The diagnostic or prognostic values of FADD in cancers based on pan-cancer analysis

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Abstract. Previous studies have determined that aberrant expression of the fas-associated death domain (FADD) contributes to the development of cancer. However, no pan-cancer analysis has been reported to explore the relationship between FADD and various cancers. Multiple databases were screened to identify cancer datasets for the present study and to validate the expression of FADD in various tumors. The association of FADD alteration with cancer prognosis, clinical features and tumor immunity was also evaluated. Reverse transcription-quantitative PCR (RT-qPCR) was utilized to confirm the expression of FADD in breast, colon, liver and gastric cancer cells. Analysis of Gene Expression Omnibus database and The Cancer Genome Atlas database indicated that FADD was highly expressed in breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma, cholangiocarcinoma, colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD) and prostate adenocarcinoma, whereas RT-qPCR results revealed that FADD was highly expressed in breast cancer and colon cancer. Further analyses demonstrated that FADD expression was significantly altered in ESCA, head and neck squamous cell carcinoma (HNSC), lung squamous cell carcinoma and BRCA. FADD expression was observed to be a risk factor of the overall survival in patients with HNSC, LIHC and LUAD as demonstrated by Kaplan-Meier and Cox regression analyses. The results of the present study demonstrated that FADD is highly expressed in numerous malignancies and can be utilized as a biomarker for the diagnosis of BRCA, COAD, LIHC and stomach adenocarcinoma. Moreover, FADD expression is a predictive risk factor for the development of HNSC, LIHC and LUAD and can potentially be used as a prognostic marker for these cancers.

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Abbreviations: FADD, fas-associated death domain; TME, tumor microenvironment; TCGA, The Cancer Genome Atlas; HPA, Human Protein Atlas; TMB, tumor mutation burden; MSI, microsatellite instability; GEO, Gene Expression Omnibus; OS, overall survival; RFS, relapse-free survival; GSEA, gene set enrichment analysis; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THYM, thymoma; THCA, thyroid carcinoma; UCS, uterine carcinosarcoma; UCEC, uterine corpus endometrial carcinoma; UVM, uveal melanoma

Key words: pan cancer, fas-associated death domain, biomarker, diagnosis

Introduction

Fas-associated death domain (FADD) is a ubiquitous adaptor protein (1). The human *FADD* gene consists of two exons and one intron and has been mapped to chromosome 11q13.3, a region strongly associated with breast invasive carcinoma (BRCA), lung cancer and esophageal carcinoma (ESCA) (2). As an important receptor protein in the tumor necrosis factor receptor family-mediated apoptosis pathway, FADD modulates its binding to death receptors of the tumor necrosis factor receptor family to transmit apoptosis initiation signals (3-5).

In addition, FADD is involved in the regulation of cell proliferation, gene expression and immunity (1,5-8). As a universal adaptor molecule, abnormal expression of FADD protein is associated with the occurrence and development of tumors in both mature and embryonic tissues.

In the last few years, major breakthroughs have been made in the treatment of cancer, including immunotherapy, which has achieved remarkable results in clinical practice (9,10). As the most important defense system of the human organism, the immune system does not only eliminate pathogenic microorganisms, but also destroys abnormal cancer cells, thus actively inhibits tumor growth (11). However, the composition of tumors and their related tumor microenvironment (TME) is relatively complex, requiring precise immune responses (11,12). Therefore, cancer immunotherapy can only achieve favorable results in specific cancer types and patients (13,14). Research to find potential targets for cancer immunotherapy and predict its efficacy is critical to achieve specificity in cancer treatment. Previous studies have demonstrated that FADD is involved in and regulates signaling complexes, including necrosomes, endosomes and inflammasomes (1,15,16). Thus, FADD plays an indispensable role in innate immunity, inflammation and cancer development (1). However, the role of FADD in tumorigenesis is not fully understood, and whether it can be used as a prognostic biomarker as well as its potential value for clinical treatment require to be further explored. In the present study, the differential expression, gene alteration, prognostic value, tumor progression and promoter methylation level of FADD in pan-cancer extent were evaluated based on The Cancer Genome Atlas (TCGA) dataset. Subsequently, the expression level of FADD in related cell lines and databases as well as its relationship with immune cell infiltration, immune checkpoint, tumor mutation burden (TMB) and microsatellite instability (MSI) were analyzed.

Materials and methods

Cell culture. Near diploid and normal human mammary epithelial cells (MCF 10A), triple-negative breast cancer cells (MDA-MB-231) and breast cancer cell (MCF-7) were purchased from Procell Life Science & Technology Co., Ltd. (https://www.procell.com.cn/). Normal human colon mucosal epithelial cell (NCM460) and human colon carcinoma cell line (SW620) were purchased from MINGZHOUBIO Co., Ltd. (https://www.mingzhoubio.com/). All cells were cultured with Dulbecco's modified eagle medium (Biological Industries) containing 10% fetal bovine serum (Biological Industries) and incubated at 37˚C in a thermostatic cell incubator containing 5% CO_2. Roswell Park Memorial Institute 1640 (Biological Industries) was used to maintain cell growth. All cells retained their original morphology throughout the study period.

Reverse-transcription quantitative PCR (RT-qPCR). Total RNA from MCF 10A, MDA-MB-231, MCF-7, NCM460 and SW620 was extracted using TRIzol reagent (Mei5 Biotechnology, Co., Ltd., https://mei5bio.com/) according to the manufacturer's instructions and converted into cDNA using M5 Sprint qPCR RT kit with gDNA remover (Mei5 Biotechnology, Co., Ltd.) according to the manufacturer's instructions. The extraction and reverse transcription were performed in an enzyme-free environment. AceQ qPCR SYBR Green Master Mix (Vazyme Biotech Co., Ltd., https://www.vazyme.com/) was used to quantify the relative expression of FADD (Sangon Biotech Co., Ltd.) in mRNA. The primers used were as follows: GAPDH forward, 5'-CAGGAGGCATTGCTGATGAT-3' and reverse, 5'-GAAGGCTGGGGCTCATTT-3'; and FADD forward, 5'-GACCGAGCTCAAGTTCCTATG-3' and reverse, 5'-GAGCATGGAGAAGAGGTCTAG-3'. The thermocycling conditions are provided in Table I.

Data acquisition and differential expression of FADD in cancer tissues. Transcriptome data and patient clinical data of 33 human cancers were obtained from TCGA database on the UCSC Xena website (xena.ucsc.edu). All gene names in the expression matrix were transformed from Ensembl ID to the Symbol format. In total, 20 datasets (GSE13057, GSE9750, GSE26566, GSE44076, GSE23400, GSE30784, GSE167093, GSE15641, GSE25097, GSE40791, GSE19188, GSE51024, GSE26712, GSE71729, GSE10927, GSE70770, GSE26253, GSE33630 and GSE63678) containing 2,778 tumor tissues and 1,821 non-tumor tissues were included from the Gene Expression Omnibus (GEO) repository (17-36). The R packages 'plyr' (version, 1.8.8; http://cran.ma.ic.ac.uk/web/packages/plyr/plyr.pdf), 'reshap2' (version, 1.4.4 http://cran.ma.ic.ac.uk/web/packages/reshape/reshape.pdf) and 'ggpubr' (version, 0.6.0; http://cran.ma.ic.ac.uk/web/packages/ggpubr/ggpubr.pdf)were used to create a box plot demonstrating FADD expression differences. Furthermore, the immunohistochemical images of FADD protein in different cancer tissues and normal tissues were obtained from the Human Protein Atlas (HPA; https://www.proteinatlas.org).

FADD alteration and promoter methylation in cancer. FADD alteration data were collected from the cBioPortal website (https://www.cbioportal.org/) for a total of 10,953 patients with cancer, including the corresponding 10,967 samples of mutation and CNA data, for analysis (37). Mutation, structural variant, amplification, deep deletion and multiple alterations of FADD were analyzed in different cancers. The University of Alabama at Birmingham Cancer (UALCAN) data analysis portal (http://ualcan.path.uab.edu) was used to explore differences in promoter methylation levels of FADD between tumor and non-tumor samples in TCGA (38). $P<0.05$ was considered to indicate a statistically significant difference.

