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FKBP5 associated CD8 T cell infiltration is a novel prognostic biomarker in luminal B breast cancer

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Abstract

Objective: To investigate the relationship between FKBP prolyl isomerase 5 (*FKBP5*) gene expression and CD8 T cells in tumour progression and immunology of the luminal B subtype of breast cancer (LBBC) using bioinformatics analyses.

Methods: The Gene Expression Profiling Interactive Analysis 2, Human Protein Atlas and breast cancer gene-expression miner v4.5 databases were used for data mining and analysing *FKBP5*, its co-expressed genes and CD8 T cell-related markers. The Tumor IMmune Estimation Resource 2.0 database was used for analysing the correlation and prognosis of *FKBP5* and CD8 T cell infiltration level in LBBC.

Results: Upregulated *FKBP5* expression was correlated with improved survival in LBBC. Upregulated *FKBP5*-related CD8 T cell markers were also demonstrated to be significantly correlated with better survival in LBBC and might play a role in the biological activity of *FKBP5*.

Conclusion: These findings suggest that *FKBP5* and its associated CD8 T cell infiltration are potential benign prognostic indicators for LBBC.

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Introduction

Breast cancer (BC) is the most common cancer in women worldwide.[1] Early diagnosis and comprehensive treatments have dramatically improved the prognosis of BC patients. In recent years, molecularly targeted therapy and immunotherapy have become new and promising options in BC.[2,3] However, it is still necessary to develop more reliable biomarkers to enhance therapeutic strategies for individual patients.

The FK506-binding protein 5 (FKBP5, also known as FKBP51) belongs to a family of immunophilins; and it has been reported to possess multiple functions with regard to the regulation of various signalling pathways and in tumorigenesis and chemoresistance.[4–7] For example, FKBP5 has been recently found to play a pivotal role in androgen receptor (AR) signalling in metastatic castration-resistant prostate cancer.[8,9] However, there still exists a lack of relevant research on FKBP5 and clinical characteristics in the luminal B subtype of breast cancer (LBBC). For the first time, this current study explored FKBP prolyl isomerase 5 (*FKBP5*) gene expression in LBBC and its relationship with clinical outcomes and co-expressed genes using bioinformatics analysis.

CD8 T cells are the major immune cells infiltrating solid tumours and detecting antigens from tumour cells in the tumour microenvironment, thus presenting an effective immunotherapy treatment response.[10] Previous studies have shown that tumour-infiltrating lymphocytes (TILs) are crucial cells involved in immunotherapeutic strategies for BC.[11] For instance, CD8 TILs have been reported to contribute to spontaneously prominent periductal fibrosis in ductal carcinoma *in situ*.[12] However, the underlying relationship between *FKBP5* and CD8 T cells in tumour progression and immunology of LBBC remains unclear.

In this current study, the Gene Expression Profiling Interactive Analysis 2 (GEPIA2) database, Human Protein Atlas (HPA) database and breast cancer gene-expression miner v4.5 (bc-GenExMiner v4.5) database were used to assess the potential variation of *FKBP5* gene expression between a BC group and an adjacent control group, as well as the relationship between *FKBP5* gene expression and clinical outcomes. Furthermore, preliminary analysis of the molecular mechanisms by which FKBP5 could be involved in LBBC was conducted by screening co-expressed genes with GEPIA2, Database for Annotation, Visualization and Integrated Discovery (DAVID) and other databases. Then, Tumor IMmune Estimation Resource 2.0 (TIMER2.0) was used to analyse the correlation and prognosis of *FKBP5* gene expression and the level of CD8 T cell infiltration in LBBC. Lastly, the association between *FKBP5*-related CD8 T cell markers and prognosis in LBBC patients was determined using bc-GenExMiner v4.5 and GEPIA2.

Materials and methods

Data mining and analysis

The GEPIA2 database (gepia.cancerpku.cn)[13] was used to evaluate the mRNA level of *FKBP5* and its co-expressed genes

in cancer specimens compared with those in normal controls using Student's *t*-test. The GEPIA2 database was also used to analyse overall survival (OS) of targeted genes in BC patients. The HPA database (www.proteinatlas.org)[14] was used to assess the immunohistochemistry outcomes of *FKBP5* and 3-hydroxyanthranilate 3,4-dioxygenase (*HAAO*) in both BC and normal tissues. The expression and correlation modules of bc-GenExMiner v4.5 (bcgenex.centregauducheau.fr)[15] were used to show the clinicopathological traits and linear dependence of the studied genes in BC patients.

Immune infiltration analysis

The relationship between *FKBP5* and the immune infiltration was explored using TIMER2.0 (timer.comp-genomics.org/).[16]The outcome module of TIMER2.0 was used to show the association between immune infiltrates and the clinical outcome of *FKBP5* in BC patients. The sCNA module of TIMER2.0 was used to explore the association between immune infiltrates and somatic copy number variation of *FKBP5* in BC patients.

