with age (P = 0.013) (Table 1).

Table 1 *CISD1* Expression in BRCA Patients with Different Clinical Parameters

Characteristic	Low Expression of *CISD1*	High Expression of *CISD1*	p
n	541	542	
T stage, n (%)			0.075
T1	148 (13.7%)	129 (11.9%)	
T2	296 (27.4%)	333 (30.8%)	
T3	80 (7.4%)	59 (5.5%)	
T4	16 (1.5%)	19 (1.8%)	
N stage, n (%)			0.012
N0	266 (25%)	248 (23.3%)	
N1	175 (16.4%)	183 (17.2%)	
N2	45 (4.2%)	71 (6.7%)	
N3	47 (4.4%)	29 (2.7%)	
M stage, n (%)			0.047
M0	451 (48.9%)	451 (48.9%)	
M1	5 (0.5%)	15 (1.6%)	
Age, n (%)			0.022
<=60	281 (25.9%)	320 (29.5%)	
>60	260 (24%)	222 (20.5%)	
Race, n (%)			< 0.001
Asian	23 (2.3%)	37 (3.7%)	
Black or African American	70 (7%)	111 (11.2%)	
White	405 (40.7%)	348 (35%)	
Pathologic stage, n (%)			0.099
Stage I	101 (9.5%)	80 (7.5%)	
Stage II	304 (28.7%)	315 (29.7%)	
Stage III	124 (11.7%)	118 (11.1%)	
Stage IV	5 (0.5%)	13 (1.2%)	
PR status, n (%)			< 0.001
Negative	145 (14%)	197 (19.1%)	
Indeterminate	3 (0.3%)	1 (0.1%)	
Positive	371 (35.9%)	317 (30.7%)	
ER status, n (%)			< 0.001
Negative	90 (8.7%)	150 (14.5%)	
Indeterminate	0 (0%)	2 (0.2%)	
Positive	430 (41.5%)	363 (35.1%)	
HER2 status, n (%)			0.647
Negative	290 (39.9%)	268 (36.9%)	
Indeterminate	6 (0.8%)	6 (0.8%)	
Positive	75 (10.3%)	82 (11.3%)	
Age, median (IQR)	60 (50, 68)	57 (48, 66)	0.013

Association Between *CISD1* Expression and Survival Prognosis of Cancer Patients

In addition, we studied the relationship between *CISD1* expression, overall survival (OS), and disease-related survival (DSS) in breast cancer patients. According to the KM diagram, patients with higher *CISD1* had a worse prognosis for OS (HR = 1.51, 95% CI: 1.09–2.09, P = 0.013) (Figure 3A), whereas for DSS, patients with higher *CISD1* still had a worse prognosis (HR = 1.66, 95% CI: 1.07–2.57, P = 0.024) (Figure 3B). Moreover, we conducted a receiver operating characteristic (ROC) curve to evaluate the applicability of *CISD1* expression in differentiating breast cancer from normal breast tissue, with an area under the ROC curve (AUC) of 0.718 (Figure 3C). We also conducted a time-dependent receiver operating characteristic (ROC) curve, in which the areas under the ROC curve (AUCs) of 1, 3, and 5 years were 0.575, 0.601, and 0.601, respectively (Figure 3D). Therefore, *CISD1* may be a promising biomarker for prognosis in breast cancer patients.

Furthermore, we performed a subgroup analysis to evaluate the effect of *CISD1* expression on OS according to T stage, N stage, M stage, pathological stage, age, PR status, ER status and Histological type and found that high

