Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

High Expression of TIMM17B Is a Potential Diagnostic and Prognostic Marker of Breast Cancer

Mingting Duan[1†], Yun Ren[2†], Jiwen Zhang[1], Tingting Zhang[3], Yanhong Wang[4*], Hongyan Jia[5*]

[1] Department of First Clinical Medicine, Shanxi Medical University, Taiyuan, China
[2] Department of Breast Surgery, Changzhi People's Hospital Affiliated to Shanxi Medical University, Changzhi, China
[3] School of Basic Medicine, Basic Medical Sciences Center, Shanxi Medical University, Taiyuan, China
[4] Department of Microbiology and Immunology, Shanxi Medical University, Taiyuan, China
[5] Department of Breast Surgery, First Hospital of Shanxi Medical University, Taiyuan, China

[†]These authors contributed equally to this work and share the first authorship

ARTICLE INFO

Original paper

Article history:
Received: January 9, 2023
Accepted: March 17, 2023
Published: March 31, 2023

Keywords:
Malignant tumor, cell cycle, TIMM17B, GPX4

ABSTRACT

Breast cancer (BC) is the most common malignant tumor in women. TIMM17B has been found to be related to the cell cycle. The purpose of this study was to explore the diagnostic and prognostic value of TIMM17B in BC and its correlation with tumor immune infiltration and ferroptosis. For this purpose, the transcription and expression profile of TIMM17B between BC and normal tissues was downloaded from The Cancer Genome Atlas (TCGA). To verify the expression of TIMM17B in BC, we analyzed it by immunohistochemical staining. The correlation between TIMM17B and clinical features was analyzed using the R package to establish a ROC diagnostic curve. The GSVA package was used to determine the relationship between TIMM17B gene expression levels and immune infiltration. The GDSC was used to predict the IC50 of the drug. Expression of TIMM17B in tamoxifen-resistant breast cancer cells was detected by protein immunoblot analysis. Results showed that the expression of TIMM17B in many kinds of malignant tumors was higher than that in paracancer, with a significantly high expression in BC ($P < 0.001$). We validated this result by analyzing tissue microarrays. ROC curve analysis showed an AUC value in TIMM17B of 0.920. The Kaplan–Meier method showed a better prognosis for patients with high expression of TIMM17B in basal BC than that of patients with low expression of TIMM17B (HR=2.32 (1.09-4.94), P=0.038). In addition, the expression of TIMM17B in BC was negatively correlated with the level of immune infiltration, Tcm cells, T helper cells, and immune targets such as CD274, HAVCR2, and PDCD1LG2. At the same time, the expression of TIMM17B in BC was significantly correlated with the drug resistance and the expression of GPX4 and other key enzymes of ferroptosis. Protein immunoblot analysis revealed high expression of TIMM17B in tamoxifen-resistant breast cancer cells. In conclusion, the expression of TIMM17B in BC was significantly increased, and it was related to immune infiltration, drug resistance and ferroptosis in BC. Our research shows that TIMM17B can be used as a diagnostic index of BC and one of the targets of immunotherapy.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.3.25

Introduction

Breast cancer (BC) is currently the most common malignant tumor in the world, and its incidence is still increasing (1, 2). According to statistics, there were 287,850 new female BC cases and 43,250 deaths in the United States in 2022 (3). With the deeper study of BC, a variety of treatments have been used in clinical settings, such as surgery, chemotherapy, radiotherapy, endocrine therapy, targeted therapy, and immunotherapy. Although the diagnosis and treatment of BC have made great progress, the mortality and recurrence rate are still high (4). Therefore, it is necessary to further explore the biomarkers that affect the prognosis of BC in order to find new therapeutic targets.

Dr. James P. Allison and Dr. Tasuku Honjo won the 2018 Nobel Prize in Physiology and Medicine for their discoveries in the immune checkpoint proteins cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed death-1 (PD-1). With the in-depth study of immunoassay targets, the successful development and application of receptor antagonists or immunoassay target inhibitors provide a new scheme for the treatment of hematological and solid tumors (5, 6). In the past 20 years, the widespread use of endocrine and targeted therapy has significantly improved the prognosis of patients with BC, but the prognosis of triple-negative BC is still poor due to the inability to receive endocrine therapy and targeted therapy. The emergence of immunotherapy provides a new direction for the treatment of triple-negative BC. At the same time, existing studies have found that combined immunotherapy can also improve the prognosis of hormone receptor (HR)-positive and Her-2 (erbb-2) receptor-positive BC (7). At present, looking for biomarkers that can predict the responsiveness of immunotherapy and the target population is a key point

* Corresponding author. Email: swallow_jhy@163.com; wangyanhong@sxmu.edu.cn

of immunotherapy.

The TIM23 complex is a transporter located on the mitochondrial membrane, which is responsible for transporting the proteins in the cytoplasm to the mitochondrial inner membrane and mitochondrial matrix (8). The complex is composed of three intimal proteins, TIM50, TIM23, and TIM17, and is powered by a mitochondrial matrix motor and ATP (9). TIMM17B, one of the subunits of TIM17, is edited by the TIMM17B gene and is responsible for recruiting sequence pre-translocase-related motors (PAM) and sorting proproteins (IMM) in the inner membrane of mitochondria (10). Some studies have shown that TIMM17B can interact with EspZ in *Escherichia coli* and inhibit cell death (11, 12). Some studies have also shown potential tumorigenicity in the TIM23 complex (13). However, there is no in-depth study on the specific role of TIMM17B in the occurrence and development of tumors.

