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Original Article

ZNF821 as a Novel Prognostic Biomarker and its Correlation with Infiltrating Immune Cells in Pancreatic Adenocarcinoma

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Abstract : The Zinc Finger Protein 821 (ZNF821) is a human protein that contains the 23 amino acid peptide repeat domain. However, ZNF821 has not been examined as a predictive biomarker. This study evaluated ZNF821, a member of the ZNF domain family, as a prognostic biomarker of various human cancers, including pancreatic adenocarcinoma (PAAD). We also identified a correlation between ZNF821 expression and immune cell infiltration in patients with PAAD. Bioinformatics analysis was performed using online databases such as the University of ALabama at Birmingham CANcer data analysis Portal database analysis, Tumor Immune Estimation Resource, Kaplan-Meier database plotter, LinkedOmics, and Gene Expression Profiling Interactive Analysis version 2. ZNF821 expression in PAAD samples was significantly lower than normal tissue. Additionally, ZNF821 expression was lower in nodal metastasis, primary tumor grade, tumor grade, and cancer stages. Lower ZNF821 expression was also associated with a poorer prognosis in PAAD. ZNF821 expression positively correlated with T cells (CD8+ and CD4+), neutrophils, macrophages, and dendritic cells. In the analysis of the ZNF821 co-expression networks, positively related genes of ZNF821 were associated with a high hazard ratio in different types of cancer, including PAAD. Regarding the biological function of ZNF821, ZNF821 mainly participates in the neuropeptide signaling pathway, monoamine transport, and protein localization in the endoplasmic reticulum. These results suggest that ZNF821 may serve as a novel prognostic biomarker for PAAD diagnosis and provide novel insights into the cancer-immune system in PAAD.

Keywords : Pancreatic adenocarcinoma, ZNF821, Prognosis, Tumor-infiltrating immune cells

INTRODUCTION

Pancreatic adenocarcinoma (PAAD) is a gastrointestinal tumor with a high mortality and incidence rate worldwide [1-3]. PAAD is associated with risk factors such as chronic pancreatitis, *Helicobacter pylori*, diabetes, obesity, alcohol consumption, and hepatitis C infection [4-6]. PAAD has a poor prognosis, rapid metastasis, and high prevalence [7]. Surgical resection is the therapy for PAAD, which increases the 5-year survival rate to 20-30% [8]. However, because of the extensive metastases, only approximately 20% of patients get surgical resection, and standard radiation and chemotherapy have a poor prognosis in patients with PAAD [9-11]. Owing to the poor prognosis of PAAD, there is a

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considerable need for therapy and research on prognostic biomarkers.

The immune system is essential in controlling tumor progression [12]. Tumor microenvironment (TME) refers to the microenvironment surrounding tumor cells, such as stromal cells, peripheral blood vessels, non-cellular components (growth factors, cytokines, etc.), immune cells, and tumor-infiltrating immune cells (TIIC) [13,14] are recruited to the cancer site and play an essential role in suppressing various tumor formation and growth through induction of angiogenesis, evasion of immune surveillance, and inhibition of apoptosis [15-19]. TME correlates with clinical outcomes of immunotherapy [20,21]. PAAD is closely associated with immune cells, blood vessels, and fibroblasts, and the TME comprises various innate and adaptive immune cells [22]. Various studies have shown that TIIC plays an important role as a prognostic marker [23].

The Zinc Finger protein (ZNF) is an essential transcription factor in the human body and plays an essential role in physiological mechanisms, such as cancer stem cell regulation and muscle differentiation [24]. Abnormal ZNF expression is associated with various diseases, including tumor formation and progression, neurodegeneration, and diabetes [25,26]. ZNF has been found to play an essential role in inflammation, invasion and metastasis, cell proliferation, drug resistance, apoptosis, and DNA methylation in various cancers, including breast cancer, lung cancer, colon cancer, hepatocellular carcinoma, breast cancer, and gastric cancer [27-29]. ZNF821, a member of the ZNF gene family, encodes a protein with two C2H2 zinc finger motifs and one 23-amino acid peptide repeat domain [30-32]. ZNF821 has been shown to have a close association with breast cancer, as parts of the DNA repair subnetwork have been found to be dysregulated [33]. In addition, the overexpression of ZNF185 has been shown to inhibit apoptosis and promote tumor growth by downregulating transcription factor proteins in pancreatic ductal adenocarcinoma [34]. However, ZNF821, which shows a relationship in various cancers, has not identified a specific mechanism related to PAAD.

In the present study, we aimed to confirm the possibility as a biomarker of ZNF821 and its correlation with TIICs in PAAD. We used the University of ALabama at Birmingham CANcer data analysis Portal (UALCAN) database analysis, Tumor Immune Estimation Resource (TIMER), Kaplan-Meier (KM) database plotter, and Gene Expression Profiling Interactive Analysis version 2 (GEPIA2) programs to analyze the correlation between ZNF821 expression in patients with PAAD and investigate the correlation between ZNF821 and TIICs. Our results potentially uncovered starting strategies for diagnosing and treating PAAD using ZNF821.

MATERIALS AND METHODS

1. TIMER analysis

TIMER (https://cistrome.shinyapps.io/timer/) provides a comprehensive web interface for exploring tumor genomics and immunology data and for visualizing systematic analyses of immune infiltrates according to different cancer types. TIMER applies a deconvolution method [14] to infer enriched TIICs from gene expression profiles, which estimates rich immune infiltrates and contains 10,897 samples spanning 32 tumor types from The Cancer Genome Atlas (TCGA). The expression of ZNF 821 has been studied in various tumor types. We also determined the correlation between ZNF 821 and TIICs (dendritic cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and B cells) in patients with PAAD.

2. UALCAN database analysis

UALCAN (http://ualcan.path.uab.edu) was used to analyze gene expression in normal and tumor samples using clinical data from 31 cancer types and TCGA RNA sequencing [35]. Various tumors were analyzed according to their clinicopathological characteristics, cancer stage, and tumor grade.

