Original Article

Prognostic and immune infiltrates for the Chromobox (CBX) protein family in human pancreatic adenocarcinoma

Qian Li[1#], Lei Fu[2#], Daoyuan Wu[3], Jufeng Wang[1]

[1]Department of Oncology, The Affiliated Cancer Hospital of Zhengzhou University, Henan Cancer Hospital, Zhengzhou, China; [2]Department of Oncology, Henan Provincial People's Hospital, Zhengzhou, China; [3]Department of Pathology, The Affiliated Cancer Hospital of Zhengzhou University, Henan Cancer Hospital, Zhengzhou, China

Contributions: (I) Conception and design: L Fu, Q Li, J Wang; (II) Administrative support: L Fu, L Qian, D Wu; (III) Provision of study materials or patients: L Qian, D Wu; (IV) Collection and assembly of data: L Fu, L Qian; (V) Data analysis and interpretation: L Fu, J Wang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Qian Li. Department of Oncology, The Affiliated Cancer Hospital of Zhengzhou University, Henan Cancer Hospital, Zhengzhou, China. Email: zlyyliqian3798@zzu.edu.cn.

Background: Globally, pancreatic adenocarcinoma (PAAD) is among the most prevalent malignant tumors. The Chromobox (CBX) protein family is a vital component of epigenetic regulatory complexes that have vital biological roles. The biological functions, immune infiltration, expression levels, and the prognostic significance of CBX proteins in PAAD have not yet been established.

Methods: Using bioinformatics tools, such as the Gene Expression Profiling Interactive Analysis (GEPIA), Oncomine, Kaplan-Meier plotter, GeneMANIA, cBioPortal, TIMER and R, we evaluated the prognostic importance, expression levels, gene alterations, risk factors, and immune cell infiltration levels of CBXs in PAAD patients. The expression levels of CBX3 in clinical-pathological samples were also confirmed by immunohistochemistry.

Results: In PAAD tissues, CBX1, CBX3, CBX5, and CBX8 expressions were high. High CBX1, CBX5, CBX6, and CBX7 levels were correlated with tumor stages. Elevated CBX2, CBX6, CBX7, and CBX8 messenger ribonucleic acid (mRNA) levels were markedly correlated with better overall survival (OS) outcomes for pancreatic cancer patients, while elevated CBX3 was markedly correlated with short OS outcomes. In particular, we have validated the differential expression of CBX3 on clinical specimens using immunohistochemical methods. A multivariate logistic analysis revealed that elevated mRNA expression levels of CBX3 and suppressed CBX8 levels were independently associated with short OS outcomes for pancreatic cancer patients. The roles of CBXs and their neighboring proteins are associated with a negatively regulated cell cycle, histone binding, polycomb group protein complexes, and the regulation of pluripotent stem cell signaling pathways. Additionally, CBX levels were found to be markedly associated with immune infiltrates, and found that the immune infiltration score of CBX3 was differentially expressed in cell lines such as CD8 T cells, NK cells, Mast cells and T helper cells.

Conclusions: CBX3/8 is a potential marker for prognostic outcomes in PAAD patients.

Keywords: Pancreatic adenocarcinoma (PAAD); Chromobox (CBX); prognostic value; bioinformatics analysis; immune infiltration

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Introduction

Clinically, pancreatic adenocarcinoma (PAAD) is one of the most poorly prognosed malignancies of the digestive system (1). Despite great advances in the diagnosis and treatment of PAAD, only 5% of patients with PAAD survive for more than 5 years due to limitations related to the late diagnosis and poor therapeutic outcomes of PAAD (2). Thus, PAAD is a highly lethal disease with an insidious onset and has become one of the most prevalent malignancies in the developed world (3,4).

Due to the lack of early symptoms and biomarkers in PAAD patients, the majority of PAAD patients are diagnosed in the mid to late cancer stages, and thus have lost the opportunity to undergo treatment (5). Thus, an in-depth exploration of the pathogenesis of PAAD and the search for new prognostic biomarkers and therapeutic targets, leading to the development of effective early screening and diagnostic methods, will be important in improving the treatment outcomes and survival of PAAD patients (6).

The Chromobox (CBX) protein family is a vital component of the polycomb group (PcG) complexes, which contain a single N-terminal chromodomain (7,8). The CBX family of proteins is reported to play roles in various biological processes (BPs), including cell-cycle regulation, gene expression, and tumor initiation. These proteins play various roles in the development and progression of various tumors (9). Based on their molecular structures, the 8 members of the CBX protein family can be assigned into the following 2 subgroups: (I) the heterochromatin protein 1 (HP1) group (which includes CBX3, CBX5, and CBX1); and (II) the PcG (which includes CBX7, CBX4, CBX2, CBX7, and CBX8) (10,11).

There is growing evidence that the CBX family of proteins plays vital roles in a variety of cancers. Abnormal expression levels of some members of the CBX family are associated with the prognosis of tumors, including breast, liver, and gastric tumors (12,13). It has also been shown that the CBX family is associated with tumour-infiltrating immune cells and may influence tumour progression and recurrence (14-16). However, the roles and prognostic significance of various CBX family members in PAAD have not yet been clearly established. Thus, we extended the field of study to PAAD by analyzing a series of major databases to investigate the possible oncogenic significance of various members of the CBX family in PAAD. We present the following article in accordance with the REMARK reporting checklist (available at https://dx.doi.org/10.21037/jgo-21-613).

Methods

Ethical approval

This study was approved by the Ethics Committee of Affiliated Cancer Hospital of Zhengzhou University (No. 2020LP00007) and informed consent was obtained from all the participating patients. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013).

Patients and specimens

We investigated PAAD tissues from 32 surgically resected cases at Henan Cancer Hospital, Zhengzhou, China between 2019 and 2020. The selected pathology specimens in each group, which included cancerous and paraneoplastic tissues, were examined. None of the patients underwent any neoadjuvant therapies, such as chemotherapy or radiotherapy, before surgery.

Immunohistochemistry (IHC) studies and scores

IHC studies were performed following routine procedures. Tissue sections (3-μm thick) were dissociated in xylene, dehydrated in ethanol, and incubated with a primary antibody overnight at 4 ℃. They were then incubated with a secondary antibody for 2 h at room temperature. The primary antibody used in this study was rabbit anti-CBX3 antibody (1:400 in PBS; Cat No.: 11650-2-AP, Wuhan Sanying Biotechnology, Wuhan, China). The secondary antibody used in this study was anti-rabbit HRP-conjugated antibody (Envision System, Cat. No.: GK500711, Dako Cytomation, Glostrup, Denmark).

After cover slipping, positive staining was determined by IHC score evaluations. CBX3 protein expression levels were determined using semi-quantitative scores as follows: 0 (positive cells: ≤10%), 1 (positive cells: 11–33%), 2 (positive cells: 34–66%), or 3 (positive cells: ≥67%) as previously reported (11).

Oncomine analysis

The gene expression array data set in the Oncomine database (www.oncomine.org), which is a publicly available database for cancer microarray, was used to analyze CBX transcription levels in various cancers (14). The expression levels of CBXs in clinical cancer samples and normal control samples were compared using the student's t test. The fold change and P

value cut-off limits were 1.5 and 0.01, respectively.

