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Systematic investigation of the clinical significance and prognostic value of the CBXs in esophageal cancer

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Abstract

Esophageal cancer (ESCA), one of the most aggressive malignant tumors, has been announced to be the ninth most common cancer and the sixth leading cause of cancer-related death in the world. Chromobox family members (CBXs) are important epigenetic regulators which are related with the transcription of target genes. The role of CBXs in carcinomas has been reported in many studies. However, the function and prognostic value of different CBXs in ESCA are still largely unknown. In this article, we first performed differential expression analysis through several methods including Oncomine and Gene Expression Profiling Interactive Analysis. The results led us to determine the differential expression of CBXs in pan-cancer, especially ESCA. Then we evaluated the prognostic value of different CBX messenger RNA (mRNA) expression in patients with ESCA through the Kaplan–Meier plotter and the Human Protein Atlas database. In addition, we used cBioPortal to explore all genetic alterations and mutations in the CBXs in ESCA. Simultaneously, the correlation between its expression and the level of immune infiltration of ESCA was visualized by TIMER. Finally, the biological function of CBXs in ESCA is obtained through Biological Enrichment Analysis including gene ontology and Kyoto Encyclopedia of Genes and Genomes. The expression levels of CBX3/4/5 and CBX8 in ESCA tissues increased significantly and the expression level of CBX7 decreased through differential expression analysis. Additionally, CBX1 is significantly related to the clinical cancer stage and disease-free survival of ESCA patients. The high mRNA expression of CBX4 is related to the short overall survival of patients with esophageal squamous cell carcinoma, and the high mRNA expression of CBX3/7/8 is related to the short overall survival of patients with esophageal adenocarcinoma, indicating that CBX1/3/4/7/8 may be a potential prognostic biomarker for the survival of ESCA patients. Besides, the expression of CBXs is significantly related to the infiltration of a variety of immune cells, including six types of CD4-positive T-lymphocytes, macrophages, neutrophils, bursindependentlymphocyte, CD8-positive T-lymphocytes cells and dendritic cells in ESCA. Moreover, we found that CBXs are mainly associated with the inhibition of cell cycle and apoptosis pathway. Further, enrichment analysis indicated that CBXs and correlated genes were enriched in mismatch repair, DNA replication, cancer pathways, and spliceosomes. Our research may provide new insights into the choice of prognosis biomarkers of the CBXs in ESCA.

Abbreviations: B cells = bursindependentlymphocyte, BP = biological processes, CBXs = Chromobox family members, CRC = colorectal cancer, CD4+ T cells = CD4-positive T-lymphocytes, DFS = disease-free survival, EAC = esophageal adenocarcinoma, ESCA = esophageal cancer, ESCC = Esophageal squamous cell carcinoma, GEPIA = the Gene Expression Profiling Interactive Analysis, GO = gene ontology, HR = hazard ratio, IHC = immunohistochemistry, KEGG = Kyoto Encyclopedia of Genes and Genomes, mRNA = messenger RNA, OS = overall survival, TCGA = the cancer genome atlas.

Keywords: bioinformatics analysis, CBX family, esophageal cancer, prognosis biomarker

1. Introduction

The Chromobox family members (CBXs) are named after the Chromobox encoded at its N terminus, which recognize and bind the modified H3K27me3 that defines PcG domains.[1,2] The proteins of CBXs involved in various biological processes (BPs) are concerning gene expression, cell fate determination and developmental programs.[3,4] In published researches, members of the CBX family were found to have prognostic values in many cancers. For example, CBX7 protein expression was significantly

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are publicly available.

TCGA and GTEx belong to public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues and other conflicts of interest.

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higher in normal adjacent cervical tissues and was not detected in cervical squamous cell carcinoma and endocervical adenocarcinoma tissues.[5] In addition, CBX3 was significantly elevated with the colorectal cancer (CRC) stage and short disease-free survival (DFS) in CRC patients.[6] Li et al[7] also found that compared with normal breast tissue, the expression of CBX7 in breast cancer tissue was down-regulated, and the expression of CBX2 is closely related to tumor stage. Then, another article showed that high messenger RNA (mRNA) expression levels of CBX3/8 were independent prognostic factors for prolonged overall survival (OS) in gastric cancer patients.[8] Additionally, it has been also known that CBX1 promotes chromatin changes to initiate DNA damage responses.[9] Obviously, these indicate that CBXs are indeed important for the research and treatment of some cancers.

Esophageal cancer (ESCA), one of the most aggressive malignant tumors, has been announced to be the ninth most common cancer and the sixth leading cause of cancer-related death in the world.[10] The main histological subtypes of ESCA include Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). The epidemiology of ESCA is eye-catching because it is the main histological type in the Western world, and the incidence of ESCA is steadily increasing.[11] It is believed that ESCA has a complex molecular mechanism to evade apoptosis by down-regulating Bax, up-regulating Bcl-2, Bcl-xl and Survivin, p53 mutations and changes in Fas expression.[12] Although there are many treatment modes for ESCA such as surgical, chemotherapy, etc., and great progress has been made, the prognosis is still very poor.[13] Among them, esophagectomy is still the most effective treatment for ESCA. However, esophagectomy is associated with a variety of postoperative complications, which will increase the risk of myocardial infarction.[14] With the development of emerging technologies such as transcriptome sequencing, deep neural networks, and gene editing technologies, unprecedented changes in bioinformatics have allowed us to study the occurrence and development of diseases in a deeper level. Therefore, comprehensive application of bioinformatics methods may discover the mechanism of occurrence and development of ESCA, screen out appropriate biomarkers and guide clinicians to diagnose and treat patients. Then, an optimal management that facilitates early detection and improvement of treatment is imperative.

Consequently, in view of the increasing role of CBXs in a variety of tumors including ESCA, at present, different methods were used to explore the mechanism of different CBXs in ESCA. Through differential expression analysis, we found that CBXs are differentially expressed in pan-cancer including ESCA. Then, the prognostic value of different CBXs mRNA expression were evaluated in ESCA patients by Kaplan–Meier plotter and the Human Protein Atlas database, showing the clinical significance of CBXs in ESCA treatment. In addition, cBioPortal were used to explore all the genetic changes and mutations of CBXs in ESCA. At the same time, the roles of CBXs in the immune microenvironment are also studied through TIMER. Finally, we constructed a protein-protein interaction (PPI) for CBX gene and bioinformatics to predict the biological function of CBX in ESCA. Therefore, this study will help to understand the molecular mechanism of the occurrence and development of ESCA, so that clinicians can more accurately carry out the prognosis and treatment of patients with ESCA.

2. Materials and Methods

2.1. Differential expression analysis

Oncomine were used to analyze the transcription levels of CBXs in various cancers.[15] The mRNA expressions of CBXs in cancer tissues specimens were compared with those in normal sample.