COSMIC and cBioPortal analysis for mutations

The COSMIC database (www.sanger.ac.uk/cosmic/)[17] and cBioPortal database (www.cbioportal.org)[18] were used to explore *FKBP5* gene mutations.

Screening for FKBP5 *co-expressed genes*

Genes co-expressed with *FKBP5* in BC were screened from the similar genes detection module of the GEPIA2 database, which identified a list of genes with similar expression patterns compared with an input gene and selected datasets. Pearson's correlation coefficient was used to determine 200 co-expressed genes.

Enrichment analysis and protein–protein interaction network

Gene Ontology (GO) analysis of *FKBP5* co-expressed genes was analysed using the DAVID database (david.ncifcrf.gov).[19] The Search Tool for the Retrieval of Interacting Genes (STRING) database (www.string-db.org)[20] was used to construct the protein–protein interaction (PPI) network of *FKBP5* co-expressed genes or CD8 T cell markers.

Statistical analyses

The prognoses of targeted genes were estimated using Kaplan–Meier curve analysis. Correlations between *FKBP5* and different immune infiltrations or CD8 T cell markers were estimated using Spearman's correlation. Other data analyses were performed and illustrations were generated using statistical algorithms within various databases. A P-value < 0.05 was considered statistically significant.

Results

Association between FKBP5 *and prognosis in breast cancer patients*

Previous findings from this current research team showed *FKBP4* was a malignant indicator in luminal A subtype of BC,[21] and a potential *FKBP4*/*5*-related ncRNA-mRNA regulatory axis contributing to BC was also identified,[22] so this current study aimed to further explore the regulatory mechanism of *FKBP5* on BC.

Based on the GEPIA2 database, *FKBP5* mRNA expression was found to be significantly down-regulated in tumour samples compared with normal samples in 16 types of cancer, including bladder urothelial cancer, BC, cervical squamous cell cancer and endocervical adenocarcinoma (see supplementary materials, Fig. S1).

Meanwhile, FKBP5 protein level in BC was assessed using the HPA database. The HPA database indicated that FKBP5 protein level was significantly reduced in cancerous tissues compared with corresponding normal tissues when using either antibody CAB009315 (see supplementary materials, Figs S2A–S2D) or antibody HPA031095 (see supplementary materials, Figs S2E–S2F).

Using the bc-GenExMiner v4.5 database, decreased *FKBP5* was found significantly related to the luminal A and luminal B subtype than the normal group rather than human epidermal growth factor receptor 2 (HER2) positive and basal-like subtype (Figure 1A). Only oestrogen receptor (ER) status was negatively correlated with *FKBP5* expression (Figure 1B), while *FKBP5* expression had no significant change when comparing progesterone receptor (PR) or HER2 positive groups with the normal group (Figures 1C and 1D). The impact of *FKBP5* expression on OS was further explored in four molecular subtypes of BC patients using GEPIA2. Upregulated *FKBP5* expression was significantly related to better survival in luminal B subtype of BC patients (hazard ratio [HR] = 0.54, $P = 0.021$), but not in luminal A (HR = 0.92, $P = 0.84$), HER2 positive (HR = 1.1, $P = 0.87$) or basal-like subtype (HR = 0.82, $P = 0.69$) (Figures 1E–1H). Taken together, down-regulated *FKBP5* expression was only correlated with worse survival in LBBC patients.

The impact of alterations in FKBP5 *gene on clinical survival*

In the COSMIC database, the pie chart described various mutation information including nonsense substitution, missense substitution and synonymous substitution. The proportion of missense substitution was the highest (14.43%) (see supplementary materials, Fig. S3A). BC mainly had 33.80% G>A, 24.65% C>T and 9.86% A>G mutations in the *FKBP5* coding strand (Fig. S3B). Alteration frequency of *FKBP5* mutations in BC was analysed by using cBioPortal. From 0.5% to 3% mutations in the patients with BC were observed (see supplementary materials, Fig. S3C). Kaplan–Meier plot and log-rank test analysis demonstrated that the alterations in *FKBP5* had no correlations to OS ($P = 0.547$) or disease-free survival ($P = 0.714$) in BC patients with/without *FKBP5* alterations (see supplementary materials, Figs S3D and S3E).

Bioinformatics analysis of FKBP5 *co-expressed genes*

A total of 200 *FKBP5* co-expressed genes collected from GEPIA2 were analysed using the DAVID database. The GO enrichment analysis comprised three categories: a biological process, a molecular function and a cellular component. The most valuable 10 pathways of each category are presented (see supplementary materials, Figs S4A–S4C), suggesting that *FKBP5* co-expressed genes might participate in multiple basic functions including the oxidation–reduction process and catalytic activity. The PPI network was displayed using the STRING database (see supplementary materials, Fig. S5); and three pairs of co-expressed genes with the highest combined scores (*HAAO*, *KYNU*, *AADAT*, *CCBL1*, *FDFT1* and *IDI1*) were collected from the PPI network (Table 1).