3. KM plotter database analysis

KM is based on an online database [36]. The effects of 54,675 genes on survival were assessed using 10,461 cancer samples, and associations between these genes and survival were identified in different types of cancer, including PAAD. KM included survival rates, such as disease-free survival (DFI), progression-free interval (PFI), disease-specific survival (DSS), overall survival (OS), and clinical data (including nodal metastasis, stage, and grade) in patients with PAAD. Correlations between ZNF821 expression and survival were analyzed and presented as 95% confidence intervals, calculated log-rank *p*-values, and hazard ratios (HR).

4. OSlihc analysis

The prognostic value of ZNF821 was confirmed using OSlihc [37]. To analyze the predictive value of genes in OSlihc, the survival terms DSS, PFI, DFI, and OS were generated; OS was measured in all cohorts and combined cohorts, while DSS, DFI, and PFI were analyzed using TCGA.

5. LinkedOmics database analysis

The LinkedOmics database (http://www.linkedomics.org/admin.php) analyzed 32 TCGA cancer-related multidimensional databases [38]. ZNF821 co-expression was statistically analyzed in scatterplots or heat maps using Pearson's correlation coefficient. Function module of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, miRNA-target enrichment, kinase-target enrichment, Gene Ontology biological process (GO_BP), transcription factor-target enrichment, and LinkedOmics analyses using gene set enrichment analysis. The rank criterion was a false discovery rate <0.05, and 500 simulations were analyzed.

6. Gene expression profiling interactive analysis version 2

Raw RNA-seq data downloaded from the ICGC and TCGA databases were recalculated using UCSC Xena to avoid inefficient differential analysis and data imbalance. Patient survival data and differential gene expression were integrated using interactive gene expression profiling analysis [39]. GEPIA2 performs survival analysis based on isoform or gene expression levels. GEPIA2 was used to generate survival curves, including disease-free survival (DFS) and OS, based on gene expression in the 33 cancer types. Recurrence-free survival (RFS) and OS were compared using the log-rank test, KM method, and a 50% (median) cutoff. A Cox proportional hazard regression model was used to obtain HR. The parameter settings were consistent in each analysis without adjustment for any *p*-value. *p*-value of less than 0.05 was considered statistically significant [40].

7. Statistical analysis

Gene expression data obtained from the TIMER database were statistically analyzed using the online tool. Survival curves were analyzed using the ZNF821 online tool, TIMER, and KM plots. Survival outcomes were generated as *p* and HR or Cox *p* in a log-rank test. The log-rank test indicated the significance of the difference in survival time, and a *p*-value less than 0.05 was considered statistically significant. The correlation between immune signature scores and gene expression was analyzed using the TIMER database and Spearman's correlation. All data in this study were extracted from a validated open database, and all analyses were performed using web tools. All results are presented as *p*-values of the log-rank test. Statistical significance was set at a *p*-value less than 0.05.

RESULTS

1. mRNA expression of ZNF821 in PAAD and various types of cancer

To determine the differences in gene expression of ZNF821 between tumor and normal tissues, ZNF821 gene expression was assessed in all tissue specimens using TIMER. These results indicate that ZNF821 expression is lower in PAAD tissues than in normal tissues. Moreover, expression in colon adenocarcinoma, glioblastoma multiforme, Kidney Chromophobe, and rectal adenocarcinoma tumors was lower than that in normal tissues (Fig. 1A). However, ZNF821 expression was higher in lung squamous cell carcinoma, lung adenocarcinoma, liver hepatocellular carcinoma, esophageal carcinoma, head and neck squamous cell carcinoma, bladder urothelial carcinoma, breast invasive carcinoma, cholangiocarcinoma, thyroid carcinoma, prostate adenocarcinoma, pheochromocytoma, and paraganglioma than in normal tissues (Fig. 1A). Based on TIMER analysis, a correlation between gene expression of ZNF821 and lymph node metastasis status, tumor grade, and tumor stage in PAAD was identified. The results showed a significant correlation between ZNF821 expression levels, tumor stage, and grade in PAAD. In the nodular metastatic state, ZNF821 was expressed at a lower level in NO than in the normal tissue (Fig. 1B-D). These results show that ZNF821 expression is downregulated in PAAD.

2. The prognostic significance of ZNF821 gene expression in PAAD

We investigated whether ZNF821 expression correlated with PAAD prognosis. Therefore, both ZNF821 gene

