Clinical Significance and Prognostic Value of the Expression of Fibronectin Type III Domain Containing 3B in Pancreatic Carcinoma

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Abstract

Background: Pancreatic carcinoma (PC) is one of the most aggressive cancers affecting human health. Identifying candidate biomarkers is essential for the early diagnosis and good prognosis of PC. Fibronectin type III domain containing 3B (*FNDC3B*) promotes many types of cancer, but its role in pancreatic cancer is not clear. The purpose of this study was to investigate the relationship between the expression of *FNDC3B* and incidence of PC.

Methods: We downloaded data related to the levels of *FNDC3B* mRNA in patients of PC from the Cancer Genome Atlas (TCGA) database, and conducted in-depth research on this data and its clinical significance through the R tools. We used GO and KEGG enrichment analyses to study the genes and signaling pathways that may be regulated by *FNDC3B*.

Results: The results showed that the level of *FNDC3B* mRNA in PC tissues was higher than that in the normal tissues, and this was associated with proliferation and lymph node metastases of PC. Increased levels of *FNDC3B* mRNA also predict poor prognosis for the patients with PC. In addition, enhanced levels of *FNDC3B* mRNA were involved in the regulation of cell junction, tissue and epithelial cell migration, and several signal transduction pathways, including notch, TGF-β, VEGF, and Wnt.

Conclusions: In short, high levels of *FNDC3B* mRNA may be associated with progression of PC and indicate a poor prognosis in patients with PC.

Background

Pancreatic carcinoma (PC) is a common gastrointestinal tumor with a high degree of malignancy and a poor prognosis. The 5-year survival rate is less than 6% [1], and its morbidity and mortality have been increasing. It is estimated that by the year 2020, the number of new cases of PC in the world will be approximately 420,000 and the number of deaths due to it will be approximately 410,000 [2]. By 2030, PC will become the second most common malignant tumor, with high mortality rate [3]. Genes and environment are two important causes of PC. Many unhealthy lifestyle choices, such as smoking, unbalanced diet, and obesity are also important risk factors for PC. Previous epidemiological studies have shown that chronic pancreatitis increases the risk of pancreatic cancer [4]. Due to the lack of identifiable clinical symptoms and specific markers at the early stages, the tumors escape early detection, grow rapidly and metastasize early. Because of this, more than 90% of patients are clinically diagnosed only when the pancreatic cancer is in the middle and advanced stages, missing the best time for treatment [1]. Therefore, early detection, diagnosis and treatment, are the key to reduce the mortality rate of the PC, and to improve the quality of life of the patients. Identification of specific markers to facilitate early diagnosis of PC is an active area of research at present [5–7].

Fibronectin type III domain containing 3B (*FNDC3B*), is a protein of the *FNDC3* family that includes *FNDC3A, FNDC3B* and *FNDC3C*, also known as factor for adipocyte differentiation 104 (*FAD104*), it contains a proline-rich region, and 9 fibronectin type III structures domain and a transmembrane region,

and is a regulator of adipocyte and osteoblast differentiation [8–12]. Fibronectin type III (FNIII) domain of *FNDC3B* serves as a tail sheath, integrates with different proteins, and plays an important role in cell adhesion and growth signaling [9, 13, 14]. Homozygous disruption of *FNDC3B* causes mice to die at birth from abnormalities in the lungs [14, 15], and has a significant effect on the adhesion, proliferation and migration of mouse embryonic fibroblasts [9, 16]. Cell adhesion is important in tumorigenesis because it provides tumor cells with the necessary contacts and cell-matrix interactions needed for cell signaling, proliferation, and migration [17]. *FNDC3B* is one of the most common genes up-regulated in cancer tissues, regardless of the cancer type and tumor origin [18]. Recent studies have found that *FNDC3B* is highly expressed in breast cancer, esophageal cancer, glioblastoma, and hepatocellular carcinoma, and is associated with cancer cell proliferation, invasion and metastasis [19–23]. Therefore, we hypothesized that *FNDC3B* promotes cell migration and tumorigenesis in PC. In this study, our bioinformatics analyses explored the role of *FNDC3B* in pancreatic cancer, as well as the processes and signaling pathways it may regulate.

Methods

Evaluation of transcription level of *FNDC3B* in PC patients

RNA-sequence data of PC patients along with individual clinical information were downloaded from the Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/). An in-depth analysis of *FNDC3B* expression at the transcriptional level in unpaired PC samples was performed by R version 3.6.2 (https://cran.r-project.org/src/base/R-3/) using the limma package version 3.8 (https://bioconductor.org/packages/release/bioc/html/limma.html) and Statistical Computing version 3.5.1 software.

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

According to the median value of *FNDC3B* mRNA expression, PC patients from the TCGA database were divided into high *FNDC3B* mRNA group and low *FNDC3B* mRNA group. Signaling pathways and molecules related to *FNDC3B* expression in PC patients were studied by GO and KEGG enrichment analysis (http://www.broadinstitute.org/gsea/index.jsp).

Evaluation of overall survival rate by KM curves

The overall survival rate in PC patients from both high and *FNDC3B* mRNA groups was assessed by KM (Kaplan-Meier) curves and the log-rank test. Patients of PC who had over five years of survival were considered as with a long overall survival (OS) had, whereas those who died or experienced recurrence in less than 2 years were classified as with a short OS. A value of $P < 0.05$ was considered statistically significant.

Statistical analysis

The mRNA levels of *FNDC3B* in patients of PC are expressed as the mean ± standard deviation. Student's t-test (unpaired) was used for comparison between two groups, and analysis of variance (ANOVA) was used to identify significant differences between multiple groups. All statistical analyses were performed with SPSS 21.0 software (IBM, Armonk, NY, USA). A P value < 0.05 was considered statistically significant.

