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ORIGINAL RESEARCH

Upregulation of ZMAT3 is Associated with the Poor Prognosis of Breast Cancer

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Background: Breast cancer is the leading cause of cancer-related deaths among women worldwide. Identifying robust biomarkers for predicting outcomes is essential for improving patient care and reducing fatalities. ZMAT3, a zinc finger protein with potential carcinogenic properties, has been associated with various cancers. However, its role in breast cancer prognosis remains unclear.

Methods: We investigated the expression level of *ZMAT3* in breast cancer tissues and its association with clinical outcomes through bioinformatics analysis and experimental validation. We examined the correlation between *ZMAT3* expression and immune characteristics. *ZMAT3* mRNA expression data from The Cancer Genome Atlas (TCGA) were analysed in relation to overall survival (OS), disease-specific survival (DSS) and progression-free interval (PFI) in patients with breast cancer. Immunohistochemistry (IHC) was performed on breast cancer tissues to assess *ZMAT3* protein levels, with findings validated using qPCR and cell experiments.

Results: *ZMAT3* mRNA levels were significantly upregulated in breast cancer samples compared to normal tissues. High *ZMAT3* expression was significantly correlated with the poor OS, DSS and PFI. A significant positive correlation was observed between high *ZMAT3* mRNA levels and the abundance of tumour-infiltrating lymphocytes (TILs), especially CD8+T cells and regulatory T cells (Tregs). Multivariate Cox regression analysis identified *ZMAT3* as an independent prognostic factor for breast cancer. IHC staining confirmed increased ZMAT3 protein expression in breast cancer tissues, which was further validated by qPCR and cell function tests.

Conclusion: Our findings suggest that *ZMAT3* is a prognostic biomarker linked to immune invasion in breast cancer. Elevated *ZMAT3* expression correlates with adverse clinical outcomes, indicating its potential role in disease progression.

Keywords: breast cancer, cancer prognosis, immunotherapy, bioinformatics, ZMAT3

Introduction

Breast cancer is a complex and heterogeneous disease that remains a significant public health issue. Its high incidence and mortality rates among women worldwide,[1,2] highlight the need for effective prognostic biomarkers to guide treatment decisions and improve patient outcomes.[3,4] Although traditional prognostic factors, such as tumour size, grade and lymph node status, provide valuable information, they often fail to capture the full biological complexity and treatment response of individual tumors.[5]

Recent research has underscored the importance of the tumour microenvironment, especially the immune environment, in influencing cancer progression and treatment efficacy.[6,7] Immune cell presence and function within the tumour microenvironment, known as immune infiltration, are crucial for prognosis and predicting responses to immunotherapy.[8,9] Tumour-filtered lymphocytes (TILs), including CD8+cytotoxic T cells and regulatory T cells (Tregs), play pivotal roles in the anti-tumour immune response and have been shown to affect the survival outcomes in various cancers.[10,11] Additionally, immunohistochemical markers such as oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) help distinguish BC subtypes and are linked to the immune microenvironment, guiding treatment strategies.[12]

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ZMAT3, a zinc finger protein known for its role in transcriptional regulation, has been implicated in cancer biology.[13] Changes in *ZMAT3* expression in malignant tumours suggest its potential role in tumorigenesis and disease progression.[14] However, the specific function of *ZMAT3* in breast cancer, particularly in relation to the immune microenvironment, remains unexplored.

Given the significance of immune infiltration in breast cancer prognosis and the potential for *ZMAT3* to modulate immune responses, this study aims to investigate the relationship between *ZMAT3* expression and immune infiltration. This study also aims to determine whether *ZMAT3* can serve as a prognostic biomarker associated with immune infiltration and to explore its impact on patient prognosis and the emerging field of cancer immunotherapy. This study integrates bioinformatics analysis of TCGA genome data with experimental validation using an independent cohort of cancer tissue samples to assess *ZMAT3* expression patterns and their association with immune signals. We also evaluate the prognostic significance of *ZMAT3* expression in immune cell infiltration and its potential as a predictor of immune response.

Materials and Methods

TCGA and GEPIA Data Processing

Gene expression profiles and clinical data for 1113 BRCA (BRCA) tumour tissues and 113 normal tissues were retrieved from TCGA. Additionally, data for 1085 BRCA tumour tissues and 112 normal tissues were obtained from GEPIA. Samples lacking OS time information were excluded. RNAseq data in TPM format from both TCGA and GEPIA were processed uniformly. The expression levels and prognosis related to *ZMAT3* were analysed.

Patients and Organisations

Ten breast cancer samples and matched non-tumour tissues were collected from the First Hospital of China Medical University. All enrolled patients provided written informed consent. The study was approved by the Ethics Committee of the First Hospital of China Medical University. For qPCR analysis, breast cancer tissues were obtained, frozen in liquid nitrogen and stored at −80° C after surgery.

Gene Enrichment Analysis

Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene set enrichment analysis (GSEA) were employed to identify genes and pathways associated with *ZMAT3*, using transcriptomic data from TCGA. Gene expression data were categorised into high expression and low *ZMAT3* expression groups for analysis using the R package (clusterprofiler plugin).

Immune Cell Infiltration

To evaluate the relative abundance of infiltrating immune cells in tumour tissues, single sample gene set enrichment analysis (ssGSEA) was performed. The "gsva" R package and an immune data set, including 24 types of immune cells, were used to analyse immune cell infiltration levels in the BRCA expression profile data. Additionally, the gene expression deconvolution method CIBERSORT (http://cibersort.stanford.edu/) was utilised to compare expression changes relative to the entire sample set.

