Comprehensive Analysis of Prognostic and Immune Infiltrates for CBX Family in Human Pancreatic Adenocarcinoma

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Comprehensive Analysis of Prognostic and Immune Infiltrates for CBX Family in Human Pancreatic Adenocarcinoma

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Background: Pancreatic adenocarcinoma (PAAD) is one of the common Malignant tumours of the digestive tract worldwide. Chromobox (CBX) family proteins are important components of epigenetic regulation complexes and play key roles in various biological processes.

However, the expression level, biological function and immune infiltration of different E2Fs in PAAD and prognostic roles of various CBX family members in Pancreatic adenocarcinoma (PAAD) remain unclear.

Methods: In this study, we used multiple bioinformatics tools including Oncomine, GEPIA, Kaplan-Meier plotter, cBioPortal, GeneMANIA, TIMER and R Tools to analyze expression levels, prognostic value, genetic alteration, immune cell infiltration and risk factors of CBXs in Pancreatic adenocarcinoma patients. Furthermore, we confirmed the expression of CBX3 on clinic pathological specimens by immunohistochemistry.

Results: The expression levels of CBX1/3/5/8 were significantly elevated in PAAD tissues, and the high expression of CBX1/5/6/7 was associated with the tumor stage of patients. Higher mRNA expression of CBX2/6/7 and CBX8 were significantly associated with favorable OS of pancreatic cancer patients, while higher expression of CBX3 was significantly associated with short OS. We verified the high expression of CBX3 in pancreatic cancer tissues on clinicopathological specimens. Multivariate logistic analysis demonstrated that high mRNA expressions of CBX3 and low expression of CBX8 were independent risk factors for shorter survival of pancreatic carcinoma patients. The functions of CBXs and its neighboring proteins are mainly related to negative regulation of cell cycle process, PcG protein complex, histone binding and regulating pluripotency of stem cells signaling pathways. We also found that the expression of CBXs was significantly correlated with immune infiltrates.

Conclusions: Our study may provide new insights into the selection of immunotherapeutic targets and prognostic biomarkers and revealed that CBX3/8 may represent a clinically useful biomarker for predicting overall survival in patients with PAAD.

Keywords: pancreatic adenocarcinoma, CBXs, prognostic value, bioinformatics analysis, immune infiltration

INTRODUCTION

Pancreatic adenocarcinoma (PAAD) is one of the most poorly prognosed malignancies of the digestive system[1]. Due to the difficulty of early diagnosis and poor treatment outcomes, only

5% of patients with PAAD survive for more than 5 years, even against a backdrop of huge advances in the diagnosis and treatment of PAAD[2]. These indicate that 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, most patients are diagnosed with PAAD only in the mid to late stages of cancer, losing the opportunity for treatment[5]. Therefore, 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 to improve the treatment outcome and patient survival in PAAD[6].

Chromobox (CBX) family proteins are canonical components of polycomb group (PcG) complexes, which contain a single N-terminal chromodomain[7, 8]. The CBX family of proteins is reported to be involved in various biological processes, such as cell cycle regulation, cell cycle regulation, gene expression, and tumor initiation. To date, CBX proteins have been widely recognized and valued for their role in the development and progression of various tumors[9]. The CBX protein family has eight members and can be divided into two subgroups based on their molecular structure, HP1 group (includes CBX1, CBX3 and CBX5) and Pc group (includes CBX2, CBX4, CBX6, CBX7, and CBX8)[10, 11].

There is growing evidence that the CBX family of proteins plays a critical role in a variety of cancers, and studies have found that abnormal expression of some members of the CBXs family is associated with the prognosis of tumors including breast, liver and gastric cancers[12, 13]. Nevertheless, the functions and prognostic roles of various CBX family members in PAAD remain unclear and elusive. Therefore, we extended the field of study to PAAD by analyzing a series of major databases, with the purpose of determining the potential oncogenic values of distinct CBX family members in PAAD.

MATERIALS AND METHODS

Ethics Statement

The study was approved by the Ethics Committee of Henan Cancer Hospital, and it was conducted according to the principles of the Helsinki Declaration.