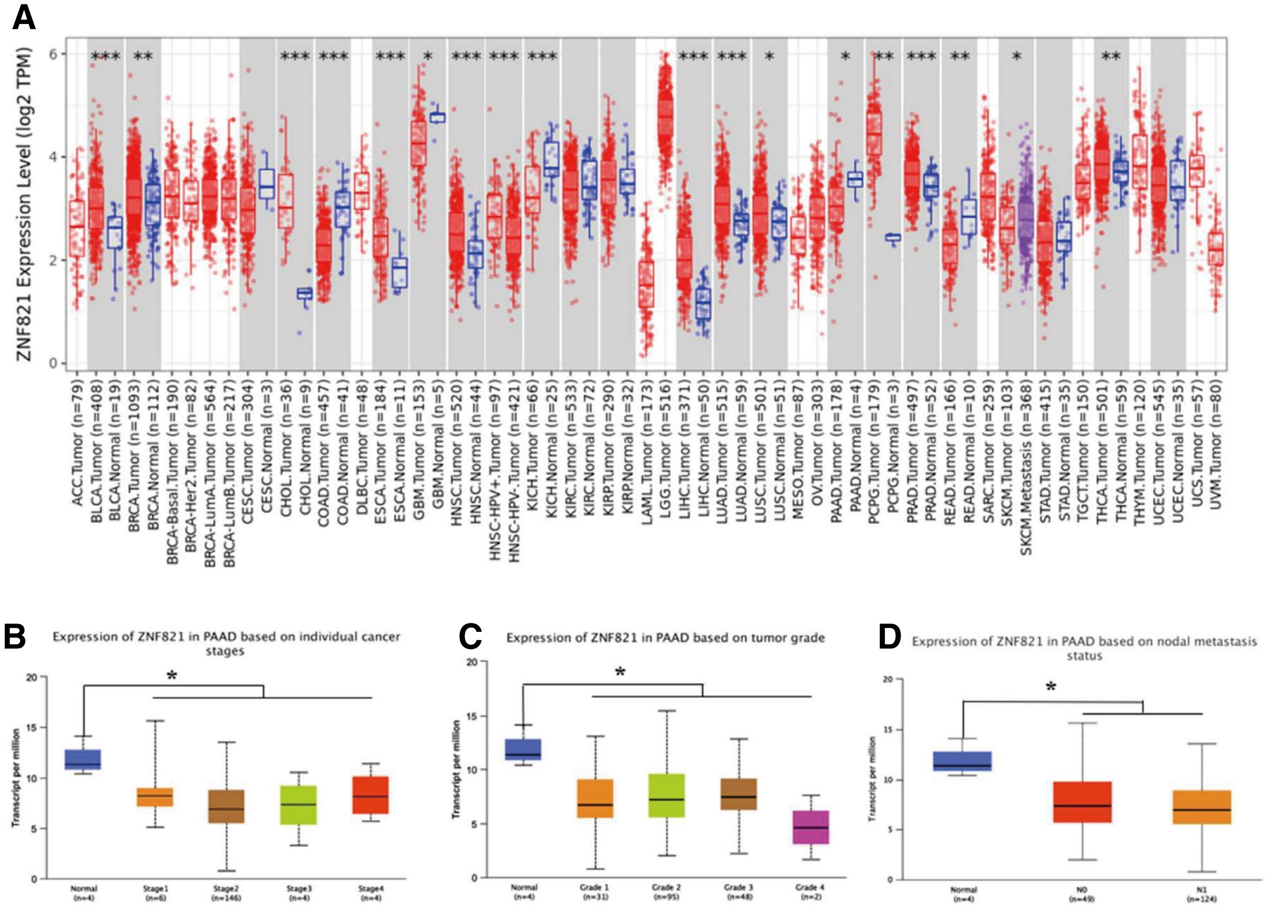

Fig. 1. ZNF821 gene expression in various types of cancer, including PAAD. (A) High or low expression of ZNF821 gene expression in different human tumor tissues compared with normal tissues using the TIMER database. The expression of the ZNF821 gene in tumor stage (B), tumor grade (C), and lymph node metastasis status (D) compared with normal tissues using the UALCAN database (*$p < 0.005$).

expression and survival rates were evaluated in PAAD using the KM plotter and OSlihc web server. The findings revealed that low gene expression of ZNF821 was associated with a poorer prognosis in PAAD (OS: HR = 0.48, $p < 0.001$; RFS: HR = 0.62, $p = 0.26$; Fig. 2A). In addition, low ZNF821 expression had a statistically significant correlation with clinical features associated with poor OS concerning DFI, PFI, and DSS (Fig. 2B).

3. Correlation between the ZNF821 gene and infiltrating immune cells in PAAD

Our results revealed that ZNF821 genes were positively associated with infiltration levels of CD4+ T cells ($r = 0.366$, $p < 0.001$), CD8+ T cells ($r = 0.31$, $p < 0.001$), B cells ($r = 0.072$, $p = 0.348$), neutrophil ($r = 0.17$, $p = 0.0263$), macrophages ($r = 0.32$, $p < 0.001$), and dendritic cells ($r = 0.241$, $p < 0.001$) in PAAD (Fig. 3A). Then, we determined whether ZNF821 was associated with prognosis and TIICs in PAAD. The results revealed that low ZNF821 gene expression combined with high infiltration levels of CD8+ T cells or dendritic cells was associated with a worse prognosis than high ZNF821 gene expression together with low CD8+ T cell or dendritic cell infiltration levels. Low ZNF821 expression and low neutrophil infiltration levels were associated with a worse prognosis than high ZNF821 expression. In addition, low ZNF821 gene expression combined with low infiltration levels of either macrophages or CD4+ T cells was associated with a worse prognosis than high ZNF821 gene expression together with high neutrophil or macrophage infiltration levels (Fig. 3B). These results suggest that low ZNF821 expression is associated with immune cell infiltration.