Analysis of survival rate and clinical association of patients with different expressions of FADD. The Kaplan-Meier plotter website (https://kmplot.com) was used to perform overall survival (OS) and relapse-free survival (RFS) prognostic analysis. According to the expression level of FADD, samples were divided into high- and low-expression groups (39). Kaplan Meier analysis was used to compare the differences between OS and RFS between high- and low-expression groups, and values with $P<0.05$ were considered statistically significant. The Cox proportional hazards model method was used to compare FADD as a continuous variable with survival status and survival time and to calculate the hazard ratio (HR) value and P-value. Values with $P<0.05$ were considered to indicate a statistically significant difference. A HR value >1 indicated that the expression of FADD was a high-risk factor

Table I. Thermocycling conditions of reverse transcription-quantitative PCR.

Stage 1	Pre-denaturation	Repeats: 1	95˚C	5 min
Stage 2	Amplification	Repeats: 40	95˚C	10 sec
			60˚C	30 sec
Stage 3	Melting Curve	Repeats: 1	95˚C	15 sec
			60˚C	60 sec
			95˚C	15 sec

Table II. Relative expression of fas-associated death domain in cancer cells.

Cell line	Mean ± SD	P-value
MCF 10A	1.1651±0.7826	
MDA-MB-231	13.5447±3.9375	0.0059
MCF-7	11.4218±4.0622	0.0127
NCM460	1.0059±0.1364	
SW620	7.8246±2.4492	0.0400

in the tumor, whereas a value <1 indicated that the expression of FADD was considered. Based on these results, a forest map was created.

Analysis of FADD expression, TME and immune cell infiltration. TME encompasses the internal and external environment in which tumors and tumor cells proliferate, develop and metastasize (40). Changes in TME contribute to the generation of tumor resistance (including immune checkpoint inhibitors resistance) and the metabolic changes in physiological processes (41). The immune infiltration in TME is highly associated with the occurrence and development of tumors and the clinical treatment outcome of patients (42,43). The Spearman correlation test between FADD expression and TME score was performed using the R packages 'ggplot2' (version, 3.4.3; https://cran.r-project.org/web/packages/ggplot2/index.html), 'ggpubr' and 'ggExtra', and the results satisfying the condition (P<0.05, correlation coefficient >0.2) were plotted for visualization. The relative content of immune cells in each sample was determined using the Sangerbox website (http://vip.sangerbox.com/home.html). The relative expression of FADD in the samples and the infiltration of immune cells [B cells, CD4 cells, CD8 cells, neutrophils, macrophages and dendritic cells (DCs)] were analyzed using TIMER2.0 tool (http://timer.cistrome.org/) (44,45).

Correlation of FADD expression with TMB and MSI. Although TMB and MSI (46) are not perfect indicators of cancer immunotherapy response, they are still important biomarkers for predicting the effect of immunotherapy (47,48). The R package 'fmsb' (version, 0.7.5; http://cran.ma.ic.ac.uk/web/packages/fmsb/fmsb.pdf) was used to analyze the correlation of the FADD expression with TMB and MSI in all cancer samples (49). P<0.05 was considered to indicate a statistically significant difference. These correlation analysis results were illustrated in a radar map. A correlation coefficient >0 indicated that FADD expression was positively correlated with TMB and MSI, whereas a correlation coefficient <0 indicated that FADD expression was negatively correlated with TMB and MSI.

Gene set enrichment analysis (GSEA). The GSEA method is useful for the discovery of genes with no significant difference in expression but key biological function (49). Using GSEA website (http://www.gsea-msigdb.org/gsea), data sets were obtained from the Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.kegg.jp/) and Gene Ontology (GO) (http://www.geneology.org) databases. The R packages 'limma' (version, 3.56.2; https://bioconductor.org/packages/release/bioc/html/limma.html), 'org.Hs.eg.db' (version, 3.17.0; https://bioconductor.org/packages/release/data/annotation/html/org.Hs.eg.db.html), 'enrichmentplot' and 'clusterProfiler' were used to perform KEGG pathway analysis and GO function annotation analysis on genes differentially expressed between high- and low-expression groups of FADD (49,50). With P<0.05 as the threshold for statistical significance, the top five most significant pathways and biological processes were displayed.

Statistical analysis. FADD expression levels in all cancer tissues and adjacent tissue samples were determined using The R Project for Statistical Computing 4.2.1 (R Foundation and R Core Team, https://www.r-project.org/). The Wilcoxon rank sum test was used to calculate the difference in FADD expression between tumor and non-tumor tissuesand the receiver operating characteristic (ROC) curve was drawn (Sangerbox website, http://vip.sangerbox.com/home.html). A hypothesis test probability (P<0.05) was considered statistically significant. With GAPDH as the internal reference gene, the $2^{-\Delta\Delta Cq}$ method was used to calculate the expression of FADD. Unpaired t-test was used to calculate the significance of the relative expression of FADD between normal breast cells and breast cancer cells. And an unpaired t test with Welch's correction was used to calculate the significance of the relative expression of FADD between colon mucosal epithelial cell and colon carcinoma cell line. Statistical Calculation and Bar Chart Drawing by GraphPad Prism 8.3.0 (Dotmatics).

Results

Expression of FADD in different cancers. The analysis results of the expression of FADD mRNA in tumor and non-tumor tissues collected from the TCGA database revealed that FADD was significantly differentially expressed in 19 cancer types. FADD was relatively highly expressed in bladder urothelial carcinoma (BLCA), BRCA, cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), ESCA, glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach adenocarcinoma STAD, and thyroid carcinoma (THCA) samples, but showed relatively

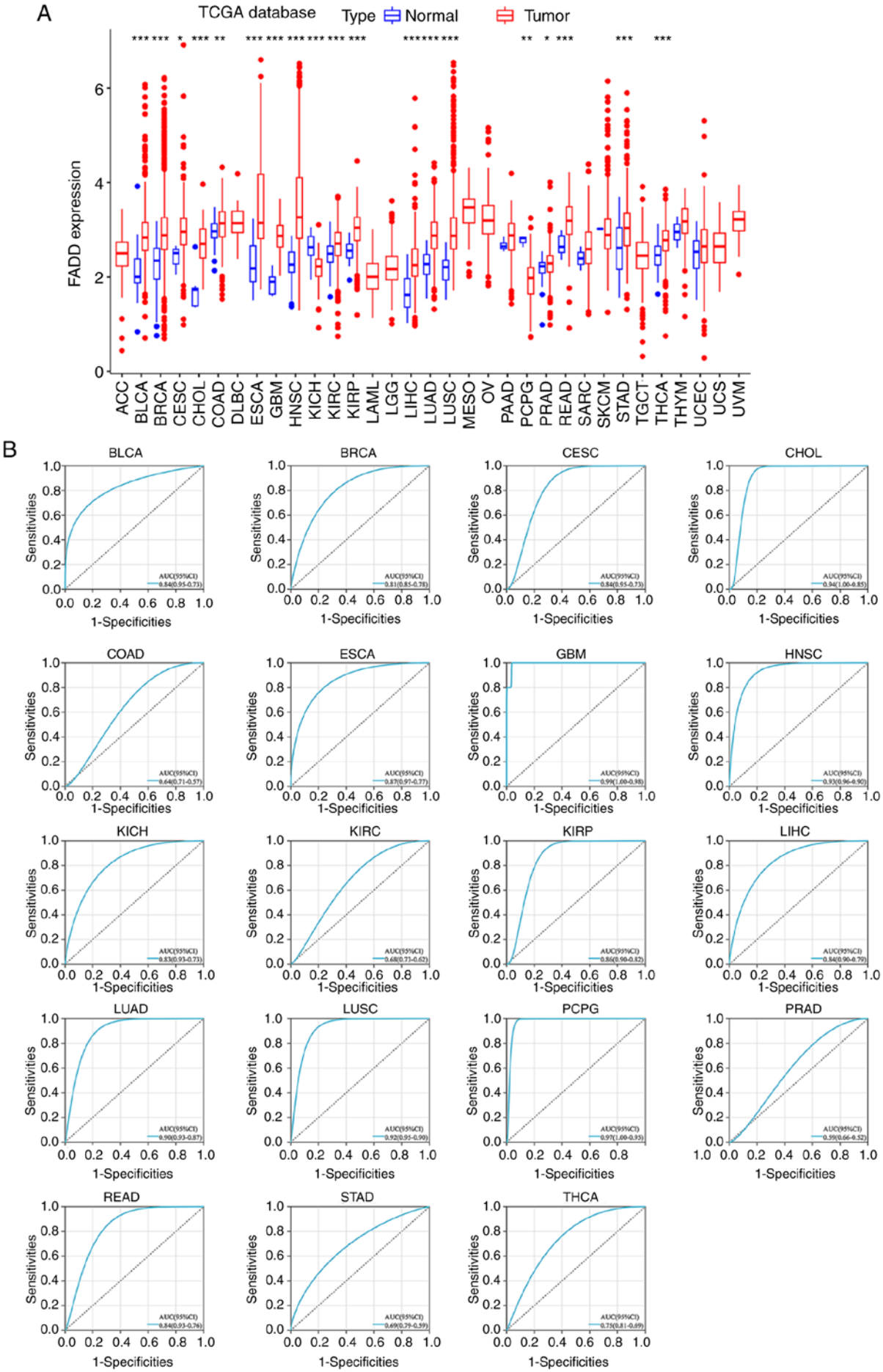

Figure 1. Continued.

