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Original Article

Comprehensive multi-omics analysis and prognostic significance of fibroblast growth factor binding protein 1 (FGFBP1) in pancreatic adenocarcinoma

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Abstract: Background: Pancreatic adenocarcinoma (PAAD) is a highly aggressive cancer with poor prognosis and limited therapeutic options. Identifying molecular markers and understanding their role in PAAD pathogenesis is crucial for developing targeted therapies. This study integrates bioinformatics and molecular experiments to investigate the diagnostic, prognostic, and therapeutic significance of FGFBP1 in PAAD. Methods: UALCAN, TNMplot, OncoDB, GEPIA2, HPA, GSCA, KM Plotter, TISIDB, TISCH2, CancerSEA, STRING, DAVID, cell culture, RT-qPCR analysis, western blot analysis, colony formation, cell proliferation, and wound healing assays. Results: Expression analyses revealed a significantly elevated FGFBP1 levels in PAAD tissues compared to normal samples. Promoter methylation analysis indicated lower methylation levels in PAAD, inversely correlated with FGFBP1 expression, suggesting epigenetic regulation. Genetic alteration analysis showed that FGFBP1 is not significantly affected by single nucleotide variants, but copy number variations are present without impacting mRNA expression. Survival analysis using KM plotter demonstrated that high FGFBP1 expression is associated with poor overall and disease-free survival. A Cox regression-based prognostic model confirmed the negative impact of elevated FGFBP1 on patient outcomes. Correlation analysis with immune-related factors indicated that FGFBP1 may contribute to an immunosuppressive tumor microenvironment, affecting immune cell infiltration and function. Single-cell analysis highlighted FGFBP1 expression in malignant, endothelial, and fibroblast cells within the tumor microenvironment. Gene enrichment analysis revealed FGFBP1's involvement in various biological processes and pathways related to cancer progression. Experimental validation using RT-qPCR confirmed high FGFBP1 expression in PAAD cell lines. FGFBP1 knockdown in HEK293T cells significantly reduced cell proliferation, colony formation, and migration. Conclusion: These findings suggest that FGFBP1 plays a critical role in PAAD pathogenesis and could serve as a potential therapeutic target for improving patient outcomes.

Keywords: PAAD, FGFBP1, prognosis, diagnosis, biomarker

Introduction

Pancreatic cancer is one of the most aggressive and lethal malignancies, characterized by a high mortality rate and poor prognosis [1]. Globally, it ranks as the seventh leading cause of cancer-related deaths, with an estimated 466,000 new cases and 432,000 deaths annually [2]. Pancreatic adenocarcinoma (PAAD) is the predominant form of pancreatic cancer, comprising about 85% of all cases [3]. Recent research indicates that PAAD constitutes 2.5% of all cancers globally, with its incidence steadily increasing [4]. Despite advancements in medical research, the five-year survival rate for PAAD remains dismally low, largely due to late diagnosis and limited effective therapeutic options [5, 6]. The identification of novel diagnostic biomarkers and therapeutic targets is crucial for improving patient outcomes. Several risk factors contribute to the development of PAAD, including smoking, chronic pancreatitis, obesity, and a family history of the disease [7]. Additionally, genetic predispositions such as

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mutations in the BRCA1, BRCA2, and KRAS genes significantly increase the risk of developing PAAD [8]. The complex interplay of these genetic and environmental factors complicates early detection and effective treatment, emphasizing the need for more in-depth research into the molecular underpinnings of PAAD.

Fibroblast Growth Factor Binding Protein 1 (FGFBP1) is a member of the FGFBP family, which plays a pivotal role in modulating the activity of fibroblast growth factors (FGFs) [9]. FGFs are critical regulators of various cellular processes, including proliferation, differentiation, and angiogenesis [10]. Dysregulation of FGFBP1 and its family members has been implicated in the progression of several cancers. For instance, FGFBP1 has been shown to enhance tumor growth and metastasis by facilitating the release and activation of FGFs, thereby promoting oncogenic signaling pathways [11].

Previous studies have demonstrated the involvement of FGFBP1 in various cancers. In colorectal cancer, elevated FGFBP1 expression has been associated with increased tumorigenicity and poor clinical outcomes [12]. Similarly, in breast cancer, FGFBP1 overexpression correlates with enhanced cell proliferation and invasion, indicating its role in cancer progression [13]. Moreover, FGFBP1 has been identified as a potential biomarker for early detection and prognosis of gastric cancer, further underscoring its clinical relevance [14]. In lung cancer, FGFBP1 has been linked to tumor growth and metastasis through the modulation of FGF signaling pathways, contributing to poor patient survival rates [15]. In prostate cancer, high levels of FGFBP1 have been associated with aggressive tumor phenotypes and resistance to conventional therapies, suggesting its role in treatment evasion [16]. Additionally, in ovarian cancer, FGFBP1 has been shown to enhance angiogenesis and tumor progression, highlighting its potential as a therapeutic target [17]. These findings collectively indicate that FGFBP1 plays a crucial role in the development and progression of a wide range of cancers, making it a significant focus for cancer research.

Despite the growing body of evidence supporting the oncogenic role of FGFBP1 in various cancers, its specific role in PAAD remains underexplored. This study aims to fill this gap by comprehensively analyzing the diagnostic, prognostic, and therapeutic significance of FGFBP1 in PAAD. Utilizing a combination of bioinformatics approaches and in vitro experiments, we seek to elucidate the molecular mechanisms by which FGFBP1 contributes to PAAD development and progression. Understanding these mechanisms will provide valuable insights into potential therapeutic strategies targeting FGFBP1 in PAAD.

Methodology

Detailed expression analysis of FGFBP1

UALCAN, TNMplot, OncoDB, GEPIA2, and the Human Protein Atlas (HPA) are prominent databases used extensively in cancer research for analyzing gene expression and protein data. UALCAN is an interactive web resource for analyzing cancer omics data, offering user-friendly access to TCGA, MET500, and CPTAC datasets to facilitate the exploration of gene expression profiles and survival data across multiple cancer types [18, 19]. TNMplot integrates data from TCGA, GTEx, and GEO, allowing users to compare gene expression levels across different tumor stages and normal tissues, aiding in the identification of potential biomarkers [20, 21]. OncoDB provides comprehensive cancer genomics data, enabling researchers to explore genetic mutations, copy number variations, and gene expression changes across numerous cancer types, thus supporting the discovery of novel oncogenic drivers and therapeutic targets [22]. GEPIA2 (Gene Expression Profiling Interactive Analysis 2) is an upgraded version of GEPIA, offering enhanced functionalities for analyzing RNA sequencing data from TCGA and GTEx, including differential expression analysis, patient survival analysis, and correlation analysis, all through an intuitive web interface [23, 24]. The Human Protein Atlas (HPA) focuses on mapping all the human proteins in cells, tissues, and organs using various omics technologies, providing invaluable insights into protein expression patterns and their implications in health and disease, particularly in cancer [25, 26]. In the current study, UALCAN, TNMplot, OncoDB, and GEPIA2 databases were utilized to analyze mRNA expression of FGFBP1 across TCGA PAAD cohorts. While the HPA database was

used for the proteomic expression analysis of FGFBP1 in PAAD tissues samples.

Comprehensive promoter methylation analysis of FGFBP1

GSCA (Gene Set Cancer Analysis) is a comprehensive web-based platform designed to facilitate the integrative analysis of gene sets in cancer [27]. It offers a wide array of analytical tools to explore gene expression, mutation profiles, and survival data across multiple cancer types. In the current work, along with UALCAN [18] and OncoDB [22] databases, GSCA was used for the promoter methylation analysis of FGFBP1 in PAAD patients.

Genetic alterations, immunolytic, and drug sensitivity analyses of FGFBP1

In this study, genetic alterations, immunolytic, and drug sensitivity analyses of FGFBP1 in PAAD were conducted using GSCA database [27].

Survival analysis and prognostic model development

KM Plotter is a widely used online tool designed for survival analysis of cancer data [28, 29]. It integrates gene expression and clinical data from various sources, including TCGA and GEO, to generate Kaplan-Meier survival plots. In this project, KM plotter was utilized for the survival analysis of the FGFBP1 in PAAD patients.

Prognostic model development methodology involves collecting gene expression and clinical survival data from datasets (GSE78229, GSE62452, and E_MTAB_6134). Data were normalized, and samples were categorized into high and low FGFBP1 expression groups. Cox proportional hazards regression was performed to compute hazard ratios (HRs), assessing the risk associated with high FGFBP1 expression. The concordance index (C-index) was calculated to evaluate predictive accuracy.

Correlation analysis of FGFBP1 with immune inhibitors, immune modulators, chemokine's, and immune subtypes of PAAD

TISIDB is an integrated repository for tumor-immune system interactions, designed to facilitate cancer immunology research [30]. It combines data from TCGA and other resources, offering insights into the relationships between tumors and immune cells, immunomodulators, and immune-related genes. TISIDB supports comprehensive analyses, including gene expression, immune infiltration, and clinical relevance, enabling researchers to identify potential immunotherapeutic targets and understand the complex dynamics of the tumor-immune environment. In this work, TISIDB database was utilized to perform correlation analysis of FGFBP1 with immune inhibitors, immune modulators, chemokine's, and immune subtypes of PAAD.

Single cell analysis

TISCH2 (Tumor Immune Single Cell Hub) is a comprehensive database designed for the analysis of single-cell RNA sequencing data in the context of tumor-immune interactions [31]. It provides detailed annotations of immune cell types and states across various cancer types. In this study, we analyzed the distribution of FGFBP1 expression in different immune cells at the single cell level using the TISCH2 database.