Patients and specimens

We evaluated PAAD tissues from 32 surgically resected cases at Henan Cancer Hospital, Zhengzhou, China. Selected pathology specimens in each column include cancerous and paraneoplastic tissue between 2019 and 2020 were examined. None of these patients received any neoadjuvant therapies before surgery, including radiotherapy or chemotherapy. This study was approved by the Ethics Committee of Henan Cancer Hospital and was in accordance with the Declaration of Helsinki.

Immunohistochemistry and IHC score

Immunohistochemistry (IHC) studies were performed following routine procedure. Tissue sections of 3- μ m thickness were dissociated in xylene and dehydrated in ethanol, and primary antibody incubation was performed overnight at 4°C, followed by appropriate secondary antibody incubation for 2 h at room temperature. Primary antibodies used in this study were rabbit anti-CBX3 antibody (diluted 1:400 in PBS; Catalog number: 11650-2-AP, Wuhan Sanying Biotechnology, Wuhan, China). The secondary antibodies used are anti-rabbit HRP-conjugated antibody (Envision System, Catalog number: GK500711, Dako Cytomation, Glostrup, Denmark).

After coverslipping, the positive staining was determined by IHC score evaluation. CBX3 protein expression levels were determined using semi-quantitative as follows: 0 ($\leq 10\%$ positive cells), 1 (11% - 33% positive cells), 2 (34% - 66% positive cells), or 3 ($\geq 67\%$ positive cells), as described previously.

Oncomine Analysis

Oncomine gene expression array dataset (www.oncomine.org) is a publicly accessible online cancer microarray database, which were used to analyze the CBXs transcription levels in different cancers[14]. Student's t test was used to compare the expression levels of CBXs in clinical cancer specimens with those in normal controls. The cut-off of P-value and fold change were defined as 0.01 and 1.5, respectively.

GEPIA Dataset

GEPIA (Gene Expression Profiling Interactive Analysis) is a newly web-based bioinformatics tool that analyzes RNA sequences extracted from TCGA and the GTEx datasets (www.gepia.cancer-pku.cn), which providing differential expression analysis, patient survival analysis, cancer type staging, similar gene detection and so on. The relation between CBXs family and PAAD was performed using the TCGA_PAAD dataset in GEPIA[15].

The Kaplan-Meier Plotter

The prognostic value of the transcription mRNA expression was evaluated using an online database, Kaplan-Meier Plotter (www.kmplot.com), which contained gene expression data and survival information for pancreatic adenocarcinoma patients (PAAD)[16, 17]. To analyze the OS and PPS of patients with PAAD, patient samples were divide into high expression groups and low expression groups according to median expression (high versus low expression) and assessed by a Kaplan-Meier survival plot, with the hazard ratio (HR) with 95% confidence intervals (CIs) and log rank p value.

TCGA Data and cBioPortal

The TCGA database contains both sequencing and pathological data for more than 30 different cancers[18]. The Pancreatic Adenocarcinoma dataset (TCGA, PanCancer Atlas), including data from 184 cases with pathological reports, was selected for further analyses the expression of CBXs using cBioPortal (www.cbioportal.org)[19, 20]. The genomic map includes data for putative copy-number alterations (CNA) from GISTIC, mRNA expression z-scores and mutations.

Enrichment Analysis

Enrichment analysis of CBXs in PAAD was performed using STRINGS[21] and DAVID[22]. We first performed a PPI network analysis on differentially expressed E2Fs using STRINGS. Thereafter, CBXs family and neighboring associated genes were analyzed using DAVID 6.8 and Metascape for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. The results were visualized with R project using a "ggplot2" package and a $p < 0.05$. Biological processes (BPs), cellular components (CCs), and molecular function (MF) were all assessed in the GO enrichment analysis.

TIMER Analysis

TIMER (www.cistrome.shinyapps.io) is a bioinformatics tool that can analysis the abundance of immune infiltrations and evaluate the clinical impact of tumor-infiltrating immune cells[23]. In this study, we used TIMER to assess the correlation between members of CBXs family and immune cell infiltration in PAAD. The analysis was performed based on the TCGA-PAAD dataset and P-value < 0.05 was considered statically significant.