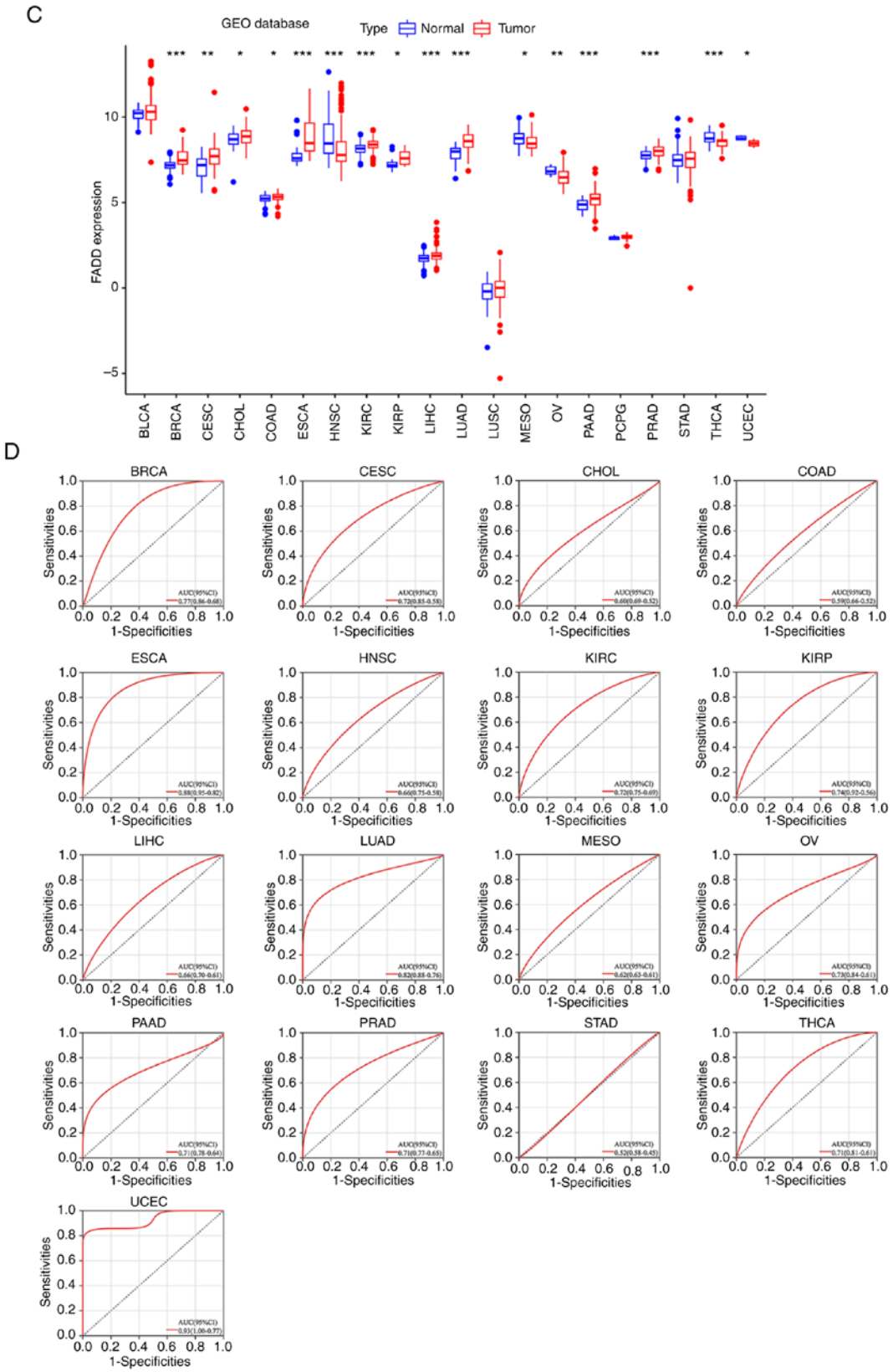

Figure 1. Expression in pan-cancer. (A) The differential expression analysis of FADD in adjacent tissues and cancer tissues from the TCGA database and (B) the ROC curve of cancer species with statistical differences. (C) The differential expression analysis of FADD in adjacent tissues and cancer tissues from the GEO database and (D) the ROC curve of cancer species with statistical differences. *P<0.05, **P<0.01 and ***P<0.001. FADD, fas-associated death domain; TCGA, The Cancer Genome Atlas; ROC, receiver operating characteristic; GEO, Gene Expression Omnibus; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; UCEC, uterine corpus endometrial carcinoma.