AUC value determination for the ROC curve

The area under the curve (AUC) value was computed for the ROC curve to assess the discriminatory ability of *FNDC3B* expression in patients of PC, with distinct clinical parameters.

Results

Gene set enrichment analysis of *FNDC3B* expression in patients of PC

To investigate the effect of upregulated *FNDC3B* expression in PC, we performed gene set enrichment analysis of *FNDC3B* expression in patients, with corresponding clinical information from TCGA datasets. Our results showed that the expression of *FNDC3B* was significantly upregulated in patients with advanced PC and distant metastasis. We also found that the mRNA level of *FNDC3B* was higher in patients of PC with good OS than in those with poor prognosis, whereas no significant difference in the expression of *FNDC3B* was observed in patients according to age, sex and lymph node metastasis. These results suggest that upregulation of *FNDC3B* is associated with distant metastasis of PC and predicts a poor prognosis for patients (Fig. 1).

Clinical and molecular characteristics of PC with high *FNDC3B* expression

We further investigated the mRNA expression of *FNDC3B* in patients with PC from the TCGA database, and found that the mRNA level of *FNDC3B* was significantly higher in the cancer cells than in corresponding noncancerous normal tissues (Fig. 2A). In grade (G stage) G2 and G3, *FNDC3B* mRNA levels were higher compared to G1 (Fig. 2D). In the TNM stage II, the level of *FNDC3B* mRNA was higher than in stage I (Fig. 2E). In patients older than 65 years, *FNDC3B* mRNA levels were higher than in younger patients (Fig. 2C). In the advanced tumor stage (T stage) T3, the *FNDC3B* mRNA level was higher than in T2 (Fig. 2F). Gender-wise, the *FNDC3B* mRNA level in male patients was comparable to that in female patients (Fig. 2B). These results showed a significant association of higher expression of *FNDC3B* with advanced age, G stage, tumor stage, and TNM stage, but not with the sex of the patient.

Upregulation of *FNDC3B* mRNA levels indicates poor prognosis for patients of PC

PC patients from the TCGA data set were divided into two groups, based on the median expression of *FNDC3B* mRNA, to investigate the prognostic role of *FNDC3B* in PC. We found that level of *FNDC3B* mRNA was higher in patients with lymph node metastasis, and poor OS, and patients who had died (Fig. 3A, 3C, 3D), whereas it was lower in patients with distant metastasis (Fig. 3B). Overall, these data

suggested that upregulation of *FNDC3B* mRNA levels indicated poor prognosis for patients with PC. This may serve as a potential indicator of prognosis in patients with PC.

The ROC curve shows that high levels of *FNDC3B* mRNA is associated with PC

Using ROC curves, we analyzed whether expression levels of *FNDC3B* mRNA can effectively distinguish PC patients with different pathological parameters, to confirm that *FNDC3B* mRNA expression is related to cancer cell proliferation and poor prognosis. *FNDC3B* mRNA expression could be used to distinguish PC tissues from normal tissues, with an AUC of 0.9510 (95% CI: 0.9253–0.9767; P < 0.0001) (Fig. 4A). We also found a significant difference in *FNDC3B* expression in PC subgroups, including subgroups of G stage (G1/G2, AUC = 0.6375, P = 0.0278; G1/G3, AUC = 0.6856, P = 0.0072), TNM stage (TNM stage I/TNM stage II, AUC = 0.6599, P = 0.0205), T stage (T2/ T3, AUC = 0.6223, P = 0.0559), status (Living/dead, AUC = 0.6236, P = 0.0052) lymph node metastasis (N0/N1 + NX, AUC = 0.6259, P = 0.0112), and distant metastasis (M0/M1 + MX, AUC = 0.6392, P = 0.0017; Fig. 4B-4I). These results confirm that higher levels of *FNDC3B* mRNA is associated with cancer proliferation, metastasis and poor prognosis in PC.

High *FNDC3B* mRNA level correlates with short OS in PC patients

KM survival analysis was used to determine the correlation of level of *FNDC3B* mRNA with OS of the patients with PC. Patients with higher levels of *FNDC3B* mRNA had shorter OS than those with lower levels (Fig. 5A, P = 0.0025). Moreover, we analyzed the association between OS and *FNDC3B* mRNA level in various clinical subgroups of PC. As shown in Fig. 5B-5G, compared with patients with low *FNDC3B* expression, males (Fig. 5B, P = 0.0130), age > 65 years (Fig. 5C, P = 0.0378), age ≤ 65 years (Fig. 5D, P = 0.0060), G1 + G2 stage (Fig. 5E, P = 0.0153), stages I + II (Fig. 5F, P = 0.0015) and T3 + T4 stage (Fig. 5G, P = 0.0371) patients with high *FNDC3B* mRNA level exhibited a shorter OS time. All these results indicate that level of *FNDC3B* mRNA correlates with short OS.

GO enrichment analysis shows that *FNDC3B* mRNA level is associated with genes involved in regulation of cell junction, tissue migration and epithelial cell migration

To identify the molecules regulated by *FNDC3B*, we performed GO enrichment analysis. We found that higher level of *FNDC3B* mRNA was associated with regulation of cell junction, tissue migration and epithelial cell migration (Fig. 6A). Furthermore, the it was also strongly associated with *HIF1A*, *ADAM17*, *CORO1C*, *ITGB1*, *LIMS1*, *MAP4K4*, *PDLIM5* and *PIK3CA* (Fig. 6B). These results indicate that high level of *FNDC3B* mRNA is associated with junction and migration of PC cells.