In this study, we analyzed the expression of TIMM17B in a variety of tumors in the TCGA database and explored the expression and prognosis of TIMM17B in BC. To verify the expression of TIMM17B in BC, we analyzed it by immunohistochemical staining. To analyze the relationship between the expression of TIMM17B in BC and the classification and prognosis of BC, and to predict the possible pathway of TIMM17B in BC. At the same time, the correlation between TIMM17B and immune infiltration and ferroptosis in BC was studied.

Materials and Methods

TCGA data set

Data on BC expression profiles and clinical information of patients were downloaded from the TCGA database(https://portal.gdc.cancer.gov/) (14). RNA sequencing data was converted from FPKM format to TPM format, and to log2. TCGA is an open public database and does not require the approval of the Ethics Committee.

Analysis of the TCGA database

The data of the TCGA database were analyzed by using the Xiantao platform (https://www.xiantao.love/), including the correlation between TIMM17B expression and clinical phenotype and diagnostic value.

Immune infiltration analysis

The correlation between immune cells and TIMM17B was calculated by the GSVA package, and that between TIMM17B and tumor immune infiltration was calculated by the estimate package.

GO/KEGG

The ClusterProfiler package was used to visualize data and the GGploT2 package was used to analyze the Kyoto gene and genomic Baike Encyclopedia (KEGG) pathway enriched by gene ontology (GO) and co-expression genes.

GSEA

Gene set enrichment analysis (GSEA) was performed using the GGplot2 R package (V3.3.3) to demonstrate important functions and pathways between the two groups. Expression levels of TIMM17B were used as phenotypic markers. Adjusted p-values < 0.05, enrichment of normalized scores (|NES|) < 1, and false discovery rate (FDR) < 0.25 were considered significantly different.

Protein-protein associated network

The network diagram of protein-protein interaction was drawn by the String database (https://cn.string-db.org/).

Correlation analysis

Correlation analysis between TIMM17B and genes using the Xiantao platform was analyzed using the GGploT2 package in R based on the expression of TCGA. Results with $P < 0.05$ was considered statistically significant.

IC50

The chemotherapeutic response for each sample was predicted based on the largest publicly available pharmacogenomics database [the Genomics of Drug Sensitivity in Cancer (GDSC), (https://www.cancerrxgene.org/). The prediction process was implemented using the R package pRRophetic. The samples' half-maximal inhibitory concentration (IC50) was estimated by ridge regression (15).

Immunohistochemistry staining and pathological correlation analysis of BC tissue microarrays

The BC tissue microarrays (F151Br01, Bioaitech, Xi'an, China) collected osteosarcoma tissue samples from 140 patients. For IHC (Immunohistochemistry) staining, BC tissue microarrays were incubated with anti-TIMM17B (1:300, 11062-1-AP, rabbit, ProteinTech, Wuhan, China). The BC tissue microarrays were analyzed by Image-Pro Plus 6.0.

Cell Culture and Transfection

The human BC cell line MCF-7 was bought from ATCC. MCF-7 cells were all maintained in Dulbecco's modified essential medium (DMEM), supplemented with 10% fetal bovine serum (FBS, Pricella, Wuhan, China), in a 5% CO_2 incubator. MCF-7-TAM was induced by 20 μM 4-hydroxytamoxifen (4-OHT, Calbiochem, Merck Millipore Billerica, MA, USA) incubation of MCF-7 for one month.

Western blot

TNBC cells or tissues were lysed by RIPA. The BSA method was used for the determination of total proteins, and 10 μL protein sample was loaded in the lane at a protein concentration of 1 mg/mL. Then, SDS-PAGE (12% gel) was performed. After adding the transmembrane onto PVDF membranes, membranes were blocked with 5% fat-free milk in TBST at room temperature and subsequently incubated with the primary antibodies of TIMM17B(1:2000) and β-actin(1:5000, GB15003, Servicebio, Wuhan, China) at room temperature for 1.5 h. Then, the PVDF membranes were incubated with HRP-conjugate secondary antibodies for 1 h at room temperature. Signals were detected using an ECL kit.

Statistical analysis

All statistical analyses used R (V 3.6.3). Paired t-test and Mann–Whitney U test were used to determine differences between BC tissue and adjacent normal tissue. Visualization was performed using the R package GGplot2 and the clusterProfiler package (version 3.14.3) (for GSEA

analysis) (16). ROC curve plotting was performed using the pROC package (17).

Results

To remove the duplicate samples and retain the samples with clinical information, we selected the information of 1065 patients with BC from the TCGA database for further analysis (Table 1). Patients were divided into two groups according to the median expression of TIMM17B: high expression group and low expression group. It was found that the expression of TIMM17B may be related to the T stage of BC. Tissues from 121 BC patients were used to validate the results of the TCGA database analysis(Table 2).

Based on the analysis of the data in the TCGA tumor database, we found that TIMM17B was overexpressed in many kinds of malignant tumors (Fig. 1A). Next, we analyzed the expression of TIMM17B in BC and found that the expression of TIMM17B in BC tissues was significantly higher than that in adjacent tissues (Fig. 1B, C). This suggests that TIMM17B may be involved in the growth and development of tumors.

Subsequently, we studied the correlation between the expression of TIMM17B and the clinical manifestations of the patients. We found that the expression of TIMM17B in T1 tumors was slightly lower than that in T2 and T3 tumors, but there was no significant relationship with the

Table 1. Clinical characteristics of TCGA.