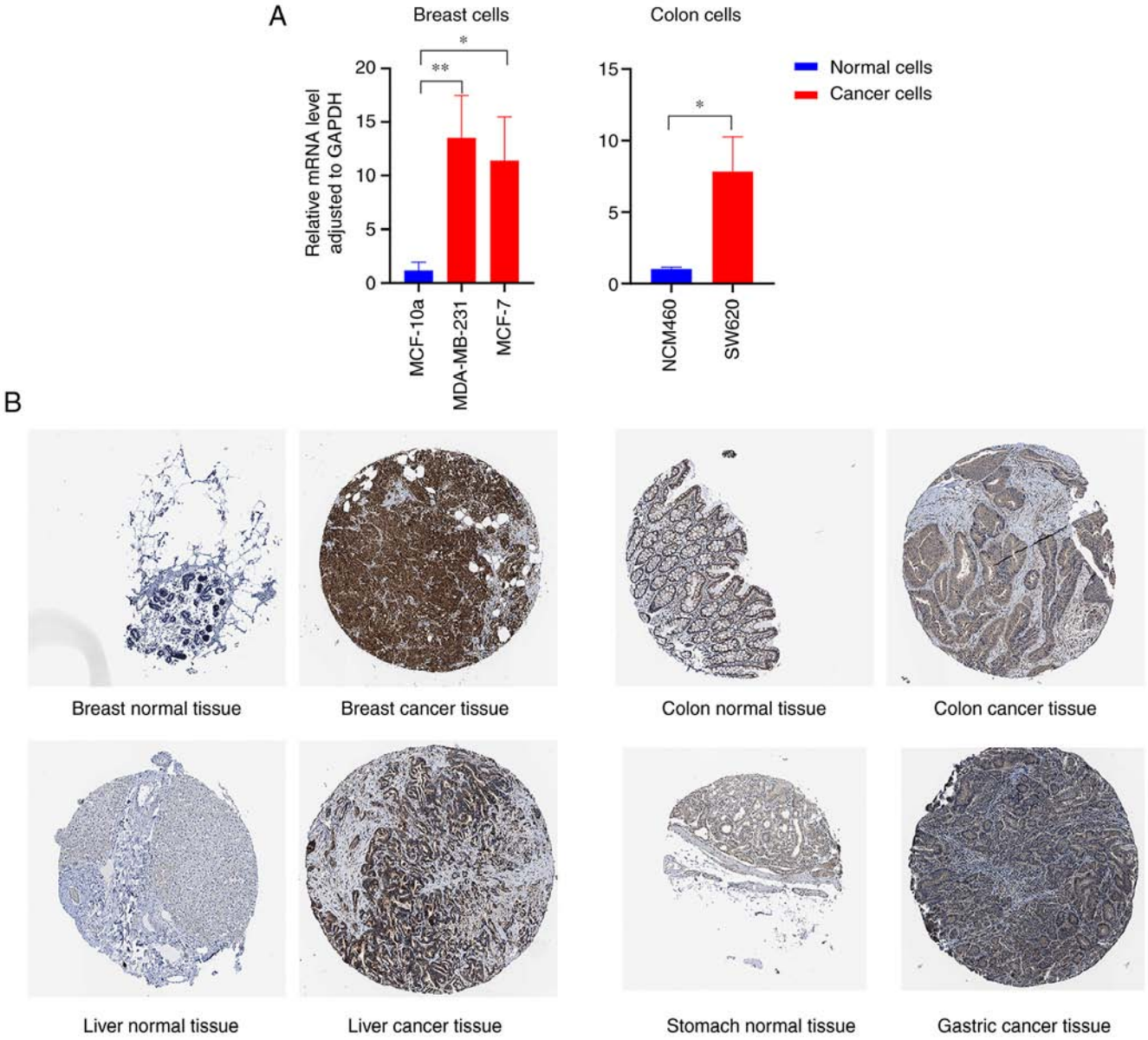

Figure 2. Expression in specific cancers. (A) Expression of FADD in different cancer cells and normal cells. (B) Immunohistochemical images of FADD protein in different cancer tissues and normal tissues from the HPA database. *P<0.05 and **P<0.01. FADD, fas-associated death domain; HPA, Human Protein Atlas.

low expression in KICH, and pheochromocytoma and paraganglioma (PCPG) samples (Fig. 1A and B). The results of mRNA expression analysis of FADD in tumor and non-tumor samples collected from the GEO database revealed that FADD was relatively highly expressed in BRCA, CESC, CHOL, COAD, ESCA, KIRC, KIRP, LIHC, LUAD, PAAD and PRAD samples, but showed relatively lower expression in HNSC, mesothelioma (MESO), ovarian serous cystadenocarcinoma (OV), THCA and uterine corpus endometrial carcinoma (UCEC) samples (Fig. 1C and D). RT-qPCR results revealed that FADD mRNA was highly expressed in breast cancer cells and colon cancer cells (Fig. 2A). The results of immunohistochemical analysis of tumor and non-tumor samples in the HPA database showed that FADD protein was relatively highly expressed in breast, colon, liver and gastric cancer tissues (Fig. 2B). Relative expression of FADD in cancer cells is demonstrated in Table II.

FADD alteration in cancer. The analysis results of the types of FADD alteration in 32 cancers in the TCGA database demonstrated that FADD changes were most frequent in patients with ESCA (Fig. 3A). Kaplan-Meier prognosis result of OS in FADD-altered and non-altered patients showed that FADD alteration was significantly associated with shorter OS in cancer patients (Fig. 3B).

FADD promoter methylation level. The analysis of tumor samples and non-tumor samples in the UALCAN database revealed that FADD promoter methylation level was relatively high in CESC (Fig. 4B), ESCA (Fig. 4C), KIRC (Fig. 4D), LUSC (Fig. 4F) and PAAD (Fig. 4G) samples, but lower in BLCA (Fig. 4A), LIHC (Fig. 4E), PRAD (Fig. 4H), sarcoma (SARC; Fig. 4I), testicular germ cell tumors (TGCT; Fig. 4J), THCA (Fig. 4K) and UCEC (Fig. 4L) samples.

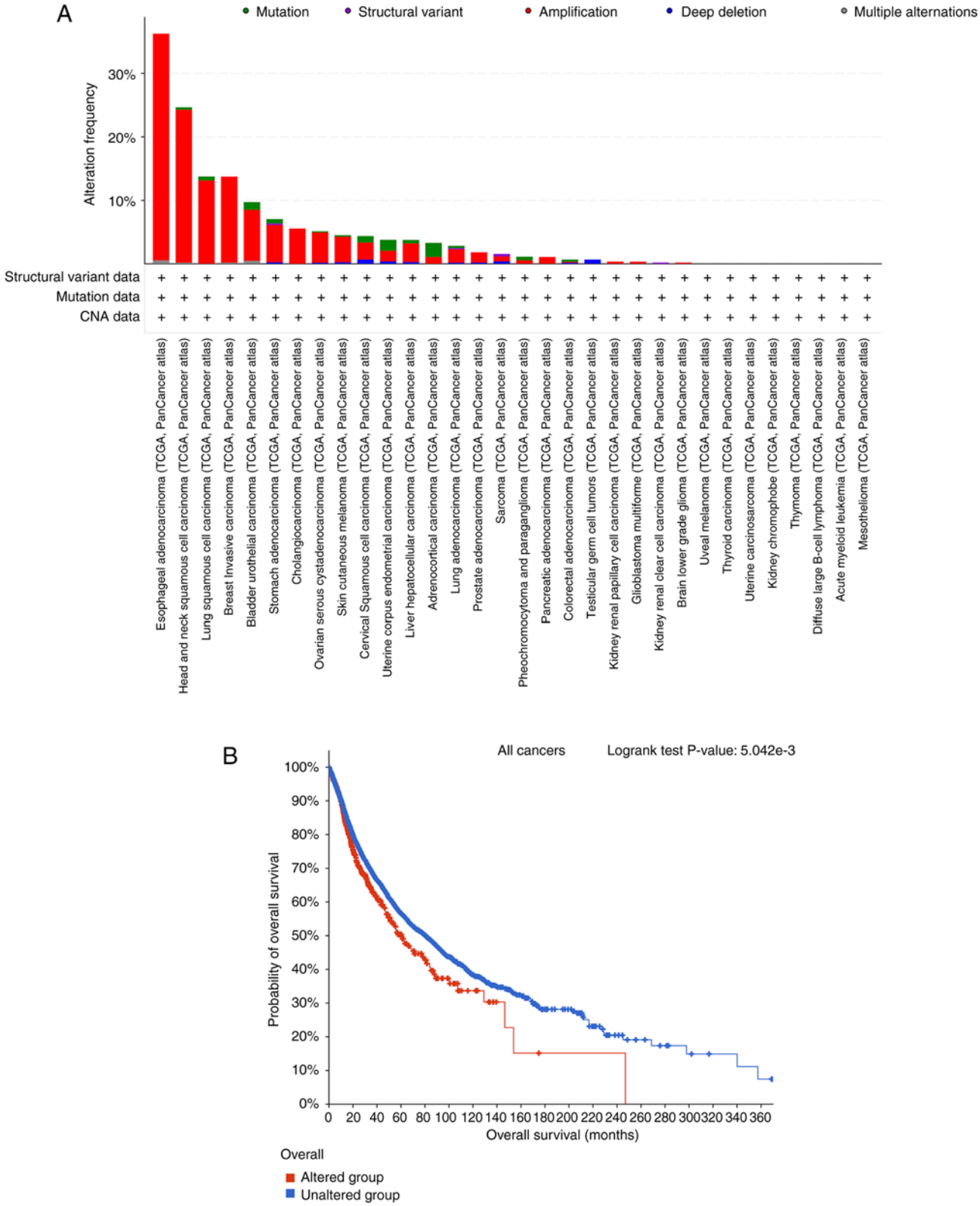

Figure 3. Alterations. (A) Alterations in FADD in various cancer types and (B) Kaplan-Meier prognosis of OS in all patients with cancer regarding a FADD altered group and a FADD unaltered group. FADD, fas-associated death domain; CNA, copy number alterations; OS, overall survival.