Based on the GEPIA2 database, *HAAO* but not *KYNU*, *AADAT*, *CCBL1*, *FDFT1* or *IDI1* mRNA expression was significantly downregulated in tumour samples compared with normal samples in various types of cancer, including BC (see supplementary materials, Figs S6–S11). The HPA database indicated that *FKBP5* co-expressed gene *HAAO* expression was significantly reduced in cancerous tissues

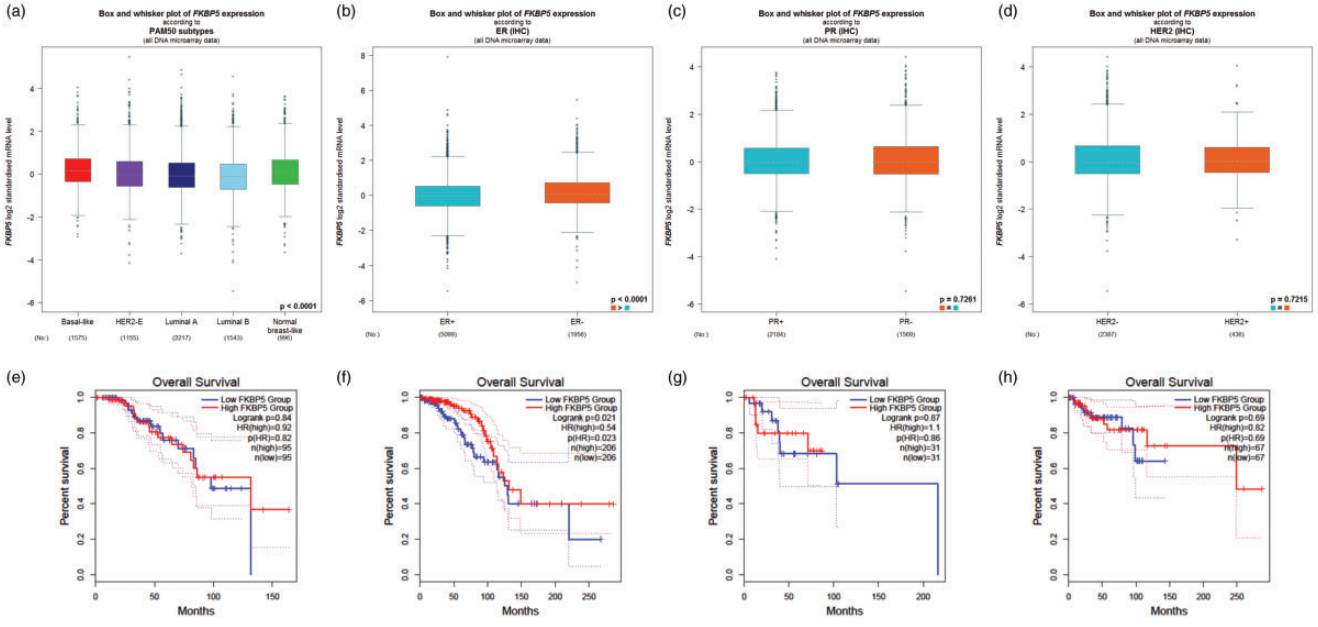

Figure 1. Relationship between FKBP prolyl isomerase 5 (*FKBP5*) gene expression with the clinicopathological characteristics and the prognostic merit in breast cancer patients. Box and whisker plots showing relationship between mRNA expression of *FKBP5* and (A) different molecular subtypes, (B) oestrogen receptor (ER), (C) progesterone receptor (PR), and (D) human epidermal growth factor receptor 2 (HER2). The central horizontal lines for each group are the medians; the extremities of the box are the 25th and 75th percentiles; the error bars represent the minimum and maximum outliers; and the asterisk(s) above and below the error bars represent extreme outlier(s).Survival curves are plotted for patients of (E) luminal A, (F) luminal B, (G) HER2 positive and (H) basal-like. The *P*-value cutoff was 0.05. A Welch's test was also performed (along with Dunnett-Tukey-Kramer's tests for pairwise comparison when appropriate).

Table 1. Top 20 pairs of FKBP prolyl isomerase 5 (*FKBP5*) co-expressed genes from the protein–protein interaction network.

Node1	Node2	Node1_string_internal_id	Node2_string_internal_id	Combined_score
HAAO	*KYNU*	4437103	4435389	0.998
AADAT	*CCBL1*	4449239	4448067	0.99
CCBL1	*KYNU*	4448067	4435389	0.988
FDFT1	*IDI1*	4451530	4445537	0.986
AADAT	*KYNU*	4449239	4435389	0.982
AFMID	*KYNU*	4440061	4435389	0.973
FDFT1	*SC5D*	4451530	4435359	0.97
ACSL1	*ACADL*	4449224	4433600	0.968
AKR1C2	*SRD5A1*	4445450	4436117	0.965
SRD5A1	*AKR1D1*	4436117	4433819	0.964
ACSL1	*FASN*	4449224	4438061	0.961
CCBL1	*AFMID*	4448067	4440061	0.959
ACSL3	*ACADL*	4441998	4433600	0.956
AADAT	*AFMID*	4449239	4440061	0.955
ACSL3	*FASN*	4441998	4438061	0.952
ACADM	*MCCC1*	4443625	4435621	0.933
ACSBG1	*ACADL*	4434660	4433600	0.927
MVK	*IDI1*	4450087	4445537	0.925
FDFT1	*MVK*	4451530	4450087	0.924
FASN	*ACSBG1*	4438061	4434660	0.92

compared with corresponding normal tissues when using antibody HPA036394 (see supplementary materials, Figs S12A–S12F).