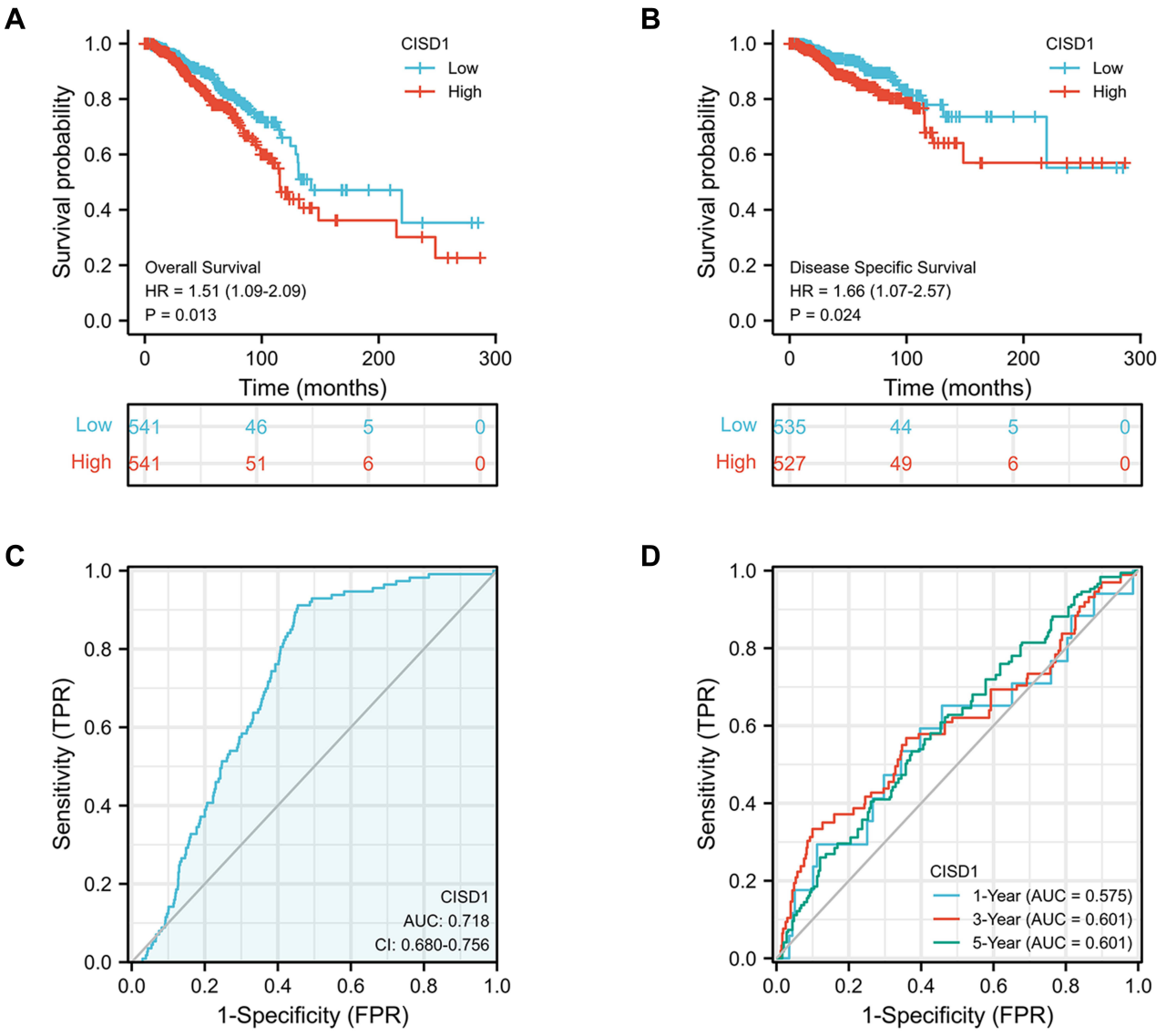

Figure 3 *CISD1* is an independent predictor of prognosis in BRCA. (**A** and **B**) Patients with low *CISD1* have significantly higher survival than patients with high *CISD1* (OS and DSS). (**C**) ROC analysis illustrated that *CISD1* expression accurately discriminated BRCA tumor tissues from normal tissues with an AUC of 0.718 (95% CI = 0.680–0.756) from TCGA-BRCA data sets. (**D**) ROC curves were used to assess the efficiency of *CISD1* for predicting 1-year, 3-year and 5-year survival rates in the TCGA-BRCA data sets.
Abbreviations: OS, overall survival; DSS, disease-specific survival; ROC, receiver operating characteristic; AUC, area under the curve.

expression of *CISD1* continued to lead to poor survival in each subgroup by T stage, N stage, M stage, pathological stage, age, PR status, ER status and Histological type (Figure 4A–H).

Correlation Between Methylation and *CISD1* Expression

We also used online tools to study the correlation between *CISD1* expression level and methylation status in breast cancer. First, we observed that most of the methylation sites in the *CISD1* DNA sequence are hypomethylated in breast cancer (Figure 5A). Moreover, the degree of methylation is related to the prognosis of patients. The total survival time of patients with low *CISD1* methylation levels was lower than that of patients with high *CISD1* methylation levels (Figure 5B).

Univariate and Multivariate Analysis of Survival

We conducted univariate and multivariate analyses to further explore the risk factors for BRCA patients, noting that univariate analysis using the Cox regression model showed that stages T3 and T4 of T stage, N-stage, M-stage, age, stages 3 and 4 of the pathological stage and *CISD1* were related to OS (Table 2). Then, we conducted a multivariate analysis to reveal independent risk factors, and we found that major M1 stage, age > 60 years and *CISD1* were independent prognostic factors for OS in hospitalized patients with BRCA.

Enrichment Analysis of *CISD1*-Related Genes

We downloaded data from the TCGA database to further study the function of *CISD1* and searched *CISD1* expression-related genes for related pathway analysis. Having obtained 20 top genes positively and negatively correlated with *CISD1* through the "cluster profile" R package for GO and KEGG enrichment analysis (Figure 6A and C), positive correlation gene analysis data