CancerSEA database

CancerSEA is a comprehensive database designed to explore the intricate landscape of cancer-associated gene sets and molecular signatures [32]. It amalgamates diverse datasets from high-throughput experiments, unveiling crucial insights into the complex biology of cancer. In our study, CancerSEA database was utilized to explore correlations between FGFBP1 gene expression and 14 diverse functional states of the PAAD.

Gene enrichment analysis

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) is a widely used database and web resource that provides information on protein-protein interactions (PPIs) and functional associations [33]. We used this database to construct the PPI network of the FGFBP1 gene in this study.

DAVID (Database for Annotation, Visualization and Integrated Discovery) is a bioinformatics resource that assists researchers in comprehensively analyzing large gene lists derived

from genomic studies [34]. It offers a suite of tools for functional annotation, including gene ontology analysis, pathway enrichment analysis, and protein domain analysis. This database was utilized in the present study for the functional enrichment analysis of the FGPBP1-associated genes.

Cell culture

In total 10 PAAD cell lines, including HEK-293T, BxPC-3, Capan-1, MiaPaCa-2, Panc-1, SU.86.86, HPAF-II, PaTu 8988T, PL45, and Hs 766T, and four control cell lines, including HPDE6-C7, HPNE, H6c7, and H6c7-β were purchased from the ATCC, USA. These cell lines were cultured under standard conditions in a humidified atmosphere with 5% CO_2 at 37°C. The growth medium for PAAD cell lines was consists of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). In contrast, control cell lines (HPDE6-C7, HPNE, H6c7, and H6c7-β) were maintained in keratinocyte serum-free medium supplemented with bovine pituitary extract and epidermal growth factor.

RT-qPCR analysis

RNA extraction utilized a total RNA extraction reagent (Vazyme Biotech), followed by reverse transcription using the GoScript Reverse Transcription System (Promega, Madison, WI, USA). Real-time quantitative PCR (qPCR) was conducted using qPCR Master Mix (Vazyme Biotech) in an Applied Biosystems 7500 Real-Time PCR System. GAPDH served as a reference gene, and expression levels were determined using the $2^{-\Delta\Delta Ct}$ method. Following primers were used to amplify GAPDH, FGFBP, FGFBP3, and FGFBP3.

GAPDH-F: 5'-ACCCACTCCTCCACCTTTGAC-3', GAPDH-R: 5'-CTGTTGCTGTAGCCAAATTCG-3'; FGFBP1-F: 5'-TGGCAAACCAGAGGAAGACTGC-3', FGFBP1-R: 5'-GGAACCCGTTCTCTTTTGACCTC-3'; FGFBP2-F: 5'-AGCAGGTGACTTCCAGCCTCAA-3', FGFBP2-R: 5'-CAGCTCTTCCATCGAGTCCTTTC-3'; FGFBP3-F: 5'-AGAGGAAGACCAACGAGGGCAA-3', FGFBP3-R: 5'-AGAGGAAGACCAACGAGGGCAA-3'.

Transfection

The HEK293T cell line underwent transfection with siRNAs directed against FGFBP1 (RuiSai, Shanghai, China) using Lipofectamine 3000 (Invitrogen, Carlsbad, USA). The miRNA sequence employed was 5'-CAGAAAGAGAGCCAAGAA-3'.

RT-qPCR and western blot analyses

To assess the gene knockdown efficiency, RT-qPCR and western blot analyses were conducted to document the expression of FGFBP1 in control and knockdown HEK293T cells. RT-qPCR was performed according to the aforementioned protocol. For western blot analysis, cellular lysis was conducted using the RIPA buffer on ice. The resulting lysate underwent centrifugation (at 12,000 g and 4°C for 15 min) to isolate supernatants. Total protein concentration was determined using a BCA assay kit following the manufacturer's guidelines. Protein samples (20-30 μg) were then subjected to separation via 10% SDS-PAGE gel electrophoresis and subsequently transferred onto a 0.45 μm polyvinylidene difluoride membrane (PVDF). These membranes were subsequently blocked in 1% TBST with 5% non-fat milk for 1 hour at room temperature, followed by overnight incubation with the designated antibodies at 4°C. The source and dilution of each antibody were as follows: anti-FGFBP1 (1:1000) obtained from Abclonal (Wuhan, China); anti-GAPDH (1:5000) sourced from Proteintech (Wuhan, China). Secondary antibodies were detected with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (1:10000, Abcam), and visualization was achieved using the ECL detection kit (Thermo Fisher).

Colony-formation and cell proliferation assay

Following transfection, around 1×10^3 cells were seeded into each well of a six-well plate for continued cultivation, with medium replacement every 3 days. Subsequently, the cells were allowed to incubate for 10-14 days before fixation using 4% paraformaldehyde and staining with crystal violet. A portion of the cells (1×10^3) was then transferred to 96-well plates for further culture. Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8) (Tongren, Shanghai, China).

Wound healing assays

For the wound healing assay, cells were first seeded into a 6-well plate, then transfected

and cultured accordingly. Next, a wound was created using a 10-μL pipette tip, followed by washing with PBS. FBS-free medium was added for cell culture, and images of the cells were captured using a microscope.

Statistics

Statistical analysis was performed using GraphPad Prism 7.0. Student's t-test was utilized to determine significant differences among groups, while Pearson's correlation analysis was employed to examine correlations. P < 0.05 was considered statistically significant.

Results

Expression landscape of FGFBP1

First, we conducted a comprehensive analysis of FGFBP1 expression across TCGA cohorts using UALCAN, TNMplot, OncoDB, and GEPIA2 database. Expression analysis via the UALCAN shows that FGFBP1 expression is markedly (p-value < 0.05) higher in PAAD samples compared to normal samples, indicating elevated level in PAAD samples (**Figure 1A**). Expression analysis via the TNMplot presents a scatter plot comparing FGFBP1 expression in normal versus PAAD tissues, with PAAD samples exhibiting notably (p-value < 0.05) higher expression (**Figure 1B**). Expression analysis using the OncoDB database further illustrates this with box plots showing significantly (p-value < 0.05) higher FGFBP1 levels in PAAD compared to normal tissues (**Figure 1C**). Expression box plots of log-transformed FGFBP1 expression values (log[TPM+1]) from the GEPIA2 database also highlights significantly (p-value < 0.05) elevated expression in PAAD samples (**Figure 1D**). UALCAN-based expression analysis provides detailed box plots stratified by clinical and demographic factors, revealing higher FGFBP1 expression in advanced cancer stages, particularly stages 3 and 4, and among different racial groups, with the highest levels in Asian patients (**Figure 1E**). Moreover, the expression of FGFBP1is slightly higher in male patients compared to females, and it increases across different age groups, peaking in patients aged 61-80 years (**Figure 1E**). Finally, the immunohistochemistry-based expression analysis using HPA database shows high levels of FGFBP1 staining in four PAAD tissue samples (**Figure 1F**). Collectively, the data underscore that FGFBP1 is significantly overexpressed in PAAD compared to normal tissues, with variations observed across clinical and demographic variables.

Promoter methylation landscape of FGFBP1

Next, we performed promoter methylation analysis of FGFBP1 using UALCAN, OncoDB and GSCA databases. **Figure 2A** shows box plots of the beta values from the UALCAN, indicating significantly (p-value < 0.05) lower promoter methylation level of FGCBP1 gene in PAAD samples relative to the control samples. **Figure 2B** plots the beta values across different genomic positions from the OncoDB, revealing inverse correlation between the expression and promoter methylation levels of FGFBP1 in PAAD samples. **Figure 2C** also illustrates the correlation between methylation and mRNA expression from the GSCA database, showing a significant (p-value < 0.05) negative correlation, suggesting that lower methylation level is associated with higher FGFBP1 expression. **Figure 2D** compares survival differences between high and low methylation levels across PAAD samples for Disease-Free Interval (DFI) and Disease-Specific Survival (DSS) using GSCA database. For FGFBP1 in PAAD, the plots show significant (p-value < 0.05) survival difference, indicating that FGFBP1 hypomethylation is significantly associated with the poor survival in patients with PAAD (**Figure 2D**).

Genetic alteration analysis of FGFBP1

Next, we analyzed FGFBP1 gene variations, including single nucleotide variants (SNVs) and copy number variations (CNVs), in PAAD samples using GSCA database. **Figure 3A** presents a heatmap of SNV percentage, indicating that there are no significant mutations in FGFBP1 among the PAAD samples, as shown by the absence of mutation frequency. **Figure 3B** shows a pie chart of CNV percentages, indicating that a substantial proportion of PAAD samples exhibit CNVs in FGFBP1, with heterogeneous amplifications (Hete. Amp.) and deletions (Hete. Del.) present, though the majority have no CNVs (None). **Figure 3C** correlates CNVs with mRNA expression levels, illustrating that there is a minimal correlation between FGFBP1 CNVs and its mRNA expression in PAAD. The Spearman correlation coefficient is