Using the bc-GenExMiner v4.5 database, decreased *HAAO* gene expression was significantly related to all molecular subtypes compared with than the normal group (Figure 2A). Both ER and PR status were negatively correlated with HAAO expression (Figures 2B and 2C), while *HAAO* expression had no significant change when comparing HER2 positive group with the normal group (Figure 2D). The correlation of *HAAO* expression and survival was further explored in all molecular subtypes of BC patients. Upregulated *HAAO* expression was only significantly related to better survival in LBBC patients (HR = 0.42, $P = 0.019$), but not correlated to those in luminal A, HER2 positive and basal-like subtypes of breast cancer patients (HR = 0.89, $P = 0.68$; HR = 0.39, $P = 0.13$; HR = 1.6, $P = 0.53$) (Figures 2E–2H). Taken together, these findings suggest that downregulated *FKBP5* co-expressed gene *HAAO* was also only correlated with worse survival in LBBC patients.

Correlation of FKBP5 *expression and immune infiltration level in breast cancer*

Tumour-infiltrating cells are independent predictors of survival in BC patients.[23,24] Therefore, this current study investigated whether *FKBP5* functioned as a tumour suppressor by interacting with immune infiltration in BC through the TIMER2 database. After the correlation adjustment by purity, results showed that *FKBP5* expression had significantly positive correlations with infiltrating levels of CD8 T cells ($r = 0.155$, $P < 0.001$), neutrophils ($r = 0.316$, $P < 0.001$), macrophages ($r = 0.072$, $P = 0.023$) and dendritic cells ($r = 0.139$, $P < 0.001$), but there was no correlation with CD4 T cells ($r = 0.025$, $P = 0.436$) or

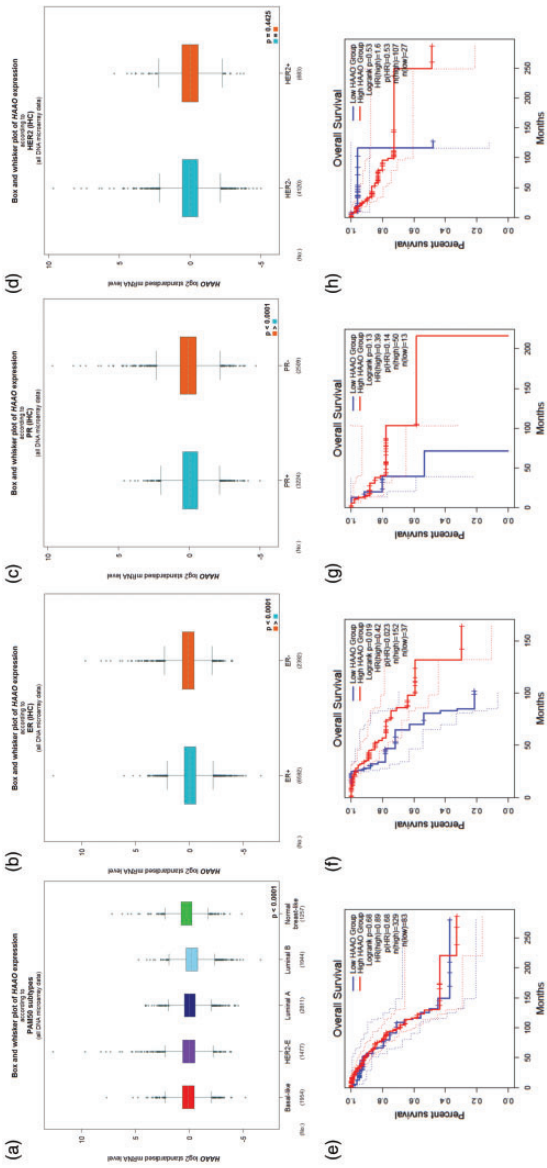

Figure 2. Relationship of *HAAO* gene expression with the clinicopathological characteristics and the prognostic merit in breast cancer patients. Box and whisker plots showing the relationship between mRNA expression of *HAAO* and (A) different molecular subtypes, (B) oestrogen receptor (ER); (C) progesterone receptor (PR); (D) human epidermal growth factor receptor 2 (HER2). The central horizontal lines for each group are the medians; the extremities of the box are the 25th and 75th percentiles; the error bars represent the minimum and maximum outliers; and the asterisk(s) above and below the error bars represent extreme outlier(s). Survival curves are plotted for patients of (E) luminal A, (F) luminal B, (G) HER2 positive and (H) basal-like. The p value cutoff is 0.05. A Welch's test is also performed (along with Dunnett-Tukey-Kramer's tests for pairwise comparison when appropriate).