Correlation between FADD expression and clinical characteristics of various cancers. To analyze the correlation between the expression of FADD and age, patients were divided into two cohorts: i) Patients aged <65 years and ii) patients aged 65 years or older. The expression level of FADD was relatively higher in elderly cancer patients with ESCA and OV and patients younger than 65 years with skin cutaneous melanoma and TGCT (Fig. 5A). In addition, FADD was highly expressed in female patients with adrenocortical carcinoma and male patients with COAD (Fig. 5B). Notably,

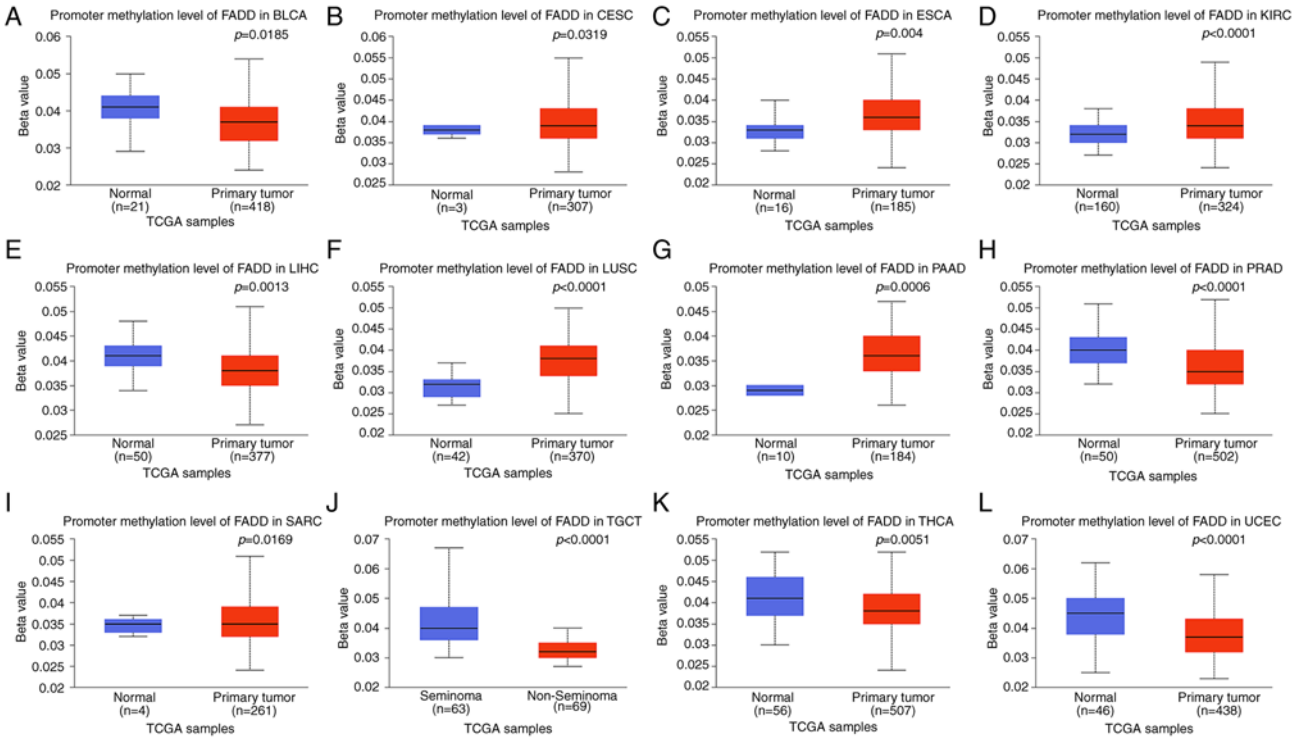

Figure 4. Promoter methylation levels. Difference analysis of the methylation levels of FADD promoter in (A) BLCA, (B) CESC, (C) ESCA, (D) KIRC, (E) LIHC, (F) LUSC, (G) PAAD, (H) PRAD, (I) SARC, (J) TGCT, (K) THCA and (L) UCEC. FADD, fas-associated death domain; TCGA, The Cancer Genome Atlas; BLCA, bladder urothelial carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; ESCA, esophageal carcinoma; KIRC, kidney renal clear cell carcinoma; LIHC, liver hepatocellular carcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; SARC, sarcoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma.

the difference in the expression of FADD among different stages of KIRC, LUAD and TGCT patients was statistically significant (Fig. 5C).

Correlation between FADD expression and prognosis of various cancers. The Kaplan-Meier-plot website was used to investigate the relationship between FADD expression and the prognosis of patients with cancer. The results demonstrated that the high expression of FADD was significantly associated with shorter OS in CESC (Fig. 6B), HNSC (Fig. 6C), LIHC (Fig. 6D), LUAD (Fig. 6E), LUSC (Fig. 6F) and PAAD (Fig. 6G), but not in STAD (Fig. 6H), and significantly longer OS in thymoma (THYM) (Fig. 6I) and THCA (Fig. 6J). The low expression of FADD in CESC (Fig. 6L), LUAD (Fig. 6M), PAAD (Fig. 6N) and TGCT (Fig. 6P) was significantly associated with shorter RFS, whereas the high expression of FADD in BRCA (Fig. 6K) and STAD (Fig. 6O) was associated with longer RFS. Cox regression analysis exhibited that FADD was a poor prognostic factor for HNSC, acute myeloid leukemia, brain lower grade glioma (LGG), LIHC, LUAD and PAAD, but a protective factor for MESO and THCA (Fig. 6A).

Effects of FADD on TME and immune cell infiltration. The results of TME analysis showed that the expression of FADD was positively correlated with the ImmuneScore of LGG (Fig. 7A), SARC (Fig. 7B), THCA (Fig. 7D), uterine carcinosarcoma (UCS; Fig. 7E) and uveal melanoma (UVM; Fig. 7F), and negatively correlated with the ImmuneScore of TGCT (Fig. 7C). The expression of FADD was positively correlated with the StromalScore of LGG (Fig. 7G) and negatively correlated with the StromalScore of MESO (Fig. 7H) and THYM (Fig. 7I). Analysis of the data obtained from the TCGA database using the TIMER method revealed that the expression of FADD was positively correlated with B cell infiltration in KIRC, KIPAN, PCPG, PRAD, THYM, THCA, LGG, LIHC, COADREAD, COAD, OV, KICH and ACC, but negatively correlated with B cell infiltration in HNSC, CESC, LUAD and ESCA. Moreover, the expression of FADD was positively correlated with CD4 cell infiltration in KIRC, KIPAN, PCPG, PRAD, LGG, LIHC, COADREAD, COAD, OV, KICH, READ, GBMLGG, GBM and BLCA. The expression of FADD was positively correlated with T cell CD8 infiltration in LIRC, KIPAN, PCPG, PRAD, THYM, THCA, LIHC, COADREAD, COAD, ACC, SKCM-P, GBMLGG, PAAD, BLCA and DLBC, and negatively correlated with T cell CD8 infiltration in HNSC, CESC and GBM. In addition, the expression of FADD was positively correlated with neutrophil and macrophage infiltration in all cancer types except LUSC and DLBC in which the expression of FADD was negatively correlated with macrophage infiltration. Furthermore, the expression of FADD was positively correlated with dendritic cells (DC) infiltration in KIRC, KIPAN, PCPG, PRAD, THYM, THCA, LGG, LIHC, COADREAD, COAD, LUAD, OV, KICH, LUSC, READ, STES, GBMLGG, GBM, KIRP, PAAD, UCEC, BLCA, DLBC and SARC (Fig. 8).

Correlation of FADD expression with MSI and TMB in cancer. FADD was observed to be positively correlated with MSI in LGG, LUAD, PAAD, SARC and UCEC, and negatively correlated with MSI in COAD, PCPG, READ

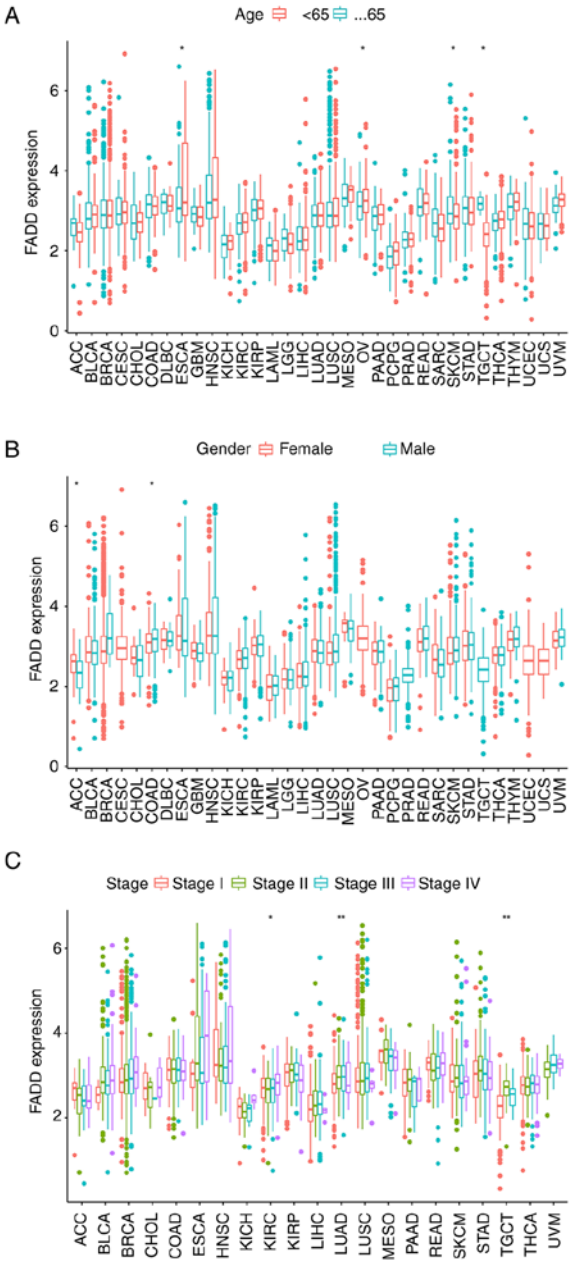

Figure 5. Clinical correlation. FADD expression correlates with (A) age, (B) sex and (C) cancer stage in patients with cancer. *P<0.05 and **P<0.01. FADD, fas-associated death domain. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, diffuse large B cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