Cox regression analysis

Hazard ratio and P-value of constituents involved in univariate and multivariate Cox regression and some parameters of the CBXs genes. Univariate and multivariate cox regression analysis was performed to identify the proper terms to build the nomogram. The forest was used to show the P value, HR and 95% CI of each variable through 'forestplot' R package.

RESULTS

Differential Expression Levels of CBXs in Patients with Pancreatic Carcinoma

To explore the distinct expression of different CBXs members in pancreatic carcinoma patients, mRNA expression was analyzed by ONCOMINE database. As were shown in Figure 1, the transcriptional levels of CBX1, CBX3, CBX5 and CBX8 were significantly elevated in pancreatic carcinoma tissues, while the transcriptional levels of CBX2 were reduced in normal tissues. For instance, CBX1 was overexpressed in pancreatic carcinoma tissues compared with normal tissues, with a fold change of 1.957 ($p = 2.04E-9$) in Badea Pancreas dataset[24]. CBX3 was also significantly up-regulated in pancreatic carcinoma tissues compared to normal tissues. The results from the Pei Pancreas dataset showed a fold change of 1.678 ($p = 3.32E-5$) increase in CBX3 mRNA expression in pancreatic carcinoma tissues[25]. Significant up-regulation of CBX5 was found in pancreatic carcinoma tissues compared to normal tissues. The result from Ishikawa Pancreas showed that there were 2.371-fold ($p=3.63E-4$) and 22.377- fold ($p=1.65E-4$) increase in CBX5 mRNA expression in pancreatic carcinoma tissues, respectively[26]. CBX8 was also significantly up-regulated in pancreatic carcinoma tissues compared to normal tissues. The results from the TCGA dataset showed a fold change of 2.269 ($p = 0.009$). While down-regulation of CBX2 was also found in pancreatic carcinoma tissues compared to normal tissues. The result from Ishikawa Pancreas dataset showed that there were -1.678-fold ($p=0.006$) decrease in CBX2 mRNA expression.

Association of mRNA Levels of different CBXs family members with clinic pathological parameters of patients with Pancreatic Carcinoma

Using the GEPIA (Gene Expression Profiling Interactive Analysis) dataset, we compared the mRNA expression of CBXs between pancreatic cancer and pancreatic tissues. The results indicated that the expression levels of CBX1, CBX3, CBX5 and CBX8 were higher in pancreatic cancer tissues than in normal tissues (Figure 2A, B). These results were almost consistent with those from Oncomine. We also analyzed the correlation between the expression of differentially expressed CBXs and the pathological stage of pancreatic cancer patients. CBX1, CBX5, CBX6, CBX7 groups significantly varied, whereas CBX2, CBX3, CBX4 and CBX8 groups were not markedly different (Figure 3).

Prognostic value of mRNA expression of CBXs in Pancreatic Carcinoma patients

We further explored the critical efficiency of CBXs in the survival of patients with pancreatic cancer. GEPIA tools were used to analyze the correlation between the mRNA levels of CBXs and the survival of patients with pancreatic cancer by using publicly available datasets. Disease-free survival (DFS) and overall survival (OS) curves are presented in Figure 4. As were shown in Figure, higher mRNA expression of CBX2, CBX6, CBX7 and CBX8 were significantly associated with favorable OS of pancreatic cancer patients, while higher mRNA expression of CBX3 was significantly associated with short DFS and OS.

Independent prognostic value of CBX family expression in terms of OS in Pancreatic Carcinoma patients

Having found that expressions of CBX2/3/6/7/8 were significantly associated with prognosis in

PAAD patients, we next analyzed the independent prognostic value of mRNA expression 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, we found that high pathologic grade (HR=1.452, 95% CI: 1.091-1.933, and $p=0.01052$), high age (HR=1.028, 95% CI: 1.007-1.051, and $p<0.01$), high expressions of CBX3 (HR= 2.004, 95% CI: 1.328-3.025, and $p<0.001$) and low expression of CBX6 (HR= 1.787, 95% CI: 1.247-2.561, and $p=0.002$), 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, and $p=0.015$) were correlated with short survival time of patients with Pancreatic Carcinoma (Figure 5A). Multivariate logistic analysis demonstrated that high mRNA expressions of CBX3 (HR= 1.691, 95% CI: 1.175-2.432, and $p=0.005$) and low expression of CBX8 (HR= 1.300, 95% CI: 1.036-1.631, and $p=0.023$) were independent risk factors for shorter survival of pancreatic carcinoma patients (Figure 5B).