Characteristic	Low expression of TIMM17B	High expression of TIMM17B	p
n	532	533	
T stage, n (%)			0.013
T1	159 (15%)	116 (10.9%)	
T2	298 (28.1%)	317 (29.8%)	
T3	58 (5.5%)	79 (7.4%)	
T4	16 (1.5%)	19 (1.8%)	
N stage, n (%)			0.335
N0	266 (25.4%)	241 (23%)	
N1	164 (15.7%)	185 (17.7%)	
N2	59 (5.6%)	57 (5.4%)	
N3	33 (3.2%)	41 (3.9%)	
M stage, n (%)			0.361
M0	470 (51.7%)	419 (46.1%)	
M1	8 (0.9%)	12 (1.3%)	
Pathologic stage, n (%)			0.062
Stage I	104 (10%)	76 (7.3%)	
Stage II	299 (28.7%)	307 (29.5%)	
Stage III	108 (10.4%)	130 (12.5%)	
Stage IV	7 (0.7%)	11 (1.1%)	
Histological type, n (%)			0.181
Infiltrating Ductal Carcinoma	366 (38.2%)	391 (40.8%)	
Infiltrating Lobular Carcinoma	109 (11.4%)	93 (9.7%)	
PR status, n (%)			0.953
Negative	167 (16.4%)	171 (16.8%)	
Indeterminate	2 (0.2%)	2 (0.2%)	
Positive	340 (33.5%)	334 (32.9%)	
ER status, n (%)			0.348
Negative	121 (11.9%)	116 (11.4%)	
Indeterminate	0 (0%)	2 (0.2%)	
Positive	388 (38.2%)	390 (38.3%)	
HER2 status, n (%)			0.091
Negative	298 (41.6%)	250 (34.9%)	
Indeterminate	4 (0.6%)	8 (1.1%)	
Positive	73 (10.2%)	84 (11.7%)	
Menopause status, n (%)			0.091
Pre	117 (12.2%)	107 (11.2%)	
Peri	26 (2.7%)	13 (1.4%)	
Post	341 (35.7%)	352 (36.8%)	
Age, mean ± SD	57.92 ± 12.92	58.79 ± 13.45	0.280

Table 2. Clinical characteristics of BC tissue microarrays.

Characteristics	overall
gender, n (%)	
female	119 (98.3%)
male	2 (1.7%)
pathologic diagnosis, n (%)	
other invasive BC	27 (22.3%)
nonspecific invasive BC	94 (77.7%)
T.stage, n (%)	
T2	98 (81%)
T3	12 (9.9%)
T4	7 (5.8%)
T1	4 (3.3%)
N.stage, n (%)	
N1	40 (33.1%)
N0	27 (22.3%)
N2	44 (36.4%)
N3	9 (7.4%)
aN	1 (0.8%)
Stage, n (%)	
IIB	33 (27.3%)
IIA	24 (19.8%)
IIIA	48 (39.7%)
IIIB	6 (5%)
IIIC	9 (7.4%)
IA	1 (0.8%)
subtype, n (%)	
Luminal A	36 (30%)
Her-2 type	33 (27.5%)
Luminal B	30 (25%)
TNBC	19 (15.8%)
*	2 (1.7%)

N stage, M stage, WHO stages age, and pathological classification of tumors (Fig. 1E-K). At the same time, we found that the expression of TIMM17B in Luminal A BC was lower than that in Luminal B and Her-2 BC (Fig. 1D). Therefore, TIMM17B may have a certain diagnostic value in BC, but its expression in BC has little correlation with the clinical phenotype of BC.

We analyzed the expression of TIMM17B in tumor tissues (Fig.2 B) and normal tissues (Fig.2 A), and found that TIMM17B was highly expressed in tumor tissues and higher in non-specific invasive BC (Fig.2 C,D). By analyzing the expression characteristics of TIMM17B, we found that TIMM17B was more highly expressed in Luminal A breast cancer, as well as in stage T3-4, stage N2, and stage III A-B breast cancers, suggesting that the high expression of TIMM17B may lead to a worse prognosis (Fig.2 E-I).

We analyzed the diagnostic value of TIMM17B expression in BC, evaluated its diagnostic value by ROC curve, and found that the AUC of TIMM17B was 0.920, which had a significant diagnostic value for BC (Fig. 3A). The results of time-dependent ROC curve showed that the expression of TIMM17B had weak prognostic value in the short term, which decreased gradually with time (Fig. 3F). Next, we analyzed the diagnostic value of TIMM17B in BC classification and found that TIMM17B had no diagnostic value in BC classification (Fig. 3B-E).

By analyzing the correlation between the survival and prognosis of BC patients and TIMM17B in the TCGA database, we found no correlation between the expression of TIMM17B and OS in BC ($P > 0.05$) (Fig. 4A). At the same time, the correlation between the expression of TIMM17B and prognosis in different types of BC was analyzed, and the expression of TIMM17B was found to have no effect on the OS of the Luminal A, Luminal B, and Her- 2 types