KEGG enrichment analysis shows that *FNDC3B* is associated with pathways related to signal transduction

To investigate the association between *FNDC3B* and signaling pathways, we performed KEGG enrichment analysis. The results showed that *FNDC3B* expression was positively associated with PC. We also found that *FNDC3B* expression was significantly enriched in four main well-studied signaling pathways in PC, including the notch, TGF-β, VEGF and Wnt signaling pathways (Fig. 7A). Moreover, the expression of

genes in these four signaling pathways, including *ACVR1*, *BMPR2*, *CTNNB1*, *ITGAV*, *MAPK1*, *NOTCH2*, *PIK3CA* and *ROCK1*, was associated with the level of *FNDC3B* mRNA (Fig. 7B). All these data suggest that level of *FNDC3B* mRNA is associated with four main signaling pathways in PC.

Discussion

PC is an aggressive disease with almost no symptoms until the cancer is well established [24, 25]. Carbohydrate antigen 19 – 9 is the only diagnostic marker approved by the FDA, but its diagnostic potential is limited due to its limited sensitivity and specificity [26, 27]. Therefore, screening new tumor biomarkers is important for the early diagnosis and targeted treatment of PC. In this study, by analyzing the TCGA database, it was found that the level of *FNDC3B* mRNA was increased in tissues involved in PC, which indicates that *FNDC3B* may be involved in PC progression.

Many studies have confirmed that *FNDC3B* promotes tumor progression. Lin et al. found that *FNDC3B* enhances the migration and metastasis of hepatocellular carcinoma cells by binding to annexin A2 (ANXA2) through the FNIII1-4 domain [14]. Cai et al. confirmed that *FNDC3B* increases the proliferation of liver cancer cells by inducing epithelial to mesenchymal transition (EMT) to activate the PI3K / AKT, Rb1 and TGF-β pathways [28]. Studies have found that miR-129-5p binds to the 3'UTR of *FNDC3B* and down-regulates its expression, leading to inhibition of the proliferation, invasion and migration of glioma cells [21]. Silencing *FNDC3B* expression in tongue squamous cell carcinoma cells can inhibit the occurrence of EMT, suggesting that *FNDC3B* plays an important role in promoting the proliferation, invasion and migration of tongue squamous cell carcinoma cells [29]. Present study found that *FNDC3B* mRNA levels had increased in PC tissues with lymph node metastasis. In addition, high levels of *FNDC3B* mRNA in PC promote cell junction, tissue migration, epithelial cell migration, and positively regulate several signaling pathways, including notch, TGF-β, VEGF and Wnt. This indicates that *FNDC3B* may promote migration of PC cells. However, Zhang et al. found that miR-143, upregulated by NF-κB transcription, inhibited the expression of *FNDC3B*, which promoted the metastasis of HBV-related liver cancer [30]. Fan et al. also found that miR-143 was significantly up-regulated during the differentiation of prostate cancer stem cells, and that the increased miR-143 could inhibit the expression of *FNDC3B* and promote the metastasis of prostate cancer [31]. Studies have shown that tumor cells can promote tumor invasion and metastasis by inducing EMT. During this process, the expression of β-catenin is up-regulated, thereby inhibiting the transcription of E-cadherin, weakening the cell-to-cell connection and spindle-shaped cell transformation, which further enhances its ability to migrate, accompanied by down-regulation of the expression of epithelial markers and up-regulation of interstitial markers [32, 33].

Overall, our results highlight the involvement of *FNDC3B* mRNA in tumorigenesis and metastasis. These results need to be further verified by testing PC tissues and in vivo and in vitro studies.

Conclusions

In conclusion, this study shows that *FNDC3B* plays a vital role in PC proliferation and metastasis, opening up new avenues for targeted treatment of PC. In addition, it can regulate biological processes, such as cell junction, tissue migration, epithelial cell migration, notch-, TGF-β-, VEGF-, and Wnt-signaling pathways, by regulating the expression of *HIF1A*, *ADAM17*, *CORO1C*, *ITGB1*, *LIMS1*, *MAP4K4*, *PDLIM5*, *PIK3CA*, *ACVR1*, *BMPR2*, *CTNNB1*, *ITGAV*, *MAPK1*, *NOTCH2*, *PIK3CA* and *ROCK1*. Since the expression of *FNDC3B* is related to the survival rate of patients, inhibiting *FNDC3B* in tumor tissue may provide an effective treatment strategy.

Abbreviations

GO
Gene ontology; KEGG:Kyoto Encyclopedia of Genes and Genomes; TCGA:the Cancer Genome Atlas; EMT:epithelial to mesenchymal transition; ANXA2:annexin A2; AUC:area under the curve; PC:pancreatic carcinoma; *FNDC3B*:Fibronectin type III domain containing 3B.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LQM, XHL, KXD and XQK designed the study, downloaded data from TCGA, performed KEGG enrichment analysis and KM survival analysis, and drafted the manuscript. JLL and HHL were responsible for GO

enrichment analysis and OS analysis. XYL and JG were responsible for ROC curve analysis. All authors read and approved the final manuscript for publication.