Further, the Gene Expression Profiling Interactive Analysis (GEPIA) was carried out to perform the differential expression, pathological stage and related prognostic analysis of CBXs in ESCA. As a newly bioinformatics analysis tool, GEPIA contains RNA expression data and provides fast and customizable functions with the data from The Cancer Genome Atlas (TCGA) and the Genotypic Tissue Expression.[16] Student's *t* test was used to generate *P* values for expression or pathological stage analysis. In addition, Kaplan–Meier curves were employed for patient survival analysis.

2.2. Prognostic analysis

Kaplan–Meier plotter was evaluated the prognostic value of the mRNA expression of distinct CBXs in ESCA patients, which contained gene expression data and survival information of ESCA patients.[17] The results are displayed in a survival curve plot using a hazard ratio (HR) with a 95% confidence interval and a log-rank *P* value.

Then, the Human Protein Atlas database (https://www.proteinatlas.org/) was used to detect the protein expression of CBXs in normal tissues and tumor tissues to explore the clinical prognostic value of CBXs for ESCA.

2.3. Analysis of gene alteration, expression and cancer pathway activity

Additionally, we carried out the analysis of genetic alterations and mutations in the CBXs gene in ESCA using CBioPortal, which is used to analyze multidimensional cancer genomics data with a visual and multidimensional way.[18] Moreover, the cancer pathway activity of the TCGA_ESCA datasets was also analyzed by employing GSCALite (http://bioinfo.life.hust.edu.cn/web/GSCALite/), a web-based analysis tool for gene set cancer analysis.

2.4. Biological enrichment analysis

GeneMANIA, a comprehensive and accurate website, is used to evaluate the function of gene lists and weight the predicted value of CBXs.[19] Herein, we used GeneMANIA to find the neighboring genes related to gene lists.

Then DAVID (https://david.ncifcrf.gov/) was used to carry out the biological enrichment analysis of the CBXs, including the analysis of gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. GO enrichment analysis reveals that the functional role of target host genes from three aspects: BP, cellular component, and molecular function. We first extracted the top 100 genes related to each member of the CBX family in GEPIA and sorted out the 50 most suitable genes. Afterwards, the CBXs and related genes were submitted to DAVID. We used the R project "ggplot2" package to visualize the results.

2.5. Immune microenvironment analysis

TIMER is an immune infiltrates analysis approach for multiple analysis modules to comprehensively evaluated the infiltration of various immune cells and clinical affect.[20] Presently, CBXs was selected to input *via* "Gene module" and generated the scatterplots to visualize the correlation of its expression with immune infiltration level in ESCA.

2.6. Statistical analysis

The result generated in Oncomine is displayed along with the *P* value, multiple change and level. The results of the Kaplan–Meier plot and GEPIA are displayed along with the likelihood

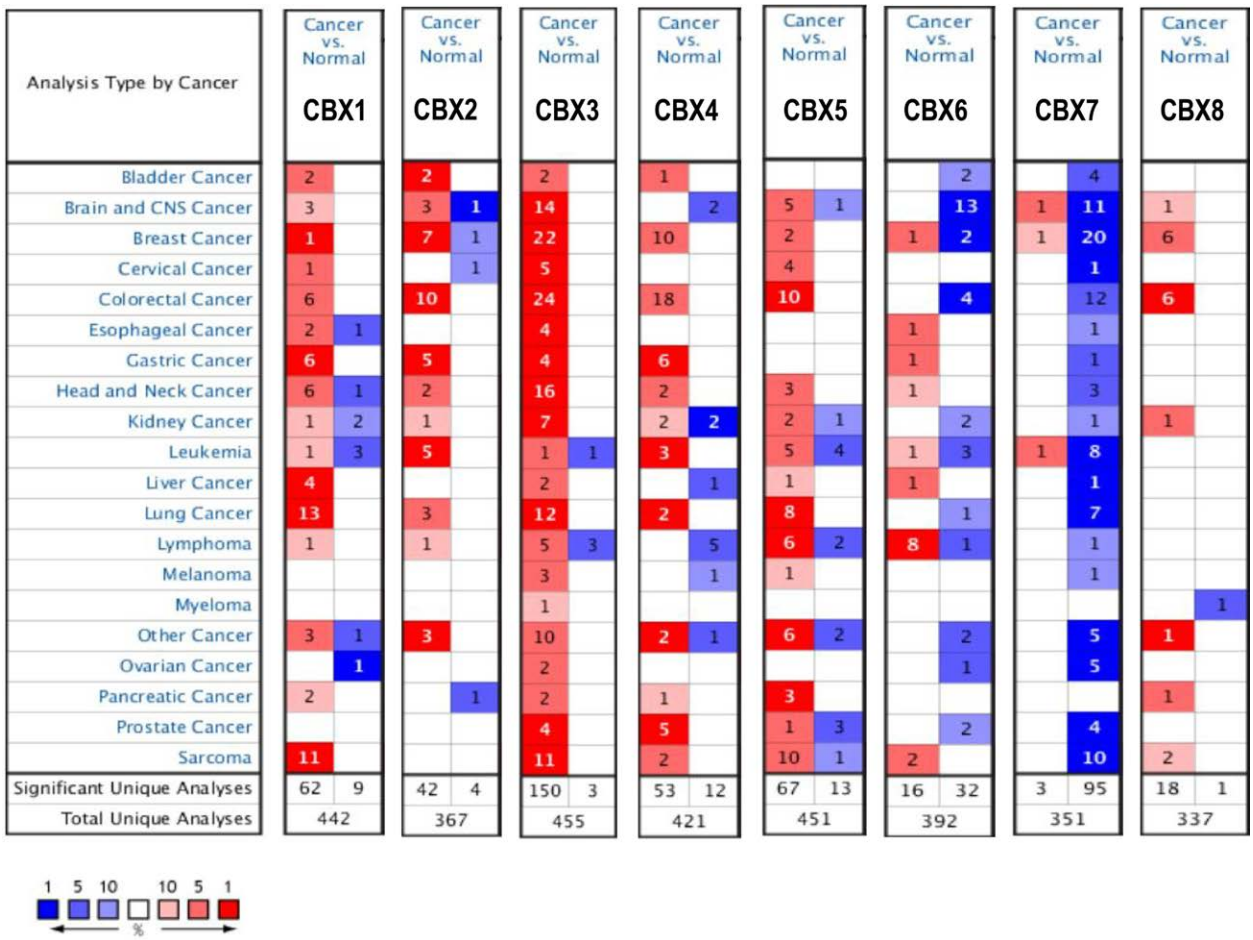

Figure 1. mRNA expression of CBXs in ESCA (Oncomine). This figure shows the difference of the transcriptional expression of CBXs in cancers: up-regulation (red) and down-regulation (blue) with the following criteria: *P* value = .05, fold Change = 1.5, gene rank = 10%, data type: mRNA. As the picture shows, the transcriptional levels of CBX1, CBX3, and CBX6 were raised, while the level of CBX7 was significantly reduced in ESCA tissues compared with the normal. CBXs = chromobox family members, ESCA = esophageal cancer, mRNA = messenger RNA.

ratio and P or Cox proportional hazards regression values from the log-rank test. The significance levels of Spearman's rank correlation coefficient were calculated using a large sample normal theory approximation that adopts a t-distribution. *P* value < .05 were considered statistically significant.