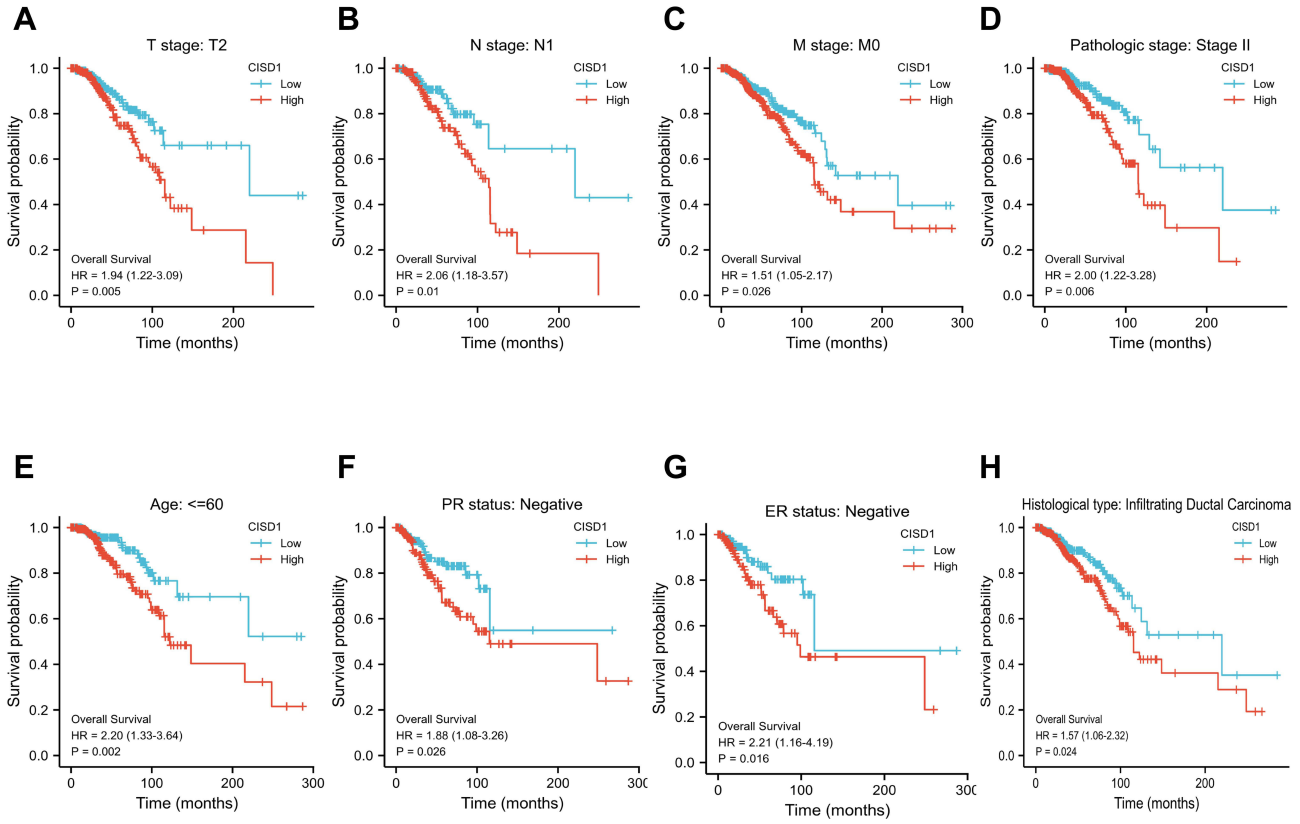

Figure 4 Subgroup analysis in BRCA. (**A**) Kaplan–Meier curves for the T2 stage subgroup. (**B**) Kaplan–Meier curves for the N1 stage subgroup. (**C**) Kaplan–Meier curves for the M0 stage subgroup. (**D**) Kaplan–Meier curves for the pathologic stage II subgroup. (**E**) Kaplan–Meier curves for the age <=60 years subgroup. (**F**) Kaplan–Meier curves for the negative PR status subgroup. (**G**) Kaplan–Meier curves for the negative ER status subgroup. (**H**) Kaplan–Meier curves for the Histological type subgroup.