B cells ($r = -0.009$, $P = 0.766$) in BC (Figures 3A–3F).

The association between survival and BC defined by immune infiltration level and *FKBP5* expression was examined. Patients with the better survival over 150 months had increased CD8 T cell infiltration with high expression of *FKBP5* ($P = 0.042$), whereas patients with increased CD8 T cell infiltration with low expression of *FKBP5* had no significant improvement in survival ($P = 0.364$) (Figure 4B). As for the influence of infiltration level of CD4 T cells, B cells, neutrophils, macrophages and dendritic cells on survival, results revealed no statistically significant correlations (Figures 4A, 4C–4F).

The association between immune infiltrates and somatic copy number variation of *FKBP5* was also explored in BC patients. Arm-level gain of *FKBP5* gene level was significantly detected among CD8 T cell, neutrophil and macrophage infiltration, but no somatic copy number variation of *FKBP5* gene was found in CD4 T cells, B cells or dendritic cells (see supplementary materials, Figs S13A–S13F). These findings strongly suggested that *FKBP5* played a specific role in CD8 T cell infiltration in BC.

Interaction between CD8 T cell related markers and FKBP5 *gene expression in luminal B subtype of breast cancer*

The CD8 T cells in the tumour microenvironment can produce various markers on their surface and secrete many immune-related markers, including *PRF1*, *CD8A*, CD69, *BTLA*, *GZMB*, *ICOS*, *CCL5* and *PTPRC*(see supplementary materials, Fig. S14), finally leading to upregulated adaptive immune pathways.[25–29]

As upregulated *FKBP5* expression was only correlated with better survival in LBBC patients, the study then examined whether *FKBP5* acted as a tumour suppressor by association with the expression of *CD8A* or other CD8 T cell-related markers among the tumour microenvironment in LBBC patients. Consistent with this hypothesis, a positive relationship between *CD8A* and *FKBP5* was observed ($r = 0.13$, $P < 0.001$) (Figure 5B). Similar results were obtained between *FKBP5* and other CD8 T cell-related markers (Figures 5A, 5C–5H).

Association between CD8 T cell related markers and prognosis in luminal B subtype of breast cancer patients

To further confirm *FKBP5*-associated CD8 T cell-related markers that might induce tumour immunosuppression in LBBC and provide prognostic insight, the expression of *PRF1*, *CD8A*, *CD69*, *BTLA*, *GZMB*, *ICOS*, *CCL5* or *PTPRC* was correlated with 150-month survival curves using data from GEPIA2.

Using the bc-GenExMiner v4.5 database, decreased *CD8A* or other CD8 T cell-related markers was found significantly related to the luminal A and luminal B subtype than the normal group rather than HER2 positive and basal-like subtype (see supplementary materials, Figs S15A–S15H). The impact of *CD8A* and other CD8 T cell-related marker expression on OS was further explored in four molecular subtypes of BC patients using GEPIA2. Upregulated *PRF1*, *CD8A*, *CD69*, *BTLA*, *GZMB*, *ICOS*, *CCL5* or *PTPRC* was solely significantly related to better survival in luminal B subtype of BC patients (HR = 0.38, $P = 0.009$; HR = 0.33, $P = 0.003$; HR = 0.22, $P < 0.001$; HR = 0.29, $P < 0.001$; HR = 0.43, $P = 0.021$; HR = 0.37, $P = 0.006$; HR = 0.3, $P = 0.001$; HR = 0.33, $P = 0.003$; respectively) (Figures 6A–6H), but not in luminal A (see supplementary materials, Fig. S16), HER2 positive (see supplementary materials, Fig. S17) or basal-like subtype (see supplementary materials, Fig. S18). Taken together, these findings show that upregulated *FKBP5*-associated CD8 T