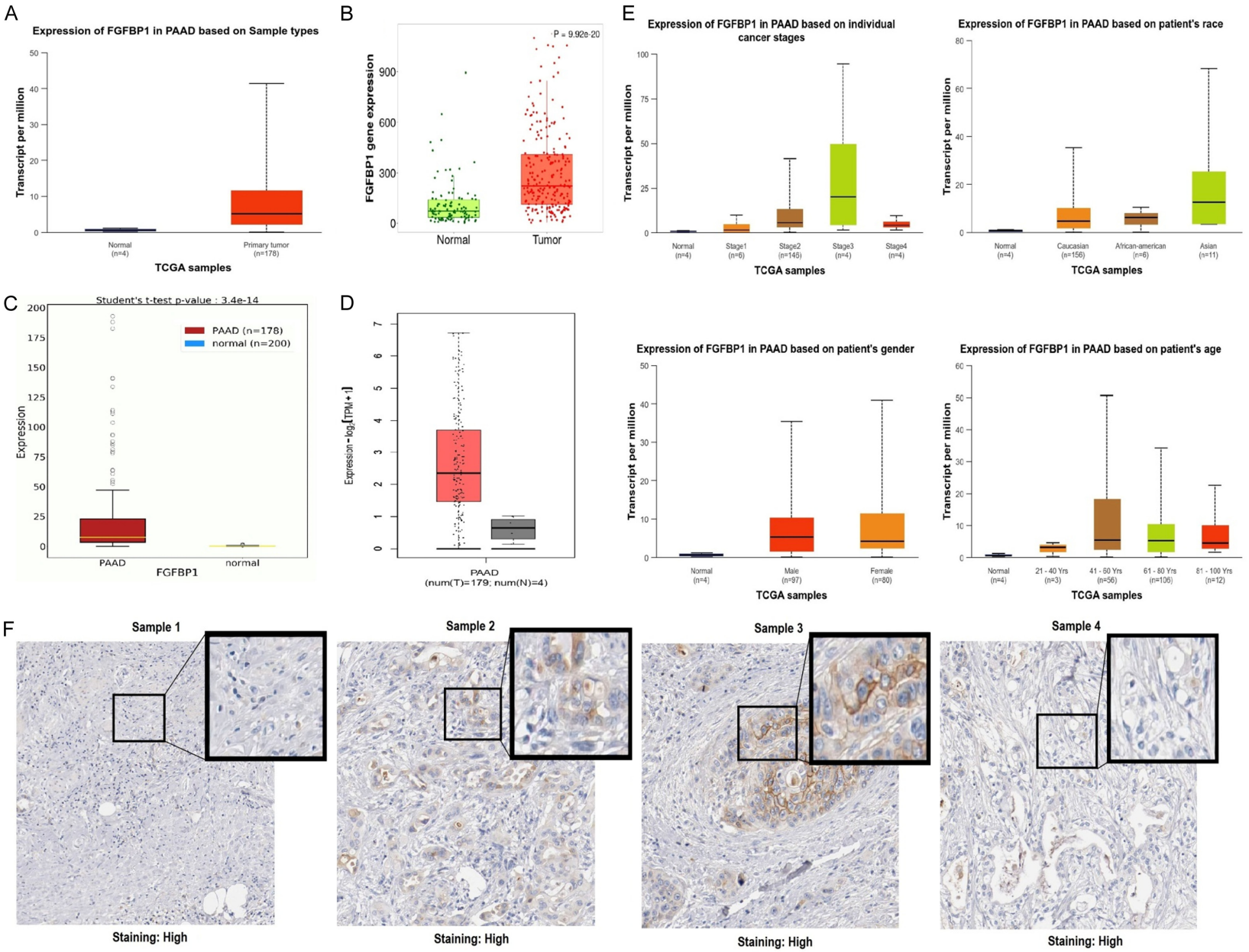

A
Expression of FGFBP1 in PAAD based on Sample types
Transcript per million
Normal (n=4)
Primary tumor (n=178)
TCGA samples
B
P = 9.92e-20
FGFBP1 gene expression
Normal
Tumor
C
Student's t-test p-value : 3.4e-14
PAAD (n=178)
normal (n=200)
Expression
PAAD
normal
FGFBP1
D
Expression = log2(TPM+1)
PAAD
(num(T)=179; num(N)=4)
E
Expression of FGFBP1 in PAAD based on individual cancer stages
Transcript per million
Normal (n=4)
Stage1 (n=6)
Stage2 (n=145)
Stage3 (n=4)
Stage4 (n=4)
TCGA samples
Expression of FGFBP1 in PAAD based on patient's race
Transcript per million
Normal (n=4)
Caucasian (n=156)
African-american (n=6)
Asian (n=11)
TCGA samples
Expression of FGFBP1 in PAAD based on patient's gender
Transcript per million
Normal (n=4)
Male (n=97)
Female (n=80)
TCGA samples
Expression of FGFBP1 in PAAD based on patient's age
Transcript per million
Normal (n=4)
21 - 40 Yrs (n=3)
41 - 60 Yrs (n=56)
61 - 80 Yrs (n=106)
81 - 100 Yrs (n=12)
TCGA samples
F
Sample 1
Staining: High
Sample 2
Staining: High
Sample 3
Staining: High
Sample 4
Staining: High

Figure 1. Expression analysis of FGFBP1 in pancreatic adenocarcinoma (PAAD) tissues compared to normal tissues. A. Box plot illustrating the transcript per million (TPM) expression levels of FGFBP1 in normal tissues (n=4) and primary PAAD tumor samples (n=178) using the UALCAN database. B. Scatter plot from the TNMplot database comparing FGFBP1 expression between normal (n=71) and PAAD tumor tissues (n=178). C. Box plot from the OncoDB database comparing FGFBP1 expression in PAAD (n=178) versus normal tissues (n=200). D. Box plot of log-transformed FGFBP1 expression values (log[TPM+1]) from the GEPIA2 database, highlighting significantly elevated expression in PAAD samples (n=179) compared to normal tissues (n=4). E. Box plots from the UALCAN database showing FGFBP1 expression stratified by clinical and demographic factors: cancer stages, race, gender, and age. F. Immunohistochemistry images from the HPA database showing high levels of FGFBP1 staining in PAAD tissue samples, supporting the elevated expression observed in the transcriptomic data. *P*-value < 0.05.

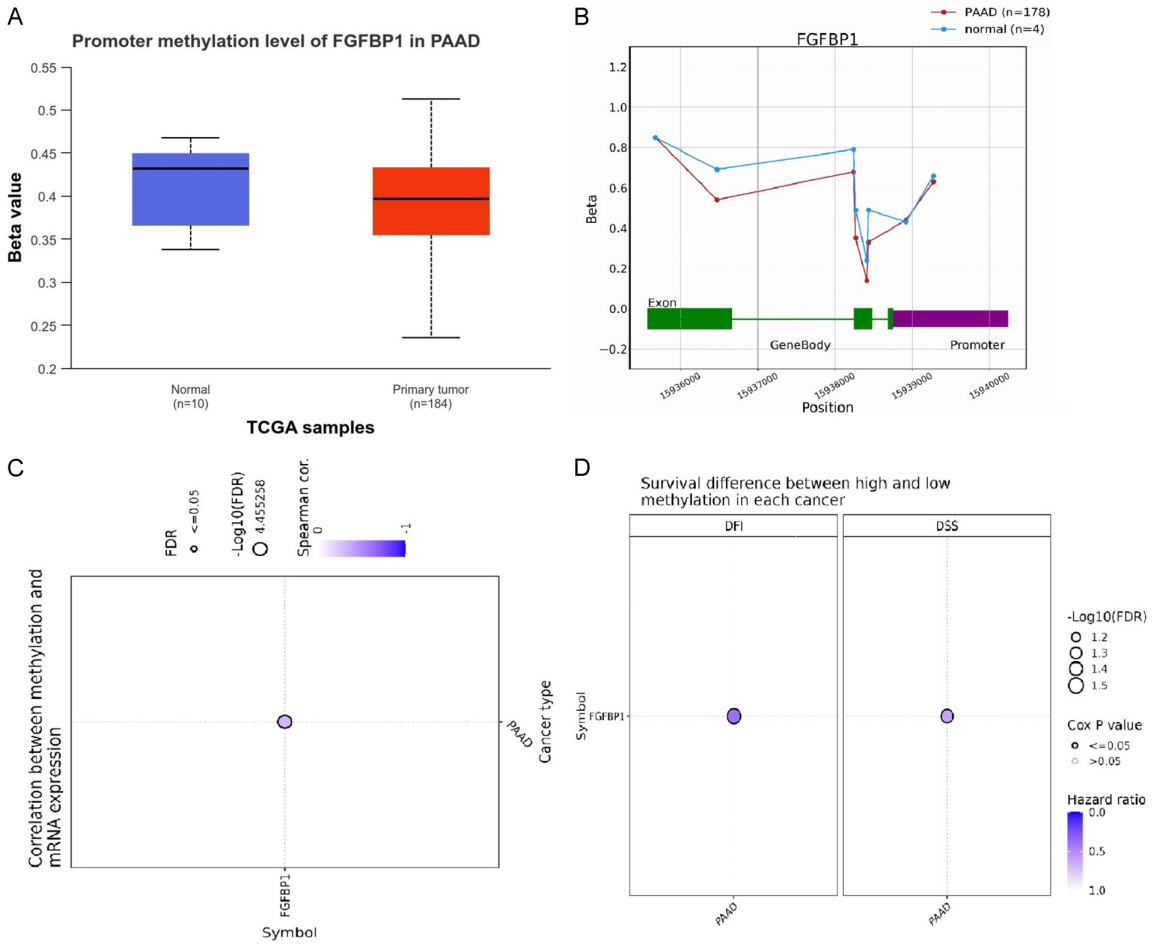


Figure 2. Analysis of FGFBP1 promoter methylation in pancreatic adenocarcinoma (PAAD) and its clinical implications. A. Box plot comparing the promoter methylation levels of FGFBP1 between normal (n=10) and primary tumor (n=184) samples in pancreatic adenocarcinoma (PAAD) based on TCGA data from the UALCAN. B. Line plot showing the beta values of FGFBP1 across different genomic regions (exon, gene body, and promoter) in both PAAD (n=178) and normal (n=4) samples from the OncoDB database. C. Correlation plot displaying the relationship between FGFBP1 promoter methylation and mRNA expression in PAAD from the GSCA database. D. Dot plot illustrating the survival difference between high and low FGFBP1 methylation in PAAD from the GSCA database. Two survival metrics are shown: Disease-Free Interval (DFI) and Disease-Specific Survival (DSS). *P*-value < 0.05.

near zero, and the false discovery rate (FDR) is above the significance threshold (> 0.05), suggesting that CNVs in FGFBP1 do not significantly impact its expression at the mRNA level (**Figure 3C**). Overall, the figure indicates that FGFBP1 is not significantly affected by SNVs in PAAD, and while CNVs are present, they do not have a notable effect on gene expression.