Cell Culture and Transfection

The MCF7 cell line, obtained from the Chinese Academy of Sciences, was cultured in MEM medium supplemented with 10% foetal bovine serum (FBS; GIBCO) and 1% penicillin-streptomycin in a 37°C incubator with 5% CO_2. Twenty four hours before transfection, MCF7 cells were seeded in a six-well plate at 50–60% confluence. SiRNA transfections were performed using Lipofectamine 2000 according to the manufacturer's instructions. The siRNA sequences used were: Si-*ZMAT3*: 5′-AAGCCCAGGCTCATTATCAGG-3′, Si-NC: 5′-AAACGTGACACGTTCGGAGAA-3′.

RNA Isolation and qPCR Analysis

RNA was extracted from tissue samples using TRIzol reagent. The extracted RNA was reverse-transcribed into cDNA using the QuantiTect Reverse Transcription Kit. qPCR was performed with SYBR-Green, and the expression levels were normalised to GAPDH. The primers used were as follows: *ZMAT3* forward primer, 5′-TATCGAAGGGAGGGGAGCAA-3′; reverse, 5′-TTAAAGGAGCCCATCTGCGG-3′.

Detection of Cell Migration and Invasion

MCF7 and si-MCF7 cells were resuspended in serum-free medium and placed in the upper chamber of a Transwell membrane filter (Corning) for migration assays, and in the upper chamber of a Matrigel-coated Transwell membrane filter (Corning) for invasion assays. The lower compartment of the chamber contained a medium with 10% FBS and 0/5/10 nM tanespimycin as a chemical attractant. After 24 hours of incubation, cells were fixed with methanol, stained with 0.1% crystal violet, imaged, and counted using a microscope.

Cell Proliferation Test

MCF7 and si-MCF7 cells were seeded in 96-well plates at a density of 5×10^3 cells per well. At various time points (0–72 hours), 10 μL of CCK-8 reagent (Beyotime) was added to each well. After 2 h of incubation at 37°C, the absorbance was measured at 450 nm. For additional proliferation assessment, MCF7 and si-MCF7 cells were seeded in six-well plates at 50–60% confluence and cultured for 24 h. Cells were then stained with EdU and DAPI (Beyotime) according to the manufacturer's instructions and imaged using an immunofluorescence microscope.

Immunohistochemistry (IHC)

BRCA samples were fixed in 10% formalin, embedded in paraffin and sectioned into 5-μm sequential sections. The sections were dewaxed with ethanol and blocked to inhibit endogenous peroxidase activity. Samples were incubated overnight at 4°C with rabbit anti-ZMAT3 (Proteintech, 10504-1-AP), followed by incubation with horseradish peroxidase-coupled goat anti-rabbit secondary antibody at 37°C for 30 min. Following incubation, the sample was stained using 3.3′-diaminobenzidine. Cell nuclei were counterstained with hematoxylin. Sections were dehydrated, cleared with xylene, and mounted. ZMAT3 expression was analysed using IHC with the streptavidin-peroxidase method, using adjacent tissues as controls. Image-Pro Plus 6.0 Software (MediaCybernetics, USA) was used for protein expression analysis and statistical evaluation.

Statistical Analysis

Statistical comparisons of *ZMAT3* expression between normal and BRCA tissues were performed using the Wilcoxon rank sum test. Patients were categorised into two categories based on the median *ZMAT3* expression level. Clinicopathological features associated with *ZMAT3* expression were analysed using the Wilcoxon rank sum test, Kruskal–Wallis test and logistic regression. Prognostic analysis was conducted using Kaplan–Meier survival analysis and Cox univariate and multivariate analyses. The receiver operating characteristic (ROC) curve was generated using the "proc" package to assess the diagnostic significance of differentially expressed genes.

Results

Expression of ZMAT3 in Breast Cancer and Its Prognostic Role

We investigated *ZMAT3* expression in breast cancer and its potential as a prognostic biomarker. Analysis using TCGA and GEPIA databases revealed that *ZMAT3* mRNA levels were significantly elevated in breast cancer tissues (Figure 1A and B). The area under the ROC curve (AUC) for *ZMAT3* expression was 0.774, indicating its ability to differentiate breast cancer tissues from normal breast tissues (Figure 1C). Prognostic analysis from the GEPIA database showed that high *ZMAT3* expression was associated with poorer outcomes (HR = 1.60 (1.09–2.08)) (Figure 1D). Similar results were observed in the TCGA database, where high *ZMAT3* expression correlated with worse OS (HR = 1.64 (1.19–2.26)), DSS (HR = 1.69 (1.09–2.62)) and PFI (HR = 1.40 (1.01–1.94)) (Figure 1E–G).