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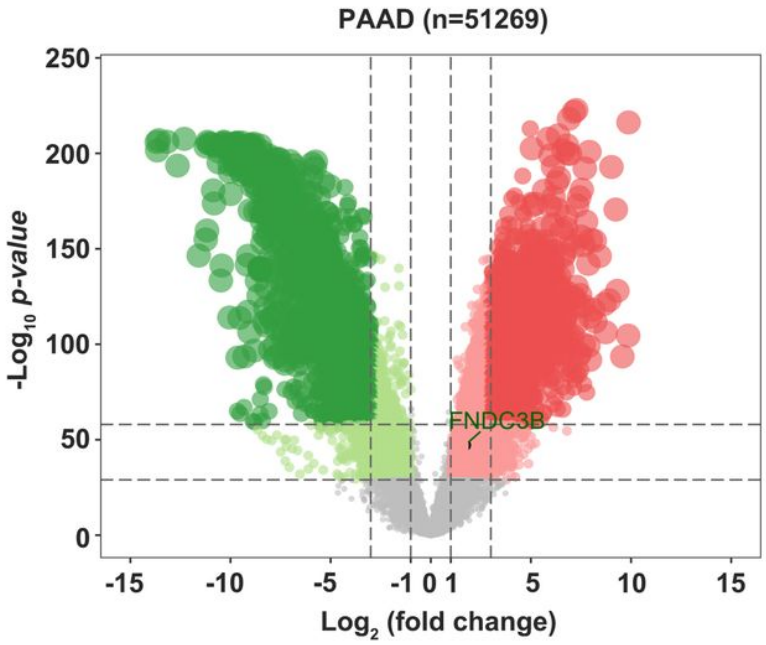
Figures

A



B

Clinical characteristics	Total (N)	Odds ratio FNDC3B expression	p-Value
Age (>65 vs. =<65)	170	0.42 (0.23-0.78)	0.006064725
Status (dead vs. alive)	170	2.04 (1.11-3.79)	0.02205951
Lymph nodes (positive vs. negative)	170	0.51 (0.28-0.94)	0.03198709
Distant metastasis (positive vs. negative)	170	2.18 (1.10-4.43)	0.02736801
Gender (male vs. female)	170	0.95 (0.52-1.75)	0.877552
Living (poor vs. good)	170	1.77 (0.96-3.26)	0.06655667

Figure 1

Odds ratio of level of FNDC3B mRNA in patients of PC with distinct clinical characteristics. PAAD: pancreatic adenocarcinoma.

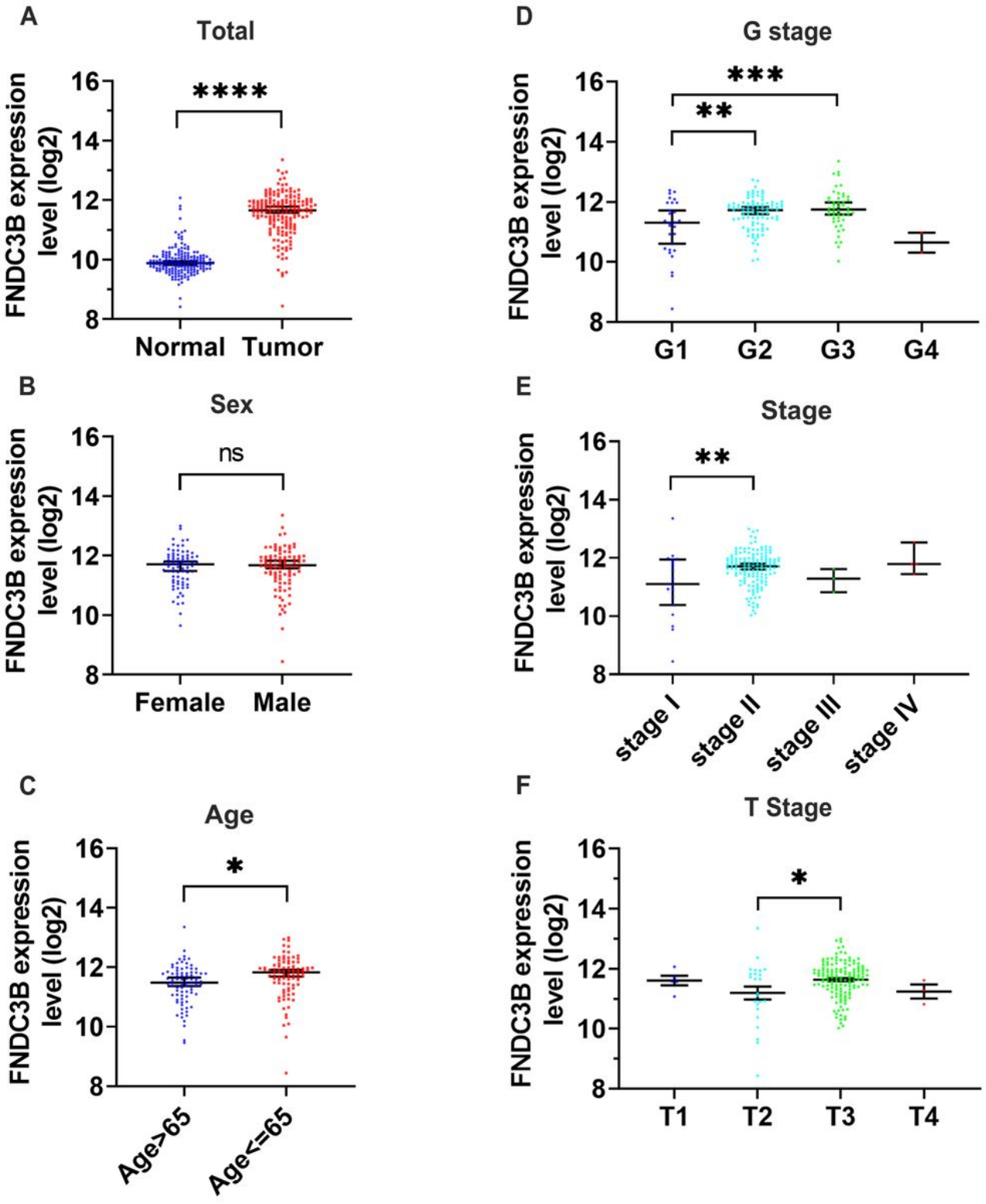


Figure 2

mRNA level of FNDC3B in patients of PC with distinct clinical characteristics, analyzed in-depth with RNA-sequence data from the TCGA database. (A) Cancer versus para-cancer, (B) sex, (C) age, (D) G stage, (E) TNM stage and (F) T stage. TCGA-CRC, The Cancer Genome Atlas colorectal carcinoma; *p < .05, **p < .01, ***p < .001, and ****p < .0001; ns denotes not significant; data were analyzed by Student's t-test or ANOVA. G stage: grade stage; G1: grade 1; G2: grade 2; G3: grade 3; G4: grade 4.