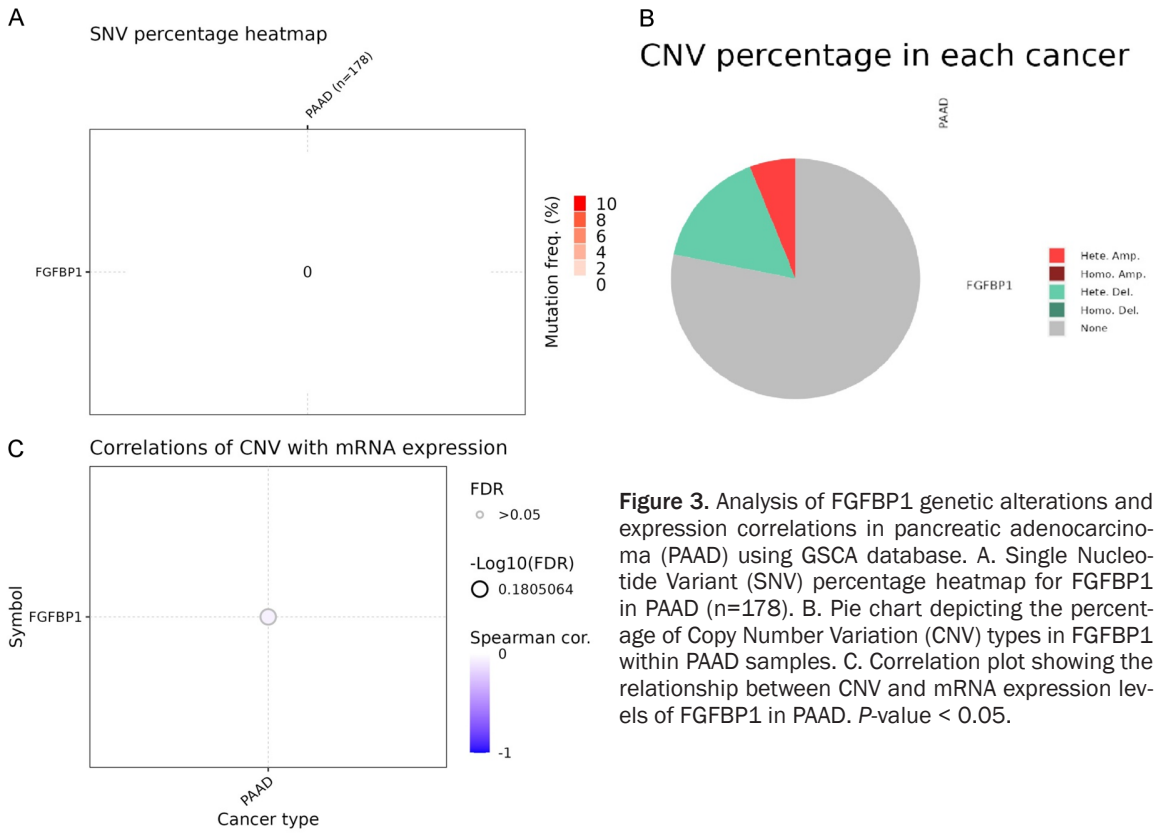


Figure 3. Analysis of FGFBP1 genetic alterations and expression correlations in pancreatic adenocarcinoma (PAAD) using GSCA database. A. Single Nucleotide Variant (SNV) percentage heatmap for FGFBP1 in PAAD (n=178). B. Pie chart depicting the percentage of Copy Number Variation (CNV) types in FGFBP1 within PAAD samples. C. Correlation plot showing the relationship between CNV and mRNA expression levels of FGFBP1 in PAAD. *P*-value < 0.05.

Survival analysis and prognostic model development

In this part of our study, we first performed survival analysis of FGFBP1 using KM plotter tool. **Figure 4A**, **4B** show Kaplan-Meier survival curves of the FGFBP1 comparing patients with high and low FGFBP1 expression. In **Figure 4A**, the hazard ratio (HR) is 2.12 (95% CI: 1.37-3.27) with a log-rank *p*-value of 5e-04, indicating that high FGFBP1 expression is associated with significantly poorer overall survival. Similarly, **Figure 4B** shows a HR of 5.15 (95% CI: 1.69-15.63) with a log-rank *p*-value of 0.0015, suggesting a strong association between high FGFBP1 expression and decreased disease free survival probability.

Next, we developed a Cox regression-based prognostic model of FGFBP1 for PAAD patients. **Figure 4C** summarizes Cox regression analysis results across three datasets (GSE78229, GSE62452, E_MTAB_6134), showing hazard ratios with confidence intervals. In this analysis, E_MTAB_6134 dataset was used as a training dataset while GSE78229 and GSE62452 datasets were used as the validation datasets. All three datasets exhibit hazard ratios greater than 1, reinforcing the negative impact of high FGFBP1 expression on patient survival (**Figure 4C**). The C-index values provided in the same panel indicate the predictive accuracy of FGFBP1 expression, with indices closer to 1 suggesting better predictive performance (**Figure 4C**). **Figure 4D** visualizes the risk categorization of FGFBP1 across the three datasets, indicating that high FGFBP1 expression consistently categorizes patients into a high-risk group. Overall, these findings demonstrate that elevated FGFBP1 expression is a robust predictor of poor prognosis in cancer patients.

Correlation analysis of FGFBP1 with immune inhibitors, immune modulators, chemokine's, and immune subtypes of PAAD

Next, we performed the correlation analysis of FGFBP1 expression with various immune-related factors in PAAD using TISIDB database. **Figure 5A** illustrates the correlation between FGFBP1 expression and immune inhibitor