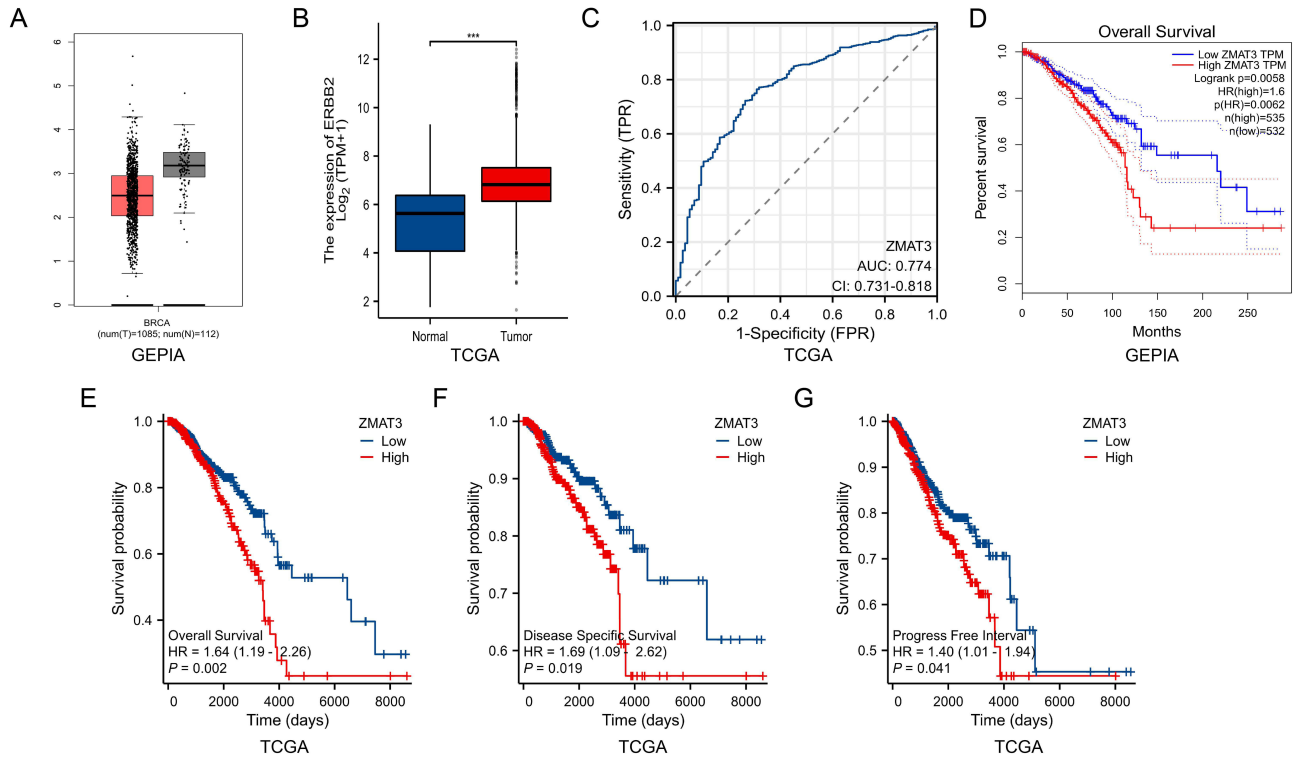

Figure 1 (**A**) Data from the GEPIA database demonstrating high *ZMAT3* expression in breast cancer tissues. (**B**) Data from the TCGA database showing elevated *ZMAT3* expression in breast cancer tissues. (**C**) ROC analysis illustrated that *ZMAT3* expression effectively distinguishes BRCA tumour tissues from normal tissues with an AUC of 0.774 (95% CI = 0.731–0.818) based on TCGA-BRCA datasets. (**D**) GEPIA database indicated that patients with breast cancer exhibiting high expression of *ZMAT3* had poor prognosis (OS). (**E–G**) TCGA database suggests that patients with breast cancer displaying high expression of *ZMAT3* have poor prognosis (OS, DSS, PFI). P<0.05 indicates statistical significance; *** P < 0.001.

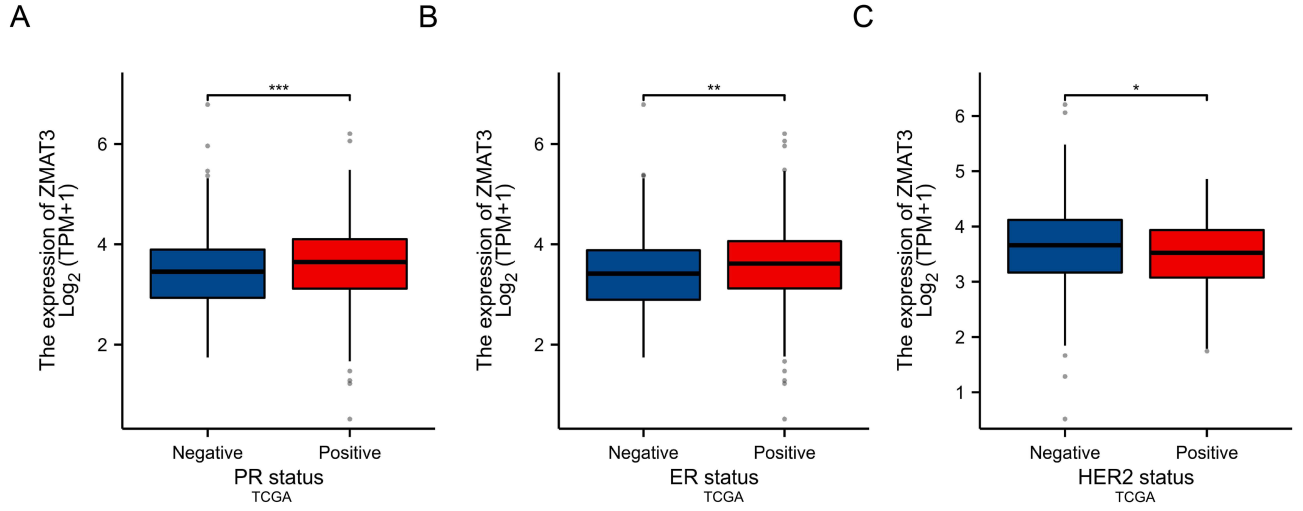

Figure 2 (**A** and **B**) Increased *ZMAT3* expression in patients with positive PR and ER status. (**C**) Decreased *ZMAT3* expression in HER2-positive patients with breast cancer. Statistical significance is indicated by P<0.05; *P < 0.05, **P < 0.01, ***P < 0.001.

Therefore, *ZMAT3* shows promise as a prognostic biomarker for breast cancer. Additionally, *ZMAT3* expression was higher in patients with positive PR status (P < 0.001) and ER status (P < 0.01) but lower in HER2-positive patients (P < 0.05) (Figure 2A–C).