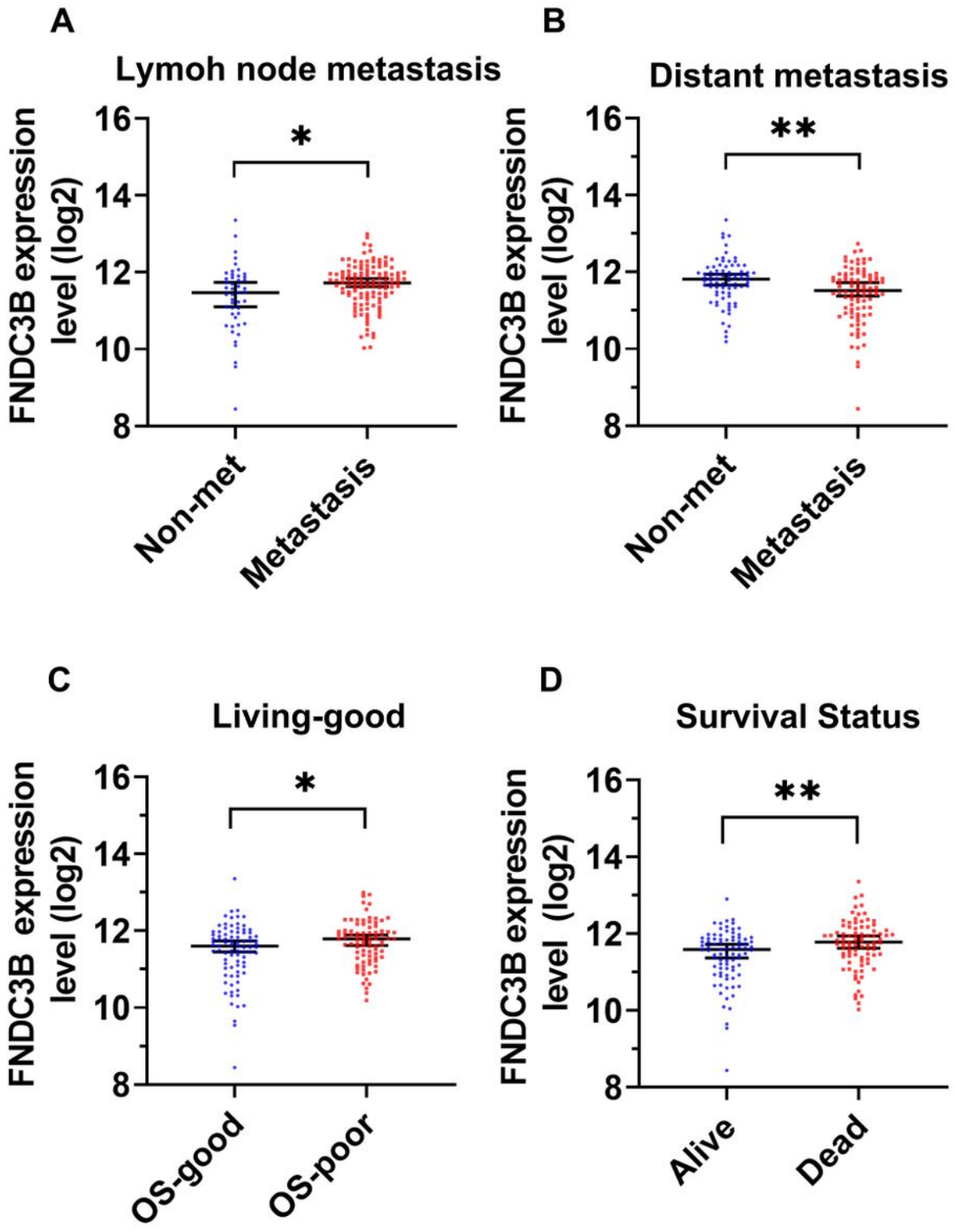


Figure 3

High levels of FNDC3B mRNA were correlated with poor prognosis in patients of PC based on the data from TCGA datasets. (A) Comparison of the levels of FNDC3B mRNA in non-metastatic PCs and PC with lymph node metastasis. (B) Comparison of FNDC3B mRNA levels between non-metastatic PC and PC with distant metastasis. (C) Levels of FNDC3B mRNA in PC with good and poor OS. (D) Comparison of levels of FNDC3B mRNA between survival and death of patients with PC. *p < 0.05, **p < 0.01; ns denotes

not significant; data were analyzed by Student's t-test. OS-good referred to patients who survived or are disease-free for ≥5 years. OS-poor referred to patients who succumbed to the disease or relapsed within two years.