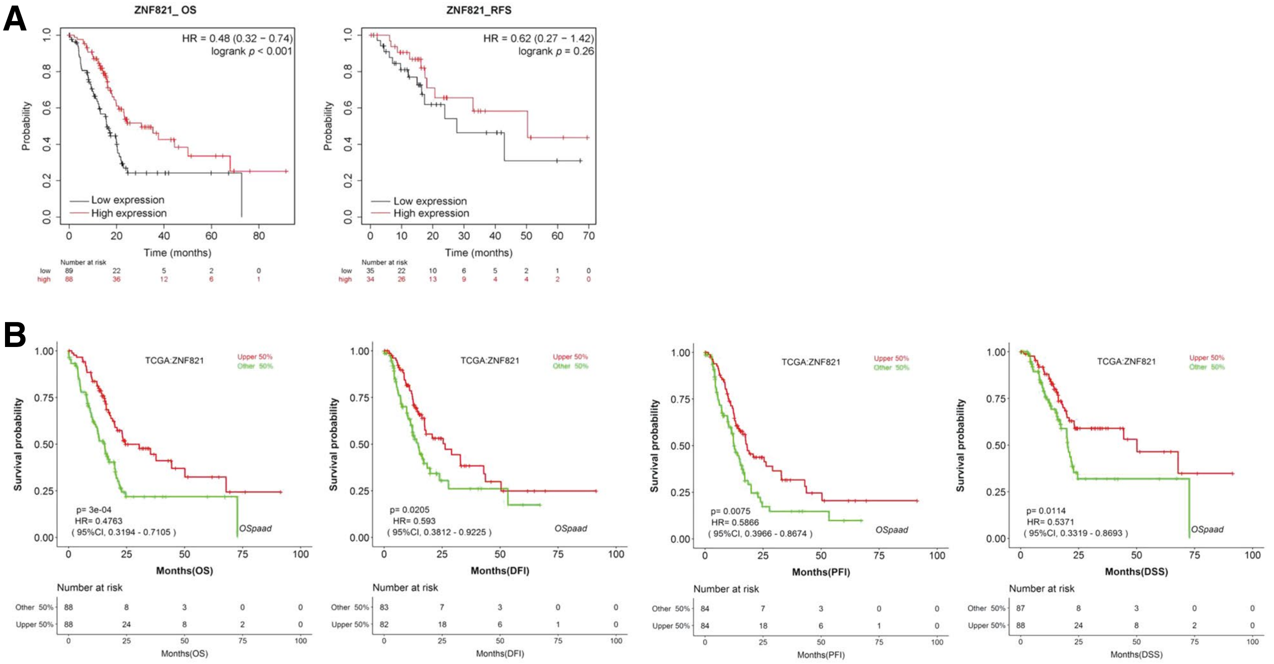

Fig. 2. The prognostic significance of low ZNF821 gene expression in PAAD. ZNF821 was analyzed for prognostic significance using the Kaplan-Meier plotter database (A) and the OSlihc (B). OS, Overall survival; RFS, Recurrence-free survival; DFI, Disease-free-survival; PFI, Progression-free interval; DSS, Disease-specific survival.

4. ZNF821 gene co-expression in PAAD

A total of 11,524 genes (dark red dots) were positively correlated with ZNF821, and 8,170 genes (dark green dots) were negatively correlated (Fig. 4A). A heat map was used to identify the top 50 genes positively correlated with ZNF821 (Fig. 4B) and negatively correlated with ZNF821 (Fig. 4C). We further analyzed the biological pathway classification of GO and found that ZNF821 and its co-expressed genes were involved in the neuropeptide signaling pathway, monoamine transport, protein localization to the endoplasmic reticulum, mitochondrial respiratory chain complex assembly, and NADH dehydrogenase complex assembly (Fig. 5A). KEGG analysis was performed and results showed that co-expressed genes were enriched in the ribosome, oxidative phosphorylation, Parkison disease, synaptic vesicle cycle, primary immunodeficiency, nicotine addiction, maturity onset diabetes of the young, neuroactive ligand-receptor interaction, other types of O-glycan biosynthesis, retrograde endocannabinoid signaling, and intestinal immune network for IgA production (Fig. 5B). ZNF821 gene showed a positive association with expression of KLHL22 ($r=0.646$, $p<0.001$), EGFL7 ($r=0.6409$, $p<0.001$), CHST8 ($r=0.6312$, $p<0.001$), RANGRF ($r=0.6278$, $p<0.001$), LOC401431 ($r=0.6258$, $p<0.001$), and PCSK1N ($r=0.6257$, $p<0.001$) (Fig. 6). These results suggest that ZNF821 plays a prognostic role in PAAD by regulating the transcriptome.

5. The predictive value of ZNF821-related gene in PAAD

We investigated the predictive value of ZNF821-related genes in PAAD using the ZNF821 database. The results of the present study showed that patients with low gene expression of ZNF821 had significantly shorter survival times than those with high expression. Lower expression of ZNF821 was associated with poorer prognosis in PAAD (OS, HR$=$0.42, $p<0.001$; DFS, HR$=$0.58, $p=0.014$; Fig. 7A). Then, we analyzed the survival map of ZNF821-related genes in various types of cancers, including PAAD. These results indicate that ZNF821-related genes are likely high-risk genes in PAAD. Thirty genes showed a low HR for OS ($p<0.05$), and 24 genes had low HR for DFS (Fig. 7B, C). In contrast, 29 of the negative genes had a high HR for OS ($p<0.05$), and 17 genes had a high HR for DFS (Fig. 7D, E).