Gene Expression Profiling Interactive Analysis (GEPIA) data set

GEPIA is a new web-based bioinformatics tool that analyzes ribonucleic acid (RNA) sequences retrieved from the Genotype-Tissue Expression (GTEx) and The Cancer Genome Atlas (TCGA) data sets (www.gepia.cancer-pku.cn), which provide differential expression analyses, cancer type staging, patient survival analyses, and similar gene detections. The analysis of the relationship between CBX family members and PAAD was performed using the TCGA_PAAD data set in GEPIA (15).

The Kaplan-Meier Plotter

The prognostic significance of the transcription messenger RNA (mRNA) expression levels was investigated using the Kaplan-Meier Plotter database (www.kmplot.com), which has survival information and gene expression data for PAAD patients (16,17). To analyze the overall survival (OS) and disease-free survival (DFS) outcomes for PAAD patients, the specimens were divided into high- and low-expression groups based on their median expression levels. The grouped samples were evaluated using a Kaplan-Meier survival plot with hazard ratios (HRs), log-rank P values, and 95% confidence intervals (CIs).

TCGA data and cBioPortal

The TCGA database has both clinical-pathological and sequencing data for over 30 cancer types (18). The PAAD data set (PanCancer Atlas) has data for 184 cases. The pathological information of these cases was used to evaluate CBX expressions by the cBioPortal (www.cbioportal.org) (19,20). The genomic map has information on putative copy-number alterations from the Genomic Identification of Significant Targets in Cancer (GISTIC), mutations, and mRNA expression level z-scores.

Enrichment analysis

The enrichment analysis of CBXs in PAAD was conducted using STRING database (https://strin g-db.org/) (21) and the Database for Annotation Visualization and Integrated Discovery (DAVID) (https://david.ncifc rf.gov/) (22). We first conducted protein-protein interaction (PPI) network evaluations on differently expressed CBXs using STRINGS. Next, members of the CBX family and the adjacent genes were evaluated using DAVID 6.8, Metascape for Gene Ontology (GO), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. The results were visualized by R project using the "ggplot2" package with a cut-off P value of <0.05. The cellular components (CCs), BPs, and molecular functions (MFs) were evaluated in the GO analysis.

TIMER analysis

TIMER (www.cistrome.shinyapps.io) was used to analyze of the abundance of immune infiltrations and evaluate the clinical effects of tumor-infiltrating immune cells (23). TIMER was used to assess correlations between members of the CBX family and immune cell infiltration in PAAD. The TCGA-PAAD data set was used for this analysis, and a P value <0.05 was considered significant.

Cox regression analysis

The P values and HRs of the constituents were subject to univariate and multivariate Cox regression analyses, together with analyses of some CBX gene parameter. Univariate and multivariate Cox regression analyses were conducted to identify proper terms for building the nomogram. Using "forestplot" in R package, a forest plot was used to determine the HR, P value, and 95% CI of each variable.

Statistical analysis

For statistical analyses we estimated risk ratios [and their 95% confidence intervals (CIs)] for each potential risk factor using Cox proportional risk regression models. Stepwise multifactorial Cox regression analysis was used to determine inclusion and exclusion criteria for type I error =0.1.

A two-sided P value of <0.05 were considered statistically significant. All statistical analyses were performed using R software for Windows, version 3.6.1.

Results

Differentially expressed CBXs in pancreatic carcinoma

The ONCOMINE database was used to assess differently expressed CBXs. As *Figure 1* shows, CBX1, CBX3, CBX5,

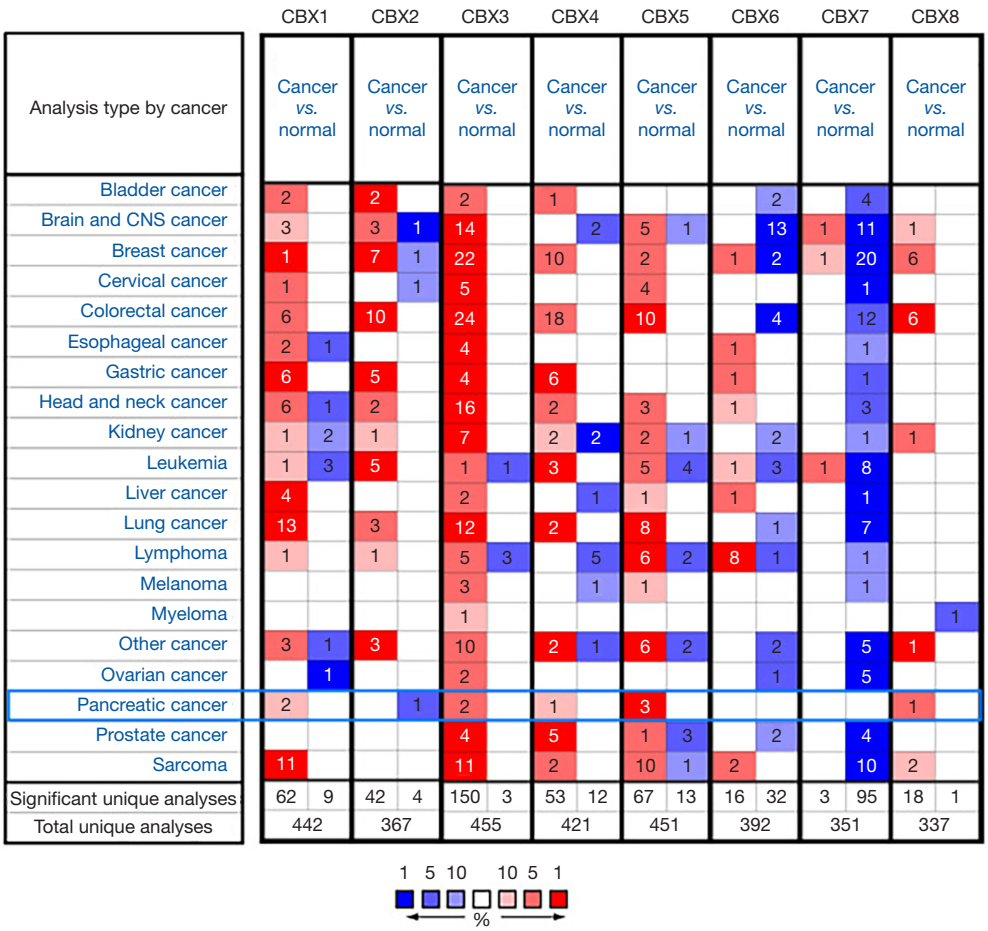

Figure 1 The transcription levels of CBXs factors in different types of cancers (Oncomine database). The threshold was designed with the following parameters: P value: 0.01, fold change: 1.5, gene rank: 10%, data type: mRNA.

and CBX8 transcription levels were markedly high in pancreatic carcinoma tissues, while the transcription levels of CBX2 were suppressed in normal tissues. In this study, CBX1 was found to be overexpressed in pancreatic carcinoma tissues relative to adjacent normal tissues (fold change in the Badea pancreas data set: 1.957; P=2.04E-9) (24). CBX3 was also significantly elevated in pancreatic cancer tissues relative to normal tissues. Findings from the Pei Pancreas data set revealed a 1.678 (P=3.32E-5) fold change increase in CBX3 mRNA expression in pancreatic carcinoma tissues (25). CBX5 was found to be significantly upregulated in pancreatic carcinoma tissues relative to normal tissues. The results of the Ishikawa Pancreas data set analysis showed that there were 2.371-fold (P=3.63E-4) increases in CBX5 mRNA expression levels in pancreatic tumor tissues (26). CBX8 was also markedly high in pancreatic tumor tissues relative to normal tissues, with the TCGA dataset results showing a 2.269 (P=0.009) fold increase in tumour tissue. CBX2 was also found to be more downregulated in pancreatic carcinoma tissues than normal tissues. The results of the Ishikawa Pancreas data set revealed that there was a 1.678-fold (P=0.006) decrease in CBX2 mRNA expression in pancreatic carcinoma tissues.