3. Results

3.1. The expression levels of the CBXs in ESCA patients

We firstly used Oncomine to explore the expression levels of the CBXs in ESCA. The results are depicted in Figure 1 and Table 1. Obviously, the transcriptional levels of CBX1, CBX3 and CBX6 were raised, while the level of CBX7 was significantly reduced in ESCA tissues compared with the normal group (Fig. 1). In detail, the expression level of CBX1 was upregulated in ESCA tissues vs normal tissues (fold change = 1.662), while that of CBX3 was significantly overexpressed in ESCA vs normal tissues. The results based on the data acquired from the dataset showed a fold change of 2.188 (*P* = 3.75E-29) (Table 1).

Afterwards, the GEPIA dataset was used to compare the mRNA levels of CBXs between ESCA and the normal groups. The results show that the transcriptional levels of CBX3 and CBX5 were higher in ESCA patients compared with normal tissues, and the expression levels of CBX7 were highly lower in the former compared with the control group (Fig. 2). We later evaluated the relation between the expression levels of CBXs and the pathological stages of ESCA patients. Clearly, the CBX1 group was significantly varied, while the CBX2, CBX3, CBX4, CBX5, CBX6, CBX7, and CBX8 groups were not obviously different (Fig. 3), demonstrating that that CBX1 is significantly associated with the progression of ESCA. Consequently, CBX1 might be considered as a pathological stage marker.

Table 1

The mRNA levels of CBXs in different types of ESCA and normal tissues.

Name	ESCA vs normal	Fold change	*P* value	*t* test	Source (Oncomine)
CBX1	ESCC	1.662	5.56E-13	8.101	Su Esophagus
	EAC	3.301	2.78E-04	3.72	Hao Esophagus
	ESCC	1.77	2.50E-04	4.158	Hu Esophagus
CBX2	ESCC	2.308	5.78E-04	6.846	Su Esophagus
CBX3	ESCC	2.188	3.75E-29	15.587	Su Esophagus
	ESCC	2.389	3.21E-10	10.596	Hu Esophagus
	EAC	2.559	1.20E-04	4.887	Kimchi Esophagus
	Barrett's Esophagus	1.629	1.06E-05	5.127	Kim Esophagus
CBX6	ESCC	2.101	1.43E-06	6.336	Hu Esophagus

CBXs = chromobox family members, EAC = esophageal adenocarcinoma, ESCA = esophageal cancer, ESCC = esophageal squamous cell carcinoma, mRNA = messenger RNA.

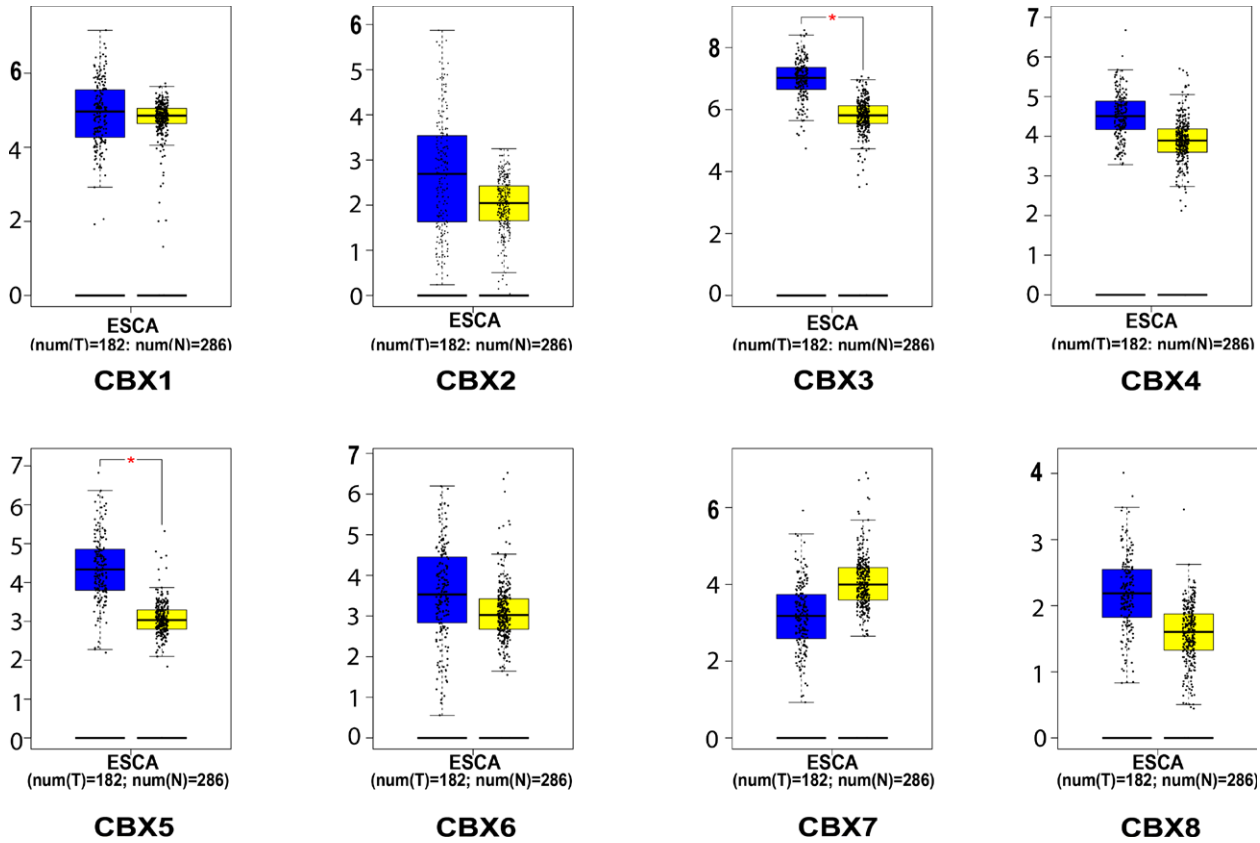

Figure 2. Transcriptional expression of CBXs in ESCA (GEPIA). The box plots show that the transcriptional levels of CBX3 and CBX5 were higher in ESCA patients compared with normal tissues, and the expression levels of CBX7 were highly lower in the former compared with the normal. CBXs = chromobox family members, ESCA = esophageal cancer.