Immunohistochemistry and Semiquantitative evaluation of CBX3 expression

Especially, we used immunohistochemistry (IHC) to detect the protein expression of CBX3 in PAAD and adjacent tissues from 32 surgically resected cases at Henan Cancer Hospital. We found that the protein levels of CBX3 are higher in PAAD tissues than in the adjacent tissues (Figure 6A). In our study, we pooled the IHC scores of both PAAD and adjacent sections. The result demonstrated that the CBX3 expression score in tumor was much higher than in paracancerous tissue (Figure 6B).

Genetic mutations and Interaction Analyses of CBXs in Pancreatic Carcinoma Patients

We analyzed genetic alteration in CBXs and their associations with OS and DFS of pancreatic cancer patients by using the cBioPortal online tool. As was shown in Figure 7B, in the 178 sequenced pancreatic cancer patients, two or more alterations were detected in nearly one-third of the samples (32.02%). In addition, CBX1, CBX4, CBX5, CBX6, CBX7 and CBX8 ranked the highest six genes with genetic alterations, their mutation rates were 11, 9, 8, 8, 8, and 8% of the pancreatic cancer samples, respectively (Figure 7A). The results of Kaplan-Meier plot and Log-rank test show that genetic alteration in CBXs was associated with shorter DFS (Figure 7C, $p=0.0208$) and OS (Figure 7D, $p=0.0232$) of pancreatic cancer patients. These results suggest that genetic alterations in CBXs may also significantly affect the prognosis of pancreatic cancer patients.

Enrichment analyses of CBX family in Pancreatic Carcinoma

Enrichment analysis was performed to explore the role of CBX family in pancreatic carcinoma. We first explored the top ten genes that correlate most highly with each member of CBX family using GEPIA. The GO enrichment analysis and KEGG enrichment analysis of CBX family and associated genes were performed using the R package cluster Profiler. Biological process (BP) analysis revealed that CBX family was associated with Negative Regulation of Cell Cycle Process, G0 to G1 Transition, Regulation of G0 to G1 Transition and Negative Regulation of G0 to G1 Transition (Figure 8A). Cellular component (CC) analysis suggested that CBX family were associated with PcG Protein Complex, Ubiquitin Ligase Complex, Nuclear Ubiquitin Ligase Complex and PRC1 Complex (Figure 8B). In addition, CBX family also prominently affected the molecular function (MF), such as Histone Binding, Modification-Dependent Protein Binding, Methylation-Dependent Protein Binding and Methylated Histone Binding (Figure 8C). As for the KEGG pathways enrichment analysis, our results suggest that CBX family participates in Regulating Pluripotency of Stem Cells Signaling Pathways (Figure 8D). We also performed a PPI

network analysis of CBXs at different transcription levels using STRING (Figure 8E).

Immune infiltrations analysis of CBX family in Pancreatic Carcinoma Patients

To investigate the relationship between immune cell level and proliferation and progression of cancer cell, The TIMER database was used to investigate the immune cell infiltration in CBX family. As shown in Figure 9A, CBX1 showed significant correlation with the abundance of B cell ($\text{cor}=0.198$, $P=9.38\text{e-}03$), CD8+ cell ($\text{cor}=0.474$, $P=5.71\text{e-}11$), macrophage ($\text{cor}=0.626$, $P=5.57\text{e-}20$), neutrophil ($\text{cor}=0.353$, $P=2.17\text{e-}06$), and dendritic cell ($\text{cor}=0.476$, $P=4.96\text{e-}11$). CBX2 was positively associated with the macrophage, neutrophils and dendritic cell in pancreatic carcinoma patients (Figure 9B). With regard to CBX3, CBX6 and CBX7, all six host immune cells (B cell, CD8+ cell, CD4+ cell, macrophage, neutrophil, and dendritic cell) have a positive correlation in pancreatic carcinoma patients (Figure 9C, F, G). Except for CD4 cell, CBX5 was positively correlated with the infiltration of the all immune cell types. Interestingly, the expression of CBX4 and CBX8 was negatively correlated with the infiltration of CD8+ T cells (Figure 9E).

