

Overexpressed FAM166B Predicts Favorable Prognosis and Associated With Metabolic Pathways and Tumor Immune Infiltrates in BRCA

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Research Article

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Abstract

Background: Breast cancer is one of the most common and lethal cancer worldwide. Though surgery, chemotherapy, endocrine therapy and immune therapy have boost patients' survival rate, to establish more molecular biomarkers that can detect the early metastasis and shed a light on breast cancer treatment still requires efforts. Based on previous studies, adipose tissue had a metabolic crosstalk in breast cancer. FAM166B is a protein-coding gene which was reported to be found in adipose tissues. Yet whether FAM166B has a role in breast cancer had not been determined.

Methods: In this study, 1109 BRCA patients and 113 adjacent cancer samples and clinical characteristics data were downloaded from TCGA data portal, R software and Strawberry Perl were used for all pre-processing processes. Kaplan-Meier plotter, univariate and multivariate Cox analysis were used to investigate FAM166B potential in BRCA prognosis. GSEA was performed to investigate the biological function of FAM166B. Furthermore, TIMER was utilized to identify the association between FAM166B and tumor-infiltrating immune cells.

Results: We found out increased FAM166B expression correlates with better prognosis in BRCA. By conducting Multivariate Cox analysis, we draw a conclusion that FAM166B could be an independent prognosis factor. Moreover, the GSEA revealed that FAM166B could possibly restrain the cell metabolism and glucose converting pathways. Also, a high expression of FAM166B was correlated with increased immune infiltration levels like CD4+ T cells and decreased macrophages, especially in luminal and basal breast cancers.

Conclusion: Our study illustrated that FAM166B is an independent prognostic factor in BRCA. A high expression of FAM166B indicates a better prognosis of breast cancer patients and it may restrain the tumor cell metabolism and likely play a role in immune cell infiltration.

Background

Breast cancer(BRCA)is the most common cancer among females worldwide and its metastasis is a crucial biological feature that leads to unfavorable patients' prognosis. According to the presence or absence of molecular markers, breast cancer is divided into four major subtypes: Luminal A, Luminal B, HER-2 positive, and triple-negative breast tumors, based on which subtype-specific therapeutics are determined[1]. The treatment for breast cancer patients includes surgery, adjuvant and neoadjuvant chemotherapy, endocrine therapy, and radiotherapy. Besides these, the presence of targeted drugs like trastuzumab has significantly increased breast cancer prognosis [2]. Although important advances in adjuvant chemotherapy and surgical techniques have improved breast cancer patient outcomes, the metastasis and recurrence after surgery are still the main causes of the failure of treatment and of poor prognosis [3]. Also, there is an increasing awareness of the importance of tumor - immune cell interactions to the therapy responses and the evolution of breast cancer. According to previous studies, adipose tissues play an important role in breast cancer development, including estrogen secretion and

providing the tumor micro environment in favor of cancer[4]. Therefore, to establish more molecular biomarkers that can detect the early metastasis and provide new ideas for breast cancer treatment that will improve survival is essential for breast cancer patients.

Family with sequence similarity 166, member B(FAM166B), is a protein-coding gene which was related with multiple symmetric lipomatosis (MSL), displaying additional positivity in the adipose tissues, adrenal gland and ciliated cells, based on previous research results[5, 6]. However, the exact expression level of FAM166B in other cancers and its clinical prognostic value are still unknown.

Therefore, in this study, bioinformatics methods were performed to predict the differential expression levels of FAM166B both in normal and in cancer tissues via several online databases. We found out that FAM166B has a prognosis potential in breast cancers. Hence, the Kaplan-Meier Plotter was used to evaluate the prognostic value of FAM166B in different subtypes of breast cancer and Cox analysis confirmed that FAM166B could be an independent prognosis factor. Moreover, the GSEA method was conducted to demonstrate the potential biological pathway that FAM166B impact. Additionally, we investigated the correlation of FAM166B with tumor-infiltrating immune cells in the various subtypes of breast cancer microenvironments by using Tumor Immune Estimation Resource (TIMER). These findings in this report shed light on the role of FAM166B in breast cancer prognosis as well as provide an underlying relationship and mechanism between FAM166B and breast cancer. Thus, FAM166B has the potential to become a new predictor to evaluate prognosis for breast cancer.