and THYM (Fig. 9A). By contrast, FADD expression was positively correlated with HNSC, KIRC, KIRP, PRAD, SARC, THCA, UCEC and UVM, while it was revealed as negatively correlated with TMB in LUAD and READ (Fig. 9B).

Analysis of different FADD expressions (GSEA). The expression of FADD was divided into two groups and GSEA analysis was performed. Furthermore, the results demonstrated that FADD was involved in different signaling pathways and biological processes in various cancers. GO enrichment results (Fig. S1) revealed that the main active biological processes associated with the high expression FADD group were the detection of chemical stimulus (in three cancers), epidermal cell differentiation (in three cancers), and epidermis development (in three cancers as well). On the contrary, the main active biological processes associated with the low expression FADD group were the detection of chemical stimulus (in 18 cancers) and the detection of stimulus involved in sensory perception (in 15 cancers). KEGG analysis results (Fig. S2) indicated that the main active signaling pathways in the high expression FADD group were olfactory transduction (in 4 cancers) and systemic lupus atherosclerosis (in 3 cancers). By contrast, the main active signaling pathways associated with the low expression FADD group were olfactory transduction (in 19 cancers) and neuroactive ligand-receptor interaction (in 10 cancers).

Discussion

Analysis of the differential expression of FADD between cancer and normal samples in the TCGA database revealed that FADD was substantially expressed in 18 of the 33 malignancies analyzed and in 11 of the 20 GSE datasets selected. The area under the ROC curve of 6 cancer types (CHOL, GBM, HNSC, LUAD, LUSC and PCPG) from TCGA database had a value of >0.9, and the area under the ROC curve of 4 cancers (ESCA, HNSC, LUAD and STAD) from GEO database had a value of >0.8. RT-qPCR exhibited that FADD mRNA was relatively significantly expressed in breast, colon, liver and stomach cancer cells, which was consistent with immunohistochemical images obtained from the HPA database. These findings showed that FADD may have diagnostic utility as a biomarker for cancer. FADD is a ubiquitous adapter protein that not only conveys apoptotic signals mediated by death receptors but also mediates inflammation and cancer (51-53). Inflammation is a hallmark of a substantial percentage of cancers, which may explain the relatively high expression of FADD in the vast majority of cancers (54,55), including oral squamous cell cancer (56). FADD alteration in the cBioPortal database demonstrated that FADD is more likely to change in more than 30, 20, 10 and 10% of patients with ESCA, HNSC, LUSC and BRCA, respectively, and amplification is the predominant FADD alteration. The human *FADD* gene is located on chromosome 11q13.3, 11q13-q14 amplification has a relatively high incidence in breast, ovary, head and neck, esophageal, melanoma and bladder tumors, which is consistent with the expression patterns of FADD in cancer (2). This suggests that FADD amplification may cause certain cancers. Methylation of FADD is also linked to cancer. A previous study has identified an association between FADD methylation and oral squamous cell carcinoma (57). The UALCAN database analysis revealed that abnormal FADD promoter methylation was associated with 12 tumor samples, indicating that both FADD mutation

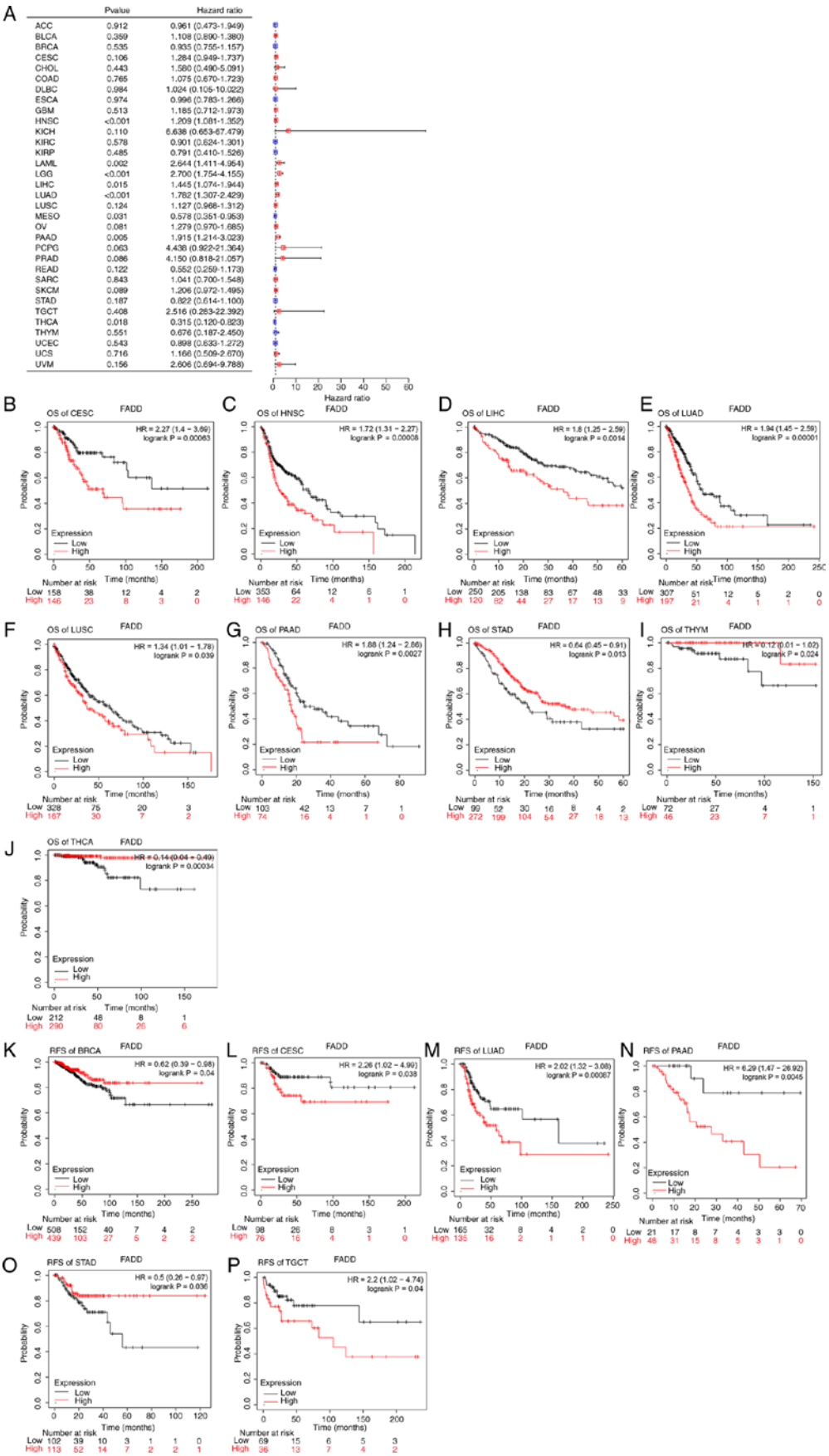

Figure 6. Prognosis analysis. (A) Cox regression analysis of 33 types of cancer. FADD expression and Kaplan-Meier prognosis analysis (based on OS) of (B) CESC, (C) HNSC, (D) LIHC, (E) LUAD, (F) LUSC, (G) PAAD, (H) STAD, (I) THYM and (J) THCA. FADD expression and Kaplan-Meier prognosis analysis (based on RFS) of (K) BRCA, (L) CESC, (M) LUAD, (N) PAAD, (O) STAD and (P) TGCT. FADD, fas-associated death domain; OS, overall survival; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; HNSC, head and neck squamous cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; STAD, stomach adenocarcinoma; THYM, thymoma; THCA, thyroid carcinoma; RFS, relapse-free survival; BRCA, breast invasive carcinoma; TGCT, testicular germ cell tumors.