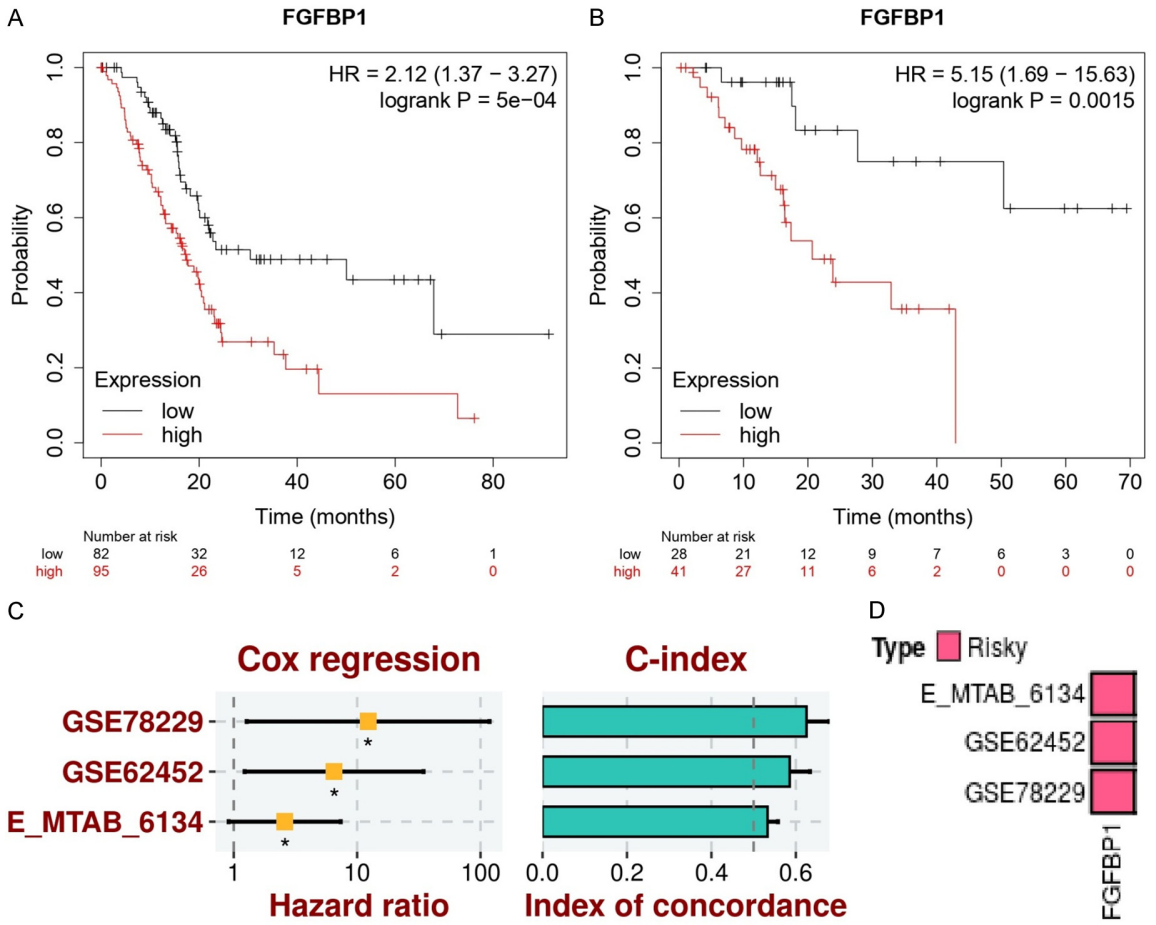
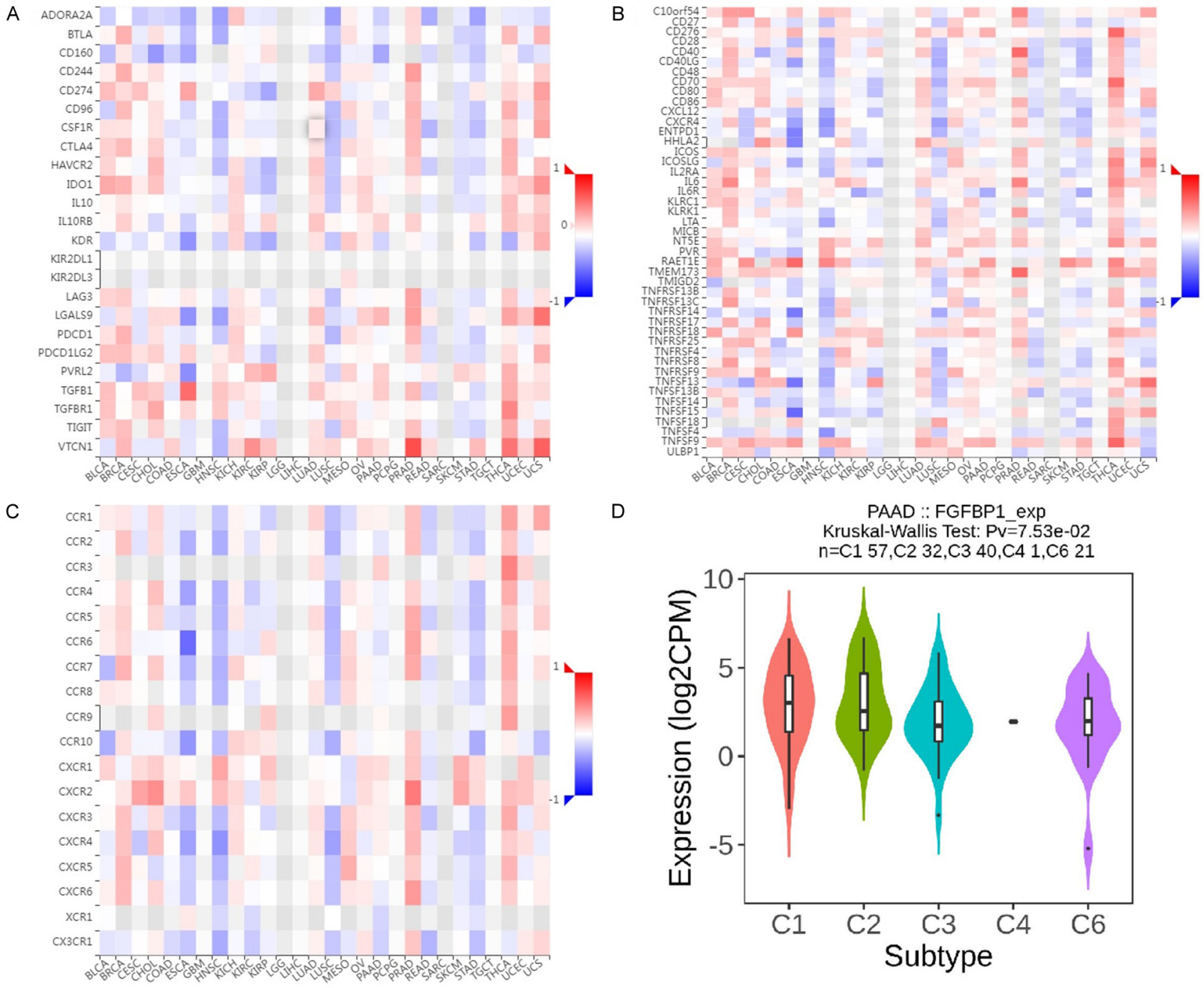


Figure 4. High FGFBP1 expression is associated with poor prognosis in pancreatic adenocarcinoma (PAAD) patients. A. KM plotter-based overall survival curves of FGFBP1 in PAAD patients. B. KM plotter-based disease free survival curves for FGFBP1 in PAAD patients. C. Cox regression analysis and concordance index (C-index) for three different datasets (GSE78229, GSE62452, E_MTAB_6134). D. Risk assessment across three datasets (E_MTAB_6134, GSE62452, and GSE78229) shows that high FGFBP1 expression is consistently associated with increased risk in these cohorts. *P*-value < 0.05.

genes, suggesting that FGFBP1 expression might be positively or negatively associated with the expression of genes involved in immune inhibition in PAAD, potentially linking high FGFBP1 expression to an immune suppressive environment. **Figure 5B** depicts the correlation between FGFBP1 expression and immune modulator genes, indicating negative correlations with some important immune modulator genes in PAAD, thus highlighting the role of FGFBP1 in altering immune regulation within the tumor microenvironment. **Figure 5C** presents the correlation of FGFBP1 with immune chemokine genes, showing negative correlations with majority of chemokine genes in PAAD. Finally, **Figure 5D** examines the correlation of FGFBP1 expression with different immune subtypes in PAAD, showing that FGFBP1 is significantly (*p*-value < 0.05) associated with immune subtypes of PAAD (**Figure 5D**). Overall, the figure underscores the potential immunomodulatory role of FGFBP1in PAAD, suggesting that FGFBP1 could impact immune evasion, immune response modulation, chemokine expression, and immune cell infiltration patterns.

Single cell analysis

In this part of our study, we analyzed the distribution of FGFBP1 expression in different immune cells and correlations of its expression with diverse functional states of the PAAD using TISIDB and CanerSEA databases. **Figure 6A** shows the expression levels of FGFBP1 across different immune cells in PAAD, with the high-


A
ADORA2A
BTLA
CD160
CD244
CD274
CD96
CSF1R
CTLA4
HAVCR2
IDO1
IL10
IL10RB
KDR
KIR2DL1
KIR2DL3
LAG3
LGALS9
PDCD1
PDCD1LG2
PVRL2
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BLCA BRCA CESC CHOL COAD ESCA GBM HNSC KICH KIRC KIRP LGG LIHC LUAD LUSC MESO OV PAAD PCPG PRAD READ SARC SKCM STAD TGCT THCA UCEC UCS
B
C10orf54
CD27
CD276
CD28
CD40
CD40LG
CD48
CD70
CD80
CD86
CXCL12
CXCR4
ENTPD1
HHLA2
ICOS
ICOSLG
IL2RA
IL6
IL6R
KLRC1
KLRK1
LTA
MICB
NT5E
PVR
RAET1E
TMEM173
TMIGD2
TNFRSF13B
TNFRSF13C
TNFRSF14
TNFRSF17
TNFRSF18
TNFRSF25
TNFRSF4
TNFRSF8
TNFRSF9
TNFSF13
TNFSF13B
TNFSF14
TNFSF15
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CCR3
CCR4
CCR5
CCR6
CCR7
CCR8
CCR9
CCR10
CXCR1
CXCR2
CXCR3
CXCR4
CXCR5
CXCR6
XCR1
CX3CR1
1
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BLCA BRCA CESC CHOL COAD ESCA GBM HNSC KICH KIRC KIRP LGG LIHC LUAD LUSC MESO OV PAAD PCPG PRAD READ SARC SKCM STAD TGCT THCA UCEC UCS
D
PAAD :: FGFBP1_exp
Kruskal-Wallis Test: Pv=7.53e-02
n=C1 57,C2 32,C3 40,C4 1,C6 21
Expression (log2CPM)
10
5
0
-5
C1
C2
C3
C4
C6
Subtype

Figure 5. Correlation analysis of FGFBP1 with immune inhibitors, immune modulators, cytokines, and immune subtypes across pancreatic adenocarcinoma (PAAD) usingTISIDB databases. A. Heatmaps displaying the correlation of FGFBP1 gene with immune inhibitor genes across PAAD. B. Heatmaps displaying the correlation of FGFBP1 gene with immune modulators genes across PAAD. C. Heatmaps displaying the correlation of FGFBP1 gene with cytokine genes across PAAD. D. Violin plots representing the expression levels of FGFBP1 (log2CPM) across different immune subtypes of PAAD. *P*-value < 0.05.

est expression observed in malignant cells (log(TPM+1) of 1.08), followed by endothelial cells, fibroblasts, and others, indicating a predominant expression of FGFBP1 in these cells compared to immune and stromal cells. **Figure 6B** displays a t-SNE plot highlighting the distribution of FGFBP1 expression at the single-cell level, with darker blue dots representing higher expression levels, suggesting heterogeneity in FGFBP1 expression within the tumor microenvironment. **Figure 6C** presents a heatmap of FGFBP1 expression across various cancer types and diverse functional states. The heatmap reveals significant positive correlation of FGFBP1 expression with some important functional states of PAAD, including angiogenesis, cell cycle, differentiation, DNAdamage, EMT, hypoxia, inflammation, metastasis, and proliferation (**Figure 6C**).

