Original Article

# Effect of caspase-1 (*CASP1*) combined with multimodal ultrasound features on the prognosis of breast cancer patients

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**Background:** Breast cancer (BRCA) is the malignant tumor with the highest incidence rate among women in the world, and its mortality rate ranks second. The purpose of our study is to explore the correlation between caspase-1 (*CASP1*) and the prognosis of BRCA patients and the potential mechanism of action, and to analyze the clinical value of *CASP1* combined with multimodal ultrasound features in early screening and prognosis of BRCA.

**Methods:** We analyzed The Cancer Genome Atlas (TCGA) database to confirm that *CASP1* was expressed in BRCA patients and determine whether its expression was correlated with patient prognosis. The relationship between *CASP1* expression and survival was measured by the clinicopathological parameters. Multivariate analysis was performed using Cox regression, and a nomogram was developed using these results for quality assurance purposes. The correlations between *CASP1* and immune cells were investigated using the Tumor Immune Estimation Resource (TIMER) and TCGA databases. Next, we performed gene set enrichment analysis (GSEA) to determine the potential mechanism of action. Finally, to analyze the effect of *CASP1* combined with multimodal ultrasonography characteristics on the prognosis of BRCA patients was studied by analyzing the clinical data of patients.

**Results:** *CASP1* expression was lower in BRCA tumor tissues than in the surrounding tissues. Patients with high *CASP1* expression had better overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI) than those with low *CASP1* expression. GSEA suggested that *CASP1* may affect the cell cycle, immune environment, inflammation, apoptosis, the HIPPOMERLIN pathway, Natural killer (NK) cell regulation of cytotoxicity, p53 expression, the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway, the mitogen-activated protein kinase (MAPK) pathway, extracellular matrix, etc., thereby influencing the biological events in BRCA. Among conventional ultrasound features and contrast-enhanced ultrasound (CEUS) features, mass margin status and blood flow grade were associated with the expression of *CASP1*. Meanwhile, patients with poor ultrasound features tended to have low *CASP1* expression.

**Conclusions:** *CASP1* may be a novel predictive marker for BRCA patients. *CASP1* combined with multimodal ultrasound features has good clinical value in the early screening and prognostic prediction of BRCA.

**Keywords:** Caspase-1 (*CASP1*); breast cancer (BRCA); multimodal ultrasound features; prognosis; bioinformatics

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## Introduction

Breast cancer (BRCA) is a common malignancy and ranks first in terms of incidence among female malignancies worldwide (1,2). As a complex heterogeneous disease, BRCA exhibits marked variability in molecular characteristics and malignancy (3). Although treatment methods for BRCA have been continuously developed in recent years, comprehensive methods including surgery, endocrine therapy, chemotherapy, radiotherapy, and targeted therapy have significantly improved the curative effect (4,5), and the overall survival (OS) status of patients with malignant tumors is better than that of other malignant tumor patients. However, 5–30% of patients still have metastasis or recurrence after surgery (6). At present, there is still a lack of effective treatment methods, especially for advanced patients (7,8). Therefore, continued investigation into the functional genes associated with BRCA incidence and progression as well as novel BRCA pathogenesis and therapeutic targets is of critical importance.

At present, relevant studies have shown that different genes play a role in biological events of BRCA (9-11). Caspase (CASP) refers to a group of cysteine aspartate-specific proteases (12). Caspase-1 (*CASP1*), as one of the earliest discovered members of the CASP family, not only participates in cytokine-mediated inflammatory responses but also has an inseparable relationship with the process of apoptosis (13,14). Research has linked *CASP1* to the onset and progression of several different cancers. For example, euchromatic histone lysine methyltransferase 2 (G9A) silences *CASP1* in non-small cell lung cancer cells, which enhances tumor cell proliferation and invasion (15); clusters differentiation (CD)44 standard isoforms and CASP—a novel interaction between the *CASP1*/interleukin-1β (IL-1β) pathway induces hepatocellular carcinoma progression (16); the tumorigenicity of prostate cancer is reduced by inhibiting cytochrome P450 family 1 subfamily B member 1 (P450 1B1), mediated by the activation of *CASP1* (17). In hypopharyngeal squamous cell carcinoma, inhibition of *CASP1* activation can increase the tumor's sensitivity to radiation therapy, and *CASP1* may inhibit endometrial cancer growth by promoting cell apoptosis (18,19). However, the role of *CASP1* in BRCA is relatively understudied.

Ultrasound, as a simple, non-radiation, non-invasive, real-time examination technology, is the first choice for screening and diagnosis of female BRCA. Conventional ultrasound, shear wave elastography, and contrast-enhanced ultrasound (CEUS), as commonly used methods for detecting breast lesions, have been widely accepted and recognized by patients and clinical doctors in the diagnosis of breast lesions and the evaluation of chemotherapy efficacy (20,21).

The primary aim of our research was to determine whether or not *CASP1* expression can be used to predict the survival rate of BRCA patients using various databases, including The Cancer Genome Atlas (TCGA). We also conducted bioinformatics analysis and validation to further investigate the impact of *CASP1* on tumor cell behavior and its possible mechanism. Finally, we pooled clinical and ultrasonographic data from BRCA patients at our hospital to investigate the clinical parameters that could assess the effect of *CASP1* on BRCA patients and predict their prognosis. Our findings suggested novel approaches for diagnosing and treating BRCA in clinical practice. We present this article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1135/rc).

### Highlight box

**Key findings**

- Elevated expression of *CASP1* can prolong the OS, DSS, and PFI of BRCA patients, and may be a novel predictive marker for these patients.

**What is known and what is new?**

- Research has linked *CASP1* to the onset and progression of several different cancers.
- This study determined whether *CASP1* expression could be used to predict the survival rate of BRCA patients, and verified the effect of *CASP1* on the behavior of tumor cells as well as its possible mechanism through bioinformatics analysis.

**What are the implications, and what should change now?**

- This study confirmed the potential of *CASP1* as a biomarker for BRCA disease progression. *CASP1* combined with multimodal ultrasound features has good clinical value in the early screening and prognostic prediction of BRCA.

## Methods

### *Data analysis using TCGA database*

We downloaded mRNA expression data for various cancer types from the TCGA database, standardized these data, and then performed differential expression analysis of *CASP1* using R packets. We used TCGA database to

explore the expression of *CASP1* expression in pan-cancer, and also analyzed the expression of *CASP1* in BRCA and adjacent tissues.

### *Relationship between CASP1 and the clinical parameters of BRCA patients in TCGA database*

We retrieved the clinical parameters of BRCA patients from TCGA database and compared and evaluated them to determine whether there is a relationship between *CASP1* and the prognosis of these patients. Based on the logarithmic rank test and Mantel Cox test for gene expression in hepatocellular carcinoma, timeROC analysis was performed to compare the predictive accuracy and risk score of *CASP1* gene.

### *Nomogram construction and evaluation*

We generated personalized predicted survival probabilities for 1, 3, and 5 years using a column chart based on the outcomes of our multivariate analysis. The creation of column charts, encompassing clinical variables associated with *CASP1* as well as calibration charts, was facilitated using the RMS R package. Model performance evaluation predominantly relies on calibration and discrimination techniques. In this study, the calibration curve was assessed visually by aligning the projected probabilities from the column plot against the actual ratios, with an optimal prediction indicated by a 45-degree line. To gauge the discriminative capability of the column chart, we employed the concord index (C-index), computed through 1,000 bootstrap resampling iterations. Furthermore, the C-index was employed to juxtapose the predictive precision of column charts against individual prognostic factors.