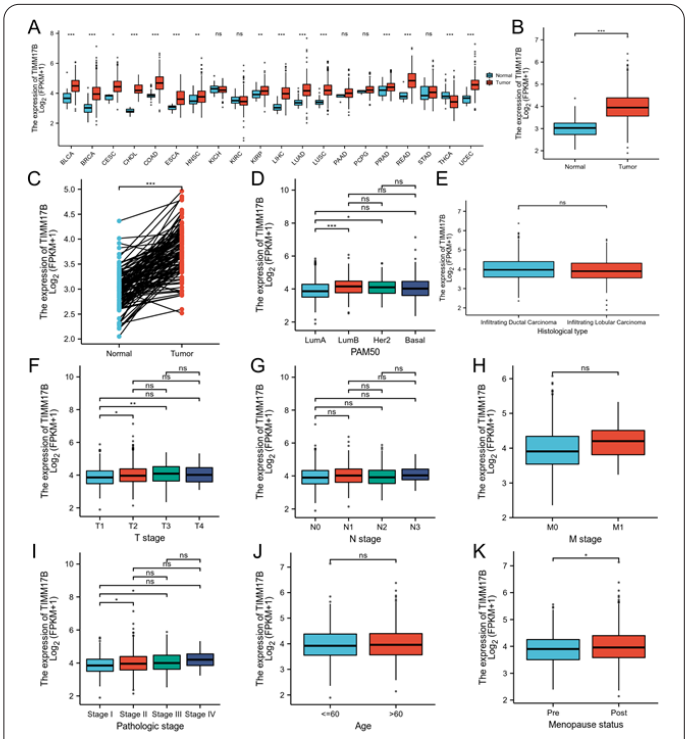

Figure 1. Expression of TIMM17B. (A) pan-cancer, (B)BRCA, (C) BRCA and their adjacent tissues, (D) PAM50, (E) histological type, (F) T stage, (G) N stage, (H) M stage. (I) pathologic stage, (J) age and (K) menopause status.

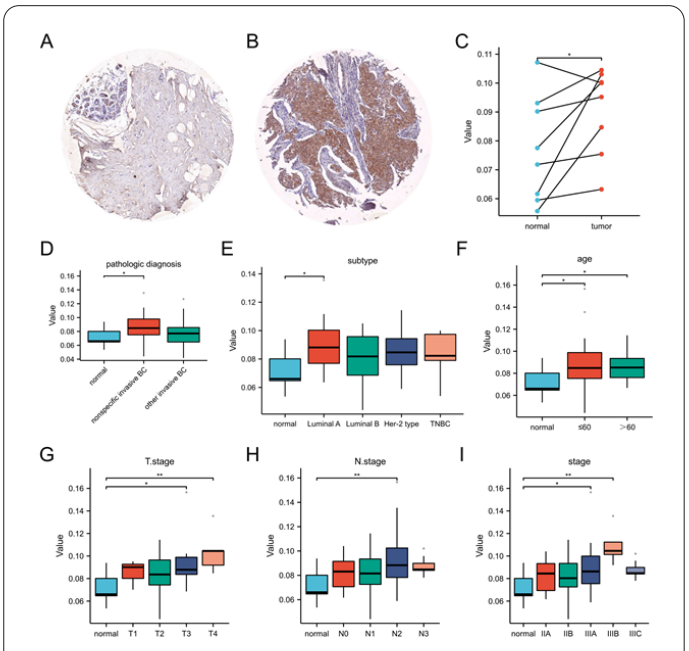

Figure 2. Expression of TIMM17B in BC tissue microarrays. (A) normal, (B) tumor, (C) BC and their adjacent tissues, (D) pathologic diagnosis, (E) subtype, (F) age, (G)T stage, (H)N stage, (I) pathologic stage.

of BC (P > 0.05) (Fig. 4B-D). In Basal BC, the high expression of TIMM17B significantly led to a worse prognosis (P < 0.05). It is suggested that TIMM17B is of significant value in predicting the prognosis of basic BC.

According to the median of TIMM17B expression data in the TCGA database, we divided TIMM17B into high-expression and low-expression groups and analyzed the difference in genes between the two groups (Fig. 5A). The differentially expressed genes with low and high expression were analyzed. The results of GO and KEGG enrichment analysis showed that TIMM17B was enriched in cell differentiation and hormone activity (Fig. 5B, C). The results of GSEA enrichment of differential genes showed that TIMM17B was significantly enriched in the cell cycle and extracellular stimulation response pathway (Fig. 5D). We also detected the proteins interacting with TIMM17B through the string database and found that TIMM17B interacted with proteins such as TIMM44 and TIMM50 (Fig. 5E). It is suggested that the expression of TIMM17B may be related to the growth of cells.

We evaluated the correlation between the expression of TIMM17B and 22 immune cells in BC by GSVA package. As shown in Fig. 6A, the expression of TIMM17B has a significant negative correlation with Tcm and T helper cells. The interstitial and immune scores of BC were evaluated by the estimation method, and the results showed a significant negative correlation between the expression of TIMM17B with interstitial scores and immune scores (Fig. 6B, C). In order to predict the correlation between

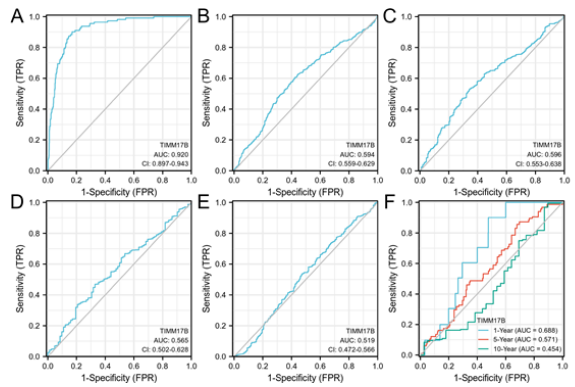

Figure 3. Diagnostic and prognostic value of TIMM17B. The ROC curve shows the AUC value of TIMM17B for (A) breast cancer. (B) Luminal A, (C) Luminal B, (D)Her-2, (E) Basal of PAM50, (F) The time-dependent ROC curve shows the AUC value of TIMM17B.