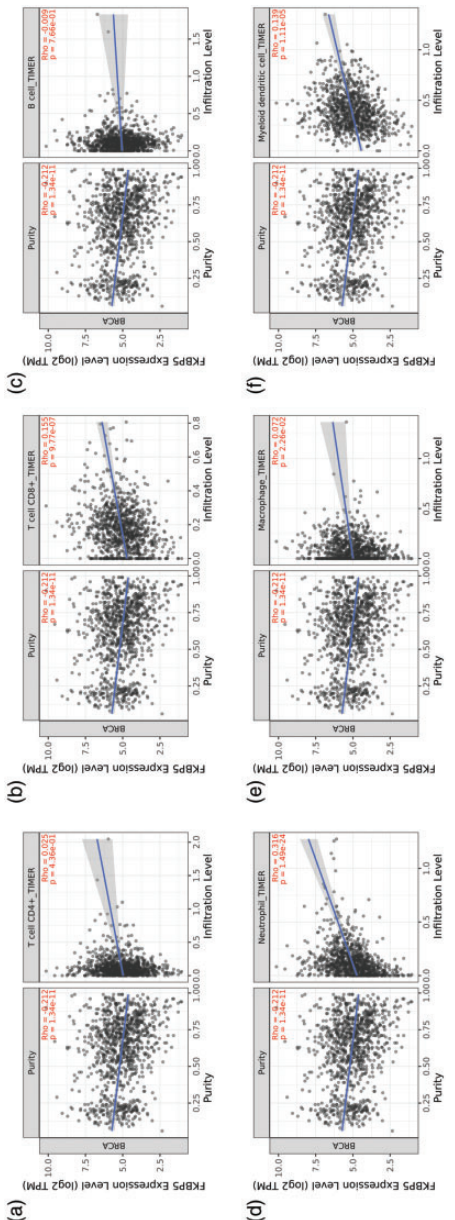

Figure 3. Correlation between FKBP proly isomerase 5 (*FKBP5*) gene expression and immune infiltration level in breast cancer. Correlation of *FKBP5* expression with infiltrating levels of (A) CD4 T cells, (B) CD8 T cells, (C) B cells, (D) neutrophils, (E) macrophages and (F) dendritic cells. The *P*-value cutoff was 0.05.

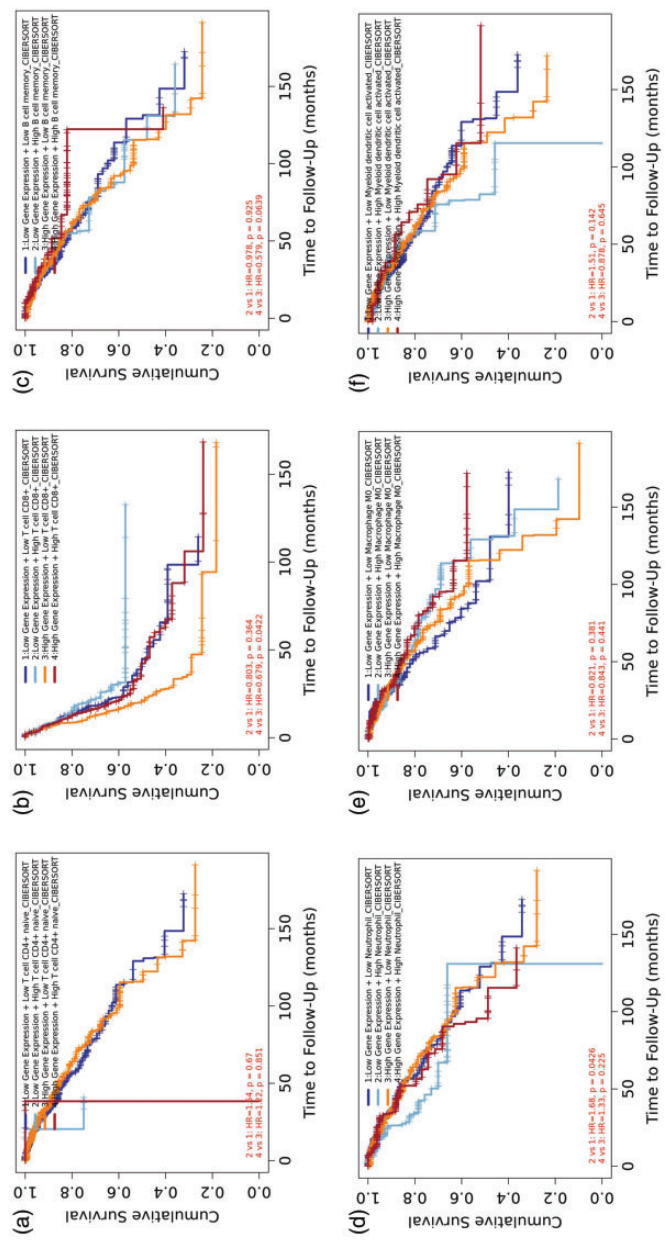

Figure 4. Overall survival stratified by a combined analysis of immune infiltration level and FKBP prolyl isomerase 5 (*FKBP5*) gene expression in Tumor IMmune Estimation Resource 2.0. Survival based on high/low *FKBP5* expression and (A) high/low CD4 T cells infiltration level, (B) high/low CD8 T cells infiltration level, (C) high/low B cells infiltration level, (D) high/low neutrophils infiltration level, (E) high/low macrophages infiltration level and (F) high/low dendritic cells infiltration level. The *P*-value cutoff was 0.05.