Correlation between the mRNA levels of different CBXs and the clinic pathological features of pancreatic carcinoma patients

Using the GEPIA data set, mRNA levels of CBXs in pancreatic tumor and normal pancreatic tissues were compared. Consistent with the Oncomine findings, CBX1, CBX3, CBX 5, and CBX8 levels were higher in in pancreatic cancer tissues than normal tissues (see *Figure 2A,2B*). We also analyzed the correlations between levels of

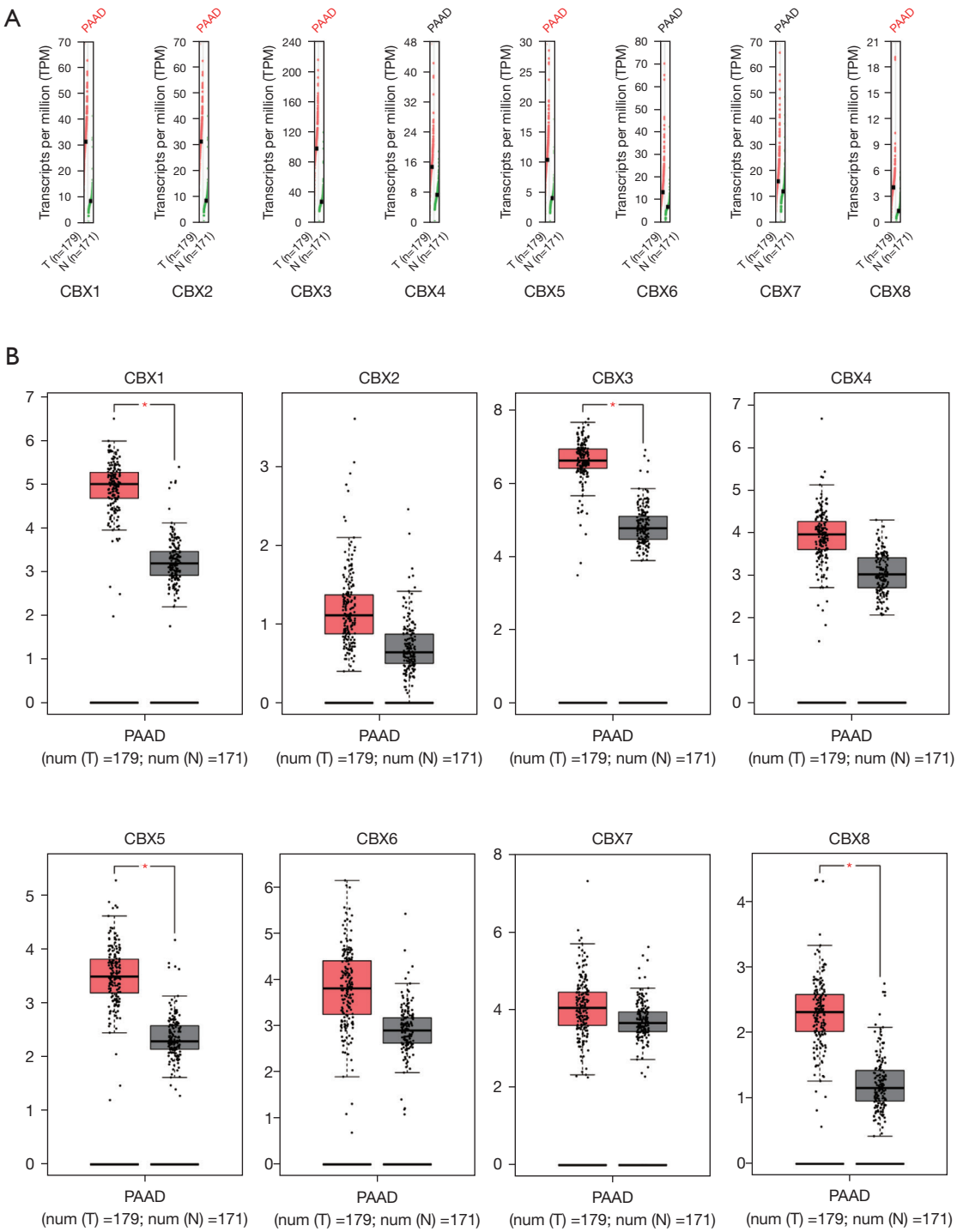

Figure 2 The expression of Chromobox (CBXs) in pancreatic adenocarcinoma (PAAD). (A) Scatter diagram; Red: overexpressed in tumor tissue; green: underexpressed in tumor tissue; black: no significant difference in expression (P>0.05). (B) Box plot; Red: tumor tissue; blue: normal tissue. *, P<0.05.

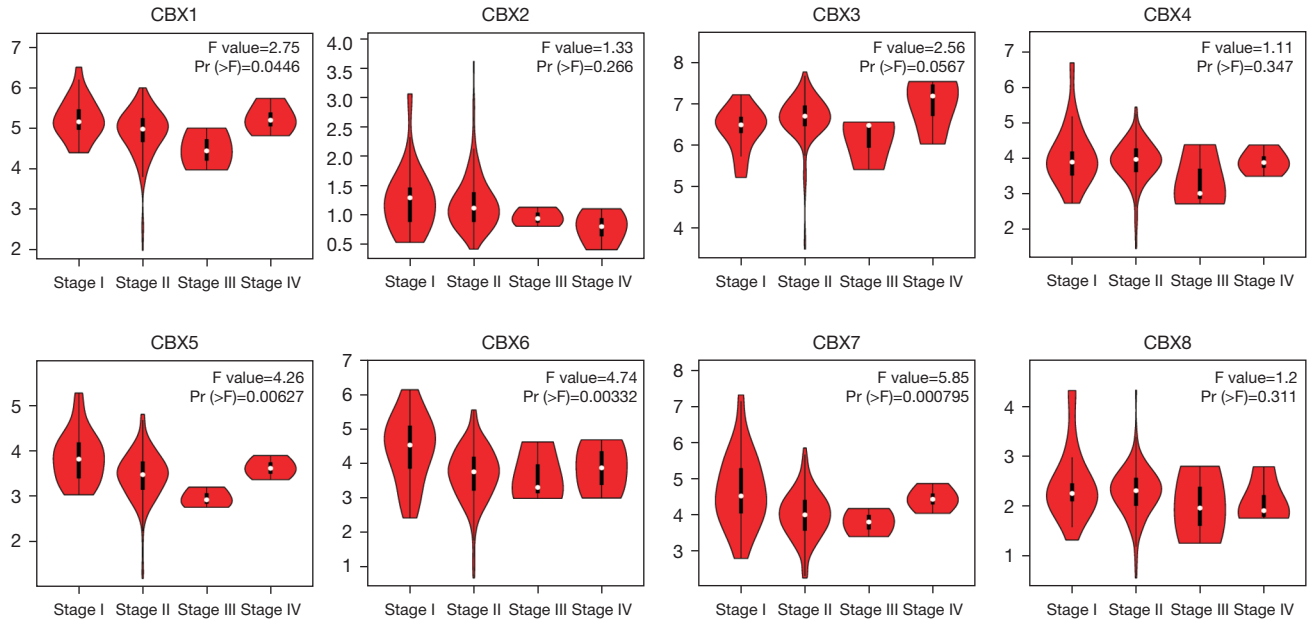

Figure 3 Correlation between CBXs expression and tumor stage in PAAD patients (GEPIA). CBX, Chromobox; PAAD, pancreatic adenocarcinoma.

differently expressed CBXs and the clinical-pathological stages of pancreatic cancer patients. Notably, the CBX1, CBX5, CBX6, and CBX7 groups varied significantly; however, the variations in CBX2, CBX3, CBX4 and CBX8 groups were not significant (see *Figure 3*).