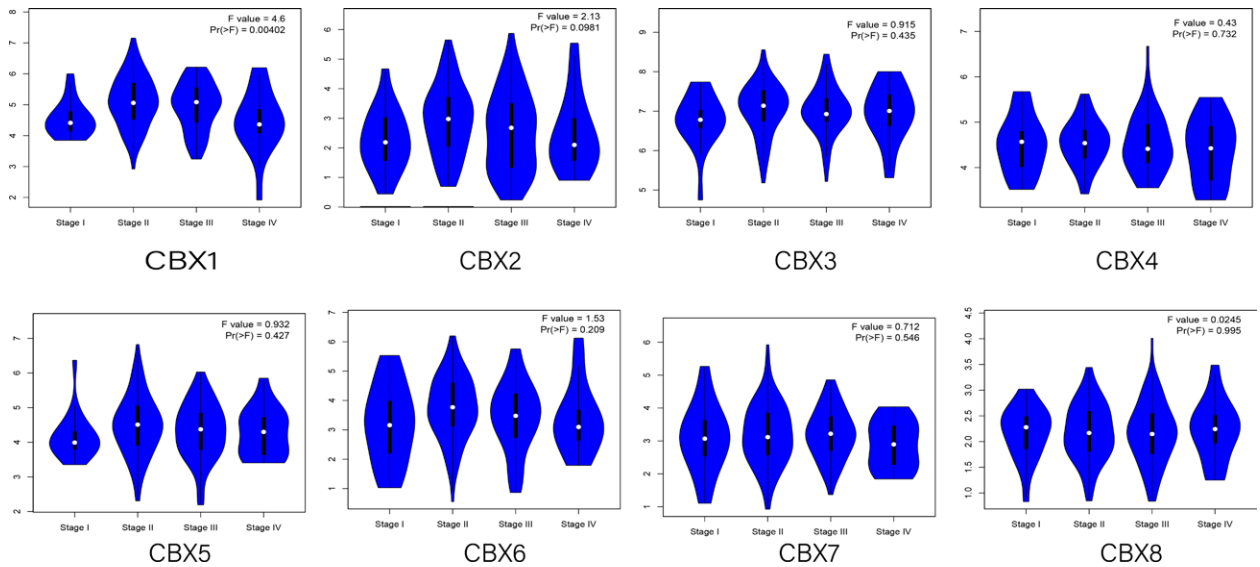

Figure 3. Correlations between CBXs expression and tumor stage in ESCA (GEPIA). Violin chart derived from the correlations between the expression of CBXs member and the tumor stage of ESCA, with *P* value = .05. CBXs = chromobox family members, ESCA = esophageal cancer, mRNA = messenger RNA.

3.2. Prognostic value of the CBXs in ESCA patients

In order to assess the prognostic value of the CBX members in the progression of ESCA, GEPIA was used to analyze the correlations between different CBXs and the clinical outcomes. The DFS and OS curves are shown in Figure 4. The transcription level of CBX1 (P = .044) was significantly correlated with short DFS. Except for CBX1, other CBX protein members have little effect on OS or DFS.

Then, the Kaplan–Meier plotter was used to analyze the prognostic values of CBXs in ESCC patients and EAC patients, which is a subgroup analysis (Figs. 5 and 6). It is obviously

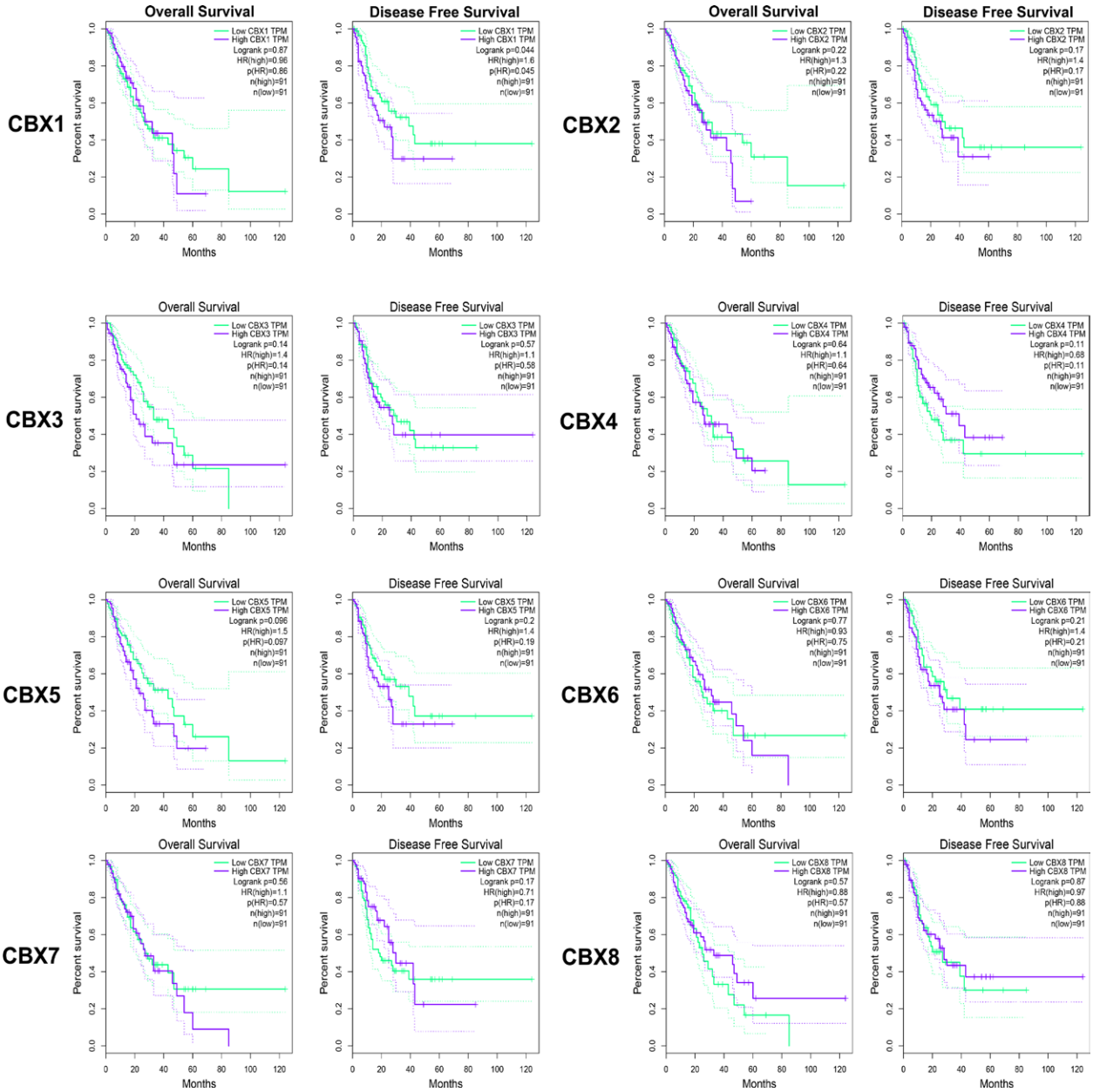

Figure 4. Prognostic value of the mRNA expression of CBXs in ESCA (GEPIA). The transcription level of CBX1 ($P = .044$) in ESCA patients was significantly correlated with short DFS. CBXs = chromobox family members, DFS = disease-free survival, ESCA = esophageal cancer, mRNA = messenger RNA.