Material And Methods

Bioinformatics mining

We identified the expression level of FAM166B gene in different types of cancers via the online database, which were The Cancer Genome Atlas (TCGA; <http://cancergenome.nih.gov/>) and Genotype-Tissue Expression (GTEx; <https://www.gtexportal.org/home/datasets>). Next, we used the OncoPrint database (<https://www.oncoPrint.org/resource/login.html>) [7] to further test the relationship between the targeted gene expression and various cancers.

Data download and preprocessing

The gene expression profiles data of 1109 BRCA patients and 113 adjacent cancer samples and clinical characteristics of matched patients were obtained from TCGA data portal. TCGA acts as a public repository that includes high-throughput microarray experimental data. BRCA sequencing data were generated by the Illumina HiSeq-RNA-Seq platform. We then performed subsequent processing of TCGA-BRCA survival information to filter out cases with insufficient or missing data. The clinical data were used for Cox regression analysis. R (<https://cran.r-project.org/>) (R software, version 3.6.2) and Strawberry Perl were used for all pre-processing processes.

The Kaplan Meier survival analysis

After confirming the correlation between FAM166B expression and breast cancer, Kaplan-Meier plotter (<http://kmplot.com/analysis/>) [8] was used to analyze the survival curves in various subtypes and statuses of breast cancers. The hazard ratio (HR) with 95% confidence intervals and log-rank P-value were also computed.

Gene set enrichment analysis

Gene set enrichment analysis (GSEA), a calculation method that could estimate whether a list of previously defined genes shows concordant differences with statistical significance between two biological processes. This study carried out the GSEA to elucidate the significant difference in survival rates observed between the low and high FAM166B groups after initially generating a sequential list of all genes according to their correlation to FAM166B expressions. For each analysis, the gene set permutations were performed 1000 times. The phenotype label was identified in the level of the FAM166B expression. In order to sort out the pathways enriched in each phenotype the Normalized Enrichment Score (NES), the nominal p value was utilized. The absolute value of NES > 1.5 and P value < 0.05 were considered with statistical significance.

Evaluation of tumor-infiltrating immune cells

TIMER, (<https://cistrome.shinyapps.io/timer/>) applies a deconvolution algorithm based on previously published statistics to infer the abundance of tumor-infiltrating immune cells (TIICs) from gene expression profiles, is a comprehensive resource for analysis of TIICs of various cancer types [9, 10]. We analyzed FAM166B expression in different types of cancer and the correlation of FAM166B expression with the abundance of immune infiltrates via gene modules. Gene expression levels against tumor purity are displayed on the left-most panel [11]. In addition, in this study, we explored the correlations between FAM166B expression and common gene markers of tumor-infiltrating immune cells via correlation modules. The gene markers of TIICs included markers of CD8 + T cells, T cells (general), B cells, monocytes, TAMs, M1 macrophages, M2 macrophages, neutrophils, natural killer (NK) cells, dendritic cells (DCs), T-helper 1 (Th1) cells, T-helper 2 (Th2) cells, follicular helper T (Tfh) cells, T-helper 17 (Th17) cells, Tregs, and exhausted T cells based on previous studies [12–14]. The correlation module generated the expression scatter plots between a pair of genes in a given cancer type, together with the Spearman's correlation and the estimated statistical significance. The threshold was identified as P-value < 0.01.

Results

The mRNA and Protein Expression Levels of FAM166B in Different Types of Normal and Tumor Tissues

To determine differences of FAM166B expression in tumor and normal tissues, the FAM166B mRNA or protein levels were analyzed by using multiple online databases. Firstly, to evaluate FAM166B expression in human tissues, we examined FAM166B expression via the TIMER website (Fig. 1A). We analyzed the

FAM166B expression profile across diverse tumors in TCGA database and found that FAM166B had a significantly lower expression relative to the corresponding normal samples (Fig. 1B). Analysis of both TCGA and GTEx datasets indicated significantly lower levels of FAM166B in 9 tumor types compared to the normal specimens (Fig. 1C). The analysis revealed that the FAM166B expression was at a significantly lower level in BRCA (Breast Cancer), KICH (Kidney Chromophobe), HNSC (Head and Neck squamous cell carcinoma), KIRP (Kidney renal papillary cell carcinoma), LIHC (Liver hepatocellular carcinoma), LUAD (Lung Adenocarcinoma), LUSC (Lung squamous cell carcinoma), PRAD (Prostate adenocarcinoma) and in KIRC (Kidney Renal Clear Cell Carcinoma), compared to adjacent normal tissues, which implied that FAM166B could play a role in cancer suppressing. To further evaluate FAM166B mRNA levels differential expression in human cancers, we analyzed it by using the OncoPrint database. This analysis revealed that the FAM166B expression was higher in Brain and CNS Cancer, Colorectal Cancer, Kidney Cancer, and Prostate cancer. However, lower expression was observed in bladder, breast, head and neck cancer, lung cancer, lymphoma, and melanoma (Fig. 1D). The fact that FAM166B shows a significantly lower expression in 2 breast cancer databases caught our attention (Fig. 1E), thus in the following studies we focused on the correlation between FAM166B and breast cancer.