### *Exploration of genes associated with CASP1 through GeneMANIA and STRING databases*

GeneMANIA database helps form hypotheses regarding gene functions, enables the analysis of gene sets, and assists in ranking genes for functional scrutiny. By inputting a gene list, GeneMANIA delves into vast genomic and proteomic datasets to identify genes of analogous functions. Furthermore, it can forecast the role of a particular gene and pinpoint others potentially having shared roles based on mutual interactions. The STRING database (https://string-db.org/) serves to identify protein-protein interactions (PPIs), encompassing both immediate physical connections and inferred functional associations between proteins. We constructed a PPI network using data from the GeneMANIA (https://genemania.org/) and STRING (https://cn.string-db.org/) databases, which included information on protein-DNA, protein-protein, genetic interactions, pathways, phenotype screening, protein domains, gene and protein expression, and physiological and biochemical reactions.

### *Comparison of the promoter methylation levels in different BRCA patients in the University of ALabama at Birmingham CANcer data analysis Portal (UALCAN) database*

We utilized the UALCAN database for Clinical Proteomic Tumor Analysis Consortium (CPTAC) analysis to assess and compare the methylation level of the *CASP1* promoter in different BRCA patients.

### *Correlation between CASP1 and immune cells in the Tumor Immune Estimation Resource (TIMER) and TCGA databases*

We comprehensively analyzed the expression of tumor infiltrating immune cells (TIICs) through TCGA database and TIMER database (https://cistrome.shinyapps.io/timer/), and analyzed the correlation between *CASP1* expression level and immune cell infiltration level or immune checkpoint expression level in BRCA. A P value of $<0.05$ is considered statistically significant.

### *Gene set enrichment analysis (GSEA)*

Metascape was used to perform online functional analysis on the differentially expressed genes.

### *Clinical data of BRCA patients in our hospital*

The clinical data of BRCA patients admitted to Jiangsu Cancer Hospital from January 2019 to January 2022 were retrospectively analyzed. The inclusion criteria were as follows: (I) aged 18–85 years; (II) patients who received a preoperative gray-scale ultrasound, color Doppler flow imaging (CDFI), and CEUS examination; (III) patients who did not undergo radiotherapy, chemotherapy, or endocrine therapy before the examination; (IV) no surgical contraindications; (V) good cognitive function and normal communication; (VI) good compliance and can cooperate

 

to complete the relevant inspections; (VII) pathologically confirmed BRCA postoperatively. The exclusion criteria were as follows: (I) combined with serious basic diseases, such as liver, kidney, cardiovascular, and cerebrovascular diseases; (II) women who were pregnant, breastfeeding, or had a prosthesis implanted; (III) patients who could not accept CEUS examination due to their own conditions.

### *Evaluation of ultrasonic features and evaluative criteria*

The LOGIQ E9 (GE, USA) and LOGIQ E20 (GE) ultrasonic diagnostic instruments were used; gray-scale ultrasound and CDFI were performed using ML6-15 high-frequency linear array probes with a frequency of 6–15 MHz; CEUS used 9L probes with a frequency of 9 MHz; and the contrast agent utilized the Sono dimension. During the CEUS imaging examination, the patient is placed in the supine position, with both hands lifted to fully expose both breasts, and is examined by gray-scale ultrasound and CDFI to determine the location of the lesion. The image report and data system grading standard observes and describes the lesion size, rear echo, tumor margin, presence or absence of microcalcification, and blood flow grading. Next, the probe is replaced and adjusted to contrast mode, and the normal breast tissue around the lesion area is selected as the area of interest. Three mL of contrast medium is then quickly injected through the cubital vein, followed by an immediate injection of 5 mL of 0.9% normal saline. The probe is kept still and continuously observed, and the mass is saved in real-time. The following aspects are analyzed during the dynamic perfusion process: the enhancement intensity, post-enhancement range, enhancement mode of the lesion, whether it is earlier than the surrounding normal tissue enhancement or regression, perfusion distribution, and whether there is a perfusion defect. The CDFI function observes the blood flow inside the tumor, implements multi slice pulse imaging sampling for local microvessels, and calculates the maximum peak systolic velocity (PSV) and blood flow resistance index (RI).

### *Statistical analysis*

GSEA was utilized to analyze the potential cellular mechanism of *CASP1*, and the statistical analysis and visualization were performed using R (version 3.6.3) (https://www.r-project.org/) software. Patient survival was determined using the Kaplan-Meier method, and statistical significance was determined using the log-rank test. According to the receiver operating characteristic (ROC) curve, the Jordan index was calculated and the optimal critical value for *CASP1* was 23%. Patients were subsequently categorized into the *CASP1* high-expression group and the *CASP1* low-expression group based on whether their *CASP1* expression values exceeded 23%. The statistical analyses in this study were automatically computed by the aforementioned online database. $P<0.05$ or log-rank $P<0.05$ was considered to indicate statistical significance.

### *Ethical statement*

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics committee of Jiangsu Cancer Hospital (No. 2022-015) and informed consent was taken from all the patients.

## Results

### *Down-regulation of CASP1 expression in BRCA*

We found that our research molecule is *CASP1* in the TCGA database. Through pan-cancer analysis, we found that *CASP1* expression differs between tumors, such as cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), and kidney renal clear cell carcinoma (KIRP). *CASP1* expression was also found to be significantly down-regulated in bladder urothelial carcinoma (BLCA), colon adenocarcinoma (COAD), BRCA, lung adenocarcinoma (LUAD), and other tumor tissues ($P<0.05$, *Figure 1A*). At the same time, in unpaired and paired BRCA tumor tissues, the expression of *CASP1* was lower than that in paracancerous tissues (*Figure 1B*,*1C*). A ROC curve was then constructed, which showed an area under the curve (AUC) of 0.630, indicating that *CASP1* has a certain predictive accuracy (*Figure 1D*).

### *Correlation between CASP1 in TCGA database and the prognosis of BRCA patients*

We found that BRCA patients with high *CASP1* expression had better OS, disease-specific survival (DSS), and progression-free interval (PFI) in TCGA database (*Figure 2*).

 