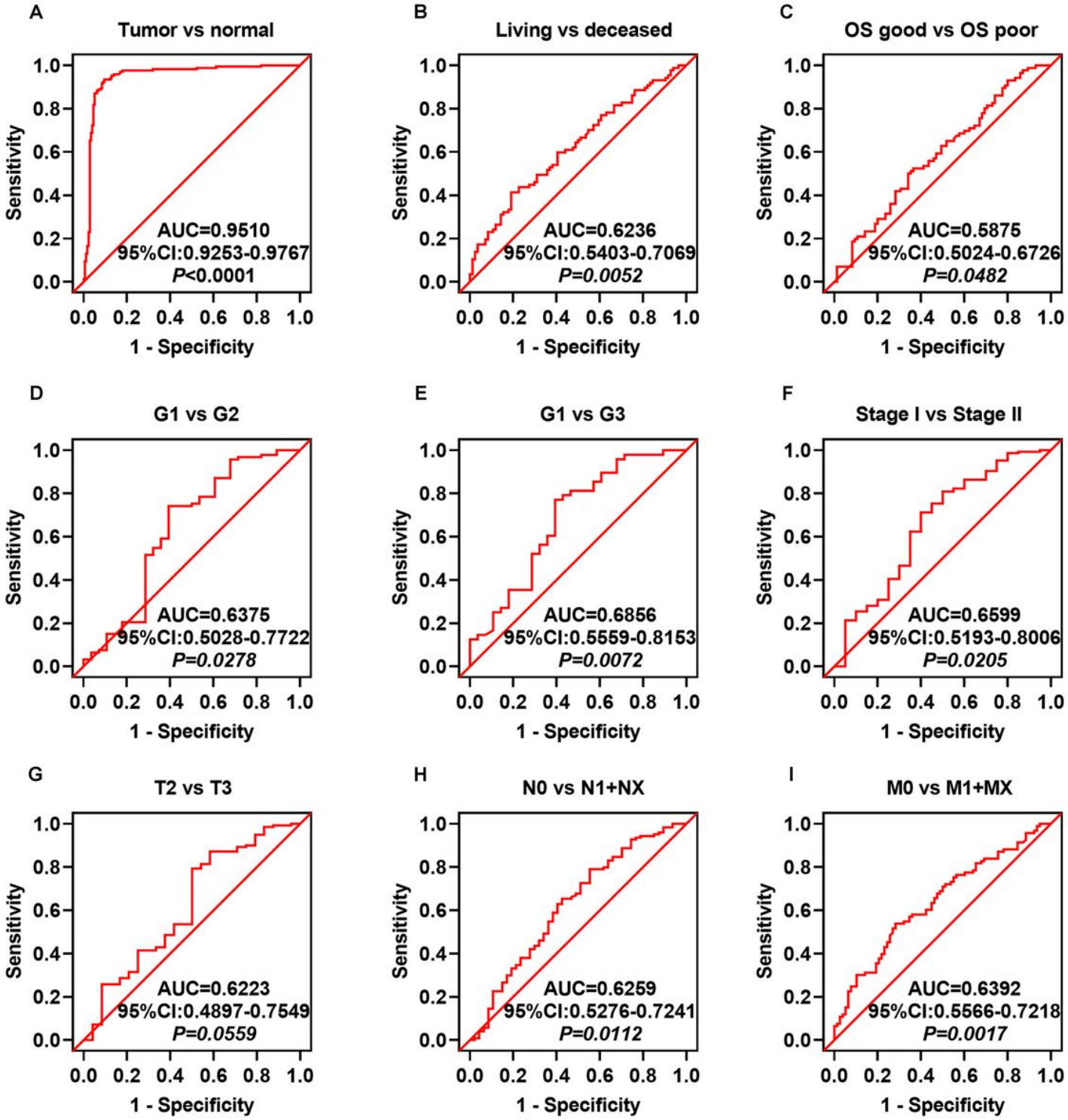


Figure 4

ROC curve analysis showing the relationship between FNDC3B mRNA levels and the indicated clinical parameters in patients of PC. (A) ROC curve analysis showing the significant difference in FNDC3B mRNA levels between PC tissues and normal tissues (AUC=0.9510; P<0.0001). (B-I) ROC curve analysis of

the mRNA expression level of FNDC3B according to subgroups of patients of PC grouped by living status (B), OS (C), G stage (D-E), TNM stage (F), T stage(G), lymph node metastasis (H), and distant metastasis (I). AUC, area under the curve; OS, overall survival; ROC, receiver operating characteristic; G1: grade 1; G2: grade 2; G3: grade 3; G4: grade 4.