DISCUSSION

Studies have shown that the dysregulation of CBXs family is significantly correlated with the occurrence and development of many tumors. Over-expression of CBXs had been found in hepatocellular carcinoma, castration-resistant prostate cancer and breast cancers [27-29]. Clermont et al. found that CBX2 mRNA expression in many cancers was higher than that in normal tissues through a genotranscriptomic meta-analysis [30]. Although it has already been demonstrated that CBXs is closely associated with multiple tumors, the prognostic role of CBXs expression in PAAD has yet to be fully elucidated.

The present study is the first time to investigate the mRNA expression and prognostic values of different CBXs in PAAD and their relationship with the prognosis and immune infiltration of PAAD patients through bioinformatics analysis. We first detected the expression pattern of CBXs family members in PAAD. CBX1, CBX3, CBX5 and CBX8 were found to be up regulated in pancreatic cancer tissues. Besides, higher mRNA expressions of CBX2/6/7/8 were significantly associated with favorable OS of pancreatic cancer patients. Interestingly, higher mRNA expression of CBX3 was significantly related to short DFS and OS. Multivariate analysis showed that high mRNA expressions of CBX3 were independent prognostic factors for shorter OS of PAAD patients. In previous studies, over-expressed of CBX3 have been proved to play important roles in a variety of cancers. Studies from Alam et al. showed that CBX3 was highly expressed in LUAD, and overexpression of CBX3 is associated with poor prognosis [31]. Liu et al demonstrated that CBX3 over-expression promoted cell proliferation of colorectal cancer cell lines by directly regulating CDKN1A and was over-expressed in human colorectal cancer [32]. Zhou et al further confirmed that higher mRNA expression of CBX3 is an independent prognostic factor for shorter OS of NSCLC [33].

However, there were few articles on the role of CBX3 in PAAD has been published. The results from our analysis revealed that the expression of CBX3 is elevated in PAAD patients and high expression of CBX3 leads to worse OS in patients. By analyzing the results of post-operative histopathologic specimen in patients with PAAD, we demonstrated that CBX3 was significantly overexpressed in PAAD tissues compared with adjacent normal tissue and the IHC scores in tumor was much higher than in paracancerous tissue. In our present study, multivariate analysis also showed that CBX3 was an independent risk factor for unfavorable OS, indicating CBX3 took part in the tumorigenesis of PAAD, which was in accordance with the findings of Lian-Yu '

studies[34].As for the mechanism of how CBX3 could lead to tumor, there are many possibilities. Studies from Shu et al showed that CBX3 was demonstrated to be highly expressed in human glioma tissues and it suppress the proliferation and colony formation ability by blocking cell arrest at G0/G1 phase and inducing apoptosis[35].Moreover, In vitro and vivo study demonstrated that miR-30a as a tumor-suppressive microRNA that targets CBX3 could suppress the growth of colorectal cancer in mouse models[36].Alam et al. also revealed that CBX3 suppressed the transcription-repressive regulators NCOR2 and ZBTB7A, which lead to activation of protumorigenic genes in lung adenocarcinoma[37].

Interestingly, there are conflicting roles for CBX8 in different types of human cancers. On the one hand, CBX8 expression is elevated in most human malignancies, including brain tumours, breast cancer and colorectal cancer. Up-regulation of CBX8 had been shown to correlate with poor prognosis. On the other hand, however, we found a small number of studies that came to the opposite conclusion. The elevation of CBX8 in tumour tissue may be a protective factor. One study found that high expression of CBX8 was associated with a low rate of distant metastases and a good prognosis for CRC patients[38].This is consistent with our findings, which COX multiple regression analysis showed that CBX8 may be an independent protective factor for the development of pancreatic cancer, and the mechanism of CBX8 needs to be further investigated.