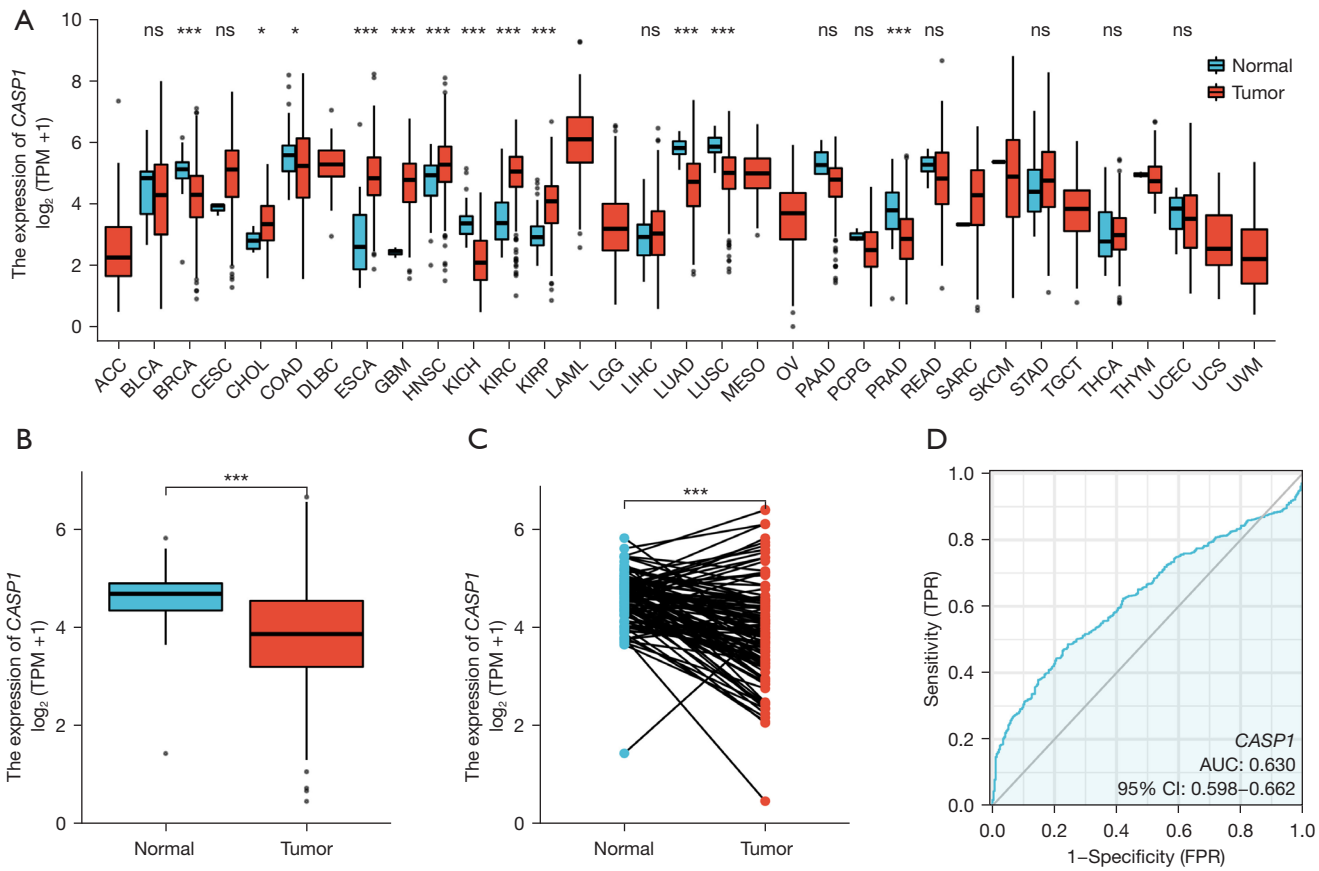


**Figure 1** Downregulation of *CASP1* expression in BRCA. (A) The *CASP1* expression level in pan-cancer in TCGA database; (B) the *CASP1* expression level in unpaired BRCA cancer tissues (n=1,109) and adjacent tissues (n=113) in TCGA database; (C) the *CASP1* expression level in paired BRCA cancer tissues (n=112) and adjacent tissues (n=112) in the TCGA database; (D) ROC curve analysis showing that *CASP1* expression in tumor and normal organizations have a certain ability to differentiate. *, P<0.05; ***, P<0.001, the difference is statistically significant; ns, not significant. *CASP1*, caspase-1; TPM, transcripts per million; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast cancer; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal clear cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate Adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; TPR, true positive rate; FPR, false positive rate; AUC, area under the curve; CI, confidence interval; TCGA, The Cancer Genome Atlas; ROC, receiver operating characteristic.

### *Nomogram construction*

According to the multivariate analysis findings of TCGA database, we built a nomogram to predict the 1-, 3-, and 5-year survival rates of BRCA patients. The C-index of the nomogram was 0.691 (0.670–0.702) (*Figure 3A*). At the same time, the predictive value of the nomogram was shown by its calibration curve (*Figure 3B*).

### *Identification of CASP1-interacting genes and proteins*

We constructed a gene-gene interaction network of *CASP1*

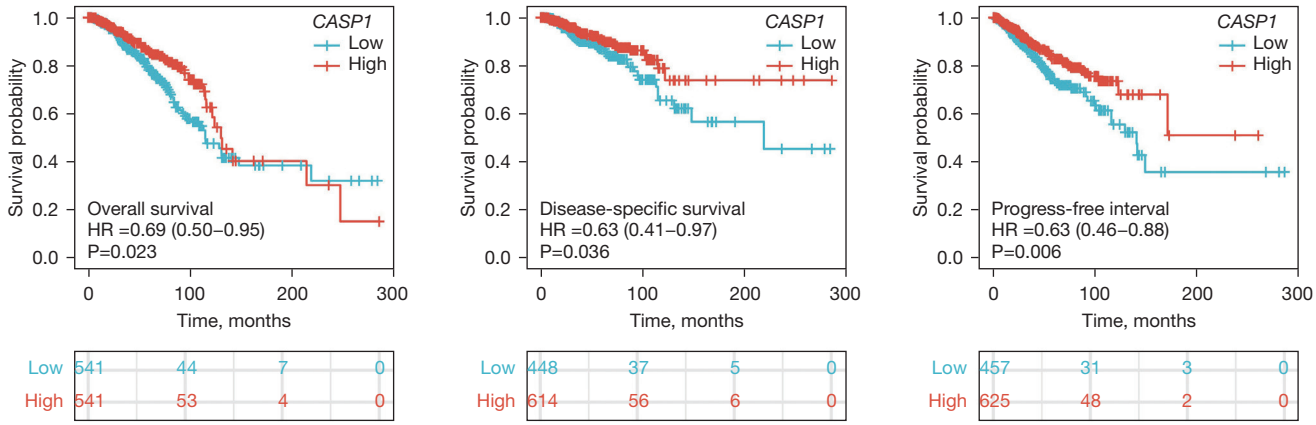


**Figure 2** Correlation of *CASP1* with BRCA patient prognosis (OS, DSS, and PFI) in TCGA database. *CASP1*, caspase-1; HR, hazard ratio; BRCA, breast cancer; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval; TCGA, The Cancer Genome Atlas.

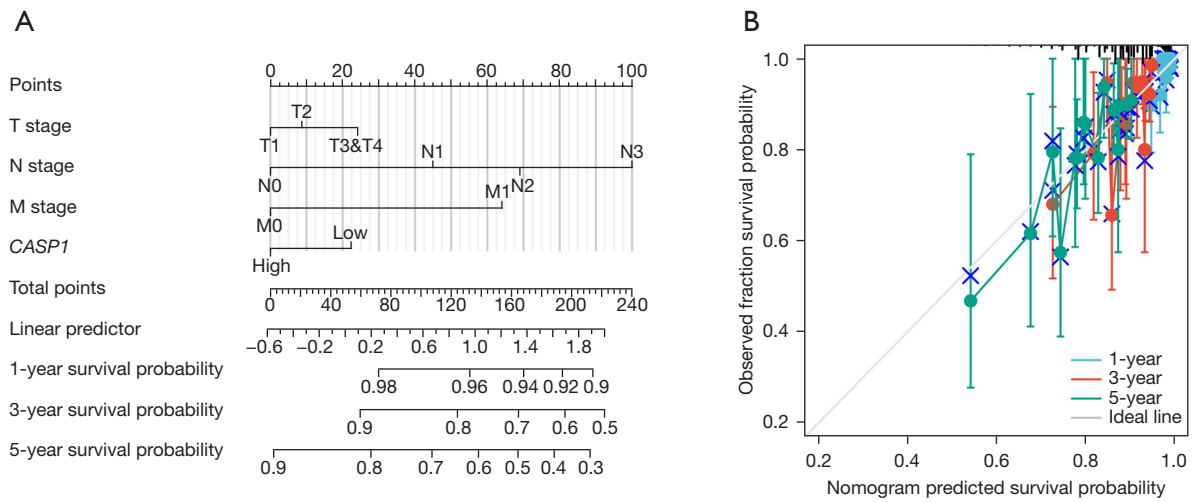


**Figure 3** Nomograms and calibration plots for BRCA patients. (A) Nomogram for predicting the 1-, 3-, and 5-year survival in BRCA patients; (B) calibration plot of the nomogram for predicting the OS likelihood. *CASP1*, caspase-1; BRCA, breast cancer; OS, overall survival.

and altered the adjacent genes using the GeneMANIA database (*Figure 4A*). *CASP1*'s PPI network was built using the STRING database (*Figure 4B*).