Correlation Between ZMAT3 Expression and Clinicopathological Variables in Breast Cancer Patients

We analysed clinical characteristics and gene expression data for 1087 patients with primary breast cancer from the TCGA database. Patients were divided into high (n=543) and low (n=544) *ZMAT3* expression groups. *ZMAT3* expression was significantly associated with race (P<0.001), PR status (P=0.002), ER status (P=0.011), HER2 status (P=0.013) and PAM50 (P<0.001) (Table 1). Univariate logistic regression analysis revealed clinicopathological differences between high and low *ZMAT3* expression groups, including T stage (OR=0.740, 95% CI=0.563–0.974, P=0.031), race (OR=2.966, 95% CI=2.175–4.043, P<0.001), PR status (OR=1.598, 95% CI=1.230–2.075, P<0.001), ER status (OR=1.560, 95% CI=1.165–2.088, P=0.003), PAM5.0 (OR=0.530, 95% CI=0.416–0.674, P<0.001) (Table 2).

Patient Characteristics and Multivariate Analysis

Both univariate and multivariate Cox regression analyses identified age and high *ZMAT3* expression as independent risk factors for breast cancer prognosis (Table 3 and Figure 3). Overall, *ZMAT3* is significantly associated with breast cancer, with high expression correlating with poor patient outcomes.

Table 1 ZMAT3 Expression in BRCA Patients with Different Clinical Parameters

Characteristics	Low Expression of *ZMAT3*	High Expression of *ZMAT3*	P value
n	543	544	
Pathologic T stage, n (%)			0.090
T1	123 (11.3%)	155 (14.3%)	
T2	324 (29.9%)	307 (28.3%)	
T3&T4	93 (8.6%)	82 (7.6%)	
Pathologic N stage, n (%)			0.527
N0	252 (23.6%)	264 (24.7%)	
N1	182 (17%)	177 (16.6%)	
N2	55 (5.1%)	61 (5.7%)	
N3	44 (4.1%)	33 (3.1%)	
Pathologic M stage, n (%)			0.895
M0	439 (47.5%)	466 (50.4%)	
M1	10 (1.1%)	10 (1.1%)	
Pathologic stage, n (%)			0.458
Stage I	82 (7.7%)	100 (9.4%)	
Stage II	316 (29.7%)	303 (28.5%)	
Stage III	128 (12%)	116 (10.9%)	
Stage IV	9 (0.8%)	9 (0.8%)	
Race, n (%)			< 0.001
Asian	40 (4%)	20 (2%)	
Black or African American	129 (12.9%)	53 (5.3%)	
White	331 (33.2%)	424 (42.5%)	
Age, n (%)			0.978
<= 60	301 (27.7%)	302 (27.8%)	
> 60	242 (22.3%)	242 (22.3%)	
PR status, n (%)			0.002
Negative	197 (19%)	145 (14%)	
Indeterminate	2 (0.2%)	2 (0.2%)	
Positive	318 (30.6%)	374 (36%)	

(*Continued*)

Table 1 (Continued).

Characteristics	Low Expression of *ZMAT3*	High Expression of *ZMAT3*	P value
ER status, n (%)			0.011
Negative	140 (13.5%)	100 (9.6%)	
Indeterminate	1 (0.1%)	1 (0.1%)	
Positive	377 (36.3%)	420 (40.4%)	
HER2 status, n (%)			0.013
Negative	254 (34.8%)	306 (42%)	
Indeterminate	10 (1.4%)	2 (0.3%)	
Positive	82 (11.2%)	75 (10.3%)	
PAM50, n (%)			< 0.001
Normal	13 (1.2%)	27 (2.5%)	
LumA	239 (22%)	325 (29.9%)	
LumB	126 (11.6%)	80 (7.4%)	
Her2	51 (4.7%)	31 (2.9%)	
Basal	114 (10.5%)	81 (7.5%)	
Menopause status, n (%)			0.183
Pre	102 (10.5%)	128 (13.1%)	
Peri	19 (1.9%)	21 (2.2%)	
Post	362 (37.1%)	344 (35.2%)	

Table 2 Univariate Logistic Regression Analysis Revealed the Clinicopathological Differences Between High and Low Expression Groups of ZMAT3

Characteristics	Total (N)	OR (95% CI)	P value
Pathologic T stage (T2&T3&T4 vs T1)	1084	0.740 (0.563–0.974)	0.031
Pathologic N stage (N1&N2&N3 vs N0)	1068	0.921 (0.724–1.170)	0.499
Pathologic M stage (M1 vs M0)	925	0.942 (0.388–2.285)	0.895
Pathologic stage (Stage IV&Stage II vs Stage I&Stage III)	1063	0.933 (0.730–1.193)	0.582
Race (White vs Asian&Black or African American)	997	2.966 (2.175–4.043)	< 0.001
Age (> 60 vs <= 60)	1087	0.997 (0.785–1.266)	0.978
PR status (Positive vs Negative)	1034	1.598 (1.230–2.075)	< 0.001
ER status (Positive vs Negative)	1037	1.560 (1.165–2.088)	0.003
HER2 status (Positive vs Negative)	717	0.759 (0.533–1.082)	0.128
PAM50 (LumB&Normal&Her2&Basal vs LumA)	1087	0.530 (0.416–0.674)	< 0.001
Menopause status (Post vs Pre)	936	0.757 (0.561–1.021)	0.068

Identification and Functional Enrichment Analysis of DEGs in Breast Cancer

A total of 217 genes were differentially expressed between the high and low *ZMAT3* expression groups, including 71 upregulated and 146 downregulated DEGs (corrected p value<0.05, | log2-FC| 1.5) (Figure 4A). GO, KEGG and GSEA were employed to analyse these DEGs. GO analysis revealed that the majority of the differential genes were associated with epidermis development, cornified envelope and structural constituent of skin epidermis (Figure 4B). KEGG analysis indicated that these genes were primarily involved in the peroxisome proliferator-activated receptor (PPAR) signalling pathway (Figure 4B). GSEA identified that the differential genes were significantly related to Non-integrin membrane-ECM interactions, PID_AVB3_INTEGRIN_PATHWAY, NABA_CORE_MATRISOME, ECM Organization and ECM Receptor interaction (Figure 4C).