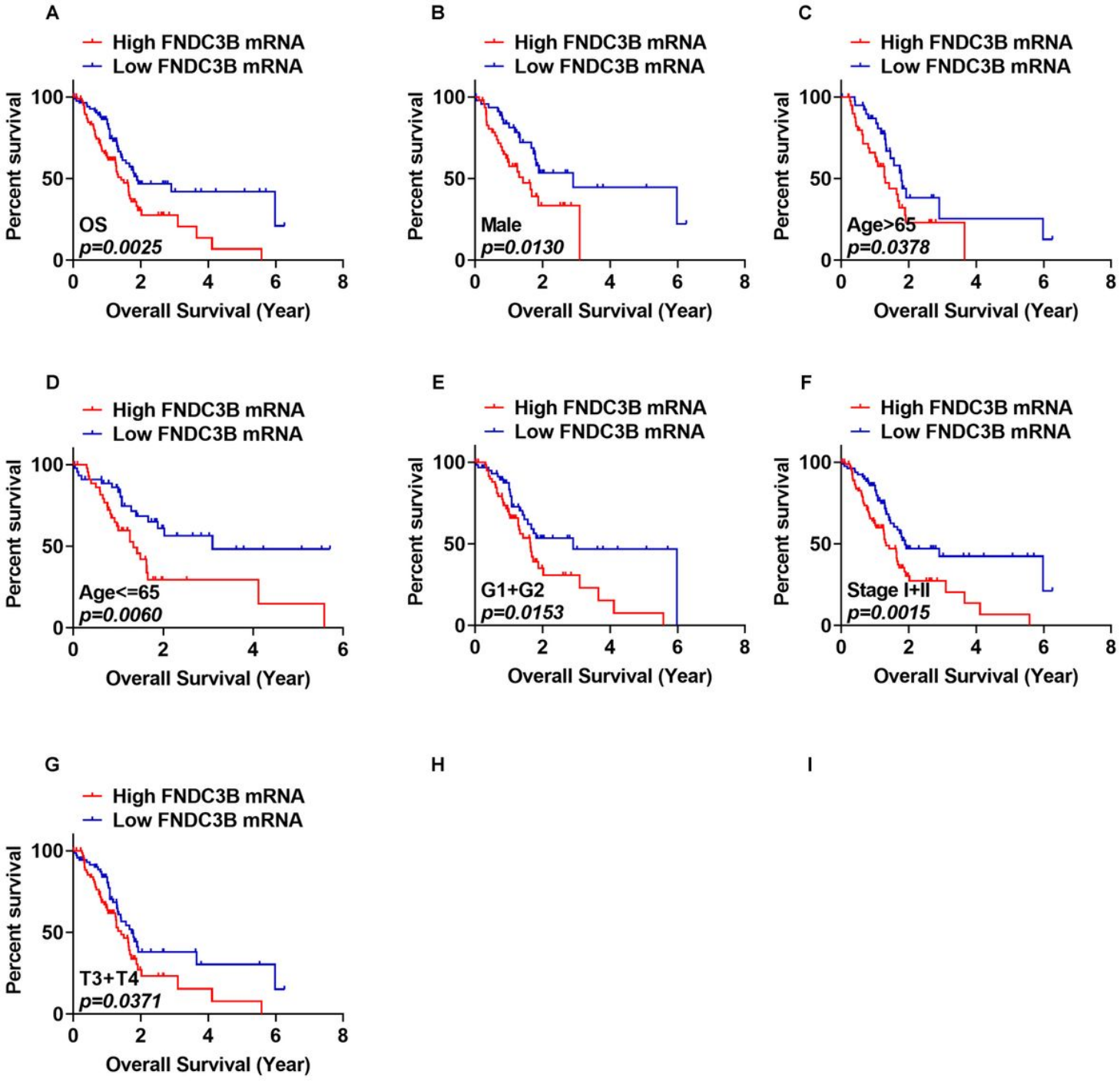


Figure 5

High levels of FNDC3B mRNA in patients of PC indicate poor prognosis. (A) Patients of PC from the TCGA database were divided into low and high FNDC3B mRNA groups, based on the median value of the FNDC3B mRNA expression. The correlation analysis by the Kaplan–Meier method between FNDC3B mRNA expression and overall survival in patients. (B-G) Overall survival analysis based on the levels of

FNDC3B mRNA expression in subgroups of patients of PC: (B) male, (C) age>65 years, (D) age ≤65 years, (E) G1+G2, (F) stages I+II, (G) stages T3+T4, (H) no metastasis, (I) N0 stage. OS, overall survival; G1+G2: grade 1 and grade 2.