Prognostic Potential of FAM166B in Breast Cancer

To further dig out the impact of FAM166B expression on BRCA, we stratified all BRCA patients in TCGA database into the FAM166B^{high} and FAM166B^{low} groups and found that increased FAM166B expression was correlated to longer OS in BRCA patients (Fig. 2E). By using the Kaplan-Meier plotter database, we then divided breast cancer patients into different subgroups, trying to get a more specific prognostic potential of FAM166B, shown in Fig. 2A-D, which indicated that a high FAM166B expression leads to a significantly better prognosis in luminal A breast cancer patients. Next, we performed univariate and multivariate Cox regression analyses on the TCGA dataset to discover the prognostic value of FAM166B expression and other clinical variables in BRCA. Univariate Cox regression analysis indicated that FAM166B expression, age, stage, tumor size, lymph nodes, metastasis were significantly correlated with the OS of BRCA patients, whereas gender did not reveal any correlation (Fig. 3A). By multivariate analysis, only two parameters were further identified as independent prognostic factors including FAM166B expression [P < 0.001, hazard ratio (HR) = 0.48] and age [P < 0.001, hazard ratio (HR) = 1.04] (Fig. 3B). We also investigated how FAM166B was involved with the prognosis of various stages of breast cancer and with the lymph nodes status, the results turned out to be insignificant (data not shown). Based on the results above, we drew the conclusion that FAM166B could be an independent factor to predict Breast Cancer patients' prognosis and an elevated expression of FAM166B indicates a better prognosis.

GSEA identifies a FAM166B-related signaling pathway

In order to better understand how FAM166B is involved in the BRCA pathogenesis, we conducted Gene Set Enrichment Analysis (GSEA) with KEGG analysis (based on absolute value of Spearman Score) to identify the signaling pathways activated between high and low FAM166B expression data sets. As we

considered that the absolute value of NES > 1.5 and P value < 0.05 were with statistical significance, four significantly enriched biological pathways caught our attention which were fructose and mannose metabolism pathway, sugar and nucleotide sugar metabolism pathway, pentose phosphate pathway and glycolysis gluconeogenesis pathway (Table 1; Fig. 4). The above biological pathways are closely related with glucose converting and cell metabolisms. Therefore, it is a reasonable deduction that FAM166B could play a role in reducing tumor progression by restraining cell metabolism.

FAM166B Expression Is Correlated with Immune Infiltration Level in Breast Cancers

According to previous studies, tumor-infiltrating lymphocytes could be an independent predictive factor of sentinel lymph node status and survival in cancers [15]. Hence, we explored whether FAM166B expression was correlated with immune infiltration levels in multiple cancers. Tumour mutation burden (TMB) and microsatellite instability (MSI) are sound biomarkers for immune therapy response in various kinds of tumor[16]. We analyzed the correlation between FAM166B and TMB. The results revealed that a significant association between targeted gene and TMB in BRCA ($p = 8.6e-05$), shown in Fig. 5A, while FAM166B has limited influence on MSI with the P value of 0.4 (Fig. 5B). Furthermore, we investigated if FAM166B expression was correlated with immune infiltration levels in different breast cancer subtypes from TIMER. Tumor purity is an essential factor that affects the analysis of immune infiltration in tumor samples, hence we selected the subtypes of breast cancer in which FAM166B expression levels have an evidential correlation with tumor purity in TIMER(Figure 5C). We found that FAM166B expression level correlates with favorable prognosis and high immune infiltration in luminal and in basal breast cancers. FAM166B expression level is significantly correlated with infiltrating levels of B cells ($P = 0.0166$), CD4 + T cells ($P = 0.0274$), macrophages ($P = 0.0007$) in BRCA-Basal sub-group. Similarly, there were significant correlations with tumor purity ($P = 0.0004$) and with infiltrating levels of CD4 + T cells ($P = 0.0185$) in BRCA-Luminal. These findings suggest that FAM166B plays a role in immune infiltration in luminal and basal breast cancers.