### *Promoter methylation of CASP1 in different BRCA types*

Previous study has shown that promoter DNA methylation affects transcriptional repression and participates in tumorigenesis and development (22). We performed a stratified analysis on BRCA patients and found that the promoter methylation level of *CASP1* varied between BRCA patients. Therefore, we speculate that the expression of transcribed *CASP1* may be due to changes in promoter methylation (*Figure 5*).

### *Correlation between CASP1 and immune cells in the TIMER and TCGA databases*

Using the TIMER database, we showed a correlation between *CASP1* expression, tumor purity, and various immune cells in BRCA. We observed that *CASP1* expression is positively correlated with six types of TIICs,

 

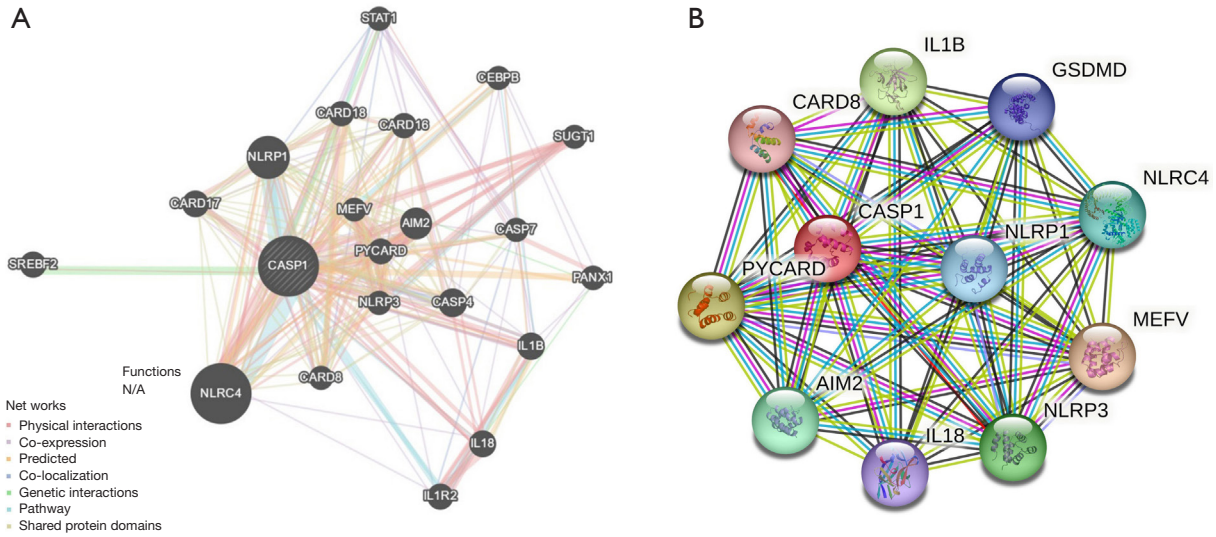


**Figure 4** Identification of *CASP1*-interacting genes and proteins. (A) Gene-gene interaction network of *CASP1* in the GeneMANIA database; (B) PPI network of *CASP1* in the STRING database. *CASP1*, caspase-1; PPI, protein-protein interaction.

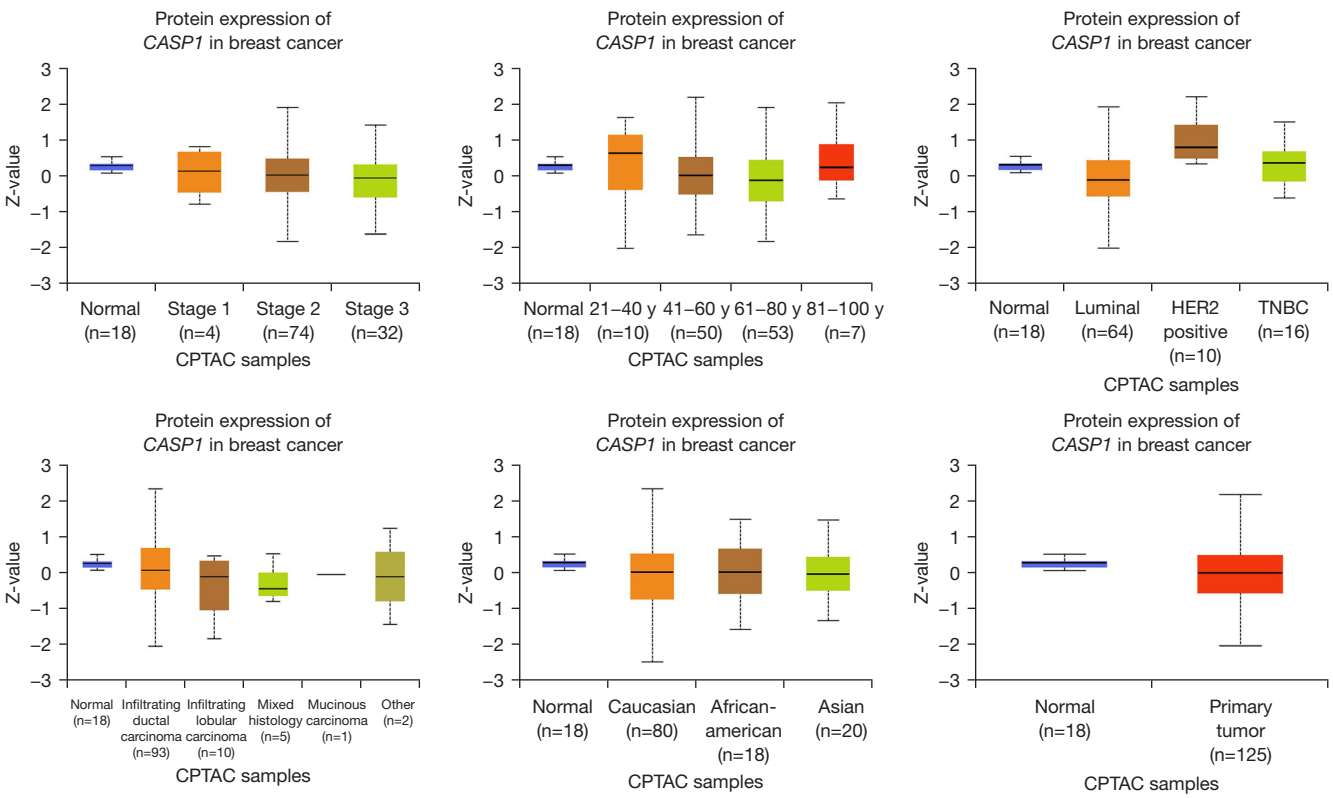


**Figure 5** Promoter methylation of *CASP1* in different BRCA types. *CASP1*, caspase-1; CPTAC, Clinical Proteomic Tumor Analysis Consortium; HER2, Erb-B2 receptor tyrosine kinase 2; TNBC, triple-negative breast cancer; BRCA, breast cancer.