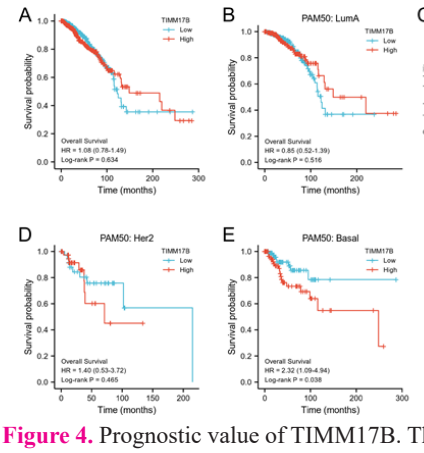

Figure 4. Prognostic value of TIMM17B. The Kaplan-Meier survival curve shows the OS of breast cancer patients. (A) BRCA, and (B) Luminal A, (C) Luminal B, (D) Her-2, (E)Basal ofPAM50.

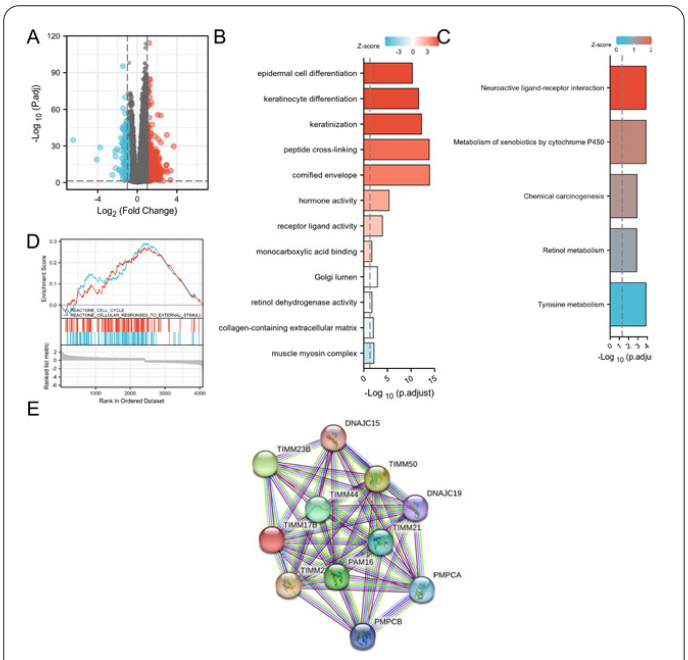

Figure 5. Function enrichment analysis of TIMM17B co-expressed genes. (A) The volcanic map shows the co-expression genes of TIMM17B in BRCA, (B) GO enrichment analysis for TIMM17B co-expressed genes, (C) KEGG enrichment analysis for TIMM17B co-expressed genes, and (D) GSEA enrichment analysis for TIMM17B co-expressed genes. (E)TIMM17B associated protein network in string database.

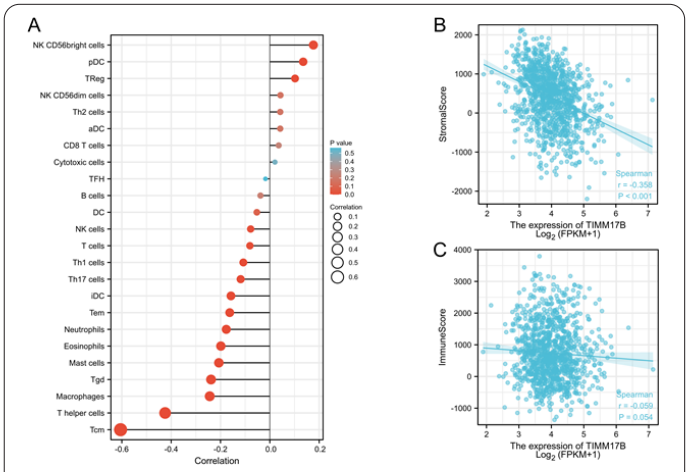

Figure 6. TIMM17B affects immune cell infiltration in BRCA patients. (A) The lollipop chart shows the relationship between TIMM17B expression and the abundance of 24 kinds of immune cells in BRCA. Correlation between TIMM17B and (B) StromalScore,(C) ImmuneScore in BRCA.

TIMM17B expression and immune response, the correlation between TIMM17B expression and immune checkpoints such as CD274, CTLA4, HAVCR2, LAG3, PDCD1, PDCD1LG2, TIGIT, and SIGLEC15 was analyzed (Fig. 7). The expression of TIMM17B was negatively correlated with CD274, HAVCR2, LAG3, PDCD1LG2, and TIGIT, and positively correlated with the expression of CTLA4, PDCD1, and SIGLEC15.

We used the GDSC database to predict the half resistance concentration (IC50) of TIMM17B with some common chemotherapeutic agents and endocrine therapeutics for breast cancer (Fig.8). We found that TIMM17B may lead to tamoxifen resistance in breast cancer when TIMM17B is highly expressed. By detecting the expres-

sion of TIMM17B in MCF-7 and its corresponding tamoxifen-resistant strain MCF-7-TAM, we found that TIMM17B was highly expressed in MCF-7-TAM($P<0.05$) (Fig.8A-C). High expression of TIMM17B was found to lead to increased IC50 for docetaxel, paclitaxel, doxorubicin, and 5-fluorouracil (Fig.8 D-G), while there was no correlation with IC50 for the aromatase inhibitor letrozole (Fig.8 H). This suggests that TIMM17B is closely associated with drug resistance in breast cancer.