To further investigate how FAM166B is related to the diverse immune infiltrating cells, we used TIMER to research the correlations between FAM166B and immune marker sets of different immune cells in breast cancer. The results were shown in Table 2. We found that the expression levels of most marker set of CD8 + T cells, B cells, T cells, and dendritic cells have positive correlations with FAM166B expression while M2 macrophages have a negative correlation with FAM166B expression in BRCA. Specifically, we found out that CCR7 of neutrophils, STAT4, TBX21 of Th1 phenotype, STAT6 of Th2 phenotype, BCL6 of Tfh are significantly correlated with FAM166B expression in BRCA ($P < 0.0001$). We further analyzed the correlation between FAM166B expression and the above markers between different subtypes of breast cancer, including luminal, basal, and Her-2 subtypes. Correlation results between FAM166B and markers of B cells, T cells, CD8 + T cells, and Th1 cells are accordant to those in BRCA. These findings suggest that FAM166B may regulate macrophage polarization and T cells in breast cancer. Based on TIMER, the results showed that high FAM166B expression relates to the high infiltration level of DCs in all types of breast cancer, DC markers such as HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1 also show significant correlations with FAM166B expression. Moreover, FAM166B expression has a strong correlation with the

high infiltration level of natural killer cells, NK markers like KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2, KIR3DL3, KIR2DS4 are positively related to FAM166B expression.

Discussion

Family with sequence similarity 166, member B(FAM166B) is a gene that hasn't been studied thoroughly previously before. In previous studies that mentioned FAM166B were more centered on its expression in multiple symmetric lipomatosis and skeletal muscle, showing FAM166B had additional positivity in the adrenal gland and ciliated cells in the present investigation but the precise function remains putative [5, 17, 18]. Here, we reported that FAM166B expression is correlated with a better prognosis of breast cancer and could serve as an independent prognostic factor. Through this study, we also discovered that FAM166B is associated with metabolism pathways and immune infiltration cells.

According to our study, FAM166B has a higher expression in corresponding normal tissues than in BRCA tumor cells. We divided the TCGA data into a high FAM166B expression group and low FAM166B expression group. An increased level of FAM166B expression can impact the prognosis of breast cancer patients which indicated that FAM166B could be a predictor of tumor treatment. Next, we conducted univariate and multivariate Cox regression analysis on FAM166B. The results showed that FAM166B had the potential to serve as an independent prognosis factor of BRCA.

Furthermore, to explore possible mechanisms how FAM166B functioned on BRCA, we investigated biological signal pathways via conducting GSEA analysis. The results revealed that FAM166B had correlations with biological metabolism pathways, including fructose and mannose metabolism pathway, sugar and nucleotide sugar metabolism pathway, pentose phosphate pathway and glycolysis gluconeogenesis pathway. As previous studies have supported, lactose and glucose metabolic pathways coordination was observed in tumor cells[19–21]. This indicates that up-regulation of FAM166B could reduce cell metabolism and cell proliferation process. Thus, FAM166B had the potential to inhibit tumor cell growth via affecting metabolic pathways.

Another essential aspect of this study is that FAM166B expression is correlated with diverse immune infiltration levels in breast cancer. Breast cancer is a heterogeneous disease that has been sub-grouped into several phenotypically diverse cancers based on specific molecular features[22]. According to previous studies, the pCR rate among different subtypes of breast cancer is related to its tumor-infiltrating lymphocytes (TILs) level [23]. The interplay between the immune system and cancer cells has critical implications on tumor progression has shown increasing evidence [24, 25]. Although the relationship between breast cancer and lymphocytic infiltration has been conflicting, some studies have revealed an association between TILs and a favorable breast cancer prognosis according to the breast cancer molecular features [26].

The abundance and composition of the immune cells infiltrating a tumor provide prediction both of prognosis and possible immunotherapy [27]. Our results demonstrate that there is a negative relationship between FAM166B expression level and macrophages infiltration level in BRCA. Based on previous