 

including neutrophils, B cells, $CD4^+$ T cells, macrophages, $CD8^+$ T cells, and dendritic cells (DCs) (*Figure 6A*). To further evaluate the effect of *CASP1* on the tumor microenvironment (TME), we analyzed the association between *CASP1* and specific immune cells. The findings demonstrated that the *CASP1* expression was linked to plasma cell like DC (pDC), T regulatory cell (Treg), DC, interdigitating DC (iDC), natural killer cell (NK), CD56dim cell, CD56bright cell, and T follicular helper (TFH) cell, and other infiltration levels were all positively correlated (*Figure 6B*,*6C*). Moreover, further analysis demonstrated that *CASP1* expression was positively linked to the immune checkpoint-related molecules cytotoxic T-lymphocyte associated protein 4 (CTLA4), programmed cell death 1 (PDCD1), and CD274 molecule, and the difference was statistically significant (*Figure 6D*). These results not only offer a foundation for future studies but also suggest that *CASP1* expression might be linked to the immune infiltration of BRCA tumors and may play a crucial role in preventing tumor cells from evading the immune system in the BRCA TME.

### *Correlation between CASP1 in TCGA database and molecularly targeted therapy drug target molecules*

At present, the treatment of tumors is becoming increasingly precise and individualized. Gene detection of mutation targets plays a critical role in improving the prognosis of tumors. Major guidelines and clinical trials are also emphasizing the importance of molecular detection of related targets, including: (I) Erb-B2 receptor tyrosine kinase 2 (*ERBB2*, like *HER2*): the detection rate of this target in BRCA is about 20–30%, and the incidence is higher in female patients under the age of 60 years. *HER2* is a relatively common type of mutation, which causes faster disease progression and a higher degree of malignancy. Also, *HER2*-positive BRCA is more likely to recur than *HER2*-negative BRCA. The symptoms of *HER2*-positive BRCA are similar to those of other types of BRCA, and there is no special bias. Trastuzumab is the most important drug for this target. According to the expressions of *HER2* targets and hormone receptors, patients are mainly divided into triple-negative BRCA, hormone receptor-positive BRCA, and *HER2*-positive BRCA. Endocrine therapy, targeted therapy, or other treatment options are usually adopted. (II) Vascular endothelial growth factor (VEGF) plays an important role in the angiogenesis induction process. The quantity of pro-VEGF and anti-VEGF in normal people is relatively balanced; however, the processes of growth and division of cancer cells require large amounts of nutrients, which elevates the expression level of pro-VEGF, promoting angiogenesis in tumor lesions and providing a suitable environment for the growth of cancer cells. (III) Poly(ADP-ribose) polymerase (PARP) refers to a group of proteins that are involved in many cellular processes in normal humans, including DNA repair, genome stability, and the regulation of programmed cell death. The BRCA gene belongs to a class of tumor suppressor genes, also known as nursing genes, and the corresponding protein encoded by it has the function of repairing DNA. PARP and BRCA jointly regulate the DNA repair processes in cells, namely base excision repair and homologous recombination repair. At present, the PARP inhibitors that have been approved for BRCA include olaparib and talzenna. In addition, there are a number of drugs under development such as CVL218 that are undergoing clinical trials. (IV) Neurotrophic receptor tyrosine kinase (NTRK) is currently the most popular pan-cancer anti-cancer agent, and there is a large gap in the positive rate of drug targets in different cancer types. In some specific cancers, such as secretory BRCA, the positive rate of NTRK can reach more than 90%. The drugs, larotrectinib and entrectinib, are both tyrosine kinase inhibitors (TKIs) agents that utilize this target. NTRK-TKIs are divided into first-generation and second-generation drugs. The first-generation drugs include larotrectinib and entrectinib, while the second-generation drugs include LOXO-195 and TPX-0005 etc. Our study found that *CASP1* was positively correlated with *VEGFC*, *VEGFB*, epidermal growth factor receptor (EGFR), BRCA2 DNA repair associated (*BRCA2*), AKT serine/threonine kinase 3 (*AKT3*), cyclin dependent kinase 6 (*CDK6*), *NTRK1*, *NTRK2*, *NTRK3*, and tumor associated calcium signal transducer 2 (TACSTD2), and negatively correlated with *ERBB2*, *BRCA1*, *AKT1*, and *AKT2*, with statistically significant differences ($P<0.05$, *Figure 7*).

### *Prediction of signaling pathways based on GSEA*

We performed GSEA functional analysis online using Metascape and found that *CASP1* may affect the mammary gland by participating in the cell cycle, immune environment, inflammation, apoptosis, the HIPPOMERLIN pathway, NK regulation of cytotoxicity, p53 expression, the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway, the

 