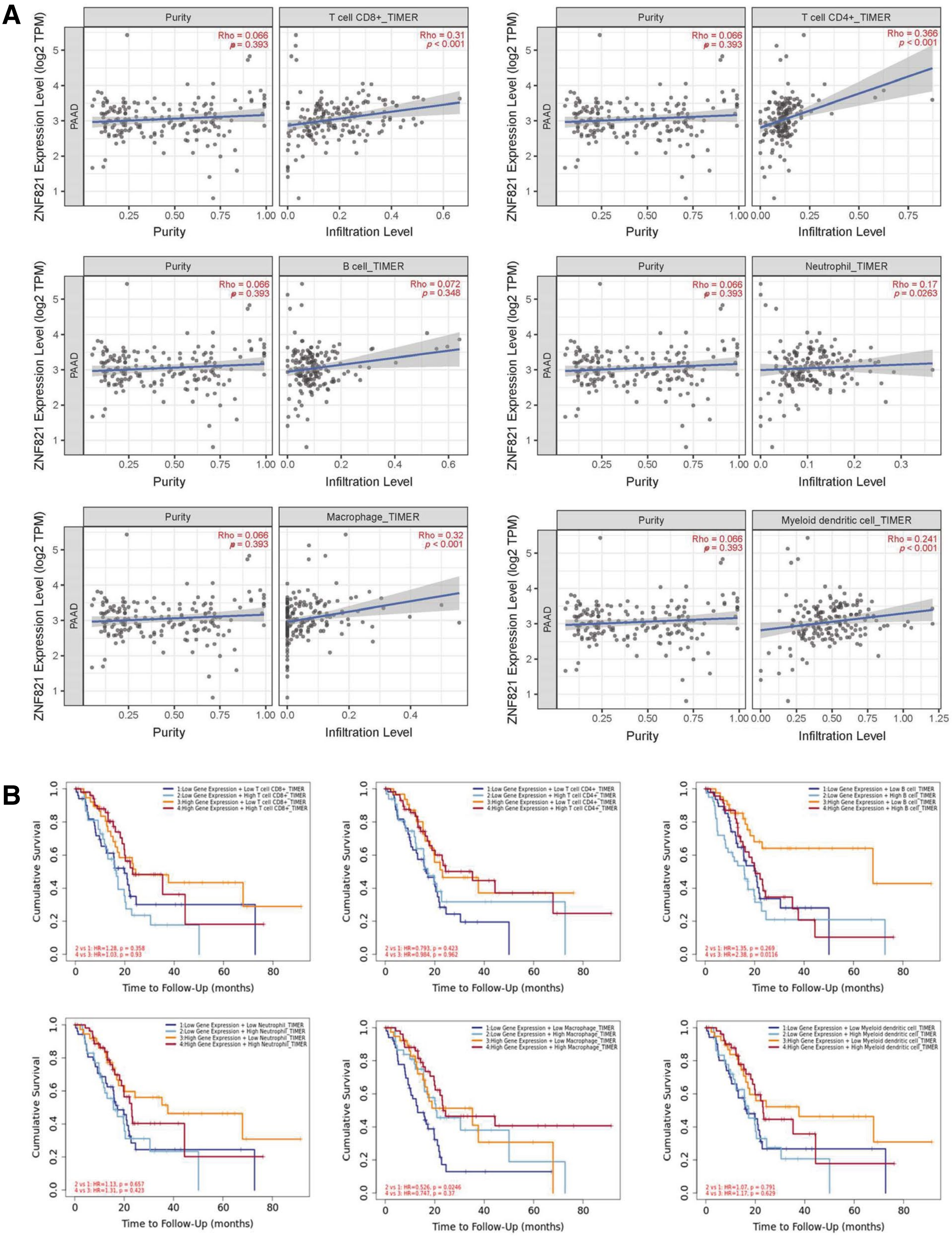

Fig. 3. Correlation between the ZNF821 gene and infiltrating immune cells in PAAD. (A) The correlation between the ZNF821 gene and infiltrating immune cells (B cells, macrophages, CD4 + T cells, dendritic cells, CD8 + T cells, and neutrophils) was analyzed using the TIMER database. (B) The predictive value between infiltrating immune cells and the ZNF821 gene was analyzed using the TIMER database.

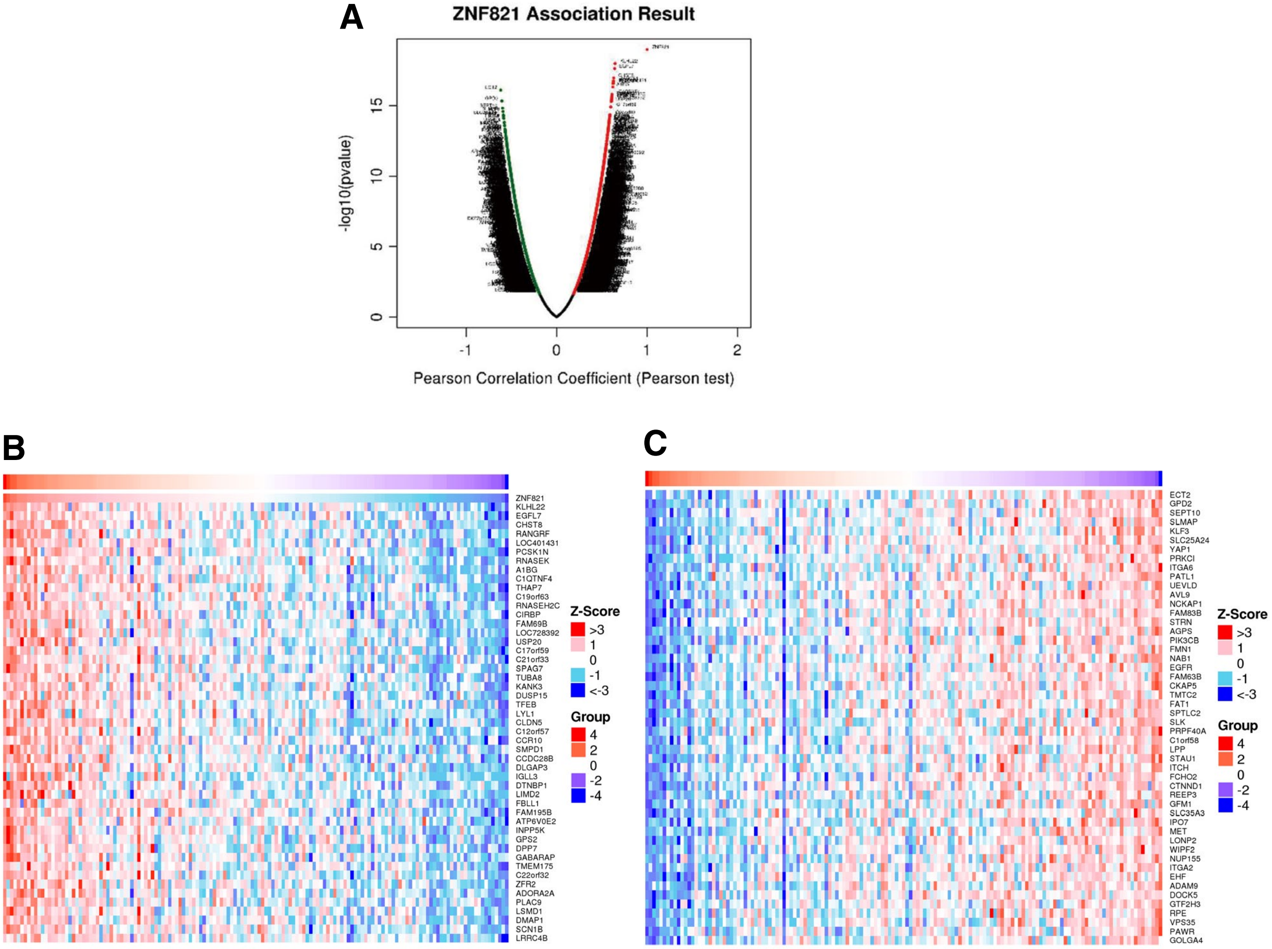

Fig. 4. ZNF821 gene co-expression in PAAD. ZNF821 co-expression genes were analyzed using the LinkedOmics database. (A) Highly correlated genes of ZNF821 were tested using the Pearson test in the PAAD cohort. Heat maps showing the top 50 genes positively and negatively correlated with ZNF821 gene in PAAD. Genes with positive correlation are shown in red (B), and genes with negative correlation are shown in blue (C).