observed that high CBX4 (HR = 2.93, $P = .008$) mRNA expression was significantly associated with short OS in patients with ESCC (Fig. 5). Consistently, CBX3 (HR = 3.12, $P = .00028$) and CBX8 (HR = 2.27, $P = .035$) mRNA expressions were highly associated with short OS in patients with EAC (Fig. 6). All these show that CBX3/4/8 have a significant effect on the prognosis of ESCA, and may provide some ideas for the research and treatment of ESCA.

In addition, in order to further explore the prognostic value of CBXs genes with prognostic value, we performed immunohistochemistry (IHC) to detect the protein expression of CBX1, CBX3 and CBX5 in normal tissues and tumor tissues. We found that the expressions of CBX1, CBX3 and CBX5 proteins in ESCA tissues are higher than that in normal tissues (Fig. 7). Therefore, we can reasonably infer that these genes play key roles in protein expression and may affect the occurrence and development of ESCA.

3.3. Genetic alteration, expression, and cancer pathway activity of CBXs in ESCA patients

To analyze the genetic alteration of CBXs in the patients with ESCA, we detected two or more alterations of CBXs in the two subtypes of ESCC and EAC in ESCA. The results show that the alterations frequency of CBXs were higher in ESCC samples (Fig. 8A). In addition, CBXs were altered in 138 samples of 927 ESCA patients, accounting for 15% (Fig. 8B). Among them, CBX3 and CBX8 are the two most frequently mutated genes.

Then, we used GSCALite to evaluate the role of the CBXs in ESCA pathway activity. And we found that most members of CBXs are involved in the activation of DNA damage response, epithelial-mesenchymal transition and hormone pathways. Also, we found that CBX6/7 are mainly related to the inhibition of cell cycle and apoptosis pathway (Fig. 8C).

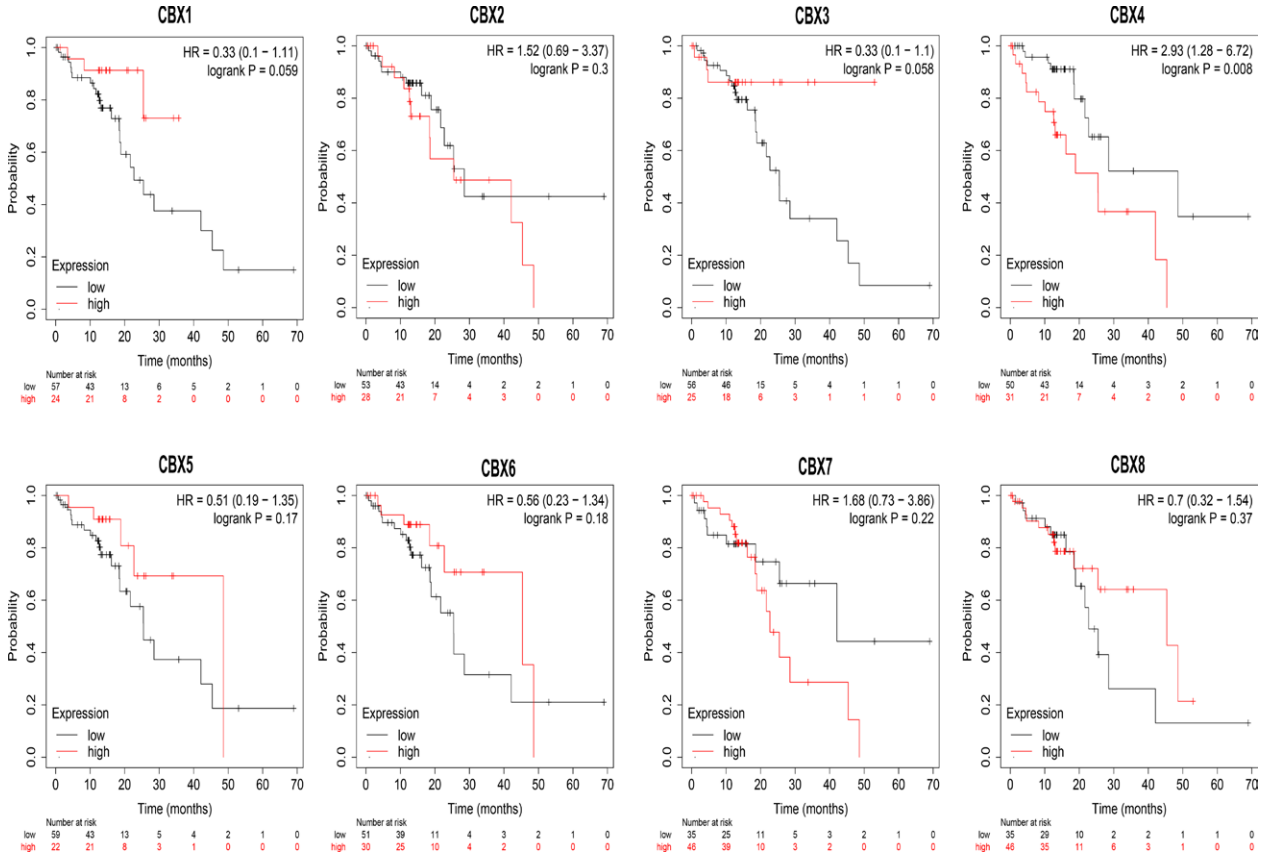

Figure 5. Prognostic value of distinct CBX members in ESCC (Kaplan–Meier plotter). high CBX4 mRNA expression was significantly associated with short OS in patients. CBXs = chromobox family members, ESCC = esophageal squamous cell carcinoma, mRNA = messenger RNA, OS = overall survival.