studies, in human breast cancers, high tumor-associated macrophages (TAM) density correlates with poor prognosis[28]. While in breast cancer progression, the roles of TAM have been identified to be capable of inducing angiogenesis, remodeling the tumor extracellular matrix to aid invasion, modeling breast cancer cells to evade host immune system and inhibiting the function of CD8 + T cell and of phagocytosis in the tumor microenvironment[29]. In this study, the expression of FAM166B has a weak correlation with TAM which revealed that the targeted gene leads to a promising breast cancer prognosis. Nevertheless, the increase in FAM166B expression positively correlates with the expression of CD4 + T cells. according to other researchers' studies, CD4 + T cells could suppress the outgrowth of tumor cells, signifying organized antitumor immunity. In breast cancer, CD4 + T cells can also act as an independent prognostic factor and predict the preoperative response to chemotherapy[30]. Previous studies have revealed that CD8 + T cells are crucial components of tumor-specific cellular adaptive immunity, and have been demonstrated that they are associated with patient survival in various cancers, including breast cancer[31, 32]. Based on this, as FAM166B has a strong correlation with the expression of CD8 + T cell markers, it may have a role in tumor immunity. Together these findings suggest that the FAM166B may play a role in the recruitment and regulation of immune infiltrating cells in breast cancer.

Still, some drawbacks of this article should not be ignored. First of all, the clinical data were obtained from TCGA data portal, which was retrospective and limited. Secondly, the mechanisms of FAM166B need further studies and verification. Thirdly, there were still some uncertainty in how FAM166B related with tumor infiltrating immune cells and therefore to affect the tumor microenvironment.

Conclusion

In this study, we illustrated FAM166B is an independent prognostic factor in BRCA, by performing multiple analyses. A high expression of FAM166B is associated with a better prognosis of breast cancer patients. Also, FAM166B may restrain the tumor cell metabolism by affecting cell metabolism pathways. Moreover, FAM166B related with the expression of B cells, CD4 + T cells and macrophages, thus likely to play a role in immune cell infiltration. This study wants to provide a new biomarker to predict BRCA prognosis and a feasible method for treating BRCA. However, further researches on FAM166B in BRCA are required to be done.

Abbreviations

BRCA: Breast cancer; MSL: multiple symmetric lipomatosis; HNSC :Head and Neck squamous cell carcinoma; KICH: Kidney Chromophobe; PRAD: Prostate adenocarcinoma; KIRC: Kidney Renal Clear Cell Carcinoma; LUAD: Lung Adenocarcinoma; KIRP: Kidney renal papillary cell carcinoma; TAMs: tumor-associated macrophages; NK cells: Natural killer cells; DCs: dendritic cells; Th1: T-helper 1 cells; Th2: T-helper 2 cells; Tfh: follicular helper T cells; Th17: T-helper 17 cells; TMB: Tumor mutation burden; MSI: microsatellite instability; NES: Normalized Enrichment Score; TIICs: tumor-infiltrating immune cells; TCGA: The Cancer Genome Atlas; GTEX: Genotype-Tissue Expression; TIMER: Tumor Immune Estimation Resource

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Xiaoan Liu designed, revised, and finalized the manuscript. Yi Zhou and Huiqin Zhu were the first authors who contributed to the drafting, editing, coordination and revision of the manuscript. Gao He prepared the figures and tables. Hongfei Zhang and Xuyu Cheng contributed to literature search. All authors read and approved the final manuscript.

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Availability of data and materials

This was not applicable to this manuscript.

Ethics approval and consent to participate

Ethical Approval is not applicable for this article.

Consent for publication

Consent for publication was obtained from all participants.

Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, table 1 PDF and table 2 jpg are only available as a download in the Supplemental Files section.