DISCUSSION

PAAD is a malignant tumor that frequently occurs in the digestive system and originates in the pancreatic ductal epithelial and follicular cells [41]. PAAD is one of the most lethal major cancers worldwide [42,43]. PAAD is a locally metastatic disease that is diagnosed at an advanced stage because of the lack of early diagnostic methods [44]. Therefore, the need for biomarkers is emerging for the effective prognostic and diagnostic evaluation of PAAD. PAAD is closely related to the immune system and regulates the development, growth, and drug responsiveness of malignant tumors [45].

Recently, TME has become more important as an immunotherapy for malignancies, and several studies have shown that functional changes and proportions of TIICs are closely related to the prognosis, incidence, and progression of PAAD [46-48]. TIICs can potentially advance tumor infiltration, and the immune system regulates cancer progression [49-51].

ZNF is an important transcription factor encoded by the human genome [29]. The diverse combinations and functions of zinc finger motifs make zinc finger proteins versatile in various biological processes, including autophagy, metabolism, differentiation, and development [52-54]. ZNF821 controls DNA repair networks and is closely associated with dysregulation of cancer development and growth [55]. ZNF821 has been associated with malignancy and has been found to be involved in dysregulation of the DNA repair subnetwork in breast cancer patients [56].

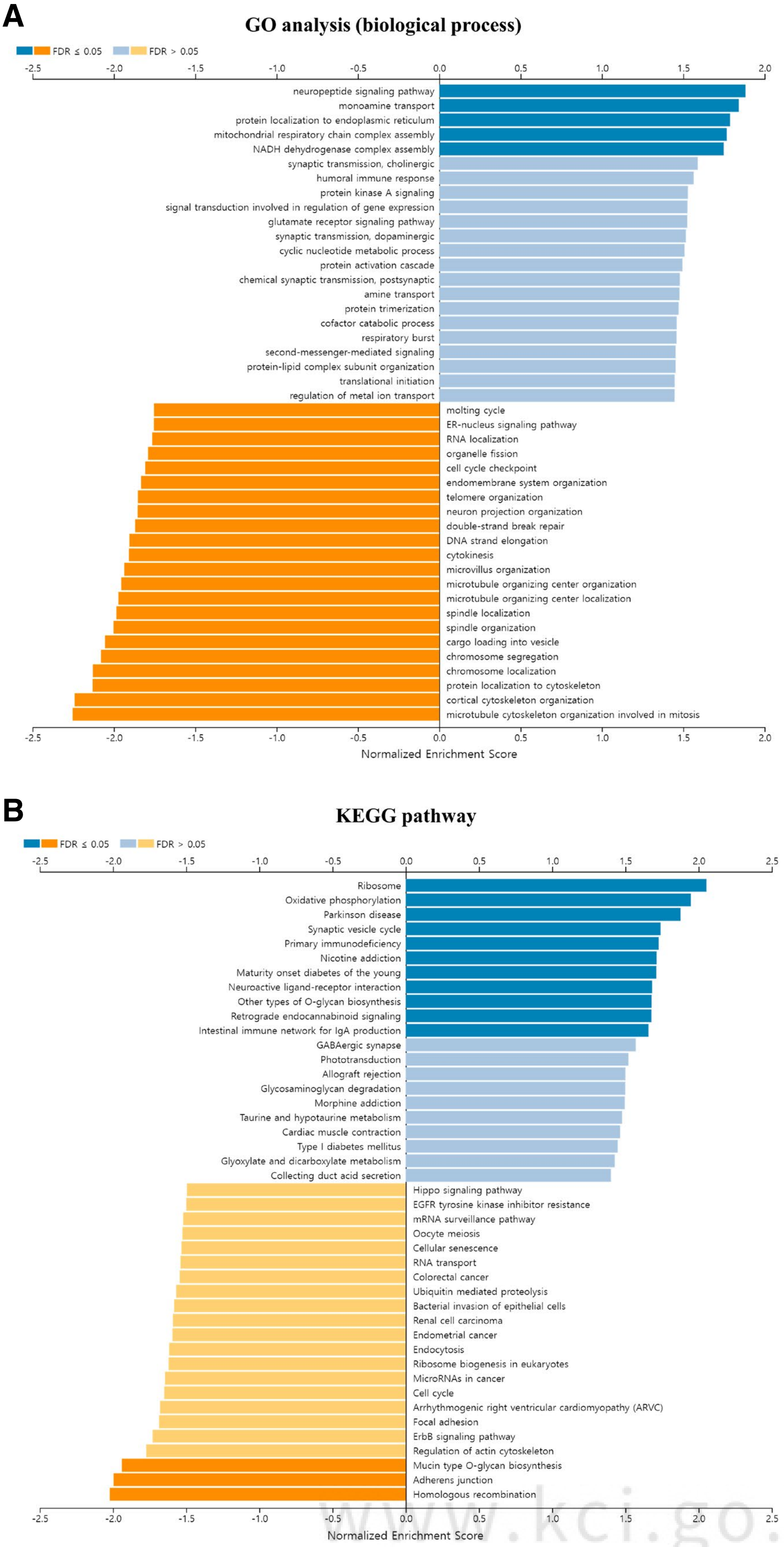

Fig. 5. Enriched GO functions and KEGG pathways of ZNF821 gene in PAAD. KEGG pathway analysis and GO functions were analyzed using the LinkedOmics database. (A) Biological process enrichment analysis of ZNF821 co-expressed genes by gene set enrichment analysis (GSEA). (B) KEGG pathway analysis of ZNF821 co-expressed genes by GSEA.