Prognostic significance of the mRNA expression of CBXs in pancreatic carcinoma patients

An analysis of the correlations between the mRNA levels of CBXs and survival outcomes for pancreatic cancer patients was conducted using GEPIA tools. *Figure 4* shows the OS and disease-free survival (DFS) curves. Notably, higher mRNA expressions of CBX2, CBX6, CBX7, and CBX8 were markedly correlated with favorable OS outcomes, while elevated mRNA expression levels for CBX3 were markedly correlated with short DFS and OS.

Prognostic significance of CBX family expressions in terms of OS in pancreatic carcinoma patients

Having established that the expressions of CBX2, CBX3, CBX6, CBX7, and CBX8 were considerably associated with prognostic outcomes in PAAD patients, we then evaluated the independent prognostic significance of mRNA levels of CBXs in pancreatic carcinoma patients. We downloaded the clinical information of 178 PAAD patients from TCGA data portal (http://firebrowse.org/api-docs/) for Cox survival regression analysis. In the univariate analysis, a high pathologic grade (HR =1.452, 95% CI: 1.091–1.933; P=0.01052), a high age (HR =1.028, 95% CI: 1.007–1.051; P<0.01), the high expression of CBX3 (HR =2.004, 95% CI: 1.328–3.025; P<0.001) and the low expression of CBX6 (HR =1.787, 95% CI: 1.247–2.561; P=0.002), CBX7 (HR =1.150, 95% CI: 1.025–1.290, and P=0.017), and CBX8 (HR =1.325, 95% CI: 1.056–1.663; P=0.015) were correlated with the short survival time of patients with pancreatic carcinoma (see *Figure 5*). The multivariate logistic analysis revealed that elevated mRNA levels of CBX3 (HR =1.691, 95% CI: 1.175–2.432; P=0.005) and the low expression of CBX8 (HR =1.300, 95% CI: 1.036–1.631; P=0.023) were independent risk factors for the short survival outcomes of patients with pancreatic cancer (see *Figure 5*).

Immunohistochemistry and semi-quantitative evaluation of CBX3 expression

IHC was used to detect protein expression levels of CBX3 in PAAD and adjacent tissues from 32 surgically resected cases at the Henan Cancer Hospital. We found that the protein levels of CBX3 were higher in the PAAD tissues than the adjacent normal tissues (see *Figure 6A*). In our study, we

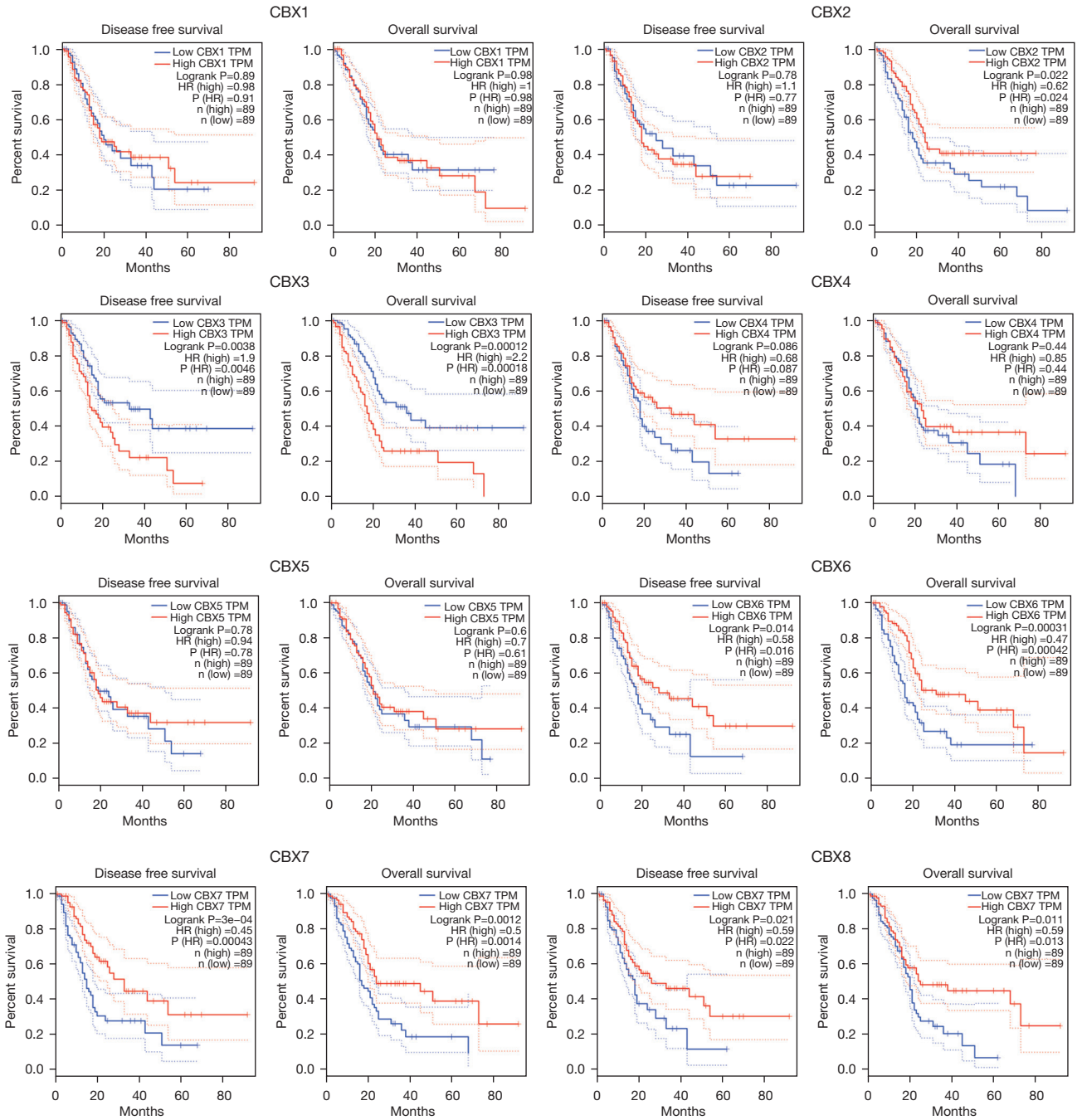

Figure 4 The prognostic value of different expressed CBXs in PAAD patients in the DFS and OS curve (Kaplan-Meier plotter). CBX, Chromobox; PAAD, pancreatic adenocarcinoma; DFS, disease-free survival; OS, overall survival.

pooled the IHC scores of both the PAAD and adjacent sections, and found that the CBX3 expression scores in tumors were much high than those in paracancerous tissue (see *Figure 6B*).