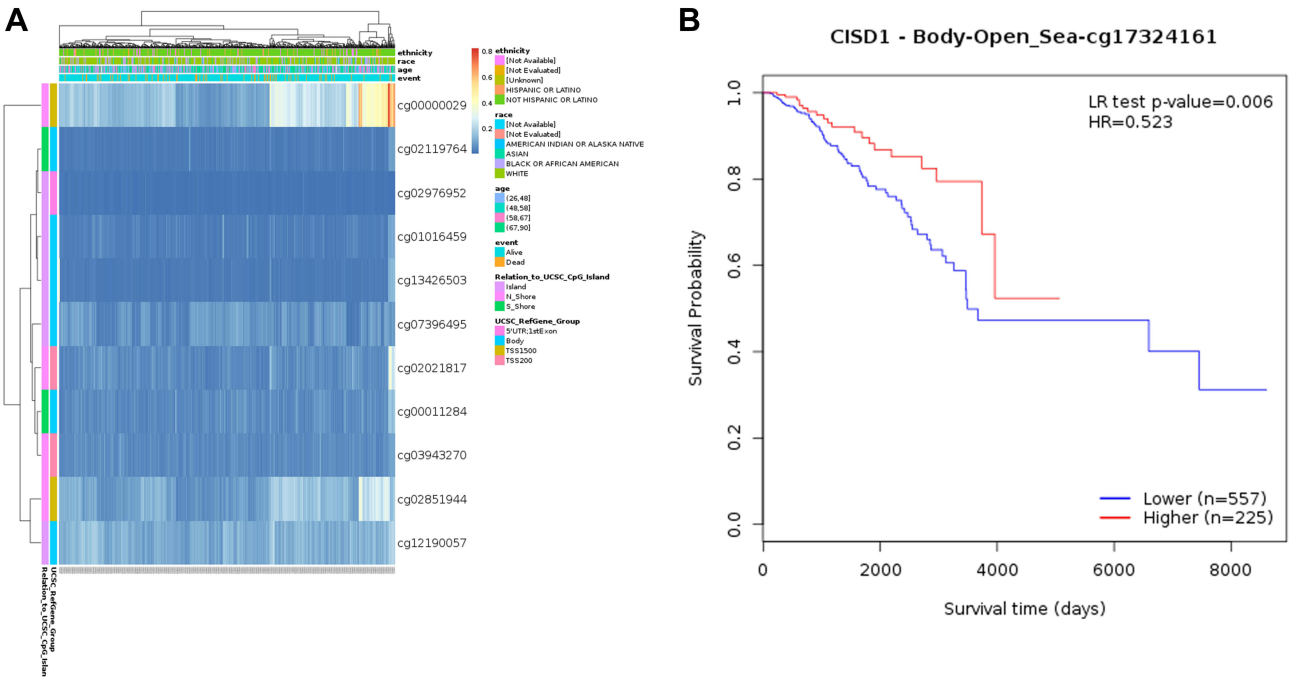

Figure 5 DNA methylation level of *CISD1* and its impact on the prognosis of breast cancer patients. (**A**) Correlation between *CISD1* mRNA expression level and methylation level. (**B**) Kaplan–Meier curves of *CISD1* methylation level.

showed that most genes were related to the rRNA metabolic process, rRNA processing and mitochondrial RNA metabolic process (Figure 6B). In turn, the data of the negative correlation gene analysis showed that most genes were related to the cell cortex part, cortical cytoskeleton, and cortical actin cytoskeleton (Figure 6D).

We uploaded the upregulated and downregulated genes to the CMap (connectivity map) database to predict potential drugs for breast cancer treatment. The top 10 drugs/molecules with positive correlations and the top 10

Table 2 Univariate Analysis and Multivariate Analysis of the Correlation Between Clinicopathological Characteristics and OS in BRCA

Characteristics	Total (N)	Univariate Analysis		Multivariate Analysis	
		Hazard Ratio (95% CI)	P value	Hazard Ratio (95% CI)	P value
T stage	1079				
T1	276	Reference			
T2	629	1.332 (0.887–1.999)	0.166	0.933 (0.468–1.863)	0.845
T3&T4	174	1.953 (1.221–3.123)	0.005	0.877 (0.369–2.083)	0.766
N stage	1063				
N0	514	Reference			
N1	357	1.956 (1.329–2.879)	<0.001	1.346 (0.779–2.328)	0.287
N2	116	2.519 (1.482–4.281)	<0.001	1.483 (0.565–3.896)	0.424
N3	76	4.188 (2.316–7.574)	<0.001	2.204 (0.862–5.632)	0.099
M stage	922				
M0	902	Reference			
M1	20	4.254 (2.468–7.334)	<0.001	5.359 (1.263–22.739)	0.023
Age	1082				
<=60	601	Reference			
>60	481	2.020 (1.465–2.784)	<0.001	2.381 (1.605–3.532)	<0.001

(*Continued*)

Table 2 (Continued).

Characteristics	Total (N)	Univariate Analysis		Multivariate Analysis	
		Hazard Ratio (95% CI)	P value	Hazard Ratio (95% CI)	P value
Pathologic stage	1059				
Stage I	180	Reference			
Stage II	619	1.697 (0.985–2.922)	0.057	1.410 (0.558–3.562)	0.468
Stage III	242	2.962 (1.664–5.273)	<0.001	2.607 (0.713–9.531)	0.147
Stage IV	18	11.607 (5.569–24.190)	<0.001		
CISD1	1082	1.514 (1.179–1.944)	0.001	1.448 (1.060–1.978)	0.020
PR status	1029				
Negative	342	Reference			
Positive	687	0.732 (0.523–1.024)	0.068	0.829 (0.452–1.519)	0.543
ER status	1032				
Negative	240	Reference			
Positive	792	0.712 (0.495–1.023)	0.066	0.594 (0.315–1.121)	0.108

drugs/molecules with negative correlations were obtained from CMap, and these drugs/molecules were ranked by score (Supplementary Tables 1 and 2). After searching these 20 drugs, we found that BMS-345541, PIK-75, etoposide, triptolide, taurocholic acid, lonidamine, 17 beta estradiol, hydrastine, and MK-1775 can be used as potential drugs to treat breast cancer patients. These data confirm the validity of the CMap database.

Relationship Between *CISD1* Expression and Immune Cell Infiltration

We further evaluated whether *CISD1* expression levels were associated with immune cell infiltration. We used ssgsea and Spearman's R from the R package to study the potential association between *CISD1* expression levels and 24 immune cell types (Figure 7). The results showed that *CISD1* expression was significantly correlated with B cells, eosinophils, macrophages, neutrophils, NK cells, T helper cells, Tregs, Th1 cells, Th17 cells, and Th2 cells. Further studies showed that the expression of *CISD1* was positively correlated with the infiltration levels of B cells, macrophages, neutrophils, T helper cells, Tregs, Th1 cells, and Th2 cells. In contrast, *CISD1* expression was negatively correlated with eosinophils, NK cells, and Th17 cells (Figure 8A–J).

We also used the TIMER database to explore the immune microenvironment and determined the correlation between the level of immune invasion in breast cancer and the expression of the *CISD1* gene (Figure 9A–L). The results showed that the expression of *CISD1* was positively correlated with CD8 T cells, CD4 T cells, neutrophils, macrophages, plasmoid dendritic cells, and MDSCs and negatively correlated with mast cells and NK cells.