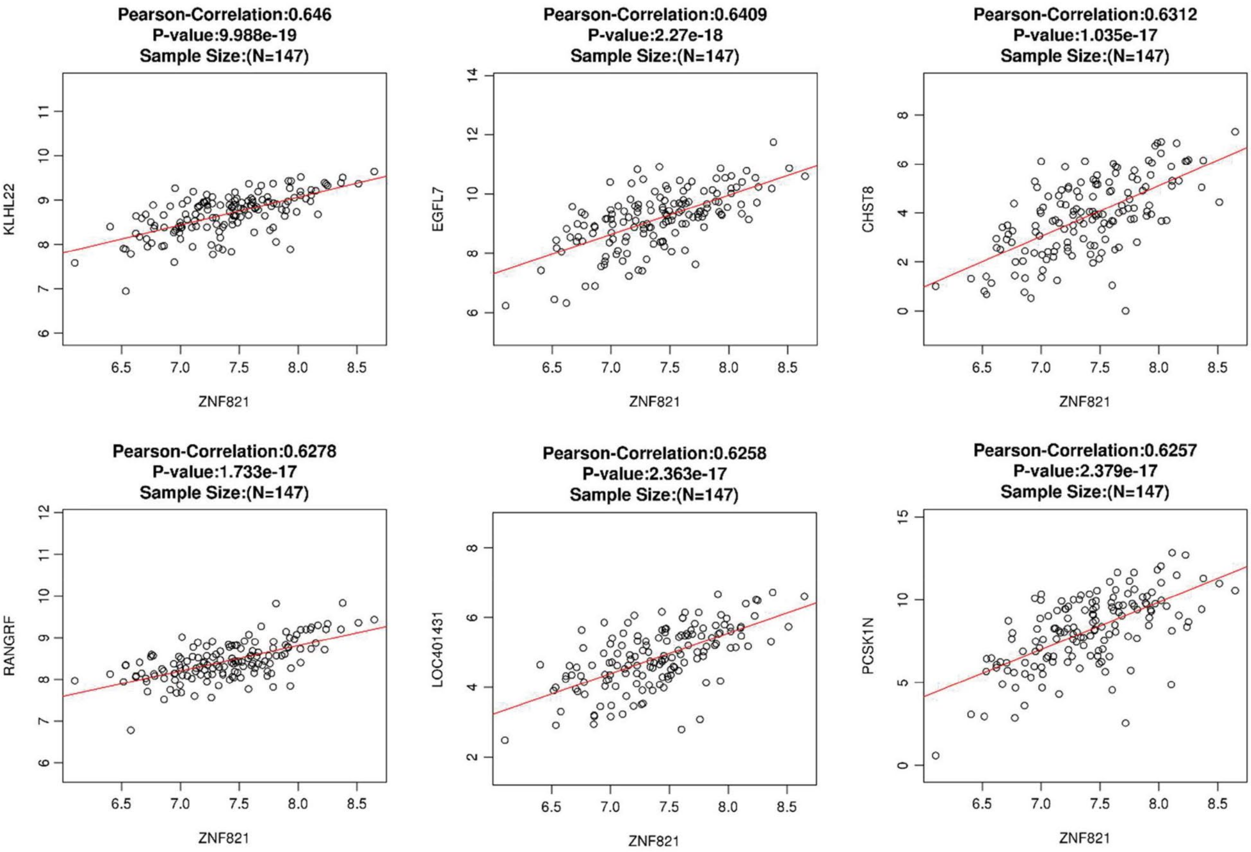

Fig. 6. Correlation with positive-related genes of ZNF821 in PAAD. Genes associated with ZNF821 positivity were identified using the LinkedOmics database. All of these genes (KLHL22, EGFL7, CHST8, RANGRF, LOC401431, and PCSK1N) were significantly correlated with the prognosis of OS in PAAD.

However, the prognostic significance of ZNF821 expression in PAAD remains unclear.

We studied the expression levels of ZNF821 in PAAD using TIMER and UALCAN databases. Based on the TIMER database, we found that the gene expression of ZNF821 was higher or lower in various cancers. It suggests that gene expression differs according to the type of cancers. Based on the UALCAN database, ZNF821 expression was lower in the cancer stages I, II, III, and IV, tumor grades I, II, III, and IV, and nodal metastasis statuses N0 and N1 in PAAD. Based on these results, we analyzed the prognostic significance of low ZNF821 expression in PAAD. Analysis of the GEPIA, OSlihc, and KM plotters revealed that low expression of ZNF 821 correlated with poor prognosis in PAAD. Low ZNF821 expression correlated with a high HR for poor survival rates. A study on genes associated with survival in patients with PAAD showed that the overexpression of centrosome and spindle pole-associated protein was associated with poor prognosis [57]. Spliced transcript variants encoding multiple isoforms for this gene control the DNA repair subnetwork and are closely associated with dysregulation of cancer development and growth [58,59].