Furthermore, a high mutation rate (32.02%) of CBXs was noticed in PAAD patients and the genetic alteration was associated with shorter DFS and OS. We also constructed a GGI network of CBXs and its 10 frequently altered neighbor genes, and analyzed the function of CBXs and its neighboring proteins by GO enrichment analysis and KEGG pathway enrichment. Biological processes such as Negative Regulation of Cell Cycle Process, Cellular component (CC) such as PcG Protein Complex, molecular function (MF) such as Histone Binding, were remarkably regulated by the CBXs mutations in PAAD patients. KEGG pathway enrichment analysis revealed that the Regulating Pluripotency of Stem Cells signaling pathways were significantly enriched. Our study also revealed the association between CBXs and immune cell infiltrations, including B cell, CD8 + cell, CD4 + cell, macrophage, neutrophil, and dendritic cell. The correlation between CBXs expression and the marker genes of PAAD immune cells indicates that CBXs may regulate PAAD tumor immunity through multiple immune cell populations.

There were some limitations in our study. First, although high/low expressions of CBX3/8 were independent prognostic factors for shorter OS in pancreatic carcinoma patients, all data analyzed in this study were obtained from online databases, so further studies with larger sample sizes are needed to validate our findings. Second, although we found that CBX3/8 may be used as a prognostic indicator of patients with PAAD, We have not explored in depth the specific mechanisms.

In conclusion, our research indicates that the expression levels of CBX1/3/5/8 are obviously up-regulated in PAAD and CBX3/8 may play a significant role in the occurrence and development of PAAD. Moreover, high mutation rate of CBXs was also observed in in PAAD patients and was associated with shorter OS and DFS. The expression of CBXs correlated significantly with the infiltration of six immune cell types in PAAD, suggesting that CBXs may be involved in the regulation of PAAD tumour immunity. Multivariate analysis showed that high mRNA expression of CBX3 was an independent prognostic factor for shortened OS in PAAD patients, while high expression of CBX8 may be an independent prognostic factor for beneficial OS in patients. However, more experimental studies are needed to confirm the clinical value of CBXs as an

immunotherapeutic or prognostic indicator in patients with PAAD.

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Statement of Ethics

This article was approved by the Ethics Committee of Henan Cancer Hospital and informed consent was obtained from participating patients.

Disclosure Statement, Funding Sources

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Data repositories

Readers could get specific data information through my email if readers need.

Author Contribution Statement

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

Conflict of interest statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work.

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Figures

Figure 1

As were shown in Figure 1, the transcriptional levels of CBX1, CBX3, CBX5 and CBX8 were significantly elevated in pancreatic carcinoma tissues, while the transcriptional levels of CBX2 were reduced in normal tissues.

A

B

Figure 2

Using the GEPIA (Gene Expression Profiling Interactive Analysis) dataset, we compared the mRNA expression of CBXs between pancreatic cancer and pancreatic tissues. The results indicated that the expression levels of CBX1, CBX3, CBX5 and CBX8 were higher in pancreatic cancer tissues than in normal tissues (Figure 2A, B).

Figure 3

These results were almost consistent with those from Oncomine. We also analyzed the correlation between the expression of differentially expressed CBXs and the pathological stage of pancreatic cancer patients. CBX1, CBX5, CBX6, CBX7 groups significantly varied, whereas CBX2, CBX3, CBX4 and CBX8 groups were not markedly different (Figure 3).

Figure 4

Disease-free survival (DFS) and overall survival (OS) curves are presented in Figure 4. As were shown in Figure, higher mRNA expression of CBX2, CBX6, CBX7 and CBX8 were significantly associated with favorable OS of pancreatic cancer patients, while higher mRNA expression of CBX3 was significantly associated with short DFS and OS.