Genetic mutations and interaction analyses of CBXs in pancreatic carcinoma patients

Gene alterations in CBXs and their correlations with the DFS and OS of pancreatic cancer patients were evaluated using the

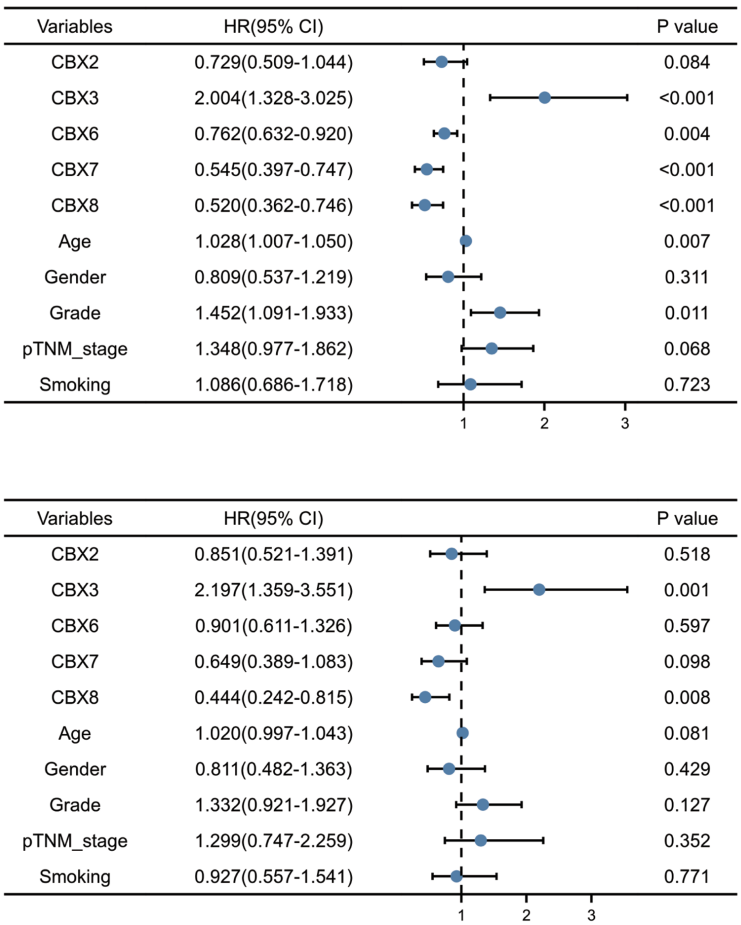

Figure 5 Univariate and multivariate analysis of overall survival in 178 PAAD specimens (top univariate analysis, bottom multivariate analysis). PAAD, pancreatic adenocarcinoma.

cBioPortal online tool. As *Figure 7A* shows, CBX1, CBX4, CBX5, CBX6, CBX7, and CBX8 genes had the highest gene alterations, with mutation rates of 11%, 9%, 8%, 8%, 8%, and 8% in the pancreatic cancer samples, respectively. Additionally, 2 or more alterations were detected in nearly 1/3 (32.02%) of the 178 sequenced pancreatic cancer patient samples (*Figure 7B*). The results of the Kaplan-Meier plot and the log-rank tests showed that gene alterations in CBXs were related to short DFS (*Figure 7C*; P=0.0208) and OS (*Figure 7D*; P=0.0232) in pancreatic tumor patients. Thus, genetic alterations in CBXs may also significantly affect the prognosis of pancreatic cancer patients.

Enrichment analyses of CBX family members in pancreatic carcinoma

An enrichment analysis was conducted to investigate the roles of CBX family members in pancreatic carcinoma. First, we explored the top 10 genes that correlated the most highly with every member of the CBX family using GEPIA. The GO and KEGG enrichment analyses of the CBX family and associated genes were conducted using the "clusterProfiler" in R package. The BP analysis revealed that the CBX family was associated with the negative regulation of the cell-cycle process and G0 to G1 transitions (see *Figure 8A*). The CC analysis suggested a relationship between the CBX family and the nuclear ubiquitin ligase complex, PcG protein complex, ubiquitin ligase complex, and protein regulator of cytokinesis 1 complex (see *Figure 8B*). Additionally, the CBX family also prominently affected MF, such as histone binding, methylation-dependent protein binding, modification-dependent protein binding, and methylated histone binding (see *Figure 8C*). The KEGG pathways analysis showed that CBX family members were involved in

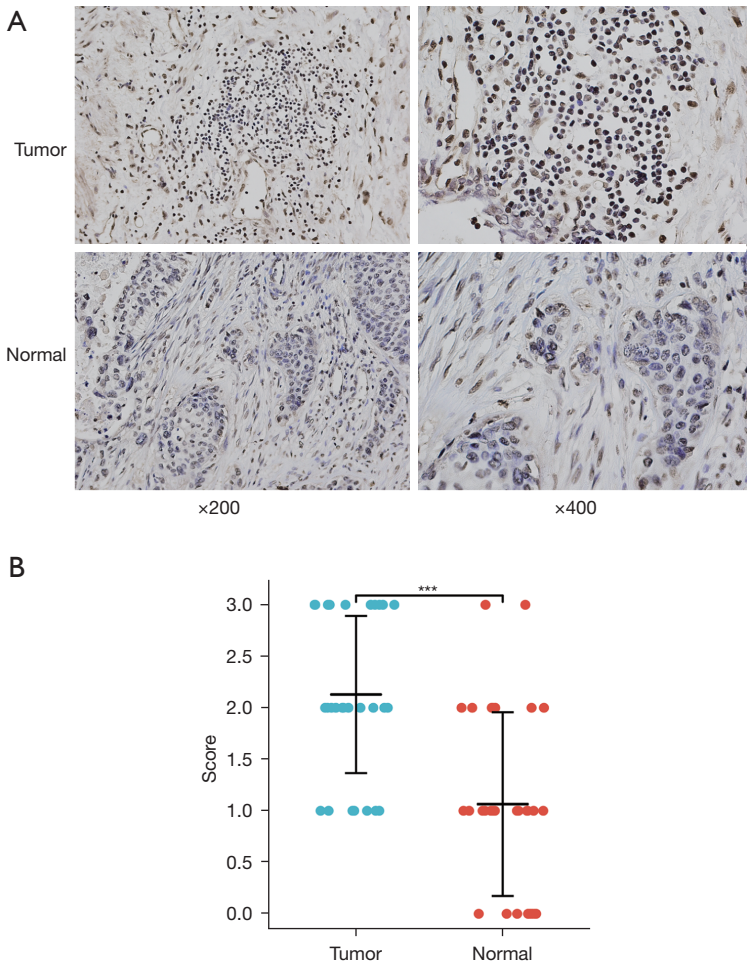

Figure 6 Representative immunohistochemistry images and IHC score of Chromobox (CBX) in pancreatic adenocarcinoma (PAAD) tissues and normal (para-carcinoma) tissues. (A) Representative image of CBX3 staining (high expression) in tumor and normal (para-carcinoma) cell nuclei (left ×200, right ×400). (B) semi-quantitative IHC score between tumor and normal (para-carcinoma) tissues (***, P<0.001).

the regulation of the pluripotency of the stem cell signaling pathways (see *Figure 8D*). A PPI network analysis of CBXs was conducted using STRING (see *Figure 8E*).