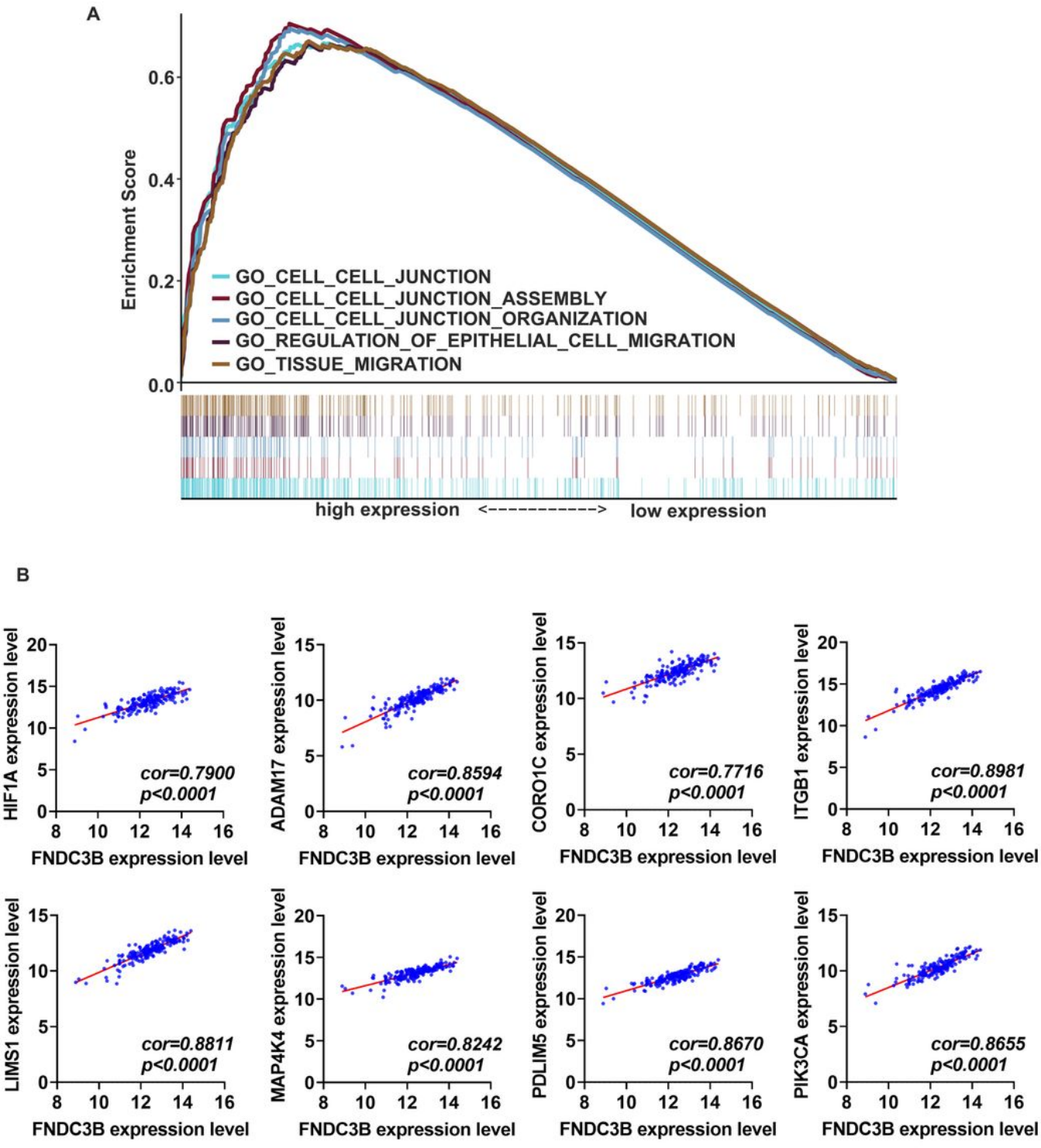


Figure 6

Gene set enrichment analysis of the correlation between level of FNDC3B mRNA and certain genes. (A) Gene set enrichment analysis of the correlation in the mRNA levels of FNDC3B and of genes involved in

cell junction, tissue migration and epithelial cell migration regulation. (B) Correlation analysis of mRNA levels of FNDC3B and of the genes involved in cell junction, tissue migration and epithelial cell migration regulation. GO, Gene Ontology

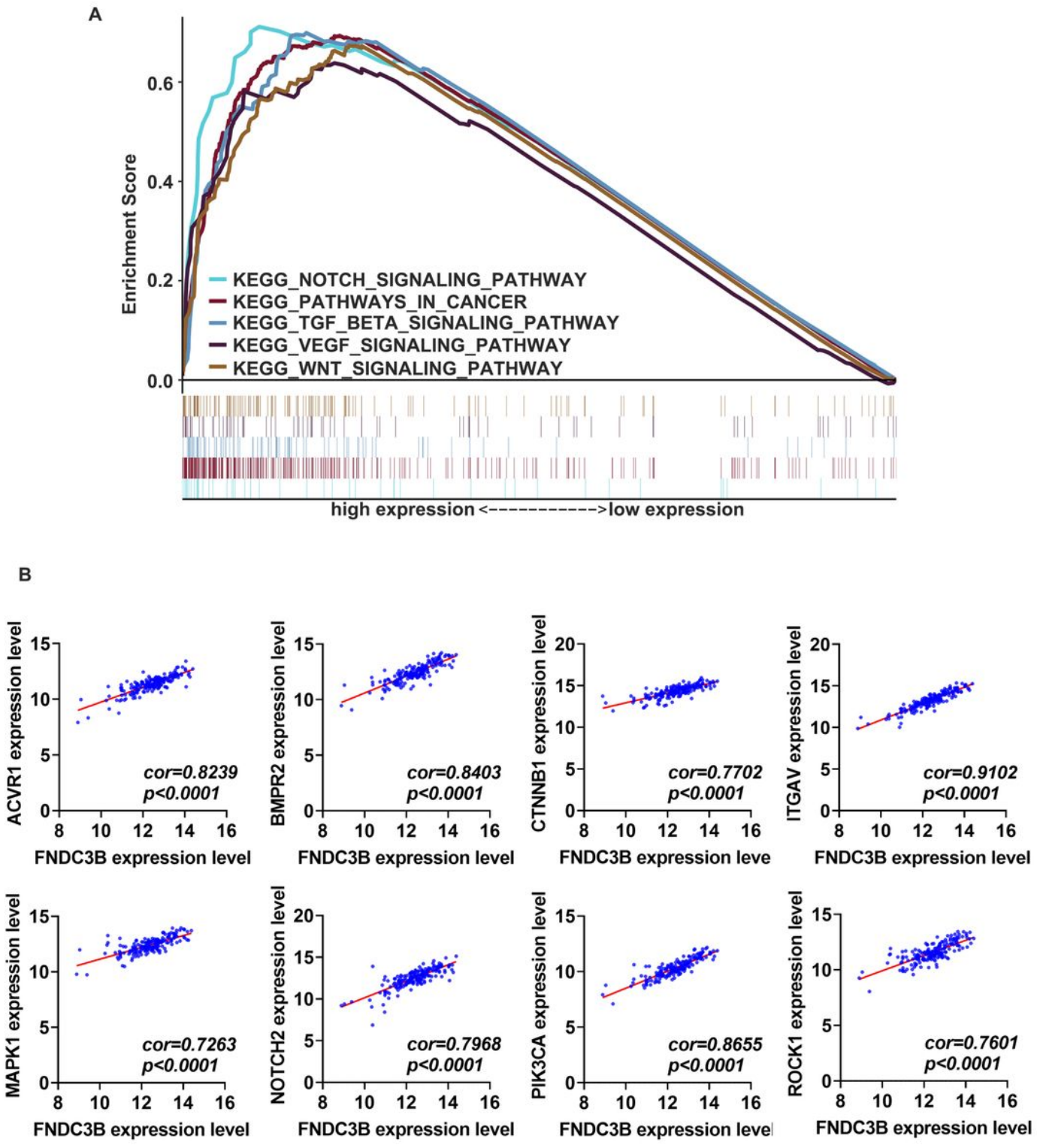


Figure 7

KEGG enrichment analysis of the correlation between level of FNDC3B mRNA and signaling pathways. (A) Gene set enrichment analysis of the correlation between the mRNA level of FNDC3B and the

expression of genes related to four signaling pathways important in PC. (B) Correlation analysis of the mRNA levels of FNDC3B and the genes related to four signaling pathways involved in PC. KEGG: Kyoto Encyclopedia of Genes and Genomes.