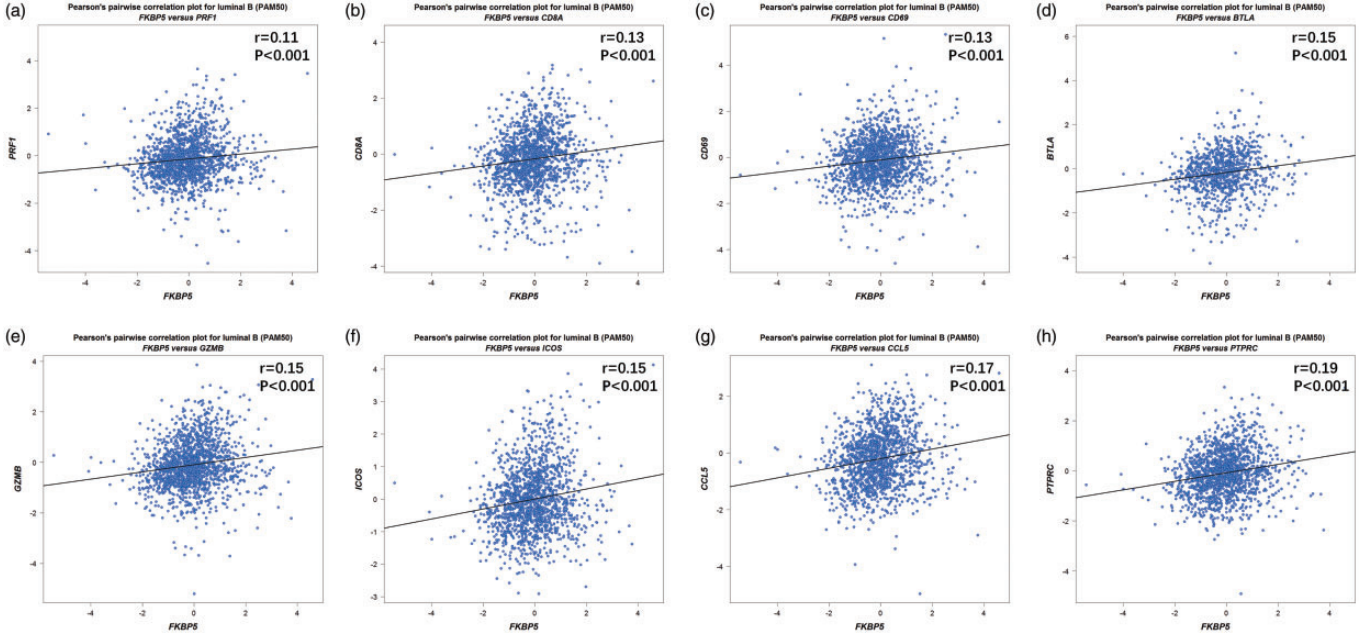

Figure 5. Pairwise Pearson's correlation analysis between CD8 T cell-related markers and FKBP prolyl isomerase 5 (*FKBP5*) gene expression in bc-GenExMiner v4.5. Scatter plots of pairwise expression between *FKBP5* and (A) *PRF1*, (B) *CD8A*, (C) *CD69*, (D) *BTLA*, (E) *GZMB*, (F) *ICOS*, (G) *CCL5* and (H) *PTPRC*. The *P*-value cutoff was 0.05.

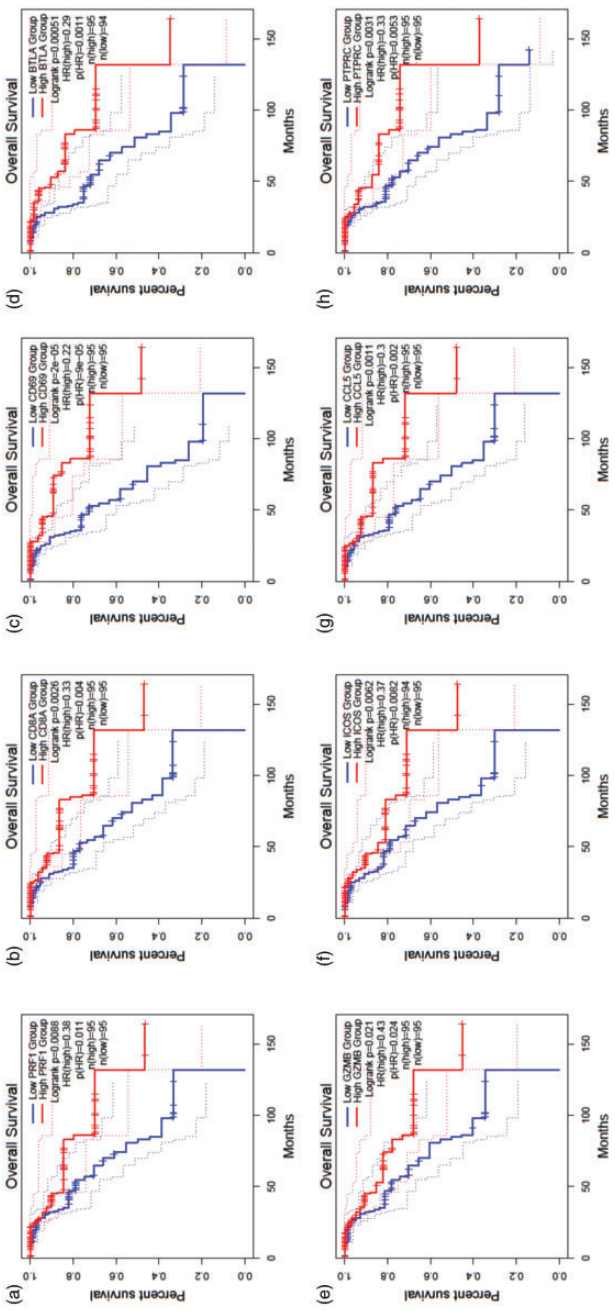

Figure 6. Relationship between CD8 T cell-related markers with the prognostic merit. Kaplan–Meier plots of overall survival based on (A) *PRF1*, (B) *CD8A*, (C) *CD69*, (D) *BTLA*, (E) *GZMB*, (F) *ICOS*, (G) *CCL5* and (H) *PTPRC* in luminal B subtype of breast cancer patients. The *P*-value cutoff was 0.05.

cell-related marker expression was only correlated with better survival in LBBC patients.