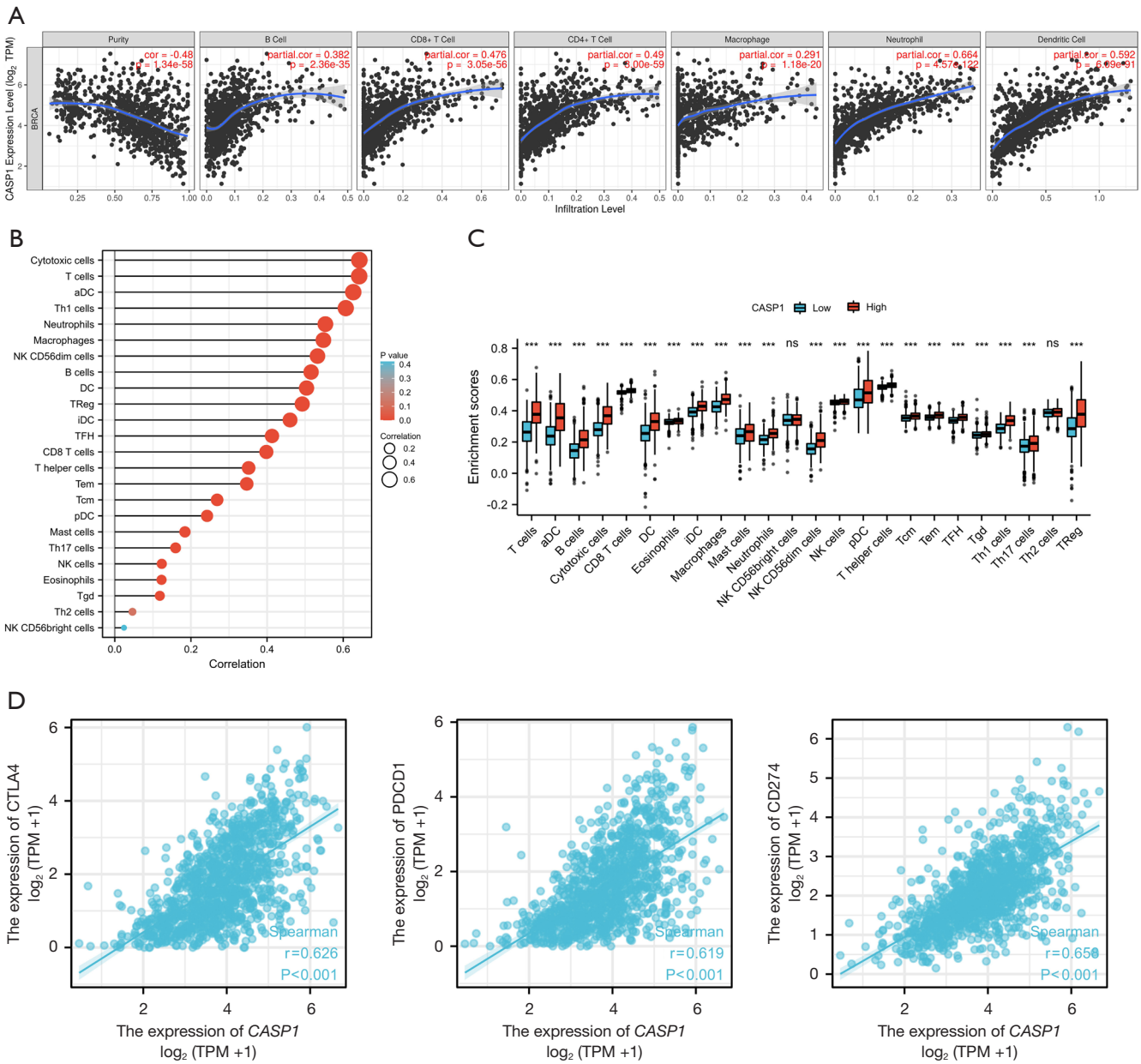


**Figure 6** Correlation between *CASP1* expression and immune molecule infiltration levels. (A) Correlation between *CASP1* and different immune cell infiltration levels of BRCA in TIMER database; (B) correlation between *CASP1* expression and different immune cell infiltration levels of BRCA in TCGA database; (C) correlation between caspase-1 expression and different types of immune cell infiltration levels of BRCA in the TCGA database; (D) *CASP1* in BRCA Scatter plot of the correlation between expression and CTLA4, PDCD1, and CD274. ***, P<0.001, the difference is statistically significant; ns, not significant. *CASP1*, caspase-1; TPM, transcripts per million; CD, clusters differentiation; aDC, activated dendritic cell; NK, natural killer cell; DC, dendritic cell; Treg, T regulatory cell; iDC, interdigitating dendritic cell; TFH, T follicular helper cell; Tem, effective memory T cell; Tcm, central memory T cell; pDC, plasma cell like dendritic cell; Th, T helper; Tgd, gamma delta T cell; CTLA4, cytotoxic T-lymphocyte associated protein 4; PDCD1, programmed cell death 1; BRCA, breast cancer; TIMER, Tumor Immune Estimation Resource; TCGA, The Cancer Genome Atlas.

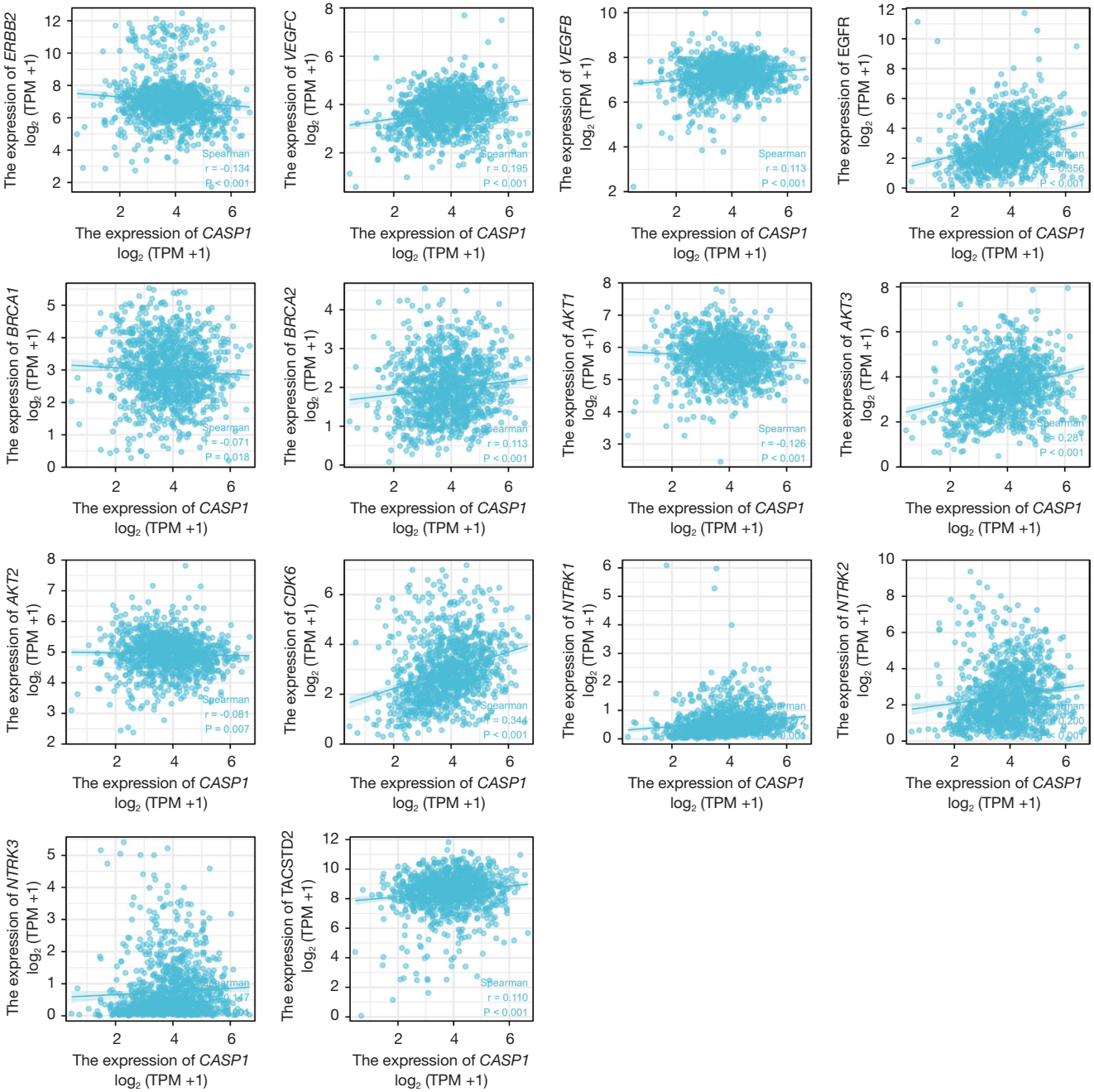


**Figure 7** Correlation between *CASP1* and molecularly targeted therapy drug target molecules in TCGA database. P<0.05, the difference is statistically significant. *ERBB2*, Erb-B2 receptor tyrosine kinase 2; TPM, transcripts per million; *CASP1*, caspase-1; VEGF, vascular endothelial growth factor; EGFR, epidermal growth factor receptor; *BRCA1*, BRCA1 DNA repair associated; *BRCA2*, BRCA2 DNA repair associated; *AKT1*, AKT serine/threonine kinase 1; *AKT3*, AKT serine/threonine kinase 3; *AKT2*, AKT serine/threonine kinase 2; *CDK6*, cyclin dependent kinase 6; *NTRK*, neurotrophic receptor tyrosine kinase; TACSTD2, tumor associated calcium signal transducer 2; TCGA, The Cancer Genome Atlas.

 