ZNF821 is closely associated with the levels of TIICs in PAAD. Based on the TIMER database, we showed a positive correlation between the expression of ZNF821 in PAAD and TIICs, such as dendritic cells, neutrophils, macrophages, CD8+ T cells, and CD4+ T cells. Moreover, we observed low ZNF821 expression in the infiltrating immune cells and poor PAAD prognosis. In particular, low gene expression of ZNF821 and high invasion levels of CD8+ T cells, neutrophils, or dendritic cells led to worse prognosis than high gene expression of ZNF821 and low invasion levels of CD8+ T cells, neutrophils, and dendritic cells. Similarly, low levels of CD4+ T cells or macrophages and low gene expression of ZNF821

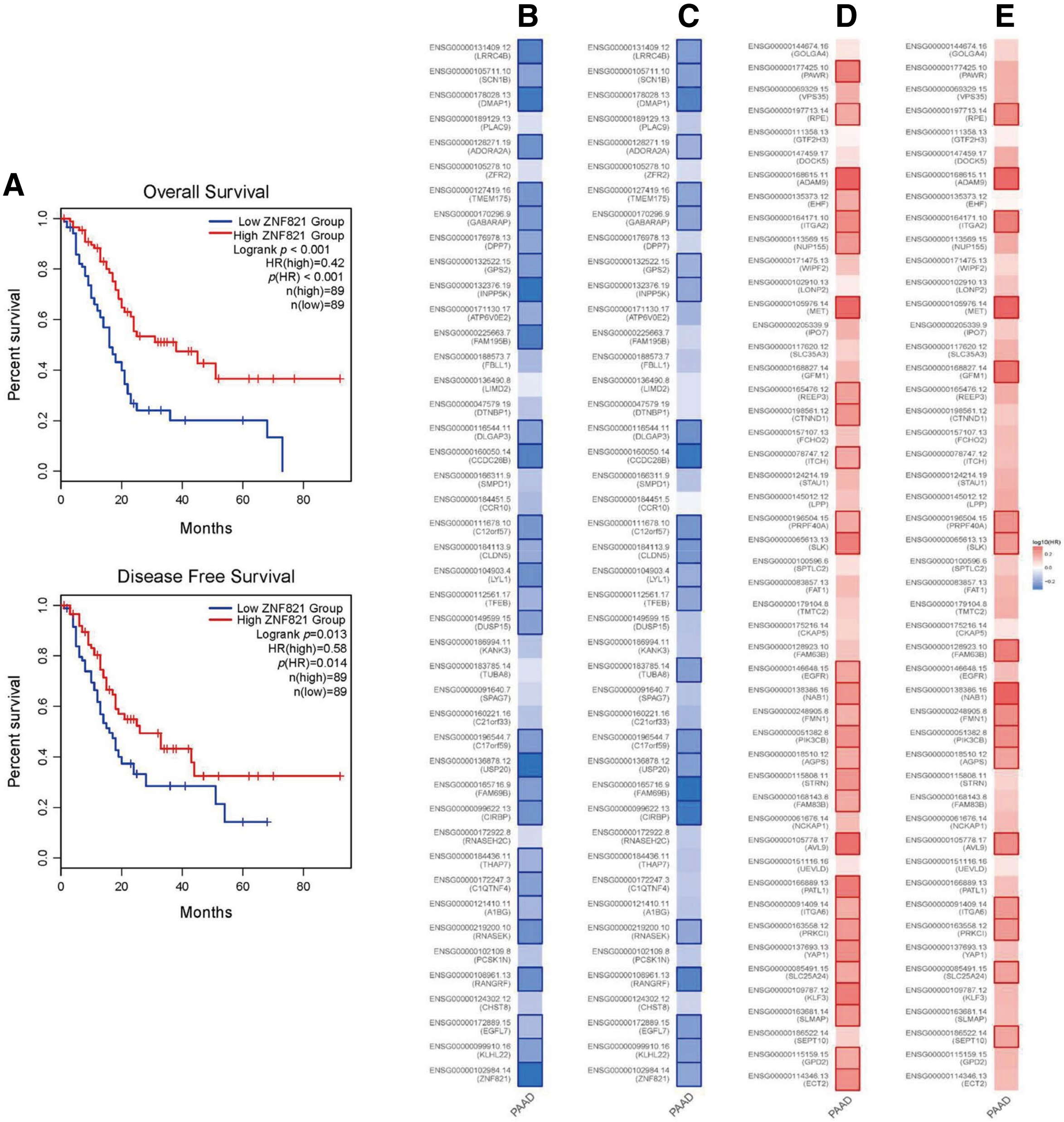

Fig. 7. The prognostic significance of ZNF821-related genes in PAAD. The prognostic significance of ZNF821-related genes was analyzed using the GEPIA2 database. (A) Survival curve of ZNF821 gene in overall survival (OS) and disease-free survival (DFS). Survival map of the negative-related genes of ZNF821 in OS (B) and DFS (C). Survival map of the positive-related genes of ZNF821 in OS (D) and DFS (E). Heatmap represents log10 (HR) of genes in PAAD. A square with a bold border indicates *p*-values less than 0.05 in survival analysis.

were associated with a worse prognosis than high levels of invasion of CD4+ T cells or macrophages and high gene expression of ZNF821. TIICs impair T regulatory cell responses in the TME [60]. In patients with PAAD, the numbers of dendritic cells, CD8+ T cells, CD4+ T cells, and neutrophils are significantly correlated [61].

We showed that ZNF821 plays a critical role in the control of immune cells and the prognosis of PAAD through TIICs. In the ZNF821 gene co-expression network analysis, 11,524 genes were positively associated with ZNF821, and 8,170 genes were negatively associated with ZNF821. ZNF821 is likely a high-risk gene for PAAD. Thirty negative genes related to ZNF821 showed high HR for OS, and twenty-four negative genes showed high HR for DFS.

Twenty-nine positive genes related to ZNF821 showed low HR in OS, whereas 17 genes in DFS showed low HR. In this study, biological process enrichment analysis showed that the ZNF821 gene was closely related to the neuropeptide signaling pathway, monoamine transport, and protein localization to the endoplasmic reticulum in PAAD, and KEGG analysis showed that it was closely related to the ribosome, oxidative phosphorylation, and synaptic vesicle cycle.

In conclusion, our findings showed that low ZNF821 expression is associated with poor prognosis and TIICs in PAAD. This study suggests that ZNF821 may play an important role in the immunotherapy and prognosis of various cancers, including PAAD. However, further studies on the potential control and immune mechanism of ZNF821 in PAAD are needed.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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