CISD1 is Highly Expressed in Breast Cancer Tissues

We used qPCR and immunohistochemistry to evaluate the potential utility of *CISD1* as a BRCA biomarker and to further verify the expression of *CISD1* in breast cancer tissues. While both qPCR and immunohistochemical results suggested that *CISD1* is highly expressed in breast cancer (Figure 10A and B), we then transfected *CISD1*-targeted siRNA into the MCF7 cell line, with CCK8 results showing a decrease in the proliferation ability of si-MCF7 cells (Figure 10C).

Discussion

Breast cancer is the most frequently diagnosed cancer among women, ranking second among the causes of cancer-related deaths in women.[16,17] It becomes necessary to find accurate biomarkers to detect and monitor disease progression early. In turn, according to previous studies, *CISD1* is overexpressed in a variety of cancers and has been identified as a prognostic factor,[18–20] but no relationship between the expression of *CISD1* and the prognosis of breast cancer has been studied.

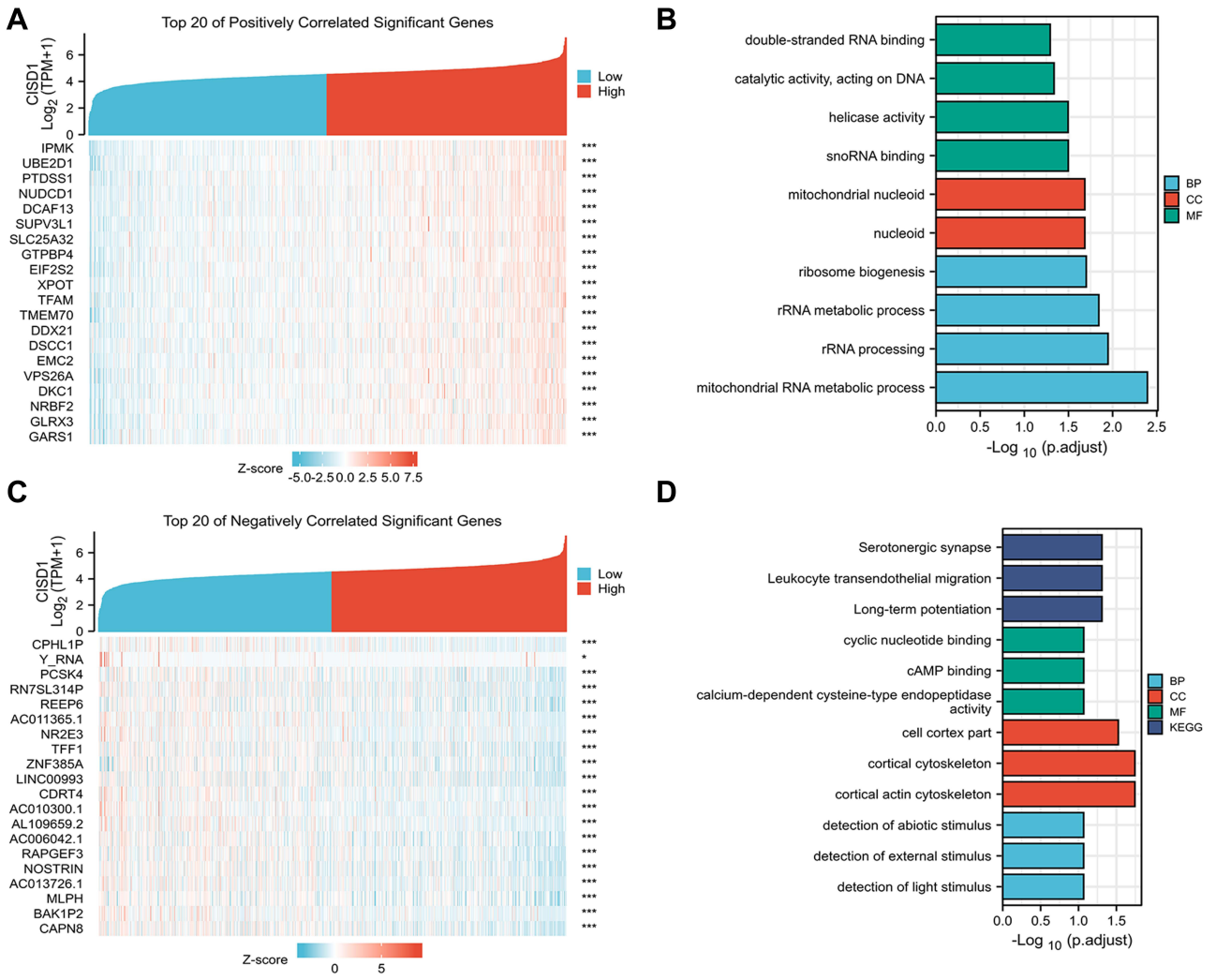

Figure 6 Gene enrichment analysis of *CISD1* in TCGA-BRCA data sets. (**A**) Heatmaps showing genes positively correlated with *CISD1* in BRCA (top 20). (**B**) Enriched GO terms and KEGG pathways of positively correlated *CISD1* genes. (**C**) Heatmaps showing genes negatively correlated with *CISD1* in BRCA (top 20). (**D**) Enriched GO terms and KEGG pathways of *CISD1* negatively correlated genes. P<0.05 indicates that the data are statistically significant; NS P>0.05, *P < 0.05, ***P < 0.001.