Table 3 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
Age	1086				
<= 60	603	Reference		Reference	
> 60	483	2.024 (1.468–2.790)	< 0.001	2.592 (1.652–4.068)	< 0.001
PR status	1033				
Negative	342	Reference		Reference	
Positive	691	0.729 (0.521–1.019)	0.065	0.902 (0.451–1.804)	0.771
ER status	1036				
Negative	240	Reference		Reference	
Positive	796	0.709 (0.493–1.019)	0.063	0.572 (0.274–1.192)	0.136
HER2 status	717				
Negative	560	Reference		Reference	
Positive	157	1.593 (0.973–2.609)	0.064	1.469 (0.885–2.439)	0.137
ZMAT3	1086				
Low	542	Reference		Reference	
High	544	1.505 (1.087–2.083)	0.014	1.603 (1.011–2.542)	0.045

Immune Infiltration Analysis

We performed an immune infiltration analysis to explore the potential association between *ZMAT3* and immune cells in breast cancer. *ZMAT3* expression was significantly positively correlated with TCM (Figure 5A). The 544 breast cancer samples were divided into high and low *ZMAT3* expression groups based on the median expression level. Figure 5B shows the relative abundance of 24 immune cell types in these groups. Specifically, the high *ZMAT3* expression group exhibited increased levels of eosinophils, IDC, TGD, TEM, TCM, T helper cells, NK cells, neutrophils, mast cells and macrophages, whereas the low. *ZMAT3* expression group showed higher levels of PDC and NK CD56bright cell infiltration (Figure 5B).

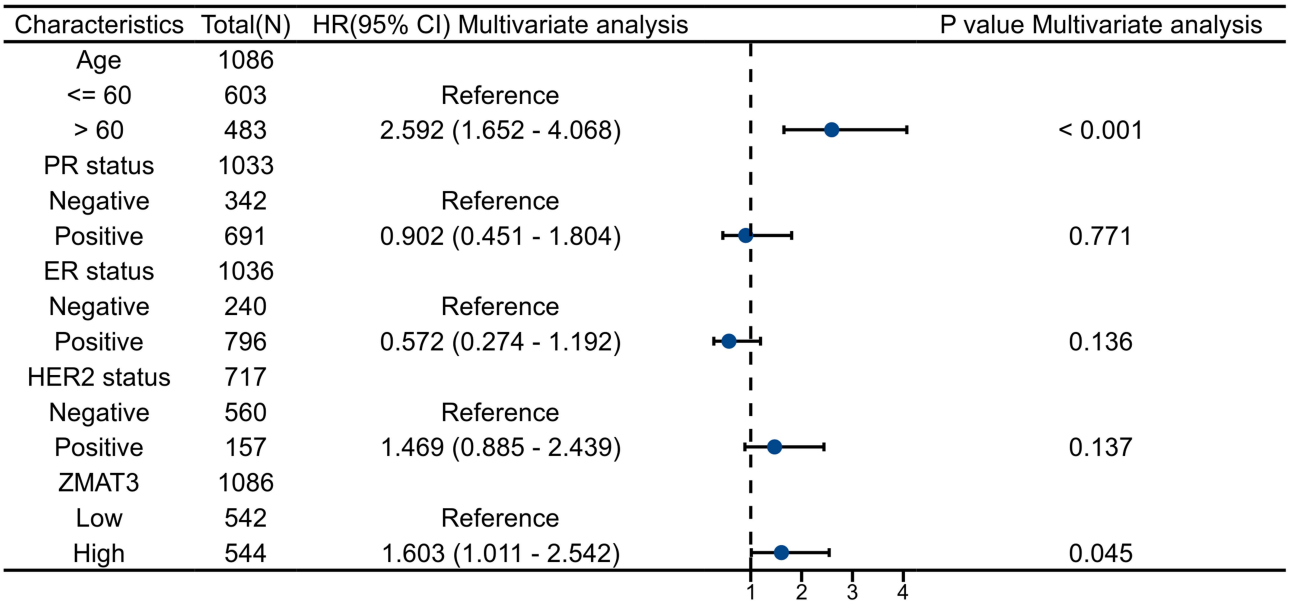

Figure 3 Age and high expression of *ZMAT3* are independent risk factors for the prognosis of breast cancer.