Ferroptosis is a newly discovered mechanism of cell death recent years, which is closely related to malignant tumors (18). To study the correlation between TIMM17B and ferroptosis, we analyzed the correlation between

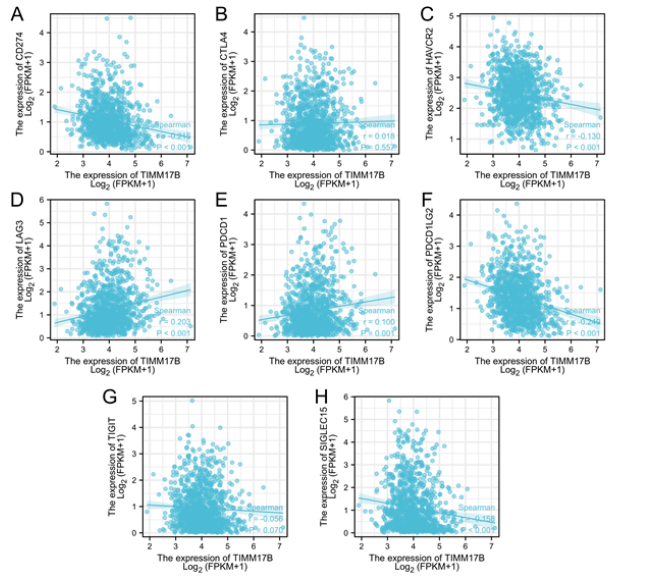

Figure 7. The relationship between TIMM17B expression and the immune checkpoints of BRCA. (A) CD274, (B) CTLA4, (C) HAVCR2, (D) LAG3,(E) PDCD1,(F) PDCD1LG2,(G) TIGIT and (H) SIGLEC15.

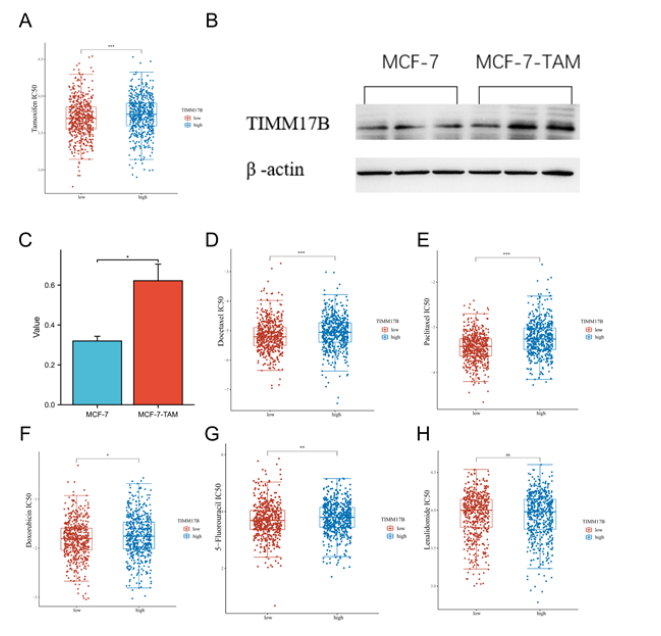

Figure 8. The relationship between TIMM17B expression and IC50. (A) Tamoxifen(B)TIMM17B expression in MCF-7 and MCF-7-TAM (C) TIMM17B was highly expressed in MCF-7-TAM (D) Docetaxel, (E) Paclitaxel, (F) Doxorubicin, (G) 5-Fluorouracil, (H)Lenalidomide.

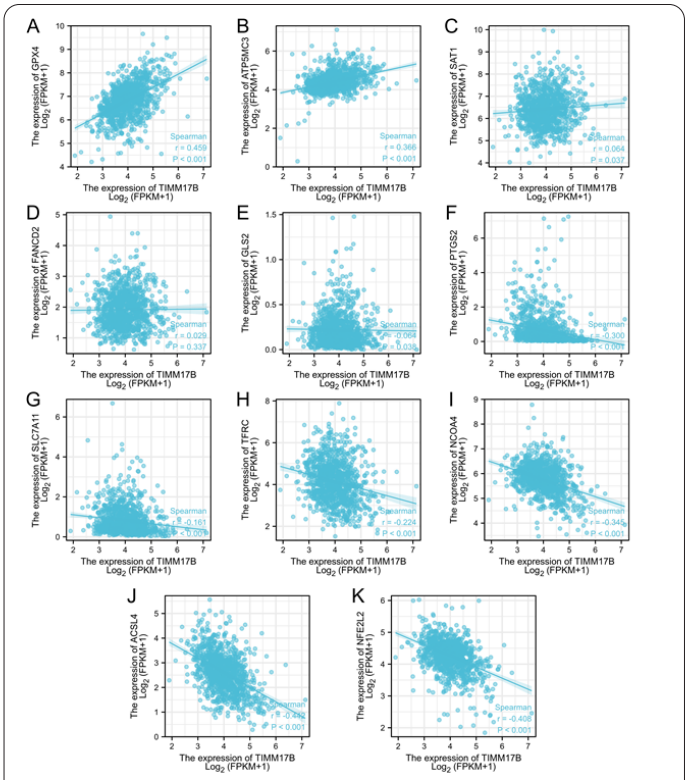

Figure 9. The relationship between TIMM17B expression and ferroptosis key enzymes (A) GPX4, (B) ATP5MC3, (C) SAT1, (D) FANCD2, (E) GLS2, (F) PTGS2, (G)SLC7A11, (H) TFRC, (I) NCOA4, (J) ACSL4, and (Q) NFE2L2.

TIMM17B and the expression of key enzymes in ferroptosis (Fig. 9). TIMM17B was positively correlated with GPX4 ($r = 0.459$, $P < 0.001$) and negatively correlated with ACSL4 ($r = 0.442$, $P < 0.001$) and NFE2L2 ($r = 0.408$, $P < 0.001$) (Fig. 9J, K). This suggests that TIMM17B may be involved in the regulation of ferroptosis.