Immune infiltration analysis of the CBX family members in pancreatic carcinoma patients

To investigate the relationship between immune cell level and cancer cell proliferation and progression, the TIMER database was used to investigate immune cell infiltration in the CBX family (*Figure 9A-9H*). As *Figure 9A* shows, there was a significant association between CBX1 and B cell (cor =0.198; P=9.38e-03), cluster of differentiation 8 ($CD8^+$) cell (cor =0.474; P=5.71e-11), macrophage (cor =0.626; P=5.57e-20), neutrophil (cor =0.353; P=2.17e-06), and dendritic cell (cor =0.476; P=4.96e-11) infiltrations. CBX2 was positively associated with macrophages, neutrophils, and dendritic cells in pancreatic carcinoma patients (see *Figure 9B*). In relation to CBX3, all 6 host immune cell types (i.e., $CD8^+$, B, $CD4^+$, neutrophils, macrophages, and dendritic cells) had a positive correlation with it in pancreatic carcinoma patients (see *Figure 9C*). Except for the CD4 cell, there was a positive correlation between CBX5 and infiltration levels of all immune cell types (see *Figure 9E*). The relationship between CBX6 and CBX7 and immune cells is largely consistent with that of CBX3 (see *Figure 9F,9G*). Interestingly, expressions of CBX4 and CBX8 were negatively correlated with $CD8^+$ T cell infiltrations (see *Figure 9D,9H*).We assessed the immune cell infiltration score of TCGA pancreatic adenocarcinoma, depending on

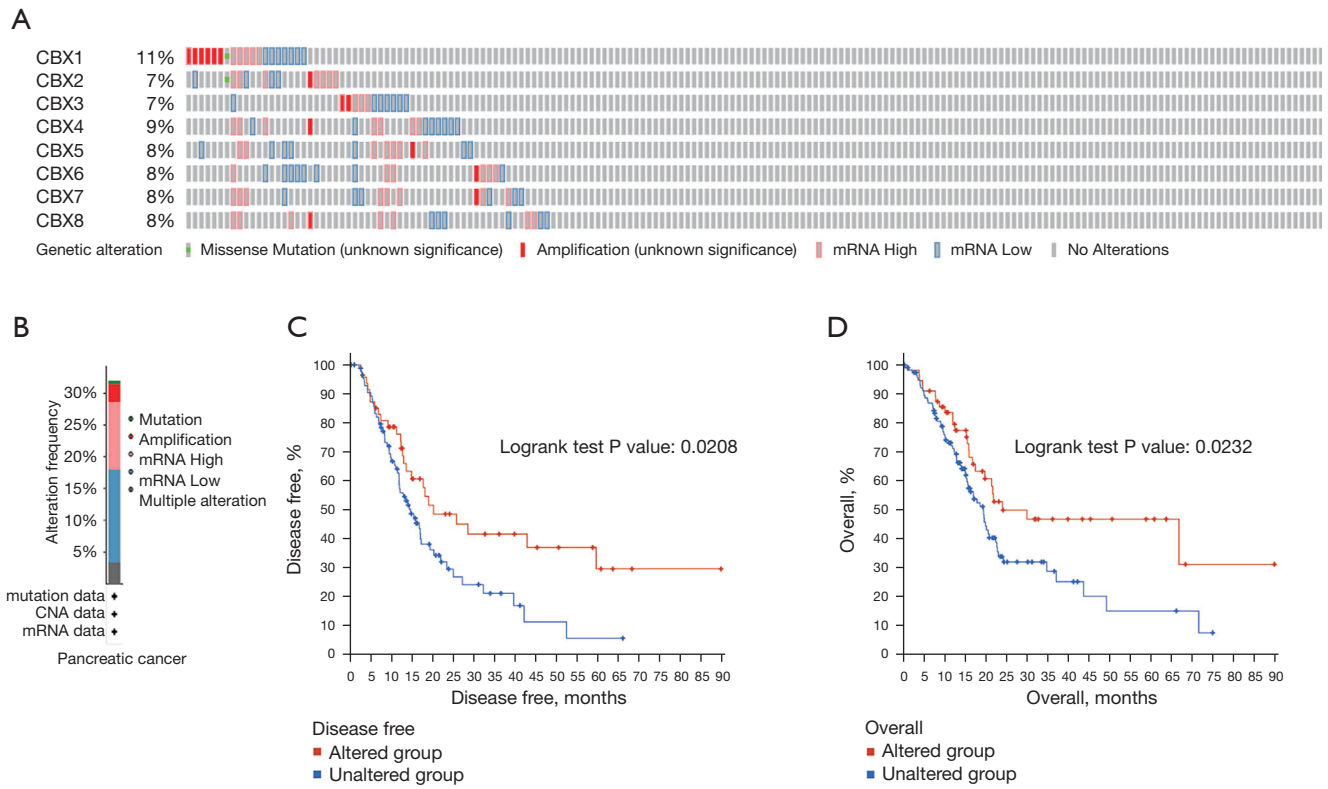

Figure 7 Genetic alteration, co-expression, neighbor gene network, and interaction analyses of E2Fs in patients with pancreatic adenocarcinoma (PAAD) (cBioPortal). (A) Oncoprint visual summary of alteration on a query of Chromobox (CBX) family members. (B) Summary of alterations in different expressed CBXs in PAAD. (C) Kaplan–Meier plots comparing overall survival (OS) in cases with and without CBXs genes alterations. (D) Kaplan–Meier plots comparing disease-free survival (DFS) in cases with and without CBXs genes alterations.

the level of CBX3 expression, and found that as shown in Figure S1. Different levels of immune infiltration of CBX3 were found to be differentially expressed in cell lines such as CD8 T cells, NK cells, Mast cells and T helper cells.

Discussion

The dysregulation of the CBX family is correlated with the development of several tumors. CBX has been found to be overexpressed in hepatocellular carcinoma, castration-resistant prostate cancer, and breast cancers (13,27,28). Clermont *et al.* reported that the expression of CBX2 mRNA is higher in many cancer tissues than normal tissues (29). CBXs have previously been shown to be closely associated with multiple tumors; however, the prognostic significance of CBX expression in PAAD had not been fully elucidated.

We evaluated the mRNA levels and prognostic significance of various CBXs in PAAD and their association with prognosis and immune infiltration in PAAD. We first detected the expression pattern of CBX family members in PAAD, and found that CBX1, CBX3, CBX5, and CBX8 were upregulated in pancreatic tumor tissues. Additionally, elevated mRNA expressions of CBX2, CBX6, CBX7, and CBX8 were markedly correlated with better OS outcomes for pancreatic tumor patients. Interestingly, elevated mRNA expression levels of CBX3 were markedly related to short DFS and OS. Our multivariate analysis revealed that elevated mRNA expressions of CBX3 were independent prognostic markers for the short OS of PAAD patients. In previous studies, the overexpression of CBX3 has been shown to play a vital role in a variety of cancers (11,13). Alam *et al.* showed that CBX3 is highly expressed in lung adenocarcinoma. The overexpression of CBX3 is correlated with poor prognostic outcomes (30). Liu *et al.* demonstrated that CBX3 overexpression promotes the cell proliferation of colorectal cancer cell lines by directly regulating CDKN1A,