Gene enrichment analysis

First, a PPI network of FGFBP1 was constructed using STRING database. **Figure 7A** shows a PPI network of various FGF proteins, highlighting complex interconnections, with FGF2 and FGFBP1 appearing as central nodes. Next, all FGFBP1 interacting partners were subjected to gene enrichment analysis using DAVID tool. **Figure 7B** depicts the cellular components (CC) where these FGFs are enriched, showing significant enrichment in the "extracellular matrix, external encapsulating structure, extracellular space, and extracellular region, with the highest fold enrichment observed in the extracellular matrix". **Figure 7C** illustrates the molecular functions (MF) of FGFs, with "type 1 and type 2 fibroblast growth factor receptor binding being highly enriched, alongside growth factor activity and binding to other proteins like S100, chemo attractants, and heparin". **Figure 7D** focuses on the biological processes (BP) influenced by FGFs, prominently featuring the "fibroblast growth factor receptor signaling pathway, cellular responses to FGF stimulus, and various regulatory processes such as protein phosphorylation, cell migration, and motility". Finally, **Figure 7E** links FGFs to disease pathways, showing their involvement in multiple pathways, including "melanoma, breast cancer, gastric cancer, signaling pathways (Rap1, Ras, MAPK, PI3K-Akt), and cellular activities like actin cytoskeleton regulation and chemical carcinogenesis".

Expression validation, immunolytic, and drug sensitivity analyses of FGFBP1

In this part of our study, the expression of FGFBP1 along with its other family members, including FGFBP2 and FGFBP3 was validated on 10 PAAD and 5 control cell lines using RT-qPCR assay. **Figure 8A** compares the FGFBP1, FGFBP2, and FGFBP3 expression levels between control cell lines and PAAD cell lines, showing a significant (*p*-value < 0.05) increase in FGFBP1, FGFBP2, and FGFBP3 expression in PAAD cells. The investigation of correlations between FGFBP1 expression and various immune cell infiltrates in PAAD using GSCA revealed a notable positive correlation with CD4 T cells, macrophages, and effector memory T cells, while showing a negative correlation with gamma delta T cells (**Figure 8B**). Next, GSCA database explores the relationship between FGFBP1 mRNA expression and drug sensitivity in PAAD. The analysis result shows a consistent positive correlation between FGFBP1 expression and resistance to multiple drugs, suggesting that higher FGFBP1 expression may predict increased resistant to these treatments (**Figure 8C**).

FGFBP1 knockdown, colony formation, cell proliferation, and wound healing assay

FGFBP1 was knockdown in HEK293T cells using siRNA. RT-qPCR and western blot results in **Figure 9A**, **9B** and Supplementary Figure 1 show that FGFBP1 expression levels significantly decrease in si-FGFBP1-HEK293T cells compared to Ctrl-FGFBP1-HEK293T cells, indicating successful knockdown. **Figure 9C** provides images from a colony formation assay, where si-FGFBP1-HEK293T cells form noticeably fewer colonies than Ctrl-FGFBP1-HEK293T

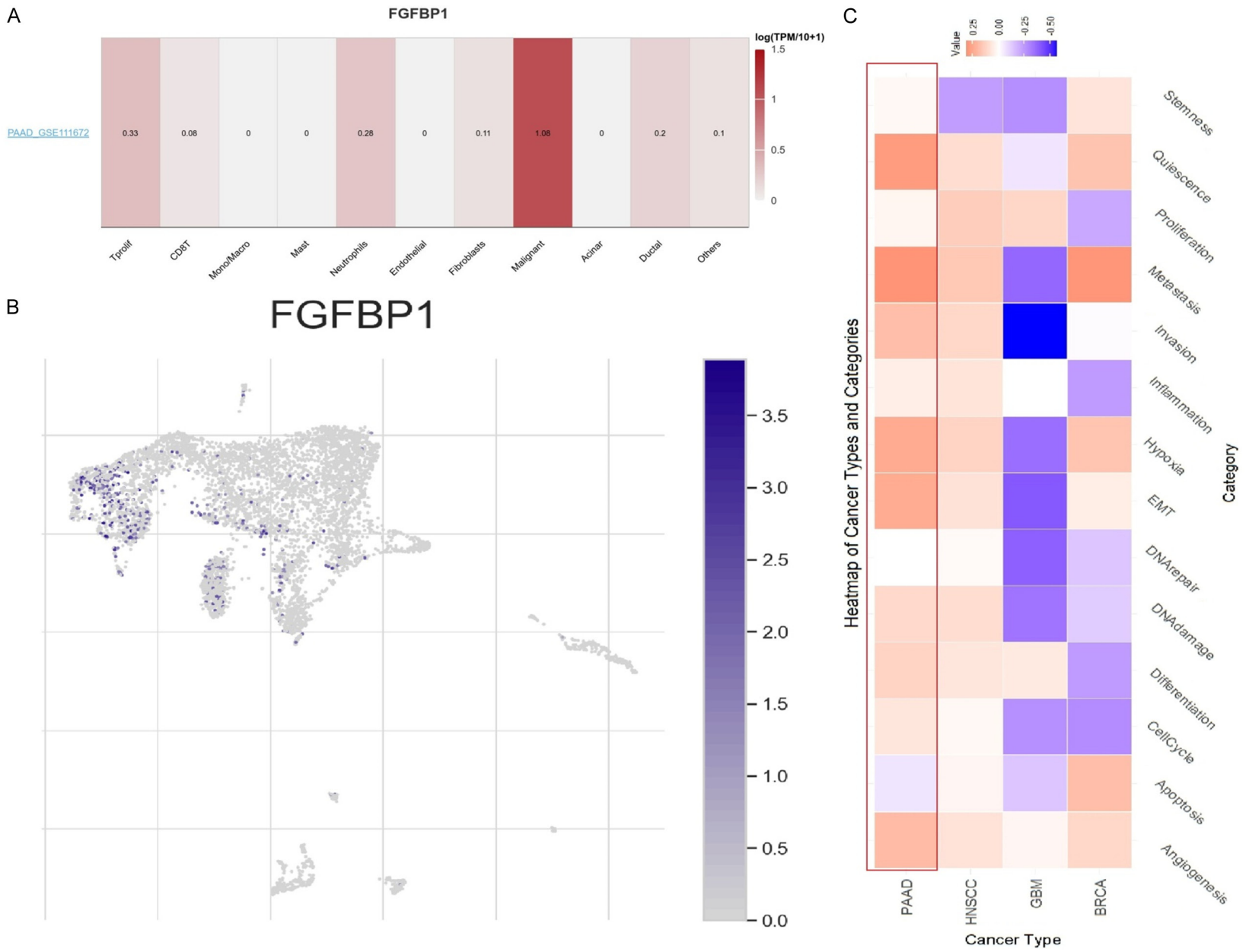
A
FGFBP1
log(TPM/10+1)
1.5
1
0.5
0
PAAD_GSE111672
0.33
0.08
0
0
0.28
0
0.11
1.08
0
0.2
0.1
Tprolif
CD8T
Mono/Macro
Mast
Neutrophils
Endothelial
Fibroblasts
Malignant
Acinar
Ductal
Others
B
FGFBP1
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
C
Value
0.25
0.00
-0.25
-0.50
Heatmap of Cancer Types and Categories
Stemness
Quiescence
Proliferation
Metastasis
Invasion
Inflammation
Hypoxia
EMT
DNArepair
DNAdamage
Differentiation
CellCycle
Apoptosis
Angiogenesis
Category
PAAD
HNSCC
GBM
BRCA
Cancer Type

Figure 6. FGFBP1 expression in different immune cell types and correlation of FGFBP1 expression with diverse functional states of different cancer. A. Bar plot showing the expression levels of FGFBP1 across various immune cell types in the PAAD_GSE111672 dataset from the TISCH2 database. B. t-SNE (t-distributed Stochastic Neighbor Embedding) plot displaying the distribution of FGFBP1 expression in a high-dimensional space from the TISCH2 database. C. Heatmap illustrating the expression of FGFBP1 across different functional states of different cancer types from the CancerSEA database. *P*-value < 0.05.