Discussion

TIMM17B, located on the X chromosome, is one of the subunits of TIM17 and forms the TIM23 complex, which is responsible for transporting cytoplasmic proteins on the mitochondrial membrane (8-10, 19). At present, the research on TIMM17B is mostly focused on the basic function of TIMM17B and cells. Some studies have shown that the function of TIMM17B is related to cellular inflammation and stress (20). According to our analysis of the data in the TCGA database, we found that the high expression of TIMM17B is associated with a variety of tumors, including lung, liver, and ovarian cancers and other malignant tumors, and that there is also a significantly high expression of TIMM17B in BC. Although the high expression of TIMM17B in BC is not related to TNM stage, genotyping, and age, we found that the expression of TIMM17B has significant diagnostic value in BC. Moreover, in triple-negative BC, we found that the high expression of TIMM17B significantly led to a worse prognosis. Therefore, targeted therapy for TIMM17B may improve the prognosis of triple-negative BC.

Advances in immunotherapy provide new possibilities for the treatment of BC, especially triple-negative BC, especially with the clinical application of PD-1/PD-L1 inhibitors, which are considered to significantly improve the prognosis of triple-negative BC (21-23). The applica-

bility of immunotherapy depends on the tumor immune microenvironment and the expression of immune targets (24). At present, no studies have shown that TIMM17B is related to immunity. In this study, we found a negative correlation between TIMM17B and immune infiltration in BC, and with the expression of multiple immune targets, indicating that TIMM17B may have an inhibitory effect on immune infiltration in BC. Therefore, targeted TIMM17B may reverse the suppression of the immune microenvironment in BC.

Drug resistance in breast cancer is one of the main causes of poor prognosis in breast cancer (25, 26). The mechanisms associated with drug resistance in breast cancer are not yet clear. By predicting the IC50 of chemotherapeutic agents commonly used in breast cancer, we found that high expression of TIMM17B may lead to resistance to doxorubicin, paclitaxel, 5-fluorouracil, docetaxel, and the hormone therapy drug tamoxifen, which are commonly used in breast cancer. This suggests that targeting TIMM17B may reverse drug resistance in breast cancer and improve the prognosis of breast cancer patients, and provides a new direction for the study of drug resistance mechanisms in breast cancer.

Ferroptosis is a newly discovered mode of cell death in recent years, which is characterized by iron-dependent lipid peroxidation (27). Evidence suggests that the occurrence of BC is related to ferroptosis, and the inhibition of ferroptosis may lead to a poor prognosis of BC (28). Glutathione peroxidase 4 (GPX4) is a key enzyme of ferroptosis, which can inhibit ferroptosis in cancer cells (29). GPX4 is important in the process of ferroptosis, but there is a significant positive correlation between the expression of TIMM17B and GPX4 in this study, indicating that TIMM17B may have a negative regulatory effect on ferroptosis. At the same time, we found a correlation between TIMM17B and a variety of key enzymes regulating ferroptosis, indicating that TIMM17B may be closely related to the occurrence of ferroptosis (30).

This study still has some limitations. First, we only studied the expression and prognosis of TIMM17B through the database, and further research is needed under *in vitro* / animal experiments and clinical sample verification. Second, we should design experiments to study how TIMM17B regulates immune infiltration and ferroptosis in BC.

In conclusion, we found for the first time that TIMM17B is significantly overexpressed in a variety of malignant tumors, including BC. TIMM17B has a high diagnostic value in BC and maybe a diagnostic marker of BC. In addition, TIMM17B may help to regulate immune infiltration and ferroptosis in BC and may induce the drug resistance of BC.

Conclusions

In summary, TIMM17B is of great significance in BC and can be used as a potential diagnostic index of BC. In addition, TIMM17B may regulate immune infiltration, drug resistance, and ferroptosis in BC.

Acknowledgements

We thank all authors of the studies included in this study. We also thank all databases of the studies for providing the free data and the free R software which was used for analysis.

Authors' contributions

MT-D and Y-R conceived and designed the study. MT-D, Y-R, JW-Z, TT-Z obtained accurate data by using the database. HY-J and YH-W revised the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the Research Project Supported by Shanxi Scholarship Council of China, China (2021-157).

Availability of data and materials

The datasets presented in this study can be found in online repositories.

Declarations

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest with the contents of this article.

References

1. Cao W, Chen HD, Yu YW, Li N, Chen WQ. Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. Chin Med J (Engl). 2021 Mar 17;134(7):783-791. doi: 10.1097/CM9.0000000000001474. PMID: 33734139; PMCID: PMC8104205.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209-249. doi: 10.3322/caac.21660. Epub 2021 Feb 4. PMID: 33538338.
3. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin 2022;72(1):7-33.
4. Zuo S, Yu J, Pan H, Lu L. Novel insights on targeting ferroptosis in cancer therapy. Biomark Res. 2020 Oct 2;8:50. doi: 10.1186/s40364-020-00229-w. PMID: 33024562; PMCID: PMC7532638.
5. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J 1992;11(11):3887-95.
6. Wei SC, Duffy CR, Allison JP. Fundamental Mechanisms of Immune Checkpoint Blockade Therapy. Cancer Discov. 2018 Sep;8(9):1069-1086. doi: 10.1158/2159-8290.CD-18-0367. Epub 2018 Aug 16. PMID: 30115704.
7. Esteva FJ, Wang J, Lin F, Mejia JA, Yan K, Altundag K, Valero V, Buzdar AU, Hortobagyi GN, Symmans WF, Pusztai L. CD40 signaling predicts response to preoperative trastuzumab and concomitant paclitaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide in HER-2-overexpressing breast cancer. Breast Cancer Res. 2007;9(6):R87. doi: 10.1186/bcr1836. PMID: 18086299; PMCID: PMC2246190.
8. Frazier AE, Chacinska A, Truscott KN, Guiard B, Pfanner N, Rehling P. Mitochondria use different mechanisms for transport of multispanning membrane proteins through the intermembrane space. Mol Cell Biol 2003;23(21):7818-28.
9. Chacinska A, Koehler CM, Milenkovic D, Lithgow T, Pfanner N. Importing mitochondrial proteins: machineries and mechanisms. Cell. 2009;138(4):628-44.
10. Chacinska A, Lind M, Frazier AE, Dudek J, Meisinger C, Geiss-