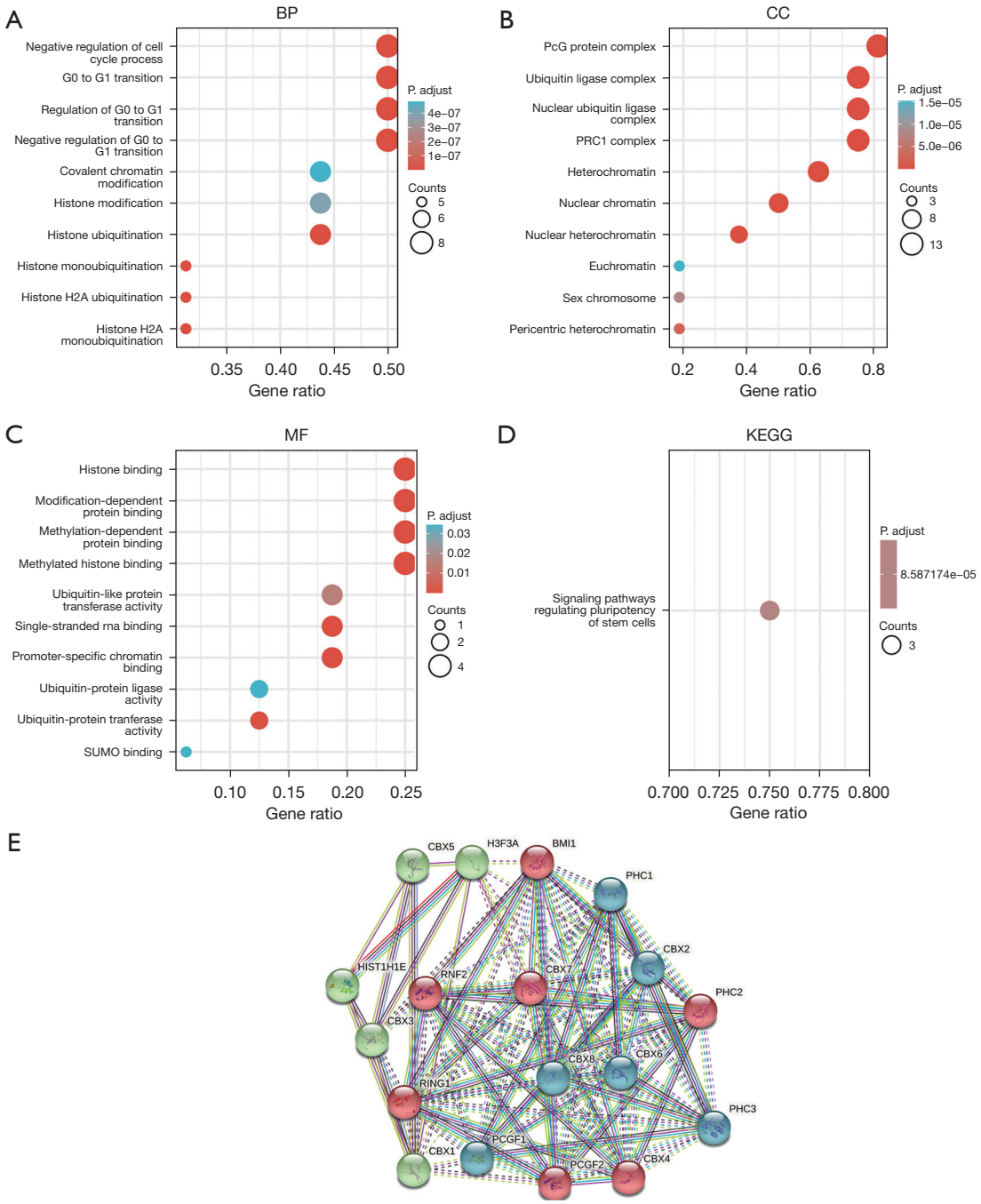

Figure 8 Correlation and functional enrichment analysis of Chromobox (CBX) family genes. (A) Biological process analysis; (B) cellular components; (C) molecular function; (D) Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. (E) Protein–protein interaction network of different expressed CBXs using STRING database.

and CBX3 is overexpressed in human colorectal cancer (31). Zhou *et al.* verified that the higher mRNA expression of CBX3 is an independent prognostic factor for short OS in non-small cell lung cancer patients (32).

However, only a limited number of studies have investigated the significance of CBX3 in PAAD. CBX3 is highly expressed in PAAD patients, and leads to worse OS outcomes. By analyzing the results of post-

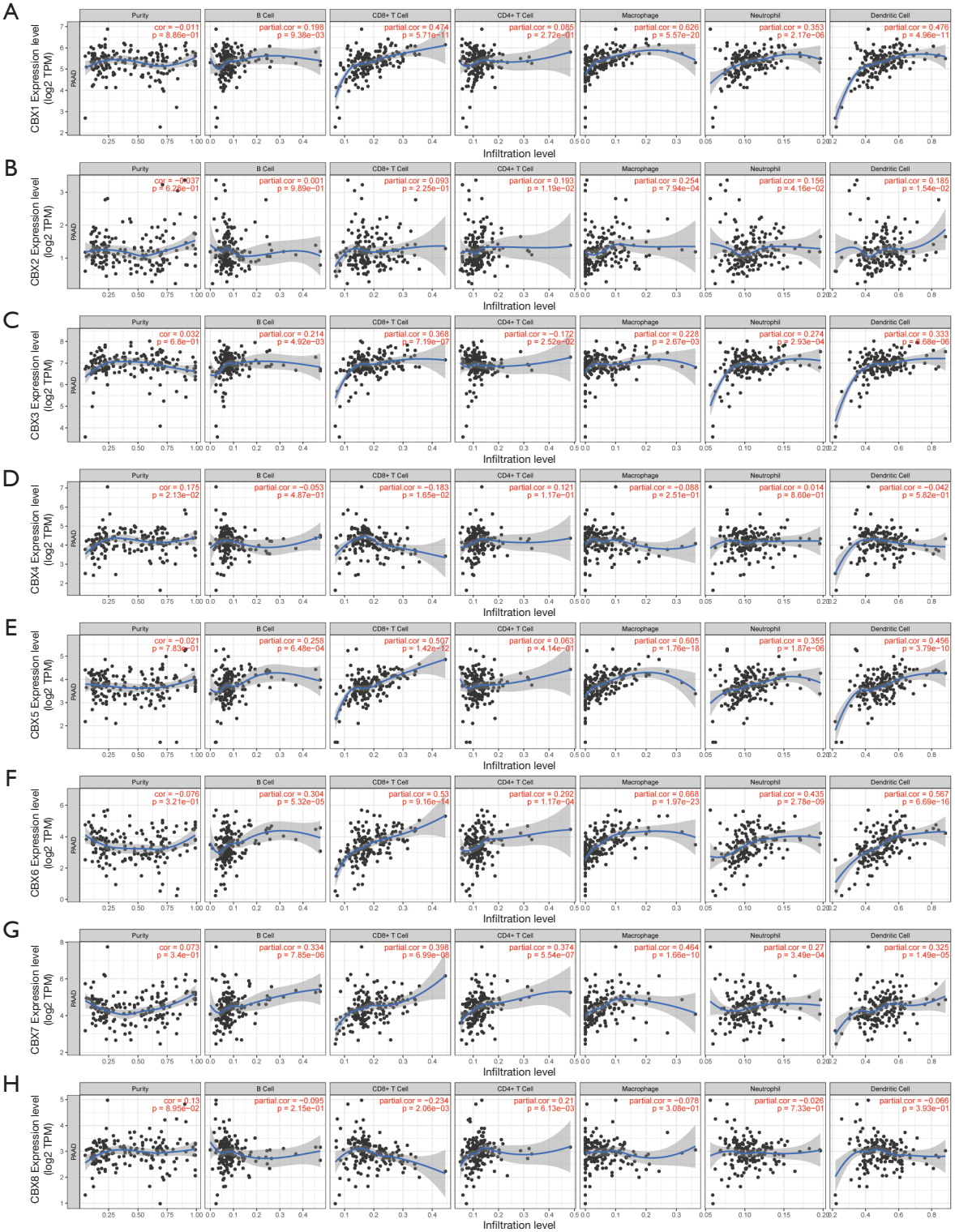

Figure 9 The Correlation between CBXs expression and the abundance of immune cells in PAAD (TIMER). (A) CBX1; (B) CBX2; (C) CBX3; (D) CBX4; (E) CBX5; (F) CBX6; (G) CBX7; (H) CBX8 in PAAD. CBX, Chromobox; PAAD, pancreatic adenocarcinoma.