Figures

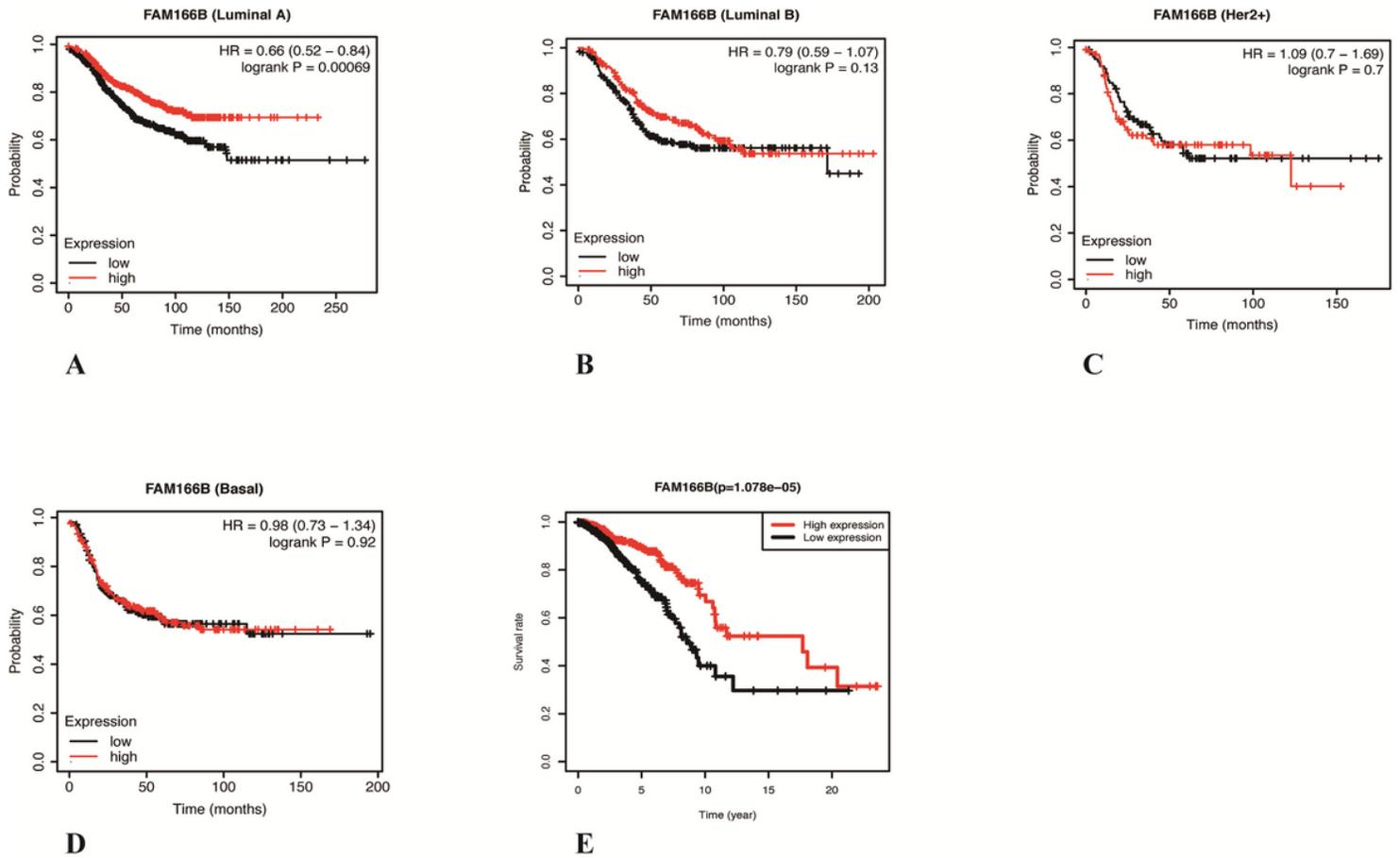


Figure 2

Overexpressed FAM166B associated with better outcome in BRCA. (A) RFS (Relapse Free Survival) of FAM166Bhigh and FAM166Blow Luminal A BRCA patients. (B) RFS of FAM166Bhigh and FAM166Blow Luminal B BRCA patients. (C) RFS of FAM166Bhigh and FAM166Blow Her2+ BRCA patients. (D) RFS of FAM166Bhigh and FAM166Blow Basal BRCA patients. (E) Overall survival (OS) of all BRCA patients stratified by FAM166B expression level in TCGA database.

A

Variable	HR	Univariate analysis		P	Multivariate analysis		
		HR	95% CI		HR	95% CI	P
Overall survival							
age	1.034	1.019-1.049	0.000	1.041	1.025-1.056	0.000	
gender	0.858	0.120-6.153	0.879	0.465	0.064-3.365	0.449	
stage	2.166	1.706-2.749	0.000	1.534	0.891-2.640	0.123	
T	1.530	1.232-1.900	0.000	1.121	0.814-1.544	0.483	
M	6.588	3.615-12.004	0.000	1.503	0.627-3.597	0.360	
N	1.679	1.392-2.025	0.000	1.277	0.941-1.733	0.116	
FAM166B	0.565	0.373-0.854	0.007	0.476	0.312-0.725	0.001	