ler A, et al. Mitochondrial presequence translocase: switching between TOM tethering and motor recruitment involves Tim21 and Tim17. Cell. 2005;120(6):817-29.
11. Shames SR, Croxen MA, Deng W, Finlay BB. The type III system-secreted effector EspZ localizes to host mitochondria and interacts with the translocase of inner mitochondrial membrane 17b. Infect Immun 2011;79(12):4784-90.
12. Shames SR, Deng W, Guttman JA, de Hoog CL, Li Y, Hardwidge PR, et al. The pathogenic E. coli type III effector EspZ interacts with host CD98 and facilitates host cell prosurvival signalling. Cell Microbiol 2010;12(9):1322-39.
13. Sinha D, Srivastava S, Krishna L, D'Silva P. Unraveling the intricate organization of mammalian mitochondrial presequence translocases: existence of multiple translocases for maintenance of mitochondrial function. Mol Cell Biol 2014;34(10):1757-75.
14. Tomczak K, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn). 2015;19(1A):A68-77. doi: 10.5114/wo.2014.47136. PMID: 25691825; PMCID: PMC4322527.
15. Lu X, Jiang L, Zhang L, Zhu Y, Hu W, Wang J, et al. Immune Signature-Based Subtypes of Cervical Squamous Cell Carcinoma Tightly Associated with Human Papillomavirus Type 16 Expression, Molecular Features, and Clinical Outcome. Neoplasia (New York, NY). 2019;21(6):591-601.
16. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Proteomics. Tissue-based map of the human proteome. Science (New York, NY). 2015;347(6220):1260419.
17. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012 May;16(5):284-7. doi: 10.1089/omi.2011.0118. Epub 2012 Mar 28. PMID: 22455463; PMCID: PMC3339379.
18. Bekric D, Ocker M, Mayr C, Stintzing S, Ritter M, Kiesslich T, et al. Ferroptosis in Hepatocellular Carcinoma: Mechanisms, Drug Targets and Approaches to Clinical Translation. Cancers. 2022;14(7).
19. Asselta R, Paraboschi EM, Gerussi A, Cordell HJ, Mells GF, Sandford RN, et al. X Chromosome Contribution to the Genetic Architecture of Primary Biliary Cholangitis. Gastroenterol 2021;160(7):2483-95.e26.
20. Ogunbileje JO, Porter C, Herndon DN, Chao T, Abdelrahman DR, Papadimitriou A, Chondronikola M, Zimmers TA, Reidy PT, Rasmussen BB, Sidossis LS. Hypermetabolism and hypercatabolism of skeletal muscle accompany mitochondrial stress following severe burn trauma. Am J Physiol Endocrinol Metab. 2016 Aug 1;311(2):E436-48. doi: 10.1152/ajpendo.00535.2015. Epub 2016 Jul 5. PMID: 27382037; PMCID: PMC5005969.
21. Emens LA. Breast Cancer Immunotherapy: Facts and Hopes. Clin Cancer Res. 2018 Feb 1;24(3):511-520. doi: 10.1158/1078-0432.CCR-16-3001. Epub 2017 Aug 11. PMID: 28801472; PMCID: PMC5796849.
22. Esteva FJ, Hubbard-Lucey VM, Tang J, Pusztai L. Immunotherapy and targeted therapy combinations in metastatic breast cancer. Lancet Oncol 2019;20(3):e175-e86.
23. Keenan TE, Tolaney SM. Role of Immunotherapy in Triple-Negative Breast Cancer. J Natl Compr Canc Netw. 2020 Apr;18(4):479-489. doi: 10.6004/jnccn.2020.7554. PMID: 32259782.
24. Loi S, Michiels S, Adams S, Loibl S, Budczies J, Denkert C, Salgado R. The journey of tumor-infiltrating lymphocytes as a biomarker in breast cancer: clinical utility in an era of checkpoint inhibition. Ann Oncol. 2021 Oct;32(10):1236-1244. doi: 10.1016/j.annonc.2021.07.007. Epub 2021 Jul 24. PMID: 34311075.
25. Bai X, Ni J, Beretov J, Graham P, Li Y. Triple-negative breast cancer therapeutic resistance: Where is the Achilles' heel? Cancer lett 2021;497:100-11.
26. Fahad Ullah M. Breast Cancer: Current Perspectives on the Disease Status. Adv Exp Med Biol. 2019;1152:51-64. doi: 10.1007/978-3-030-20301-6_4. PMID: 31456179.
27. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell. 2012;149(5):1060-72.
28. Sui S, Xu S, Pang D. Emerging role of ferroptosis in breast cancer: New dawn for overcoming tumor progression. Pharmacol Ther 2022;232:107992.
29. Zhang X, Sui S, Wang L, Li H, Zhang L, Xu S, et al. Inhibition of tumor propellant glutathione peroxidase 4 induces ferroptosis in cancer cells and enhances anticancer effect of cisplatin. J Cell Physiol 2020;235(4):3425-37.
30. Garg H, Digital twin technology: Revolutionary to improve personalized healthcare, SPR, 2021, Volume 1, issue 1, Page No.: 32 – 34