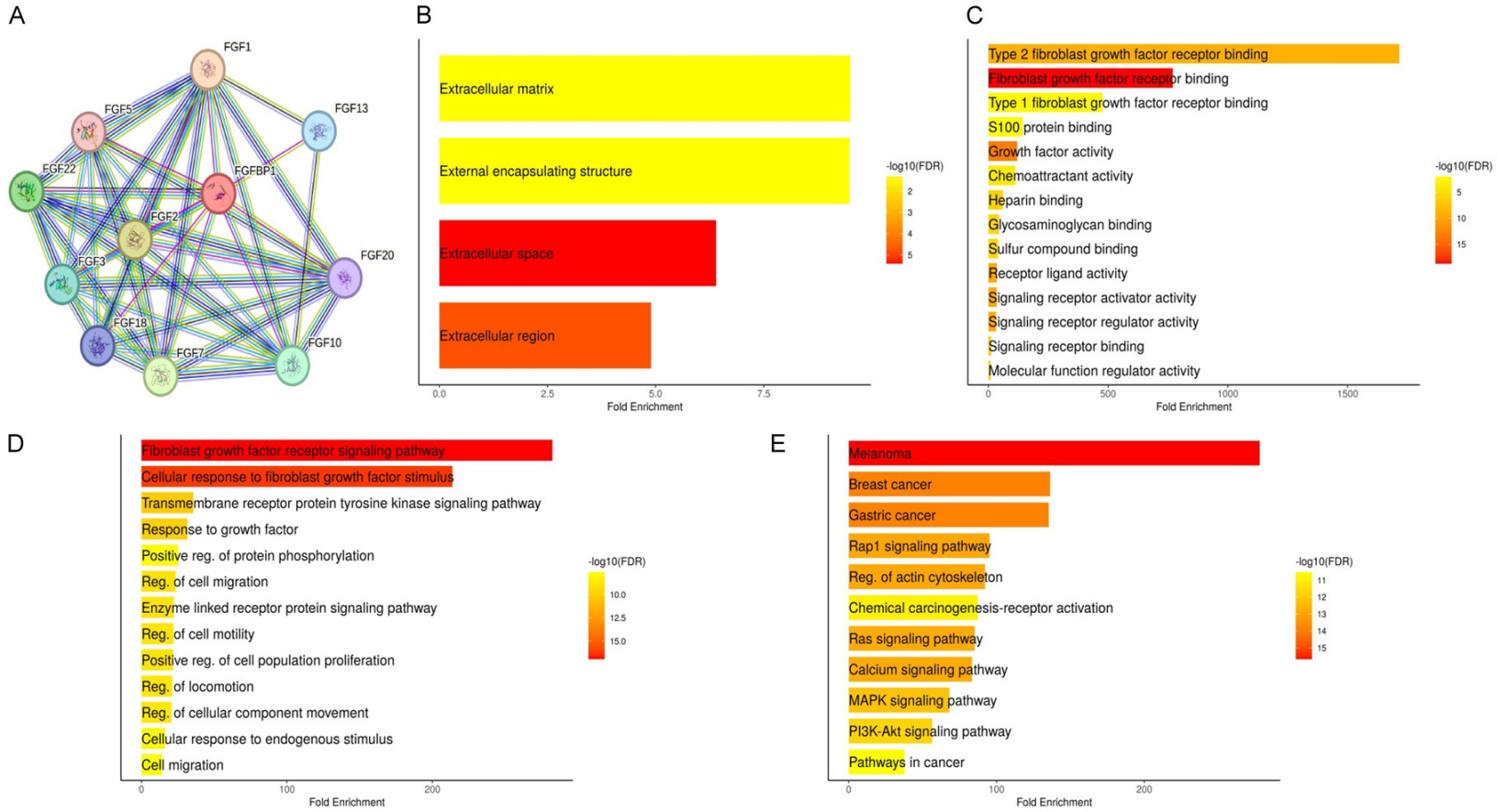

Figure 7. Functional enrichment analysis of FGF family proteins and associated pathways. A. Protein-protein interaction network of FGFBP1, illustrating interactions among different FGF members. B. Cellular component (CC) enrichment analysis showing the fold enrichment of FGF family proteins in various cellular components. C. Molecular function (MF) enrichment analysis depicting the fold enrichment of molecular functions associated with FGF family proteins. D. Biological process (BP) enrichment analysis showing the fold enrichment of biological processes related to FGF family proteins. E. Pathway enrichment analysis illustrating the fold enrichment of pathways involving FGF family proteins. *P*-value < 0.05.

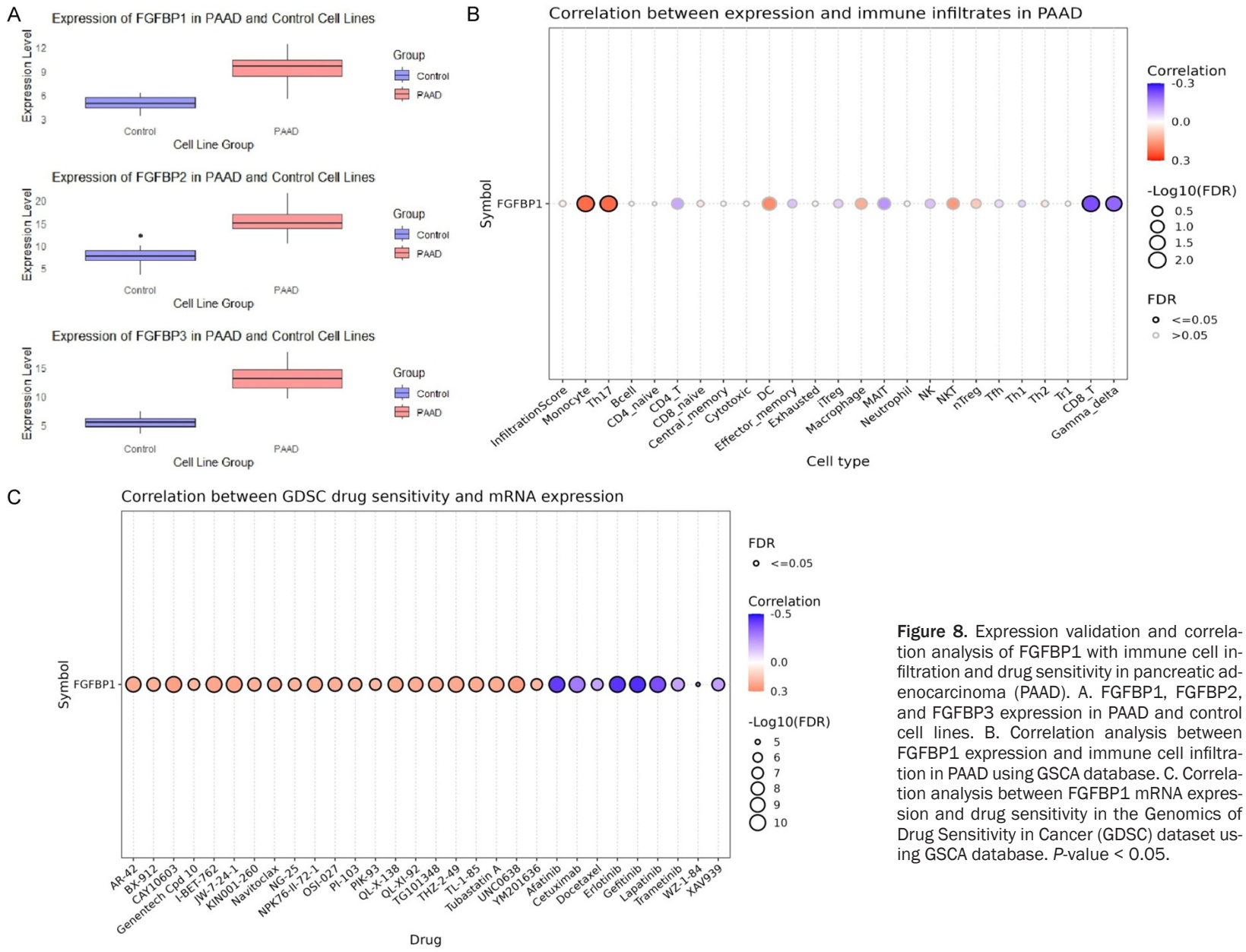

Figure 8. Expression validation and correlation analysis of FGFBP1 with immune cell infiltration and drug sensitivity in pancreatic adenocarcinoma (PAAD). A. FGFBP1, FGFBP2, and FGFBP3 expression in PAAD and control cell lines. B. Correlation analysis between FGFBP1 expression and immune cell infiltration in PAAD using GSCA database. C. Correlation analysis between FGFBP1 mRNA expression and drug sensitivity in the Genomics of Drug Sensitivity in Cancer (GDSC) dataset using GSCA database. *P*-value < 0.05.