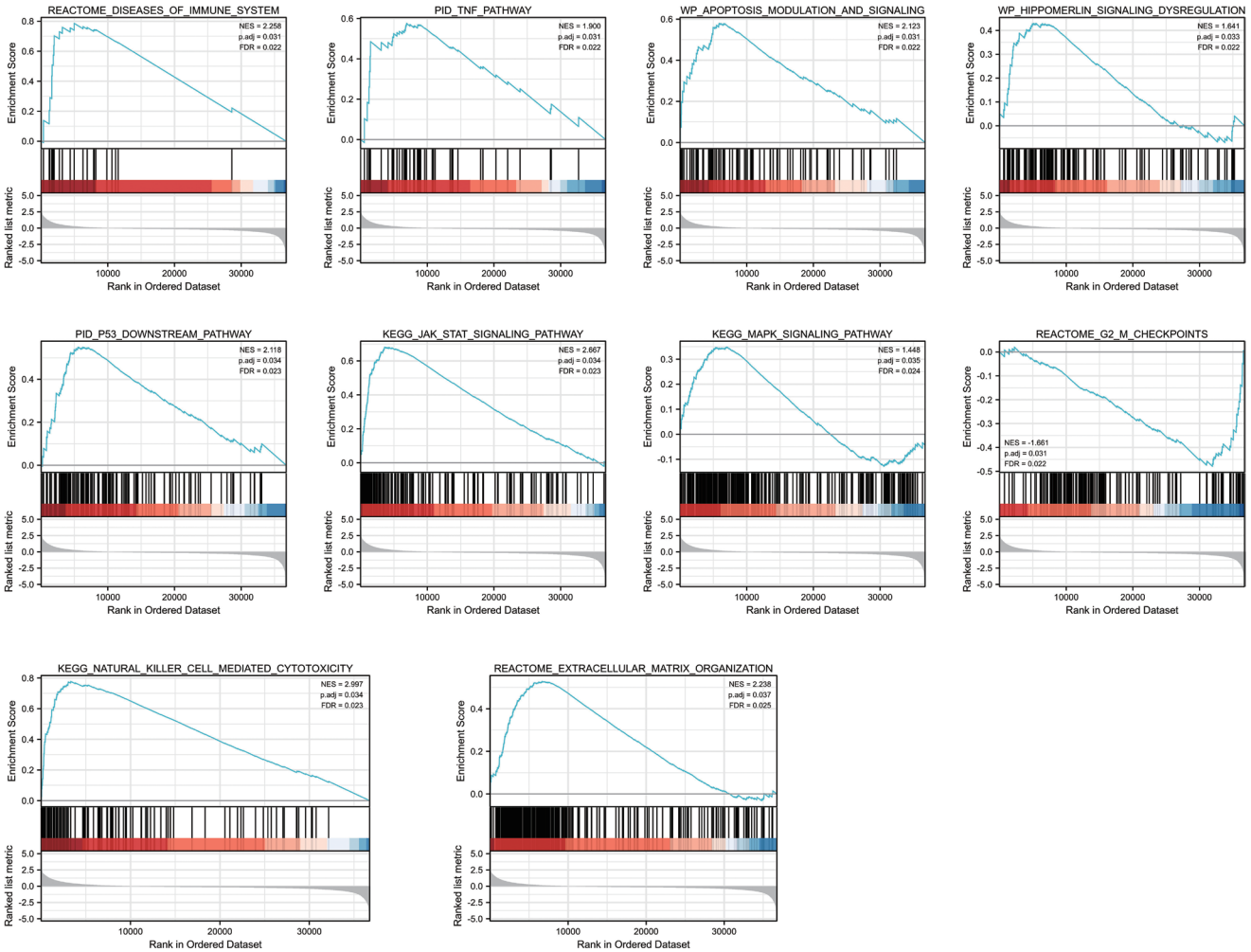


**Figure 8** Potentially relevant pathways with statistical significance in the GSEA. The gene ear biology biological process gene set from MSigDB was used. A total of 1,400 random sample permutations were performed. NES, normalized enrichment score; FDR, false discovery rate; GSEA, gene set enrichment analysis.

mitogen-activated protein kinase (MAPK) pathway, and the extracellular matrix. The biological events of cancer lead to different BRCA prognoses, which also provides a reference for our basic experimental research in the future (*Figure 8*).

### *Relationship between the general information of the enrolled patients and routine ultrasound characteristics and the expression of CASP1*

Finally, we included the clinical data of 86 BRCA patients (age range, 22–61 years old), including 58 cases of invasive ductal carcinoma, 19 cases of ductal carcinoma *in situ*, 5 cases of mucinous glands, 2 cases of solid papillary carcinoma, and 2 cases of encapsulated papillary carcinoma. The expression of *CASP1* among the patients was determined by immunohistochemistry, Then, based on the ROC curve, the Jordan index was calculated, and the optimal critical value for *CASP1* was 23%. These patients were divided into *CASP1* high expression group and *CASP1* low expression group based on whether the *CASP1* expression value was greater than 23%. The patients were then divided into high and low *CASP1* expression groups according to whether the *CASP1* expression value was greater than 23%. We found that in the routine ultrasound features, the mass margin status and blood flow grade (23)

 

**Table 1** Relationship between routine ultrasound features and expression of *CASP1*

| Routine ultrasound features | Low *CASP1* expression | High *CASP1* expression | $\chi^2$ value | P value |
|---|---|---|---|---|
| Largest diameter of the lesion | | | 0.880 | 0.348 |
| <2 cm | 24 | 8 | | |
| ≥2 cm | 45 | 9 | | |
| Echo pattern | | | – | >0.999 |
| No attenuation | 66 | 16 | | |
| Attenuation | 3 | 1 | | |
| Margin | | | 6.561 | 0.010 |
| Circumscribed | 36 | 3 | | |
| Not circumscribed | 33 | 14 | | |
| Microcalcification | | | 2.158 | 0.142 |
| No | 27 | 10 | | |
| Yes | 42 | 7 | | |
| The blood flow classification | | | 7.401 | 0.007 |
| 0, I | 17 | 10 | | |
| II, III | 52 | 7 | | |

P<0.05, the difference is statistically significant. *CASP1*, caspase-1.

were related to the expression of *CASP1*. Moreover, we observed that the number of patients with low *CASP1* expression was higher in BRCAs with adverse ultrasound features, which is also consistent with the previous results confirming *CASP1* as a tumor suppressor gene in TCGA database (see *Table 1* for details).

### *Relationship between CEUS features and CASP1 expression*

Among the CEUS features, we also found a higher number of BRCA patients with low *CASP1* expression among BRCAs with poor ultrasound features [the blurry edge, irregular shape, and large blood circulation of the lesion indicate the possibility of malignant lesions (24)] (*Table 2*).

### *Prediction of CASP1 expression using multimodal ultrasound features*

*Table 3* shows the assignment of the respective variables. We set the expression of *CASP1* as the dependent variable and incorporated the characteristics with P<0.05 as the independent variables into a multivariate analysis through binary logistic single-factor analysis. The results showed that tumor margin (25,26) and blood flow grade (21) were independent prognostic factors affecting the expression of *CASP1* in BRCA (see *Table 4*).

## Discussion

Globally, the incidence of BRCA ranks first among malignant tumors in women. The differences in gene expression profiles lead to differences in the biological behavior, treatment, and prognosis of BRCA (27,28). Therefore, the basis and principle of current BRCA therapies is to formulate individualized precision treatments based on the differences in BRCA gene expression profiles. Breast imaging plays an important role in the clinical evaluation of breast diseases. Ultrasonography is the most commonly used imaging technique worldwide, with a sensitivity of 80–90%. Compared with X-ray, the sensitivity of ultrasound is less affected by age and can detect some BRCAs that are missed by X-ray (29). For the diagnosis of non-palpable breast disease, ultrasound diagnosis will increase the detection rate of early BRCA, thereby improving the OS rate of patients (30). Ultrasound is suitable for all age groups and has the advantage that

 

**Table 2** Relationship between CEUS features and *CASP1* expression

| CEUS feature | Low *CASP1* expression | High *CASP1* expression | $\chi^2$ value | P value |
|---|---|---|---|---|
| Enhancement | | | 0.748 | 0.739 |
| Non to low enhancement | 4 | 2 | | |
| High enhancement | 65 | 15 | | |
| Enhanced range | | | 2.640 | 0.196 |
| Consistent | 12 | 6 | | |
| Increase | 57 | 11 | | |
| Perfusion method | | | 2.191 | 0.283 |
| Early enhancement, late fading | 62 | 13 | | |
| Early fading, late enhancement | 7 | 4 | | |
| Distribution | | | 0.149 | 0.700 |
| Even | 32 | 7 | | |
| Uneven | 37 | 10 | | |
| Enhancement mode | | | 2.108 | 0.273 |
| Radial | 59 | 12 | | |
| Centrifugal | 10 | 5 | | |
| Perfusion defect | | | 2.125 | 0.251 |
| No | 49 | 15 | | |
| Yes | 20 | 2 | | |

P<0.05, the difference is statistically significant. CEUS, contrast-enhanced ultrasound; *CASP1*, caspase-1.

magnetic resonance imaging (MRI) cannot match women under 40 years old. Although the signal in the malignant area can be significantly enhanced on MRI examination, this examination method is costly and in clinical practice, patients spend a lot of time and make appointments for examination. Some benign lesions are detected by MRI as suspicious malignant lesions, increasing the likelihood of patients undergoing surgery (31,32).