B

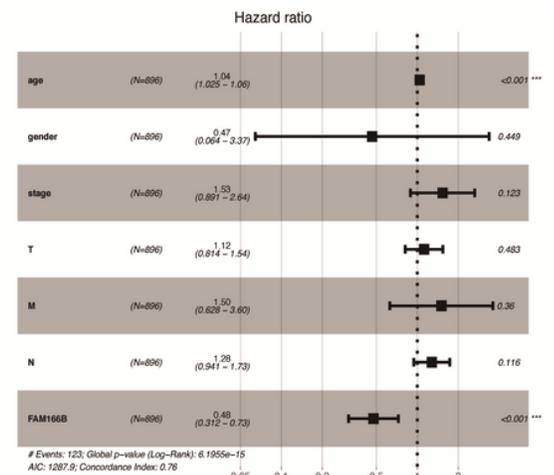


Figure 3

Prognostic value of FAM166B expression in BRCA. (A) Cox regression analysis of FAM166B expression level as a prognostic indicator of FAM166B by using the TCGA database. (B) Forest plot illustrating results of the multivariate Cox regression analysis of FAM166B expression and clinical parameters in predicting the OS of BRCA patients in the TCGA dataset.

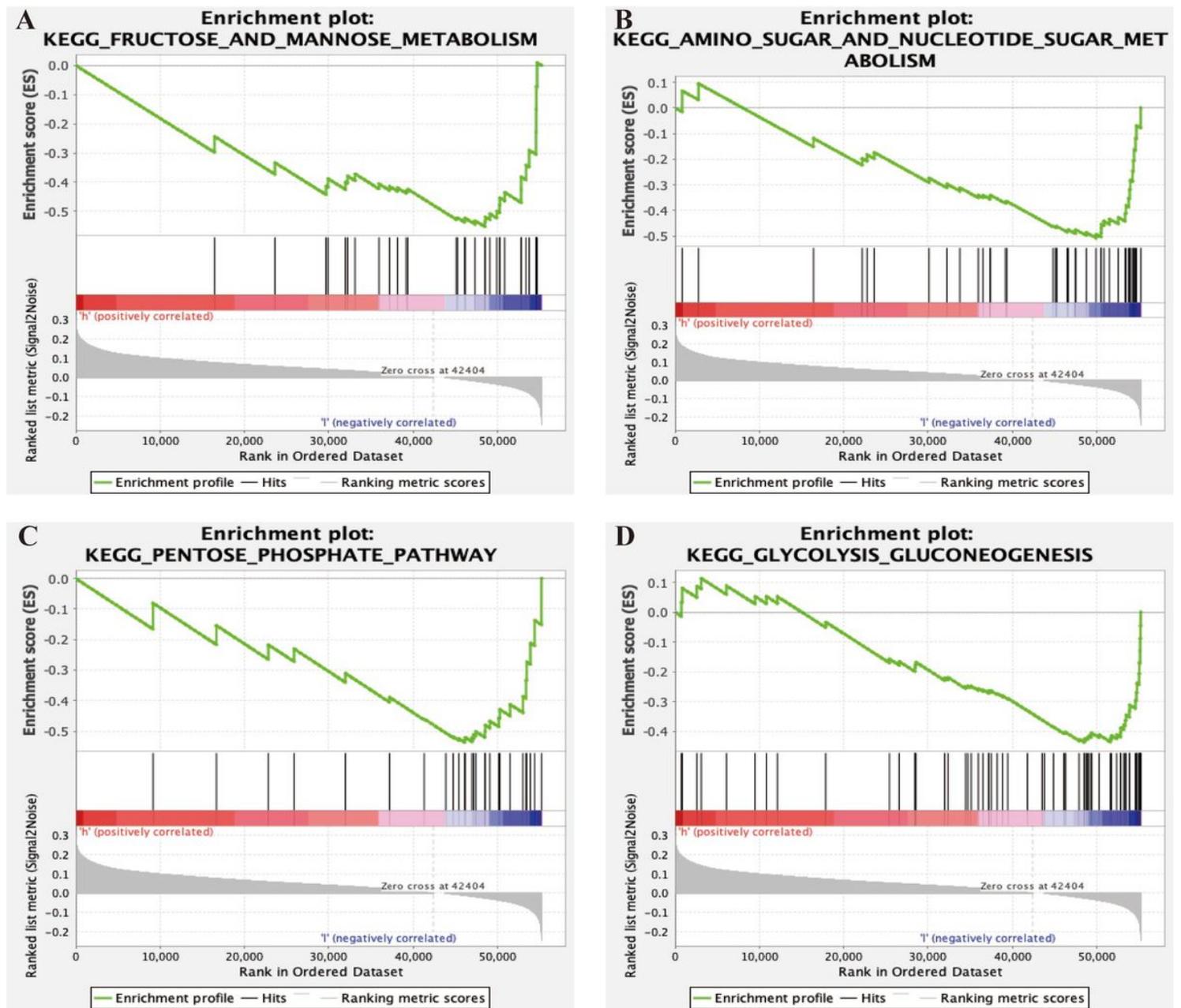


Figure 4

Enrichment plots from gene set enrichment analysis (GSEA); (A) fructose and mannose metabolism pathway; (B) sugar and nucleotide sugar metabolism pathway; (C) pentose phosphate pathway; (D) glycolysis gluconeogenesis pathway.