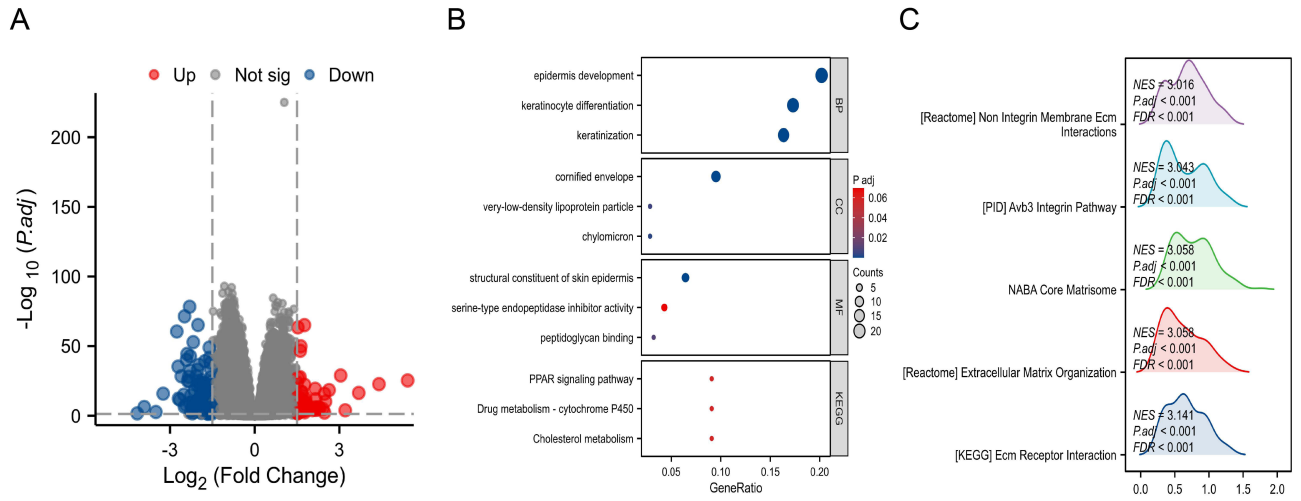

Figure 4 Gene enrichment analysis of *ZMAT3* in TCGA-BRCA datasets. (**A**) Volcano plot depicting differentially expressed genes (DEGs). (**B**) Enriched GO terms and KEGG pathways of DEGs. (**C**) GSEA of DEGs.

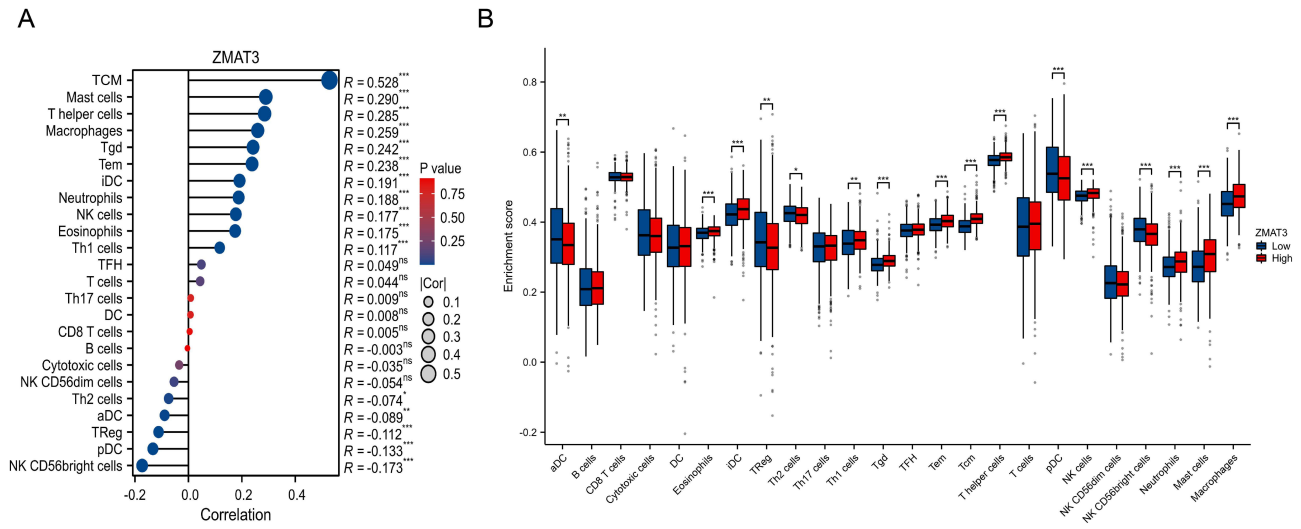

Figure 5 (**A**) The association between *ZMAT3* expression and 24 types of tumour infiltrating lymphocytes. (**B**) High *ZMAT3* expression is associated with increased infiltration of eosinophils, IDC, TGD, TEM, TCM, T helper cells, NK cells, neutrophils, mast cells and macrophages. Conversely, *ZMAT3* high expression is associated with reduced infiltration of PDC and NK CD56bright cells. P<0.05 indicates statistical significance; *P < 0.05, **P < 0.01, ***P < 0.001.

Construction and Verification of Nomogram Based on Independent Factor

To predict the prognosis of patients with breast cancer, a nomogram incorporating independent factors associated with OS was developed. Higher total points on the nomogram correlated with poorer prognosis (Figure 6A). The calibration curve demonstrated that the nomogram provided accurate predictions for OS in patients with breast cancer (Figure 6B), indicating its suitability for clinical use.

ZMAT3 is Highly Expressed in Breast Cancer and Promotes Breast Cancer Progression

The role of *ZMAT3* in breast cancer was further investigated through in vitro experiments. qPCR, immunohistochemistry and H score confirmed that *ZMAT3* was highly expressed in breast cancer tissues (Figure 7A and B). CCK8 and EdU assays revealed decreased proliferation of MCF7 cells following *ZMAT3* knockout (Figure 7C and D). Additionally, Transwell assays demonstrated reduced migration and invasion abilities of MCF7 cells post-*ZMAT3* knockout (Figure 7E and F).