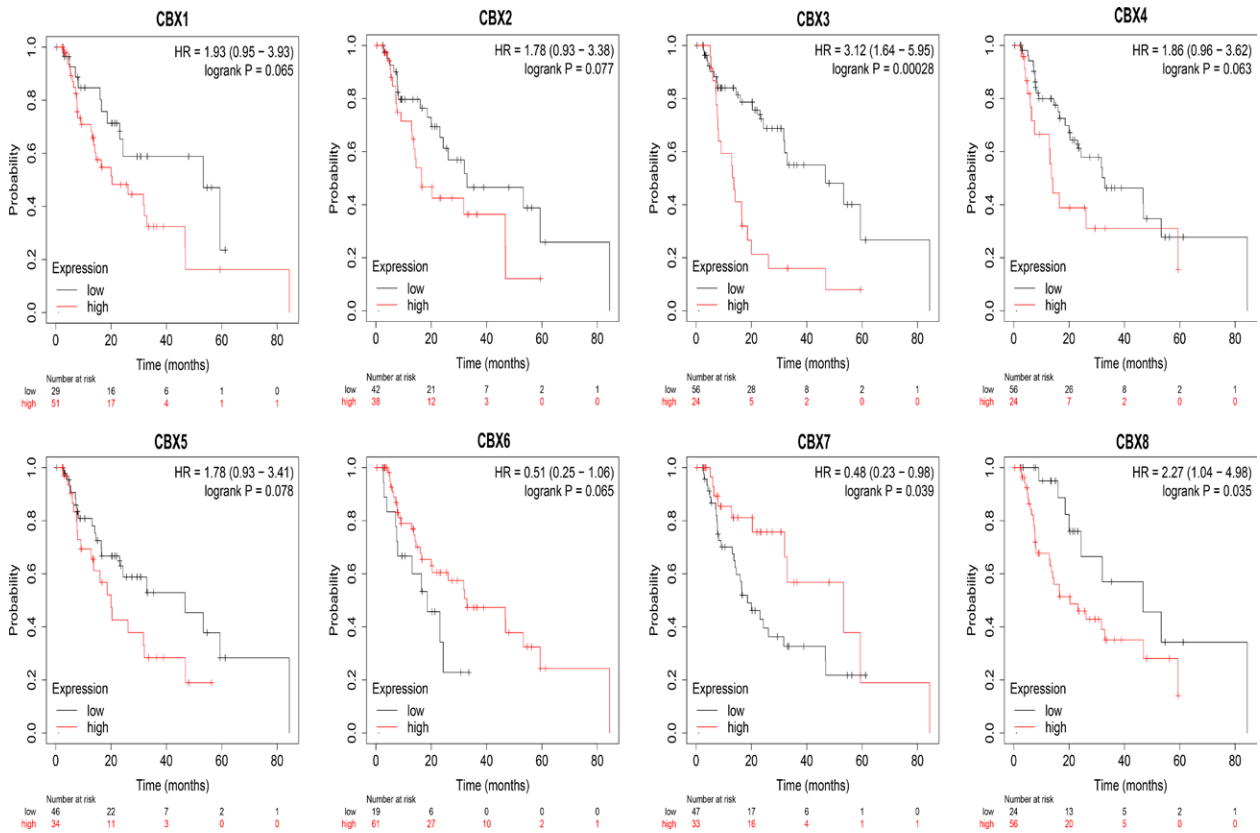

Figure 6. Prognostic value of CBXs in EAC (Kaplan–Meier plotter). CBX3 and CBX8 mRNA expressions were highly associated with short OS in patients with esophageal adenocarcinoma. CBXs = chromobox family members, EAC = esophageal adenocarcinoma, mRNA = messenger RNA, OS = overall survival.

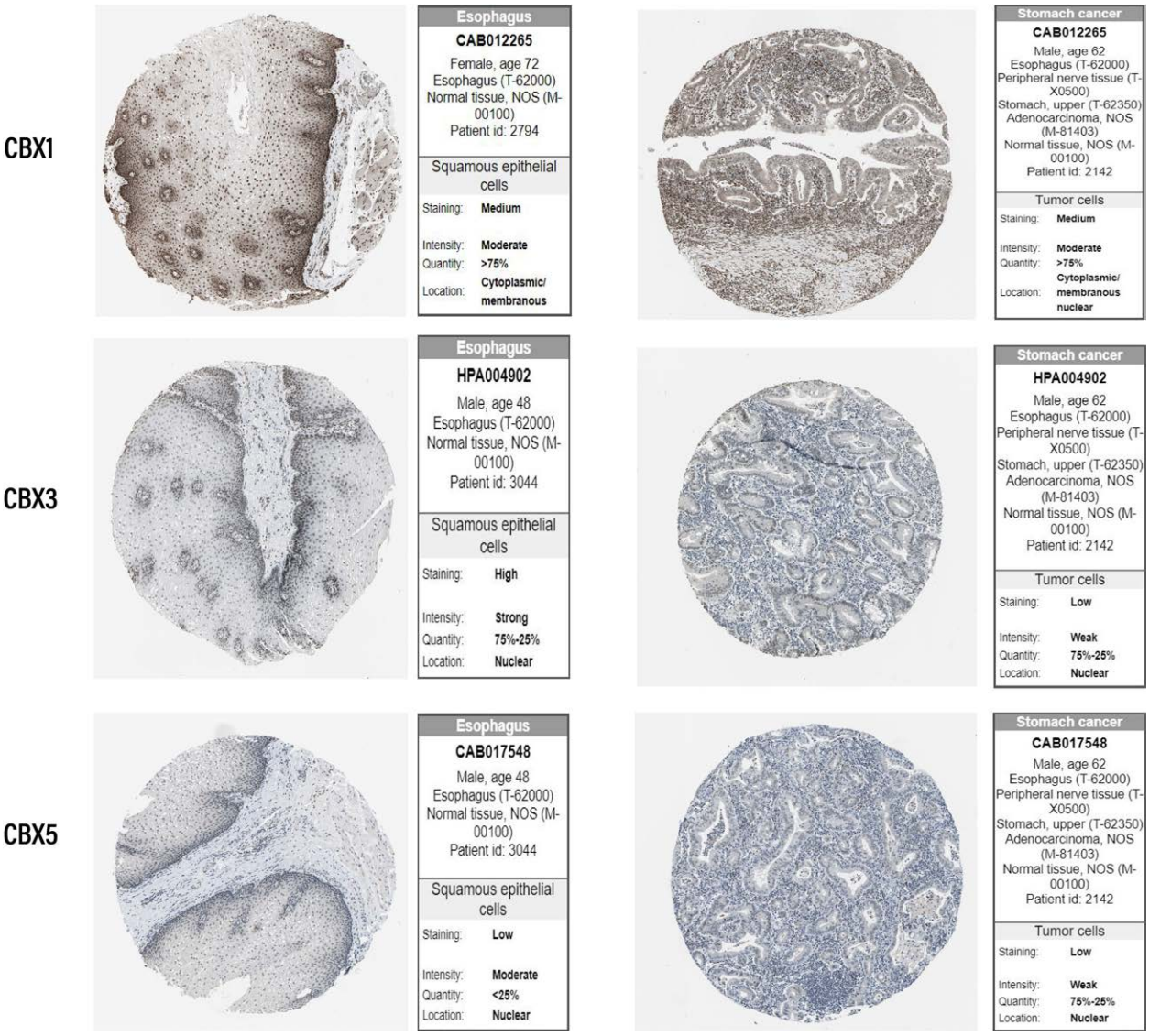

Figure 7. IHC analysis of CBX1, CBX3 and CBX5. The differentially expressed proteins of CBX1, CBX3 and CBX5 have prognostic value in ESCA tissues and normal tissues based on the human protein atlas database. CBXs = chromobox family members, ESCA = esophageal cancer, IHC = immunohistochemistry.