Discussion

Breast cancer is a heterogeneous tumour, showing variable morphological and biological features.[30] Although early detection and personalized treatments have decreased the mortality rate based on traditional molecular classification of BC, identifying novel drivers and biomarkers are still necessary for improving the prognosis of BC patients. This current study found that *FKBP5* might play a significantly benign role through modulating its co-expressed *HAAO* gene expression in LBBC.

The *FKBP* family members in human and other mammals have been reported to exert important roles in multiple biochemical pathways including cardiac function, neurodegenerative disorders and cancer development.[31] For instance, lack of *FKBP12.6* leads to sever cardiac dysfunction via a calcium signalling pathway, resulting in early death of the gene deletion mice shortly after birth.[32] In Alzheimer's disease, *FKBP4* is the most extensively studied protein binding to hyperphosphorylated Tau (a microtubule associated protein) and it blocks its ability to induce microtubule assembly.[33] In melanoma, upregulated *FKBP8* has been found to reduce cancer cell growth by involvement in mTOR signalling activity.[34]

Recent research suggests that *FKBP5* expression is also strongly associated with various types of cancers, for example, oesophageal adenocarcinoma,[35] prostate cancer,[36] acute lymphoblastic leukaemia,[37] melanoma[5] and neuroglioma.[38] Particularly in prostate cancer, where *FKBP5* has been well studied in relation to AR; with the overexpression of *FKBP5* stimulating AR transcriptional level and promoting cell proliferation.[39] Because of the lack of a more systematic study between *FKBP5* and breast cancer, this current study was conducted to assess the clinical role and molecular regulatory importance of *FKBP5* in LBBC.

In the GEPIA2 and HPA databases, both the mRNA and protein levels of *FKBP5* were observed to be significantly downregulated in the BC group compared with the adjacent normal group in the current study. In addition, the lower *FKBP5* expression was found in both luminal A and luminal B subtypes, but was irrelevant to the HER2 positive or basal-like subtype in BC; and downregulated expression of *FKBP5* was only significantly correlated with ER status. Furthermore, the bcGenExMiner v4.5 database was used to demonstrate that downregulated mRNA expression of *FKBP5* was associated with unfavourable survival for LBBC patients. More research is warranted to find out whether *FKBP5* plays a crucial part in ER-related signalling pathways in LBBC.

A total of six co-expressed genes of *FKBP5*, namely *HAAO*, *KYNU*, *AADAT*, *CCBL1*, *FDFT1*and *IDI1*, were screened in the current study. With the exception of *AADAT* and *IDI1*, the other co-expressed genes are all involved in many biological processes of tumorigenesis. *HAAO* has been shown to be a significant methylated gene in prostate cancer;[40]*KYNU* can suppress BC cell proliferation;[41] and *CCBL1* and *FDFT1* are both good prognostic markers for survival prediction in patients with colorectal cancer.[42,43] In the current study, only lower *FKBP5* co-expressed gene *HAAO* was found in the BC group compared with the adjacent normal group at the mRNA and protein levels. Moreover, downregulated *HAAO* significantly correlated with ER and PR status and predicted a shorter survival time in LBBC patients. Therefore, these current findings suggest that *HAAO* might interact with *FKBP5* in LBBC, though specific molecular

mechanisms remain to be further explored and clarified.

Another important aspect of this current study was that *FKBP5* expression was found to be positively correlated with various immune infiltration levels in LBBC, especially the infiltration level of CD8 T cells. In addition, these current results indicated that the increase in *FKBP5* expression positively correlated with the expression of CD8 T cell-related markers. For example, *CD69*, a strongly increased marker of CD8 T cell activation,[44] was highly correlated with *FKBP5* expression in LBBC. These above-mentioned correlations could indicate the potential mechanism by which *FKBP5* regulates CD8 T cells within the tumour microenvironment of LBBC. This current study has for the first time demonstrated longer survival for $FKBP5^{high}$/CD8 $T\ cells^{high}$ patients with LBBC. These findings suggest future opportunities for the improvement of novel immunotherapies for LBBC patients depending upon screened credible biomarkers.

Author contributions

Writing – Original Draft Preparation: F.T., H.C. X., J.Z. and G.L.L.; Writing – Review & Editing: W.J.X., J.Z., F.T., D.J.H., J.D.C. and Q.D.; Supervision: Q.D. and H.C.X. All authors have reviewed the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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