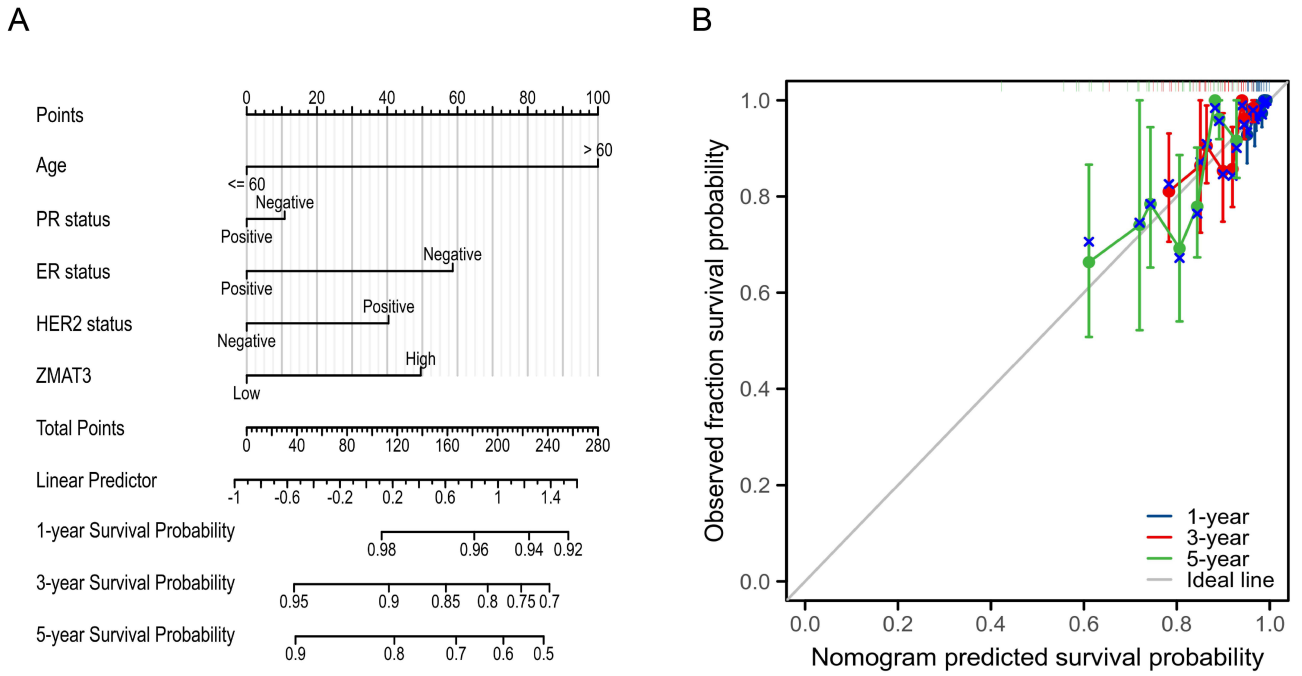

Figure 6 A nomogram and calibration curves for prediction of one-, three- and five-year overall survival rates of patients with breast cancer. (**A**) Nomogram for the prediction of one-, three- and five–year overall survival rates of patients with breast cancer. (**B**) Calibration curves of the nomogram prediction scores for one-, three- and five-year overall survival rates of patients with breast cancer.

Discussion

This study explored the expression pattern of *ZMAT3* in breast cancer, its association with immune invasion and its prognostic value using comprehensive bioinformatics analysis and experimental validation. Our results indicate that *ZMAT3* is not only highly expressed in breast cancer, but also closely related to immune invasion and patient prognosis, suggesting its potential as a prognostic biomarker.

ZMAT3, an RNA-binding zinc finger protein, is involved in the post-transcriptional regulation of gene expression and is widely expressed across various tissues.[15] Recent studies have shown that *ZMAT3*, similar to p53 target genes, plays a significant role in regulating cell proliferation and cell survival by modulating p53 and p21 mRNA levels.[16,17] However, the precise role of *ZMAT3* in breast cancer remains elusive.

Our bioinformatics analysis of TCGA data revealed significantly elevated *ZMAT3* expression in breast cancer tissues compared to normal breast tissues, with high *ZMAT3* expression correlating with poorer prognosis. This finding underscores the potential of *ZMAT3* as a valuable and therapeutic target. Further clinical correlation analysis indicated that elevated *ZMAT3* expression is associated with clinicopathological features such as PR status, ER status and HER2 status, positioning *ZMAT3* as an independent risk factor for adverse outcomes in patients with breast cancer. The association of *ZMAT3* with various immune markers and the degree of immune invasion supports its role as an indicator of breast cancer malignancy.

Experimental validation confirmed high *ZMAT3* expression in independent breast cancer tissue samples through qPCR and immunohistochemical staining. *ZMAT3* knockdown led to decreased proliferation, invasion and migration of breast cancer cells. Previous research has suggested that *ZMAT3* may serve as a prognostic indicator of reduced survival in several solid tumours, including lung, liver, colorectal, malignant mesothelioma and prostate cancer.[18–21] Moreover, *ZMAT3* has been implicated in tumour progression through its effects on various signalling pathways, including p53, Myc and Ras signalling pathway.[18,22,23] Our study further suggests that *ZMAT3* may influence the immune microenvironment of breast cancer through its interaction with the PPAR signalling pathway, which is involved in glucose and lipid metabolism and immune regulation.[24–26] This finding indicates that *ZMAT3* might modulate immune responses in breast cancer, highlighting its potential role in tumour progression and immune modulation.