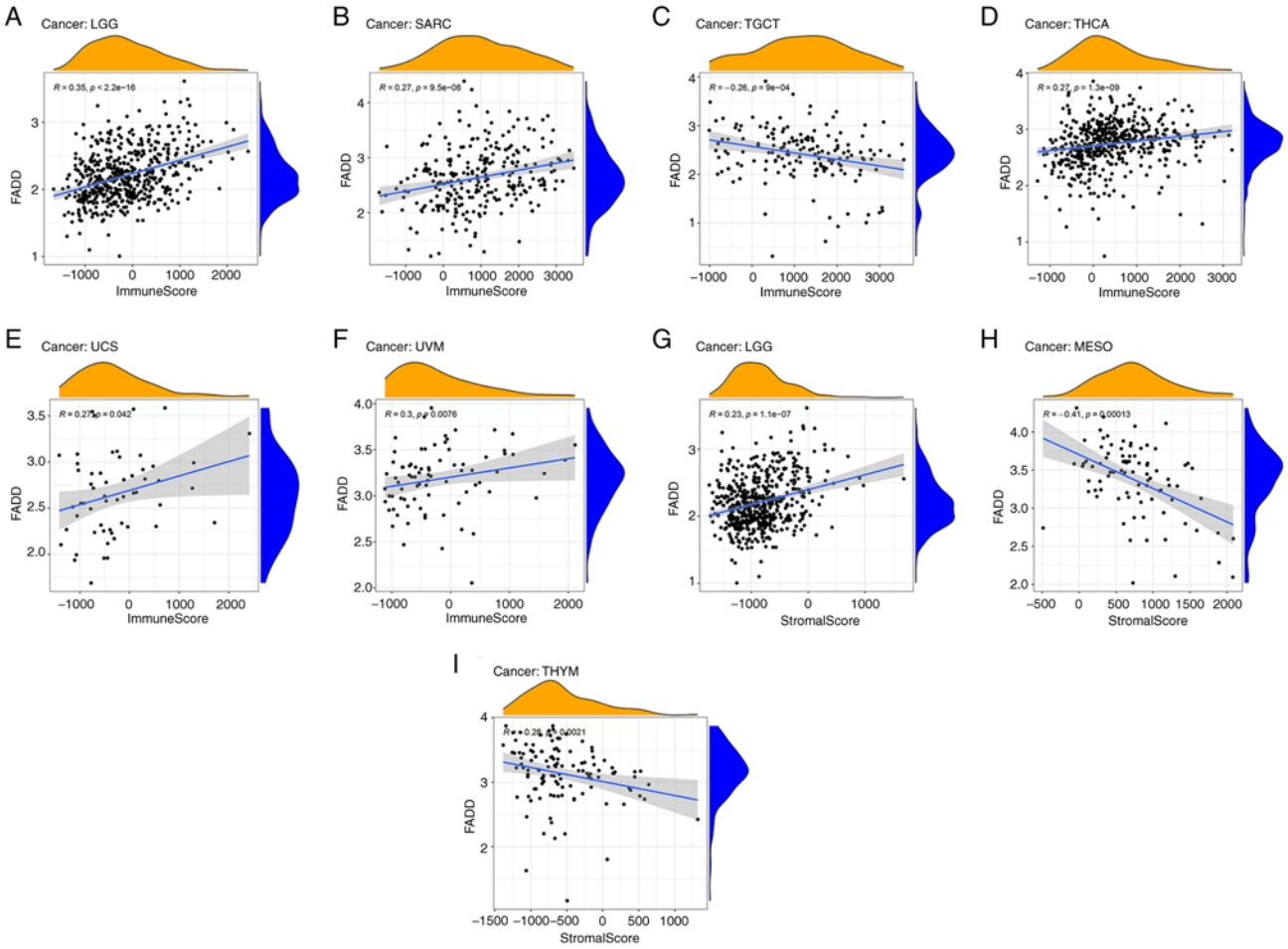

Figure 7. Tumor microenvironment. Correlation between FADD expression and ImmuneScore of (A) LGG, (B) SARC, (C) TGCT, (D) THCA, (E) UCS and (F) UVM; the correlation between FADD expression and StromalScore of LGG (G), MESO (H) and THYM (I). FADD, fas-associated death domain; LGG, brain lower grade glioma; SARC, sarcoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; MESO, mesothelioma; THYM, thymoma.

and promoter methylation are associated with malignancy. Compared with normal tissue, FADD promoter methylation levels were significantly reduced in primary tumors of BLCA, LIHC, PRAD and THCA, and differential analysis revealed that FADD mRNA was significantly highly expressed in these cancer tissues. The high expression of FADD mRNA in BLCA, LIHC, PRAD and THCA may be related to the decrease of the promoter methylation level. FADD mRNA was also highly expressed in CESC, KIRC and LUSC, but FADD promoter methylation levels were significantly lower in primary tumor tissues than in corresponding normal tissues in these cancers. This may be due to the low expression or no expression of FADD mRNA in the corresponding normal tissue. Even if the methylation level of the promoter is increased, the expression of FADD in primary tissue remains significantly higher than that in normal tissue. This suggests that FADD is reliable as a biomarker for the diagnosis of these cancers.

High expression of FADD was significantly associated with shorter OS in six types of cancer patients and RFS in four types of cancer patients, as exhibited by Kaplan-Meier analysis. FADD expression was a risk factor for numerous cancers (6 types) and a protective factor for fewer cancers (2 types), according to Cox regression analysis. For instance, Kaplan-Meier prognostic analysis and Cox regression analysis of patients with HNSC, LIHC and LUAD revealed that FADD expression was a risk factor for these malignancies. A recent study has demonstrated the predictive utility of *FADD* gene can in the prognosis of lung adenocarcinoma in women (58). Because FADD amplification occurs in high frequency in HNSC, numerous studies have investigated its potential as a biomarker of HNSC (59,60). Additionally, the immunohistochemical results of FADD overexpression were substantially linked with poor OS in patients with HNSC, according to a previous meta-analysis (61). As one of the apoptosis-related factors, FADD is associated with the occurrence of LIHC, but further research is needed to confirm its prognostic value for patients with LIHC (62-64). Different prognostic analysis approaches have demonstrated that FADD predicts poor OS in LIHC patients, indicating that FADD may be employed as both a diagnostic and prognostic biomarker for patients with LIHC. Analysis of TME and immune cell infiltration revealed that FADD expression influences tumor immunity in a number of malignancies, particularly various tumors where neutrophil and DC infiltration are strongly positively associated. The neutrophil is a crucial cell that regulates inflammation and immune response, whereas DC is an antigen-presenting cell with a significant effect on tumor immunity (65-68). This suggests that FADD may influence tumor immunity by