3.4. Immune infiltration of CBXs in ESCA patients

In addition, we also used the TIMER to explore the relation between the CBXs and immune infiltration. The results depict that CBX1 is significantly correlated with the abundance of bursindependentlymphocyte (B cells) (cor = 0.071, P = 3.48e-01) and macrophages (cor = 0.171, P = 2.20e-02) (Fig. 9). Also, we found that the CBX2, CBX5, CBX6 and CBX8 expression levels have positive correlations with B cells, CD4-positive T-lymphocytes (CD4+ cells), and macrophages. Besides, CBX6 is also significantly related to the abundance of dendritic cell (cor = 0.03, P = 6.92e-01) (Fig. 9). Interestingly, we found that CBX7 is positively correlated with these 6 immune cells. Meanwhile, the abundance of other immune cells (CD4+ cells and dendritic cells) is negatively correlated with CBX3 and CBX4. All these results reveal that CBXs may have a certain effect on immunotherapy of ESCA.

3.5. Enrichment and PPI analysis of CBXs in ESCA

Further, to explore the biological functions of CBXs in ESCA, the GO and KEGG analysis were also carried out. We first used GEPIA to investigate the top 100 genes associated with each CBX member, and screened out the 50 most frequently alterd adjacent genes. Then, we submitted the CBX family and related genes to DAVID for enrichment analysis. The results reveal that the CBXs participated in various BPs, including the negative regulation of RNA polymerase II transcription through promoters, DNA replication, negative regulation of transcription, and mRNA splicing through spliceosome (Fig. 10A). With respect to the cellular component, the CBXs are involved in nuclear chromatin, nucleus, nuclear ribonucleoprotein complex, and nucleoplasm (Fig. 10A). In addition, molecular function analysis disclosed that the CBX-related genes were enriched in chromatin binding, protein binding, ATP binding, DNA binding, and enzyme binding (Fig. 10A). Besides, the results of KEGG show that the CBXs and related genes are enriched in mismatch repair, DNA replication, cancer pathways, and spliceosomes (Fig. 10B). Obviously, these signaling pathways are involved in the tumorigenesis and pathogenesis of ESCA.

Additionally, in order to show the direction and degree of association between different nodes of CBXs and related genes, and to indicate the type and intensity of interaction between CBXs and related genes, we constructed a PPI network. The

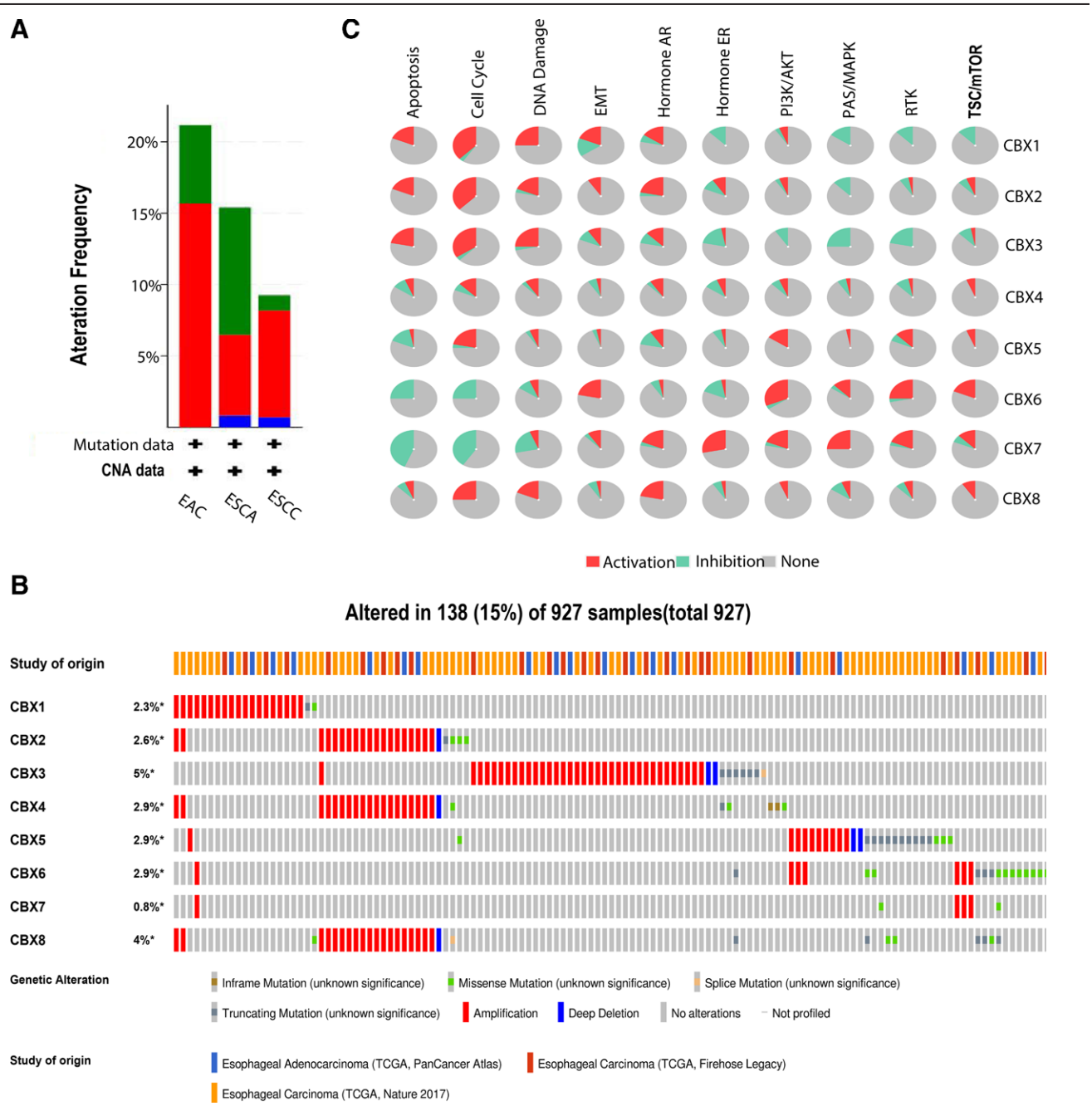

Figure 8. CBX gene mutation and cancer pathway activity in ESCA (cBioPortal and GSCALite). (A and B) The alterations frequency of distinct CBX members in ESCA. CBXs were altered in 138 samples of 927 ESCA patients, accounting for 15%. (C) The role of CBXs in famous cancer-related pathways. CBXs = chromobox family members, ESCA = esophageal cancer.

results show that the CBXs is related to nuclear chromatin, histone blinding, PcG protein complex, chromatin, transcriptional coactivator activity, and nuclear ubiquitin ligase complex (Fig. 11). All these pathways are closely related to gene coding and transcription. Consequently, we can speculate that CBXs play certain roles in the generation and development of ESCA.