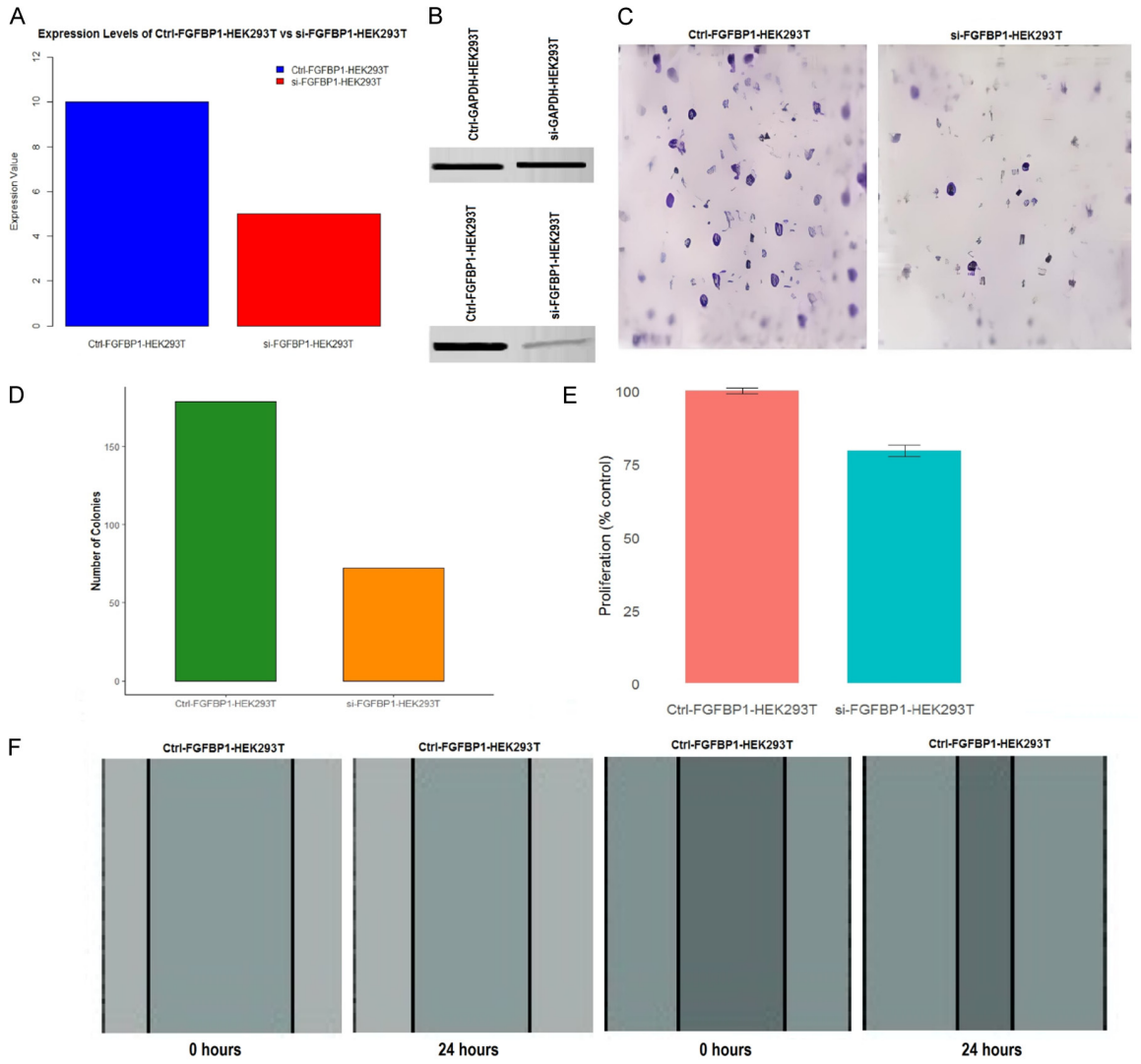

Figure 9. Impact of FGFBP1 knockdown on HEK293T cell proliferation, colony formation, and migration. A. Expression levels of FGFBP1 in control and siRNA-treated HEK293T cells. B. Western blot analysis of FGFBP1 protein levels in control and siRNA-treated HEK293T cells. C. Representative images of colony formation assays for control and siRNA-treated HEK293T cells. D. Quantification of colony formation in control and siRNA-treated HEK293T cells. E. Proliferation assay of control and siRNA-treated HEK293T cells. F. Wound healing assay for cell migration in control and siRNA-treated HEK293T cells. Images were taken at 0 and 24 hours post-scratch.

cells, quantitatively supported by **Figure 9D** which shows a marked reduction in the number of colonies in si-FGFBP1-HEK293T cells. **Figure 9E** indicates a reduction in cell proliferation in si-FGFBP1-HEK293T cells to approximately 80% of the control, demonstrating that FGFBP1 knockdown inhibits cell proliferation. Lastly, **Figure 9F** depicts wound healing assay results, showing that si-FGFBP1-HEK293T cells exhibit fast wound closure compared to Ctrl-FGFBP1-HEK293T cells over 24 hours. Overall, these results collectively indicate that FGFBP1 knockdown in HEK293T cells significantly reduces their proliferation, colony formation, and migration capabilities.

Discussion

Pancreatic adenocarcinoma (PAAD) is one of the most aggressive and lethal forms of cancer, characterized by poor prognosis and limited treatment options [35, 36]. Despite advances in medical research, the survival rate for PAAD remains dismally low [37, 38]. A growing body of evidence suggests that the FGFBP1 plays a crucial role in the progression and malignancy

of various cancers [39, 40]. This study aimed to elucidate the diagnostic, prognostic, and therapeutic role of FGFBP1 gene in PAAD.

Our comprehensive expression analysis across multiple databases, including UALCAN, TNMplot, OncoDB, and GEPIA2, consistently showed that FGFBP1 expression is significantly elevated in PAAD tissues compared to normal tissues. This overexpression was further corroborated by immunohistochemistry data from the HPA database, which indicated high levels of FGFBP1 staining in PAAD samples. Overexpression of FGFBP1 drives cancer progression through several key mechanisms. FGFBP1 binds to and mobilizes fibroblast growth factors (FGFs) from the extracellular matrix, enhancing their bioavailability and promoting angiogenesis, thereby ensuring a sufficient blood supply to the tumor, which facilitates its growth and metastasis [41]. FGFBP1-mediated release of FGFs activates FGF receptors (FGFRs) on cancer cells, triggering downstream signaling pathways such as MAPK/ERK and PI3K/AKT, which are critical for cell proliferation, resulting in increased cancer cell division and growth [42]. Additionally, FGFs and their receptors, when activated by FGFBP1, promote cell survival by activating anti-apoptotic pathways like PI3K/AKT, allowing cancer cells to evade programmed cell death and accumulate [15]. FGFBP1 also facilitates epithelial-mesenchymal transition (EMT), enabling epithelial cells to gain migratory and invasive properties typical of mesenchymal cells, a critical step in cancer metastasis [43, 44]. Furthermore, FGFBP1 modifies the tumor microenvironment by altering the extracellular matrix and influencing the behavior of stromal and immune cells, creating a more permissive environment for tumor invasion and an immunosuppressive environment that allows cancer cells to evade immune surveillance [16]. By enhancing angiogenesis, cell proliferation, EMT, and modifying the tumor microenvironment, FGFBP1 significantly increases the metastatic potential of cancer cells [13]. Its interactions with other signaling pathways and oncogenes, such as VEGF, WNT, and TGF-β, further amplify oncogenic signals and drive tumor progression, making FGFBP1 a critical player in cancer biology and a potential target for therapeutic intervention [45]. Moreover, our findings also align with previous studies that reported elevated FGFBP1 expression in various malignancies, such as breast cancer, gastric cancer, and melanoma [15, 46, 47], where it is associated with tumor progression and poor prognosis.

Promoter methylation analysis revealed that FGFBP1 is hypomethylated in PAAD samples, which is inversely correlated with its expression. This suggests that epigenetic modifications might play a significant role in the up-regulation of FGFBP1 in PAAD. Hypomethylation of FGFBP1 was also found to be associated with poorer survival outcomes, indicating its potential as a prognostic marker. These results are consistent with other studies highlighting the role of DNA methylation in the regulation of gene expression in cancers [48, 49].

Survival analysis using Kaplan-Meier plots and Cox regression models demonstrated that high FGFBP1 expression is associated with significantly poorer overall survival and disease-free survival in PAAD patients. This reinforces the potential of FGFBP1 as a prognostic biomarker. Our findings are in line with studies on other cancers, where FGFBP1 overexpression has been linked to adverse clinical outcomes [50, 51].

Correlation analysis with immune-related factors revealed that FGFBP1 expression might modulate the immune environment in PAAD. High FGFBP1 expression was associated with an immune suppressive milieu, as indicated by its correlation with immune inhibitors and modulators. This immunomodulatory role of FGFBP1 suggests that it could be involved in immune evasion mechanisms, further contributing to PAAD malignancy. Previous research has shown similar immune-related associations in other cancers, where FGFBP1 influences tumor-immune interactions [51, 52].

Single-cell analysis highlighted that FGFBP1 is predominantly expressed in immune cells within the tumor microenvironment and is positively correlated with various functional states of PAAD, including angiogenesis, cell cycle progression, and metastasis. These findings suggest that FGFBP1 not only promotes tumor growth and spread but also contributes to the complex biology of the tumor microenvironment.

Gene enrichment analysis identified FGFBP1 interacting partners and linked FGFBP1 to key cellular processes and pathways, including fibroblast growth factor receptor signaling, cell migration, and actin cytoskeleton regulation. These pathways are crucial for tumor development and metastasis, highlighting the multifaceted role of FGFBP1 in cancer biology. Previous studies have also identified FGFBP1 as a central player in these pathways, further validating our findings [53, 54].

Finally, functional assays demonstrated that FGFBP1 knockdown in HEK293T cells significantly reduced cell proliferation, colony formation, and migration. This indicates that FGFBP1 is not only a marker of poor prognosis but also a potential therapeutic target. Targeting FGFBP1 could disrupt its role in promoting tumor growth and metastasis, offering a new avenue for therapeutic intervention in PAAD.

Conclusion

In conclusion, our study provides robust evidence that FGFBP1 is significantly overexpressed in PAAD and is associated with poor clinical outcomes. The hypomethylation-driven up-regulation of FGFBP1, its impact on the immune environment, and its role in critical oncogenic pathways emphasize its potential as both a prognostic biomarker and a therapeutic target. Further research is warranted to explore the mechanisms underlying FGFBP1 regulation and its potential in clinical applications for PAAD management.

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Disclosure of conflict of interest

None.

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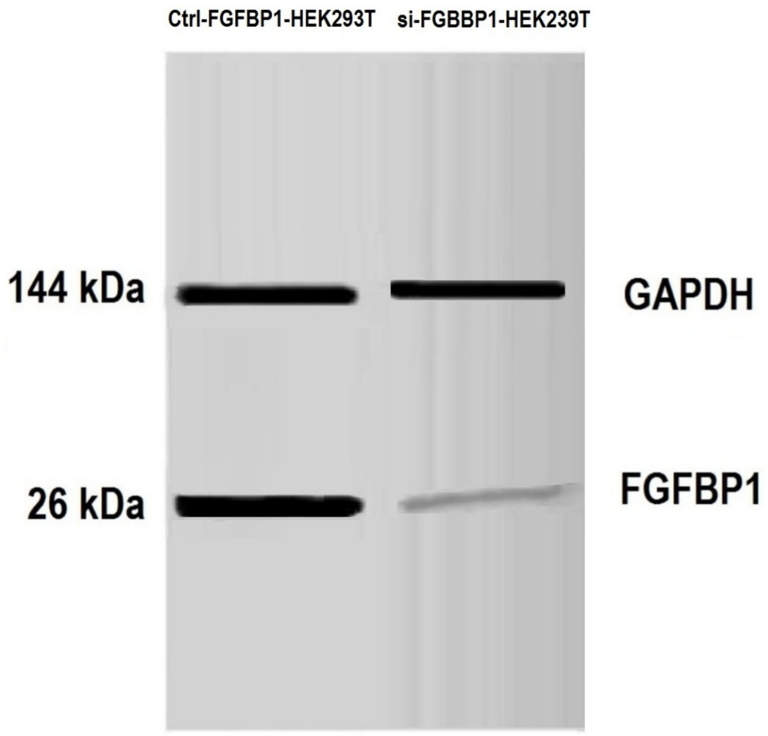

Supplementary Figure 1. Uncut bands of GAPDH and FGBP1 proteins in the western blot analysis.