In this study, we explored the potential mechanism of *CISD1* in promoting breast cancer and its feasibility as a molecular biomarker. In the pancancer analysis, while the higher expression of *CISD1* was associated with a decrease in the overall survival (OS) of breast cancer patients, we also found that *CISD1* was upregulated in most cancer types. Similarly, analysis of different clinical stages found that *CISD1* was significantly correlated with clinical stages, and univariate and multivariate Cox analyses showed that *CISD1* was an independent factor predicting the prognosis of patients. All these results, including the ROC analysis, suggest that *CISD1* may be a prognostic biomarker for breast cancer patients.

Ferroptosis is a kind of cell death that plays a vital role in inhibiting tumorigenesis by removing cells lacking or excessive key nutrients or damaged by environmental pressure.[21] Unlike autophagy and apoptosis, ferroptosis is a form of cell death that depends on iron (FE) and reactive oxygen species (ROS). It regulates cell death through excessive production of phospholipid hydroperoxide, and its mechanism is different from autophagy and apoptosis.[22] Recent studies have shown that ferroptosis can affect cell metabolism, redox status, degenerative diseases, and ischemic reperfusion injury.[23] Ferroptosis plays an important regulatory role in the occurrence and development of tumors, providing a promising treatment strategy for BRCA.[24,25] CDGSH iron sulfur domain 1 (*CISD1*) is a mitochondrial protein located in the outer membrane that plays an

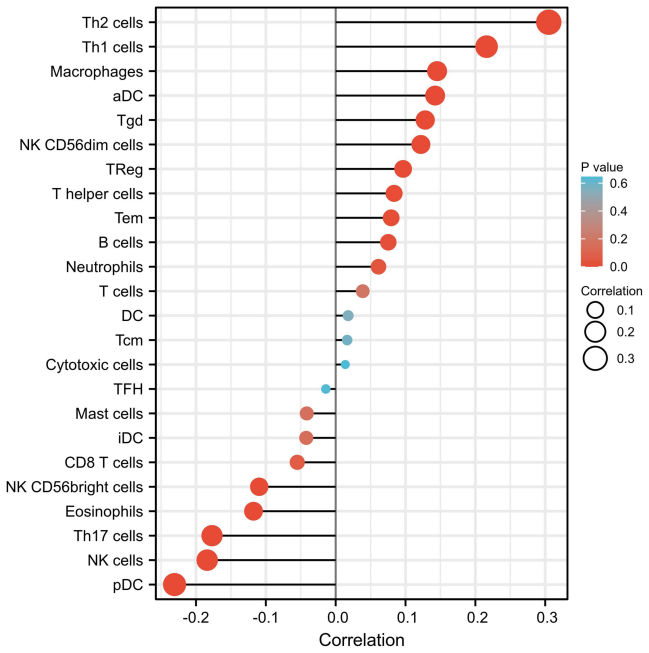

Figure 7 The association between *CISD1* expression and 24 tumor-infiltrating lymphocytes.