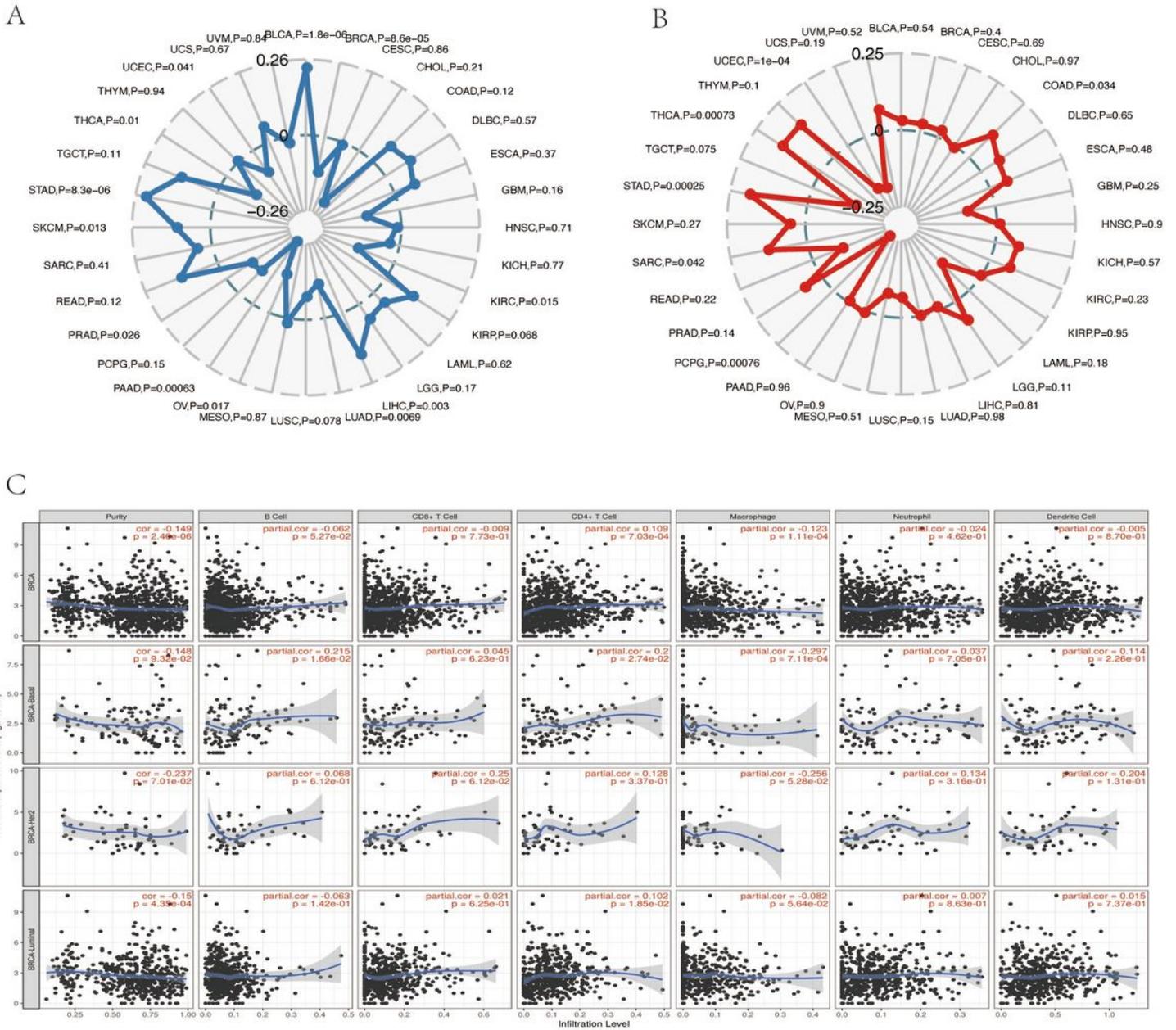


Figure 5

FAM166B associated with TMB (A) and MSI (B). (C) Correlations between FAM166B expression and immune cells of different types of BRCA were analyzed by TIMER.

Supplementary Files

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