A

B

Figure 5

In the univariate analysis, we found that high pathologic grade (HR=1.452, 95% CI: 1.091-1.933, and $p=0.01052$), high age (HR=1.028, 95% CI: 1.007-1.051, and $p<0.01$), high expressions of CBX3 (HR= 2.004, 95% CI: 1.328-3.025, and $p<0.001$) and low expression of CBX6 (HR= 1.787, 95% CI: 1.247-2.561, and $p=0.002$), 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, and $p=0.015$) were correlated with short survival time of patients with Pancreatic Carcinoma(Figure 5A).Multivariate logistic analysis demonstrated that high mRNA expressions of CBX3 (HR= 1.691, 95% CI:1.175-2.432, and $p=0.005$) and low expression of CBX8 (HR= 1.300,95% CI: 1.036-

1.631, and $p=0.023$) were independent risk factors for shorter survival of pancreatic carcinoma patients(Figure 5B).

Figure 6

We found that the protein levels of CBX3 are higher in PAAD tissues than in the adjacent tissues (Figure 6A). In our study, we pooled the IHC scores of both PAAD and adjacent sections. The result demonstrated that the CBX3 expression score in tumor was much higher than in paracancerous tissue (Figure 6B).

Figure 7

We analyzed genetic alteration in CBXs and their associations with OS and DFS of pancreatic cancer patients by using the cBioPortal online tool. As was shown in Figure 7B, in the 178 sequenced pancreatic cancer patients, two or more alterations were detected in nearly one-third of the samples (32.02%). In addition, CBX1, CBX4, CBX5, CBX6, CBX7 and CBX8 ranked the highest six genes with genetic alterations, their mutation rates were 11, 9, 8, 8, 8, and 8% of the pancreatic cancer samples, respectively (Figure 7A). The results of Kaplan-Meier plot and Log-rank test show that genetic alteration in CBXs was associated with shorter DFS (Figure 7C, $p=0.0208$) and OS (Figure 7D, $p=0.0232$) of pancreatic cancer patients. These results suggest that genetic alterations in CBXS may also significantly affect the prognosis of pancreatic cancer patients.

Figure 8

Biological process (BP) analysis revealed that CBX family was associated with Negative Regulation of Cell Cycle Process, G0 to G1 Transition, Regulation of G0 to G1 Transition and Negative Regulation of G0 to G1 Transition (Figure 8A). Cellular component (CC) analysis suggested that CBX family were associated with PcG Protein Complex, Ubiquitin Ligase Complex, Nuclear Ubiquitin Ligase Complex and PRC1 Complex (Figure 8B). In addition, CBX family also prominently affected the molecular function (MF), such

as Histone Binding, Modification-Dependent Protein Binding, Methylation-Dependent Protein Binding and Methylated Histone Binding(Figure 8C). As for the KEGG pathways enrichment analysis, our results suggest that CBX family participates in Regulating Pluripotency of Stem Cells Signaling Pathways(Figure 8D). We also performed a PPI network analysis of CBXs at different transcription levels using STRING (Figure 8E).

Figure 9

To investigate the relationship between immune cell level and proliferation and progression of cancer cell, The TIMER database was used to investigate the immune cell infiltration in CBX family. As shown in Figure 9A, CBX1 showed significant correlation with the abundance of B cell ($\text{cor}=0.198$, $P=9.38\text{e-}03$), CD8+ cell ($\text{cor}=0.474$, $P=5.71\text{e-}11$), macrophage ($\text{cor}=0.626$, $P=5.57\text{e-}20$), neutrophil ($\text{cor}=0.353$, $P=2.17\text{e-}06$), and dendritic cell ($\text{cor}=0.476$, $P=4.96\text{e-}11$). CBX2 was positively associated with the macrophage, neutrophils and dendritic cell in pancreatic carcinoma patients (Figure 9B). With regard to CBX3, CBX6 and CBX7, all six host immune cells (B cell, CD8 + cell, CD4 + cell, macrophage, neutrophil, and dendritic cell) have a positive correlation in pancreatic carcinoma patients (Figure 9C, F, G). Except for CD4 cell, CBX5 was positively correlated with the infiltration of the all immune cell types. Interestingly, the expression of CBX4 and CBX8 was negatively correlated with the infiltration of CD8+ T cells (Figure 9E).