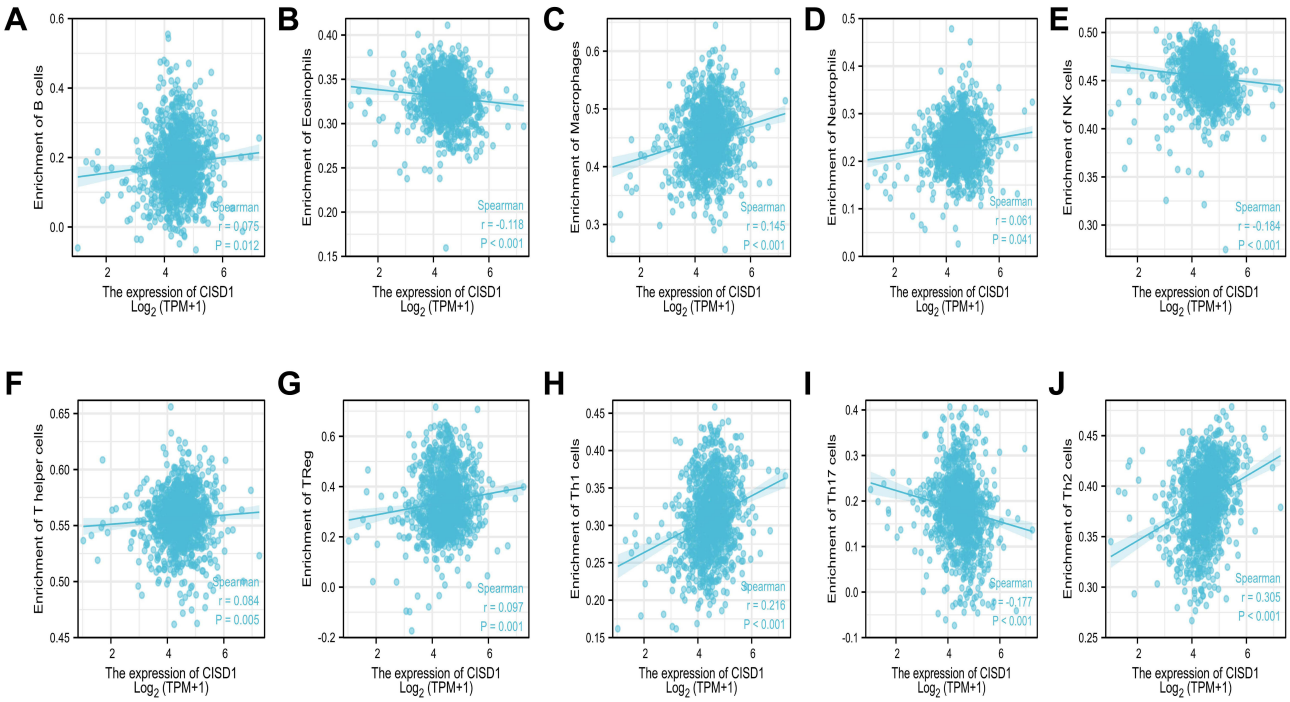

Figure 8 The correlation of *CISD1* expression with the immune infiltration level of tumor-infiltrating lymphocytes. (**A**) B cells. (**B**) Eosinophils. (**C**) Macrophages. (**D**) Neutrophils. (**E**) NK cells. (**F**) T helper cells. (**G**) Tregs. (**H**) Th1 cells. (**I**) Th17 cells. (**J**) Th2 cells.

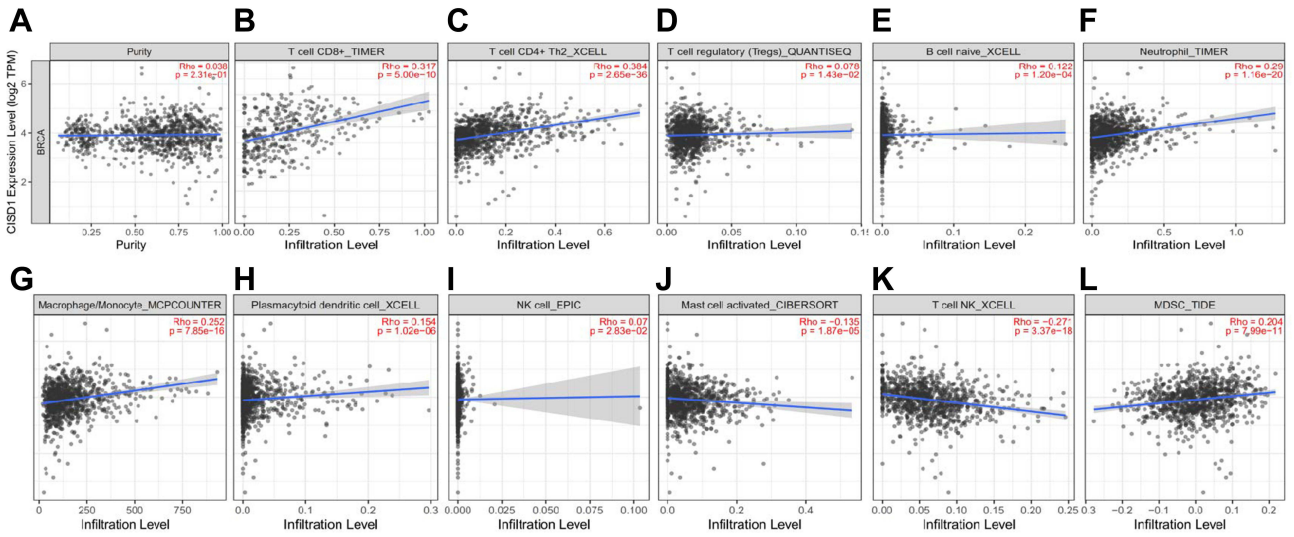

Figure 9 (**A**–**L**) Scatter plot of the correlation between the expression of the *CISD1* gene and the level of immune invasion in breast cancer.

important role in mediating the crosstalk between mitochondrial iron uptake and oxidative stress in normal and cancer cells. Upregulation of *CISD1* protein expression in cancer cells limits autophagy activity.[26]

In turn, the tumor microenvironment (TME) plays an important role in tumor progression, metastasis, and therapeutic drug resistance.[27] Tumor-infiltrating immune cells affect the tumor microenvironment and tumor proliferation, invasion, and migration. Our gene enrichment analysis shows that the biological function of *CISD1* involves an immune response, which likewise confirms that *CISD1* expression was associated with immune cell infiltration.

While studies have shown that eosinophils can produce cytotoxicity by releasing particles or regulate the immune response, especially by attracting CD8+ T cells,[28] and eosinophils can also inhibit the growth of colorectal cancer through IL-33,[29] there is a correlation in breast cancer between high baseline eosinophil counts and better response to treatment or survival rate.[30] For peripheral NK cells, the increase in NK cytotoxic activity is positively correlated with the decrease in cancer risk,[31] whereas in BRCA, the abundance of NK cells can also reflect a good survival rate.[32]

Recent studies have shown that Th17 cells are usually associated with a variety of cancers, including lung cancer, breast cancer, prostate cancer, colon cancer, and melanoma.[33] In BRCA, the increase in Th17-cell number enhances the antitumour immune response in BC tissue,[34] with the abundance of Th17 cells increasing and BRCA having a better prognosis.[35] Our study found that the expression of *CISD1* in breast cancer is negatively correlated with eosinophils, NK cells, and Th17 cells, suggesting that *CISD1* may play an important role in regulating the breast cancer immune microenvironment. Additionally, we also conducted PCR and immunohistochemical studies on the clinical samples of breast cancer, and the results suggested that *CISD1* is highly expressed in breast cancer and that the proliferation of MCF7 cells with *CISD1* silencing also decreased.