The present research primarily used data from TCGA and other databases to obtain the differentially expressed gene, *CASP1*, in BRCA and then investigated the expression of this molecule and its significance in predicting the survival rate of BRCA patients. At the same time, we also performed bioinformatics analysis and verification to determine the effect of *CASP1* on tumor cell behavior. Then, we evaluated *CASP1* in tandem with the multimodal ultrasonography characteristics of study participants with BRCA to further assess its prognostic efficacy (33,34).

The expression of *CASP1* in BRCA tissues was lower than that in surrounding tissues, and patients with high *CASP1* expression had a longer OS, DSS, and PFS than those with low *CASP1* expression. The TME primarily comprises tumor cells and neighboring immune and inflammatory cells. It also includes tumor-associated fibroblasts, nearby stromal tissues, microvasculature, as well as different cytokines and chemokines. This microenvironment can be categorized into two distinct parts: the immune sector, dominated by immune cells, and the non-immune sector, primarily influenced by fibroblasts. Researches indicate that activated *CASP1* has crucial biological functions (35-37). On the one hand, activated *CASP1* can process pro-IL-1β and pro-IL-18 to promote their maturation and release, thereby regulating the innate and adaptive immune functions of the body. Mature IL-1β and IL-18 can directly affect innate immune cells such as neutrophils, monocytes, and macrophages inducing their activation. In addition, IL-1β and IL-18 can also act on $CD4^+$ T cells and B cells, regulating their differentiation and function (35,36). On the other hand, activated *CASP1*

 

**Table 3** Assignment of variables for multimodal ultrasound features

| Feature | Assigned value |
|---|---|
| Tumor diameter | |
| <2 cm | 0 |
| ≥2 cm | 1 |
| Rear echo | |
| No attenuation | 0 |
| attenuation | 1 |
| Edge of mass | |
| Smooth | 0 |
| Not smooth | 1 |
| Microcalcification | |
| No | 0 |
| Yes | 1 |
| Blood flow classification | |
| 0, I | 0 |
| II, III | 1 |
| Enhancement | |
| Non to low | 0 |
| High enhancement | 1 |
| Enhanced range | |
| Consistent | 0 |
| Enhanced | 1 |
| Perfusion method | |
| Late enhancement, early fading | 0 |
| early enhancement, late fading | 1 |
| Distribution | |
| Even | 0 |
| Uneven | 1 |
| Enhancement mode | |
| Radial | 0 |
| Centrifugal | 1 |
| Perfusion defect | |
| No | 0 |
| Yes | 1 |

can also mediate programmed cell death (37). Six different types of TIICs ($CD4^+$ T cells, neutrophils, B cells, macrophages, $CD8^+$ T cells, and DCs) were found to be positively correlated with *CASP1* expression in the TIMER database. Positive correlations were also observed with the immune checkpoint-related molecules CTLA4, PDCD1, and CD274. These results suggested that *CASP1* expression is associated with BRCA tumor immune infiltration and that *CASP1* plays a critical role in preventing the immunological escape of tumor cells within the BRCA TME. Next, we explored the relationship between *CASP1* and the targets of molecularly targeted therapy drugs and found that *CASP1* was positively correlated with *VEGFC*, *VEGFB*, EGFR, *BRCA2*, *AKT3*, *CDK6*, *NTRK1*, *NTRK2*, *NTRK3*, and TACSTD2, and negatively correlated with *ERBB2*, *BRCA1*, *AKT1*, and *AKT2*. These two findings suggest that *CASP1* may play a role in the selection and effectiveness of targeted medicines in BRCA, which is important given its association with tumor immune infiltration. These findings further demonstrate the scientific and clinical significance of our investigational molecule-*CASP1*.

We then performed a predictive analysis of the related mechanism pathways and found that *CASP1* may affect the cell cycle, immune environment, inflammation, apoptosis, the HIPPOMERLIN pathway, NKs regulate cytotoxicity, p53 expression, the JAK-STAT pathway, the MAPK pathway, and the extracellular matrix, thereby impacting the biological events of BRCA. Related study has been conducted in this area, including on the role of NLR family pyrin domain containing 3 (NLRP3)/*CASP1*/gasdermin D (GSDMD)-dependent pyroptosis in BPA-induced apoptosis of human neuroblastoma cells (38). Negative regulation of the interaction between tumor suppressor protein p53 and B-cell CLL/lymphoma 6 (BCL6) by controlling *CASP1* expression (39); secoisolaciresinol diglucoside induces pyroptosis by activating *CASP1* to cleavage GSDMD in colorectal cancer cells (40). These results provide a good reference for our *in vivo* and *in vitro* experiments in the future.

The high heterogeneity of BRCA determines its biological behavior, prognosis and individualized treatment. Different molecular subtypes of BRCA have significant differences in treatment methods, and individualized treatment for different molecular subtypes is particularly

 

**Table 4** Logistic regression analysis results of multimodal ultrasound features and *CASP1*

| Index | Factor | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|---|
| | | P value | OR | 95% CI | P value | OR | 95% CI |
| *CASP1* | Edge of mass | 0.017 | 0.196 | 0.052–0.745 | 0.025 | 0.207 | 0.053–0.817 |
| | Blood flow classification | 0.009 | 4.370 | 1.440–13.263 | 0.016 | 4.134 | 1.300–13.142 |

P<0.05, the difference is statistically significant. *CASP1*, caspase-1; OR, odd ratio; CI, confidence interval.

important in clinical practice. Multimodal ultrasound has also been considered to play an important role in the diagnosis of BRCA and different subtypes. For example, one study suggested that spiculation is common in BRCA of low histological grade and is a kind of self-protection against tumor metastasis (41). A study believe that Luminal tumor imaging mode mostly shows radioactive convergence, HER2 overexpression BRCA can up regulate VEGF, and is more prone to perfusion defects (42). However, considering the incompleteness of subtype data of BRCA specimens in this study, we tentatively analyze the role of *CASP1* in BRCA. We found that *CASP1* has good research potential related to prognosis, immunotherapy, and targeted therapy of BRCA in multiple databases, such as TCGA. We also further examined the data from our hospital, including the clinical information of 86 BRCA patients, and determined the expression of *CASP1* in different patients by immunohistochemistry. We try to avoid using preoperative puncture pathology for patients to reduce deviations caused by uneven internal structure of malignant tumor tissue, as well as small tissue quantity, cell fragmentation, and morphological distortion. We observed that in the conventional ultrasound features and CEUS features, mass margin status and blood flow grade were associated with the expression of *CASP1*, and patients with poor ultrasound features, tended to have lower *CASP1* expression.

## Conclusions

This research identified multiple sources of evidence supporting *CASP1*'s role in BRCA development and its potential as a biomarker of BRCA disease progression. The therapeutic use of *CASP1* in conjunction with multimodal ultrasonography features for the early detection and prognostic evaluation of BRCA was also verified. However, our findings need to be confirmed by multi-center clinical studies in the future to ensure their accuracy and practicability.

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## Footnote

*Reporting Checklist:* The authors have completed the TRIPOD reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1135/rc

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*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics committee of Jiangsu Cancer Hospital (No. 2022-015) and informed consent was taken from all the patients.



 

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