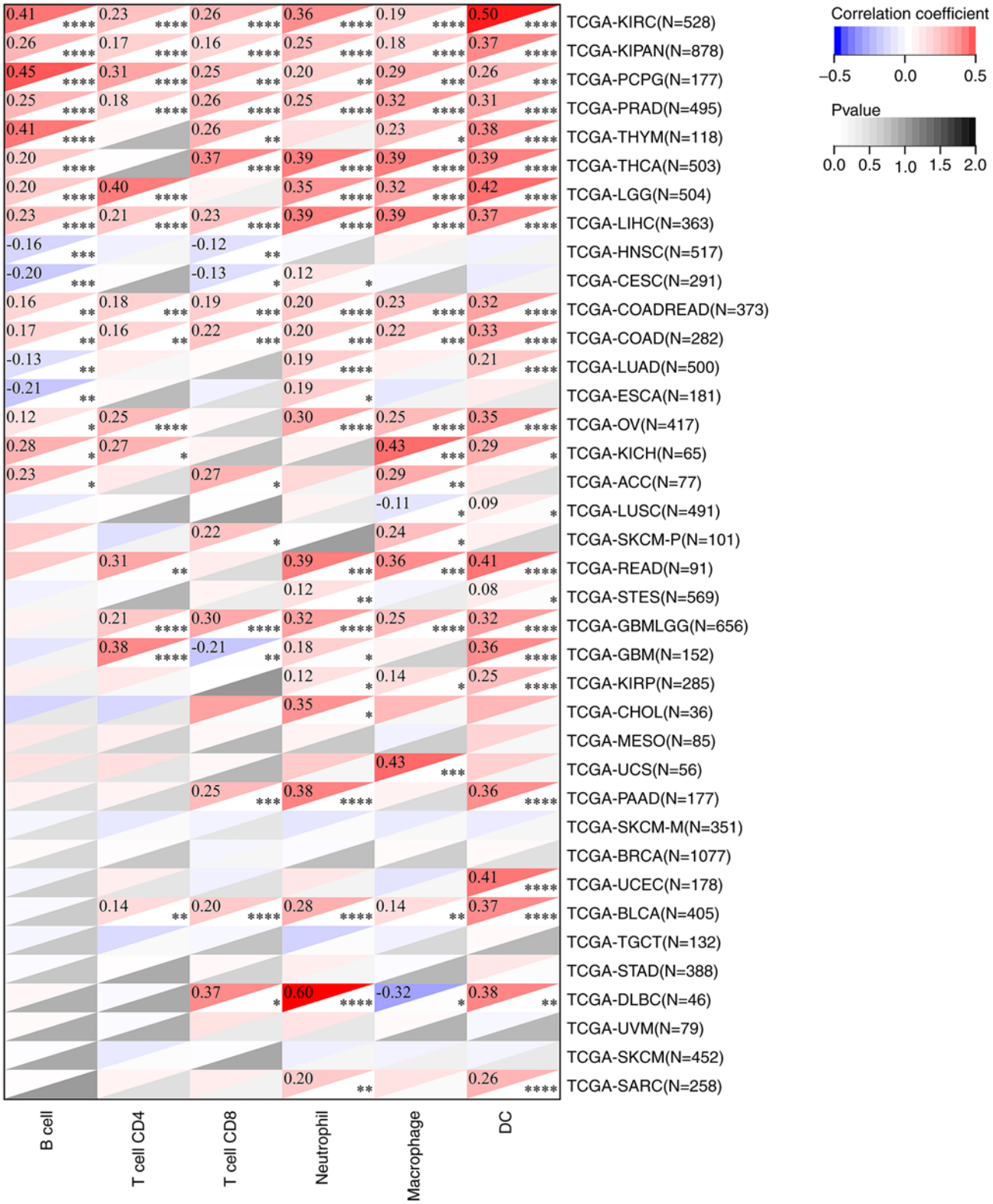

Figure 8. Immune cell infiltration. The correlation between FADD and immune cell infiltration in cancer. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. FADD, fas-associated death domain. DC, dendritic cells.

boosting neutrophil and DC infiltration into tumors. FADD expression was substantially related to MSI in 9 malignancies and TMB in 10 tumors, suggesting its potential as an immunotherapy marker. Bowman *et al* (69) revealed that the phosphorylation of FADD promoted the proliferation of lung cancer cells, suggesting that FADD indeed plays a role in tumorigenesis and development, and it is necessary to conduct in-depth research on it in the future.

Because of inconsistencies between the GEO and TCGA databases, expression data for 33 tumors was not gathered to verify the differential expression of FADD. The present study is limited to the expression and clinical relevance of

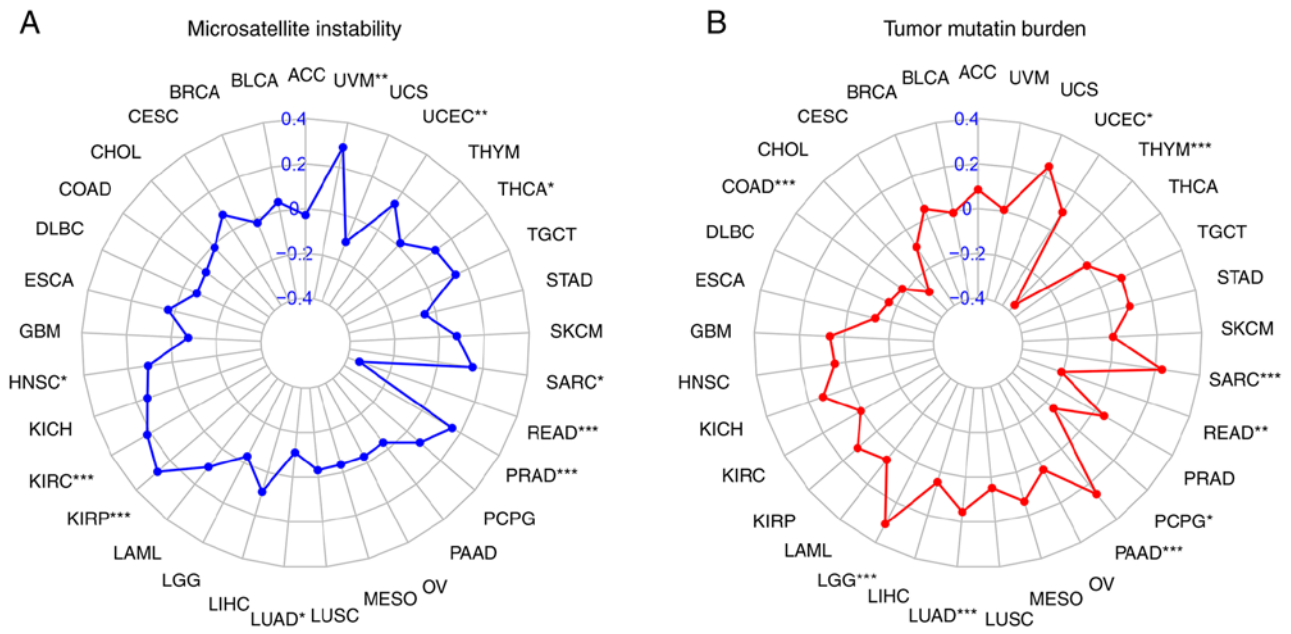

Figure 9. MSI and TMB. Correlation of FADD expression with (A) MSI and (B) TMB in cancer *P<0.05, **P<0.01 and ***P<0.001. FADD, fas-associated death domain; MSI, microsatellite instability; TMB, tumor mutation burden. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, diffuse large B cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

FADD in different cancers, and lacks clarification of the specific role of FADD in tumorigenesis and progression, which is necessary to explore FADD as a therapeutic target. Although FADD expression was identified as a potential diagnostic and prognostic biomarker for specific cancers, its clinical application and applicability in clinical practice need to be rigorously evaluated and verified by large-scale clinical trials.

The present study carefully evaluated the expression of FADD in various malignancies and its effect on the prognoses of patients with cancer. Analysis of various databases revealed that FADD was highly expressed in BRCA, CESC, CHOL, COAD, ESCA, KIRC, KIRP, LIHC, LUAD and PRAD. Moreover, the high expression of FADD was confirmed in BRCA, COAD, LIHC and STAD using RT-qPCR, supporting the potential utility of FADD as a prognostic biomarker for patients with LIHC. In conclusion, FADD is highly expressed in numerous malignancies and can be utilized as a diagnostic biomarker for BRCA, COAD, LIHC, and STAD. FADD expression is a predictive risk factor for HNSC, LIHC, and LUAD patients and has potential value as a prognostic marker for these tumors.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in TCGA (https://portal.gdc.cancer.gov/), UCSC Xena website (xena.ucsc.edu/), Kaplan Meier plotter portal (https://kmplot.com/), GEO database (https://www.ncbi.nlm.nih.gov/), The Molecular Signatures Database (https://www.gsea-msigdb.org/gsea/msigdb), The Human Protein Atlas (https://www.proteinatlas.org/), cBioPortal database (http://www.cbioportal.org/), UALCAN portal (ualcan.path.uab.edu/) and sangerbox website (http://vip.sangerbox.com/home.html).

Authors' contributions

XJ and CW conceived the study. ZX and QZ comprehensively collected relevant data. XJ and CW contributed in data

analysis and in drafting the manuscript. XJ and CW confirm the authenticity of all the raw data. CW revised the manuscript and HC reviewed the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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