In conclusion, we demonstrated that *CISD1* expression was upregulated in breast cancer and was significantly associated with poor survival, while *CISD1* may also participate in the development of breast cancer by affecting the level of immune cell infiltration. The present study thus reveals the role of *CISD1* in breast cancer and identifies a promising biomarker for prognosis. The mechanism by which *CISD1* affects the tumor immune microenvironment and tumor progression in BRCA remains unclear and needs further basic and clinical trials to fully elucidate its biological effects.

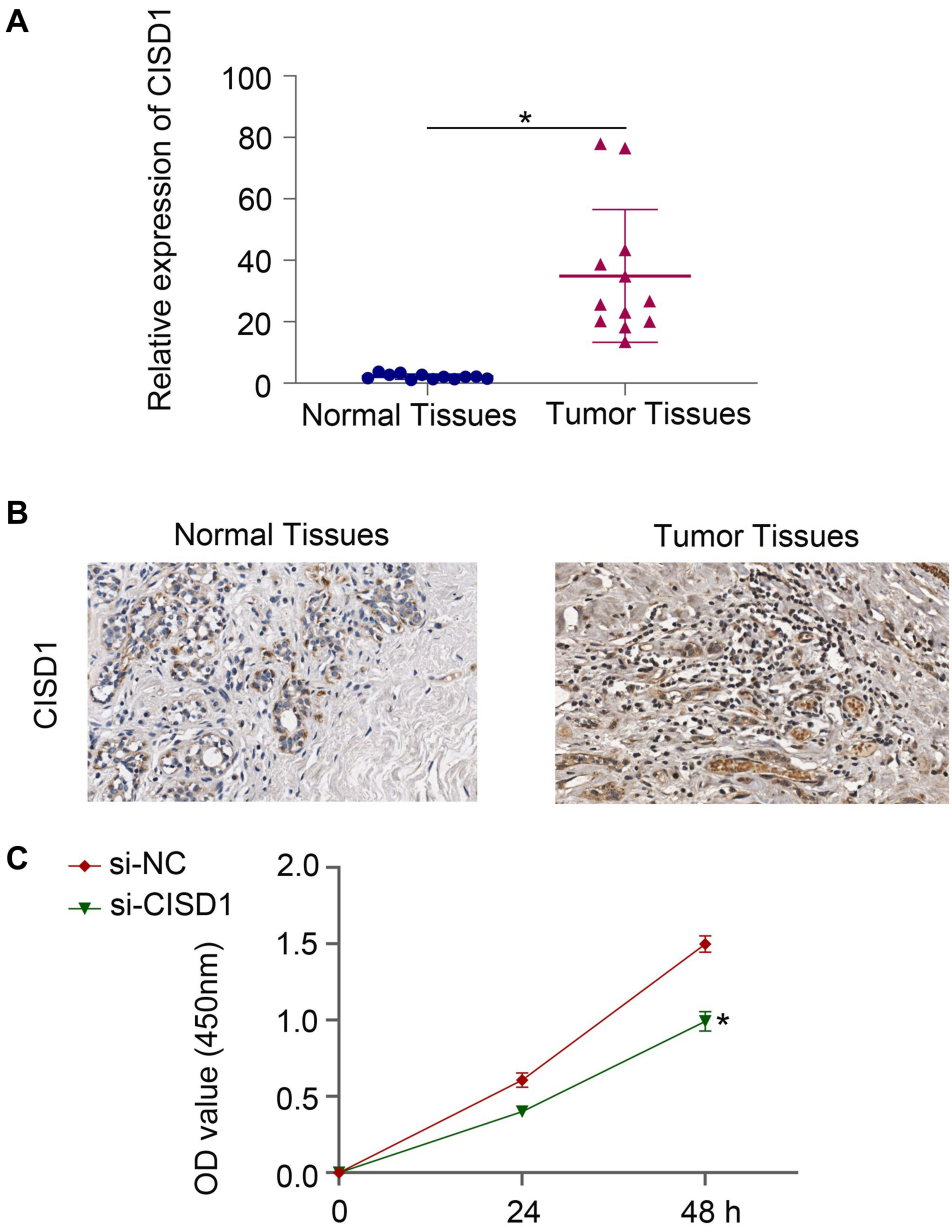

Figure 10 (**A** and **B**) The expression level of *CISD1* in BRCA breast cancer tissues and matched nontumor tissues. (**C**) Expression of the *CISD1* gene in MCF7 cells was silenced using RNA interference technology. Proliferation was significantly reduced in the si-*CISD1*. P<0.05 indicates that the data are statistically significant; *P < 0.05.

Conclusion

Overall, the high expression of *CISD1* was associated with prognostic significance. *CISD1* was negatively correlated with eosinophils, NK cells, and Th17 cells, which may be related to immune infiltration and can therefore be used as a prognostic factor in patients with BRCA.

Data Sharing Statement

The data supporting the findings of this study are available through OPEN ACCESS, as well as from the corresponding author upon request.

Ethics Approval and Informed Consent

The study was conducted in accordance with the declaration of Helsinki. The study was also approved by the Ethics Committee of Liaoning Cancer Hospital, number: 2021-239.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that there are no conflicts of interest.

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