operative histopathologic specimens in PAAD patients, we demonstrated that CBX3 was markedly overexpressed in PAAD tissues relative to adjacent normal tissues, and the IHC scores of tumor tissues were much higher than those of paracancerous tissues. In our present study, the multivariate analysis also showed that CBX3 was an independent risk factor for unfavorable OS, which indicates that CBX3 is involved in the tumorigenesis of PAAD. These findings support those of Chen *et al.* (33). As to the mechanism by which CBX3 leads to tumor, there are many possibilities. Zhao *et al.* showed that CBX3 is overexpressed in glioma tissues and appears to inhibit proliferation and colony formation abilities by blocking cell arrest at the G0/G1 phase, thereby inducing apoptosis (34). Moreover, an *in-vitro* and *in-vivo* study demonstrated that miR-30a is a tumor-suppressive microRNA that targets CBX3 to suppress colorectal cancer growth (31). Alam *et al.* also revealed that CBX3 suppresses the transcription-repressive regulators of ZBTB7A and NCOR2, leading to pro-tumorigenic gene activation in lung adenocarcinoma (30).

Interestingly, CBX8 appears to play conflicting roles in different types of human cancers. For example, CBX8 expression is elevated in most human malignancies, including brain tumors, breast cancer, and colorectal cancer, and the up-regulation of CBX8 has been shown to be correlated with poor prognosis (9,11,13). Conversely, a small number of studies (35) have drawn the opposite conclusion. The elevation of CBX8 in tumor tissues may be a protective factor. High expressions of CBX8 are associated with low rates of distant metastases and good prognostic outcomes for colorectal cancer patients (35). This is consistent with our findings. Our study revealed the contradiction that although CBX8 expression was elevated in the tumour tissue, the results of the Cox multiple analysis and KM curves suggested that elevated CBX8 reduced the risk of death and prolonged survival in patients. Specifically, our Cox multiple regression analysis showed that CBX8 might be an independent protective factor in the development of pancreatic cancer. Thus, the mechanism of CBX8 needs to be further investigated. According to previous reports, CBX1/2/3/6/8 may all be prognostic biomarkers for survival in tumour patients, for example in renal and hepatocellular carcinoma. However, in PAAD patients, we found that only CBX3/8 is a potential prognostic marker, which may be related to the different role status of the individual components of the CBX family in different tumours, but the exact mechanism needs to be further investigated (13,36,37).

High mutation rates (32.02%) of CBXs in PAAD patients and genetic alterations were associated with short DFS and OS. We established a Gene-gene interaction(GGI) network of CBXs and their 10 frequently altered neighbor genes, and evaluated the significance of CBXs and their neighboring proteins by GO and KEGG pathway enrichment analyses. BPs, such as the negative regulation of the cell-cycle process, CC, such as the PcG protein complex, and MF, such as histone binding, were regulated by CBX mutations in PAAD patients. The KEGG pathway enrichment analysis revealed that the regulating pluripotency of stem cells signaling pathways were significantly enriched. Further, there was an association between CBXs and immune cell infiltrations, such as B cells, $CD8^+$ cells, $CD4^+$ cells, macrophages, neutrophil, and dendritic cells. The association between the expression levels of CBXs and marker genes for PAAD immune cells implies that CBXs may regulate PAAD tumor immunity through multiple immune cell populations.

This study had a number of limitations. First, all the data evaluated in this study were obtained from online databases; thus, our findings should be verified by studies with large sample sizes. Second, we did not evaluate the mechanisms for CBX3 or CBX8, which are potential prognostic indicators in PAAD.

In conclusion, the expression levels of CBX1, CBX3, CBX5, and CBX8 in PAAD are elevated, and CBX3 and CBX8 may play vital roles in PAAD development. Additionally, elevated mutation rates for CBXs in PAAD patients are associated with short DFS and OS. The expression levels of CBXs were markedly correlated with the abundance of 6 immune cell types in PAAD, which suggests that CBXs are involved in the regulation of PAAD tumor immunity. Elevated CBX3 mRNA levels were shown to be an independent prognostic factor for short OS in PAAD patients, while the high expression of CBX8 may be an independent prognostic factor that is beneficial to OS in patients. Future studies should seek to confirm the clinical value of CBXs as immunotherapeutic or prognostic indicators in patients with PAAD.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Ethics Committee of Affiliated Cancer Hospital of Zhengzhou University (No. 2020LP00007), and informed consent was obtained from all the participating patients. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013).

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Supplementary

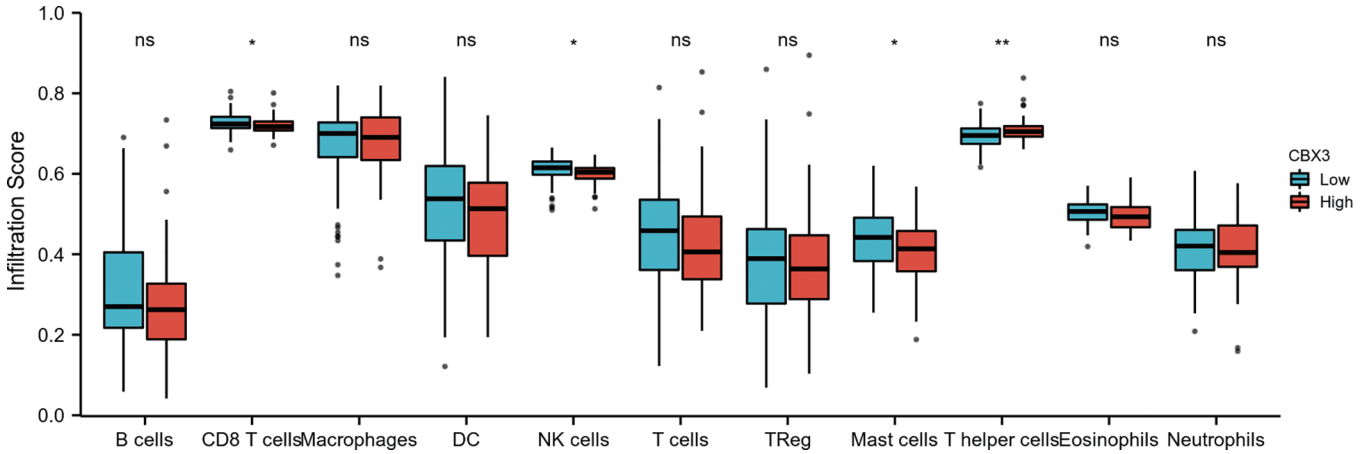

Figure S1 Immune cell infiltration level in the high CBX3 expression group and low CBX3 expression group in TCGA cohort.

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