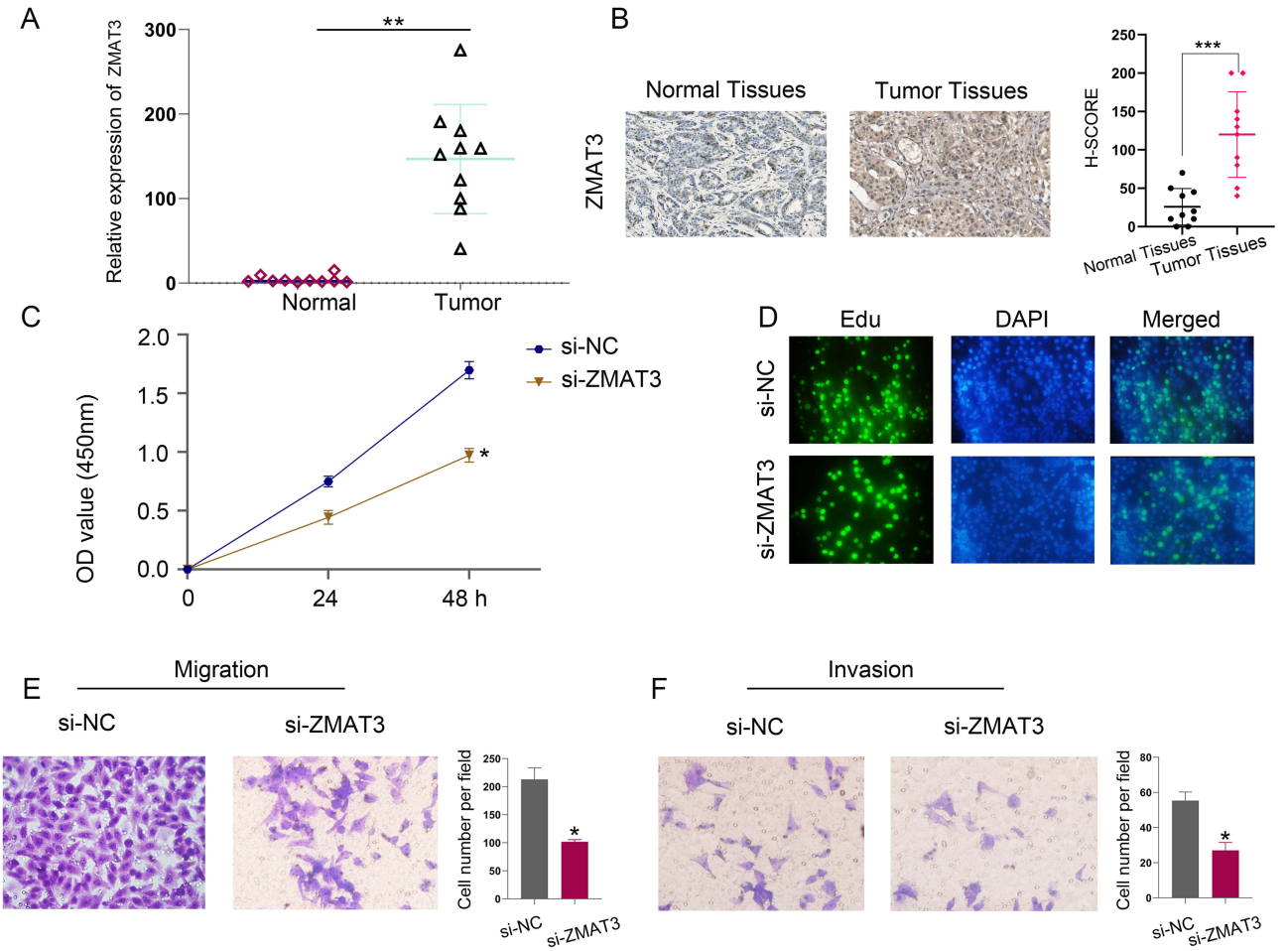

Figure 7 (**A**) *ZMAT3* mRNA expression levels in BRCA tissues versus matched non-tumour tissues. (**B**) ZMAT3 protein expression levels and H score in BRCA tissues versus matched non-tumour tissues. (**C** and **D**) Expression of the *ZMAT3* gene in MCF7 cells was silenced using RNA interference technology. Proliferation was significantly reduced in the si-*ZMAT3*. (**E** and **F**) Expression of the *ZMAT3* gene in MCF7 cells was silenced using RNA interference technology. Migration and invasion were significantly reduced in the si-*ZMAT3*. P<0.05 indicates statistical significance; *P < 0.05, **P < 0.01, ***P < 0.001.

The prognostic value of tumour infiltrating immune cells in solid malignant tumours has been well established, with outcomes influenced by the type, density and location of immune cells.[27–29] Additionally, the presence of infiltrating immune cells has been shown to predict responses to neoadjuvant chemotherapy and immune checkpoint inhibition (ICI) therapy.[30,31] Therefore, assessing immune cell infiltration in breast cancer can not only enhance the application of ICI treatments but also serve as a potential predictive marker for ICI efficacy. Given *ZMAT3*'s role in immune regulation, we investigated the correlation between *ZMAT3* expression and immune cell infiltration. Our results revealed that *ZMAT3* overexpression was positively correlated with the infiltration of TCM cells, mast cells and T helper cells, and negatively correlated with the infiltration of NK CD56 bright cells. Activated TCM cells, mast cells and T helper cells, as innate immune components, have been demonstrated to inhibit the growth of breast cancer cells.[32–34] Conversely, the presence of NK CD56 bright cells is linked to improved prognosis in patients with breast cancer.[35,36] These results suggest that *ZMAT3* overexpression may influence breast cancer progression and prognosis by modulating immune cell infiltration.

Despite these promising results, several limitations to this study warrant further investigation. First, although we validated the expression of *ZMAT3* in independent samples, larger clinical cohorts are needed to corroborate these findings. Second, the specific functional mechanisms of *ZMAT3* in breast cancer remain unclear, necessitating additional functional studies to elucidate its role in tumour occurrence, development and immune regulation. Future research should also explore the potential for combining *ZMAT3* with other biomarkers or therapeutic targets to refine prognostic assessments and treatment strategies.

This study elucidates the expression pattern of *ZMAT3* in breast cancer and its association with immune invasion and prognosis, offering new insights into the evaluation of prognosis and treatment strategies for breast cancer. However, further studies are required to validate these findings and further explore the functional mechanisms of *ZMAT3* in the occurrence and progression of breast cancer. Future investigations should focus on expanding the sample size, exploring the potential synergistic effects of *ZMAT3* with other biomarkers and evaluating its feasibility and effectiveness in clinical settings. Continued research and validation could establish *ZMAT3* as a valuable reference for prognostic assessment and treatment decision-making in patients with breast cancer.

Conclusion

In conclusion, this study highlights the potential of *ZMAT3* as a prognostic biomarker associated with the immune invasion of breast cancer. With further research and validation, *ZMAT3* could offer new insights and methods for prognosis evaluation and treatment selection in patients with breast cancer.

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 the First Hospital of China Medical University.

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. Meng Wu and Shuang Wu contributed equally to this work.

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Disclosure

The authors declare that there are no conflicts of interest in this work.

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