4. Discussion

ESCA, a common malignant tumor in the digestive system, has been announced to be the ninth most common cancer and the sixth leading cause of cancer-related death in the world.[7,21] Since the lack of reliable biomarkers and specific genes to guide individualized treatment, the morbidity and mortality of ESCA have not significantly been improved despite the progress in its pathogenesis and clinical treatment.[22] In addition, there have been no clinical trials specifically evaluating the epigenetic treatment of human ESCA, but esophageal malignant tumors have attracted the attention of experts and clinicians as clinically relevant model targets.[23] With the development and wide application of gene-related technologies such as gene chips, deep neural networks, and gene editing technologies, we can directly obtain a large amount of core slice data stored in public databases. Obviously, sorting and in-depth analysis of these data sets can provide great help for our research. Although the diverse genetic drivers and distinct prognostic factors have been broadly explored, the prognosis of ESCA is still very poor.[24] Therefore, the urgent need for new biomarkers related to the prognosis and treatment of ESCA is necessary.

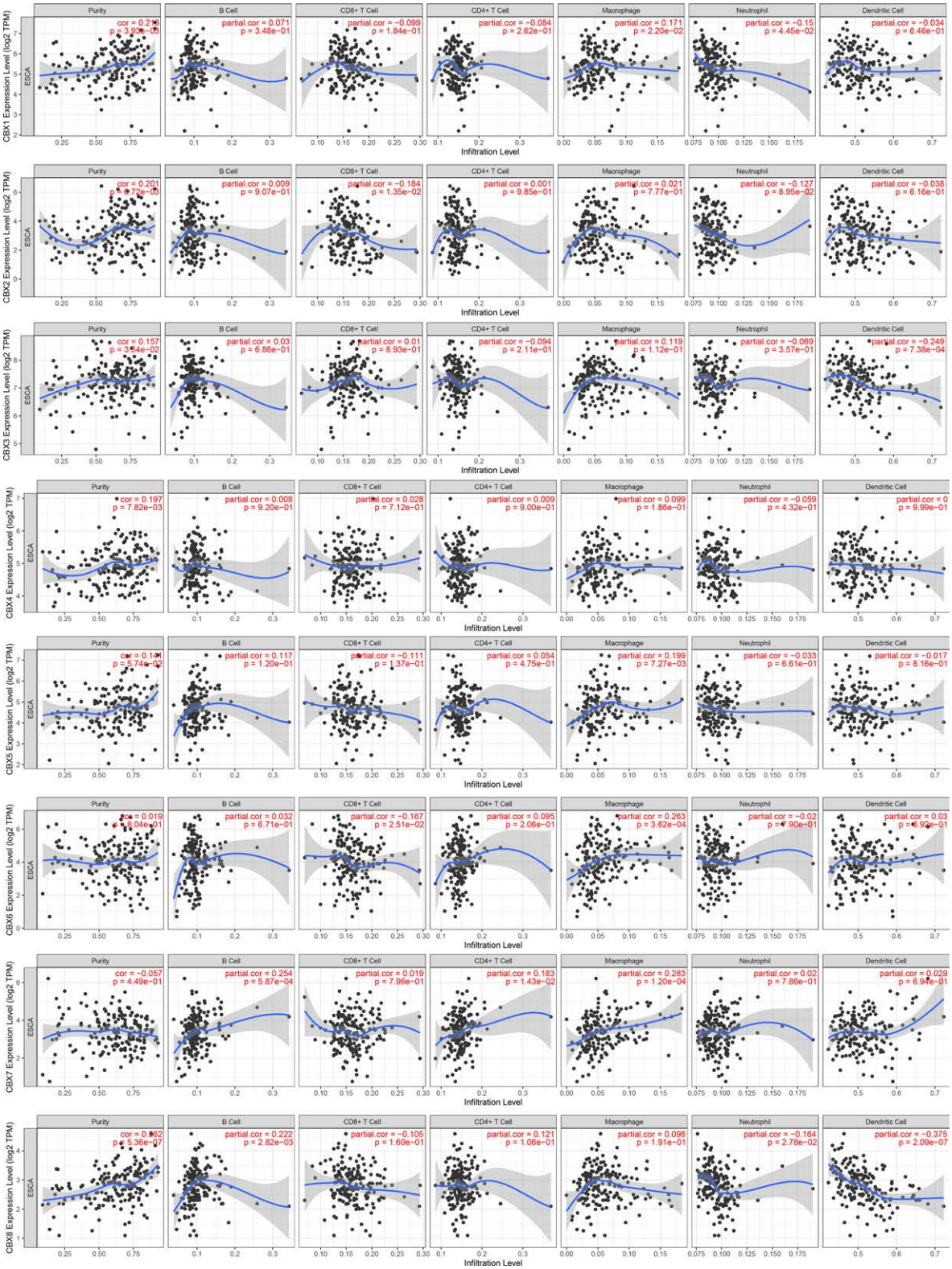

Figure 9. Correlations of CBXs expression with immune cell infiltration in ESCA (TIMER). Correlations between the abundance of immune infiltration cells and the expression of distinct CBX members. CBXs = chromobox family members, ESCA = esophageal cancer.

Although the CBX family has been shown to play a key role in various cancers, the unique role of CBXs protein in ESCA remains to be expound. Currently, we conducted a comprehensive analysis of the expression and prognostic value of the CBXs in ESCA. As a result, the levels of CBX3 and CBX6 in ESCA tumor tissues were up-regulated, while the levels of CBX7 were down-regulated (Fig. 1). Compared with the normal tissues, the expression of CBX1 in ESCA tissues is slightly higher, and its expression is significantly related to the clinical tumor staging of patients with ESCA (Fig. 3). In addition, ESCA patients with high CBX1 transcription levels were highly associated with short DFS (Fig. 4). These results show that CBX1 might

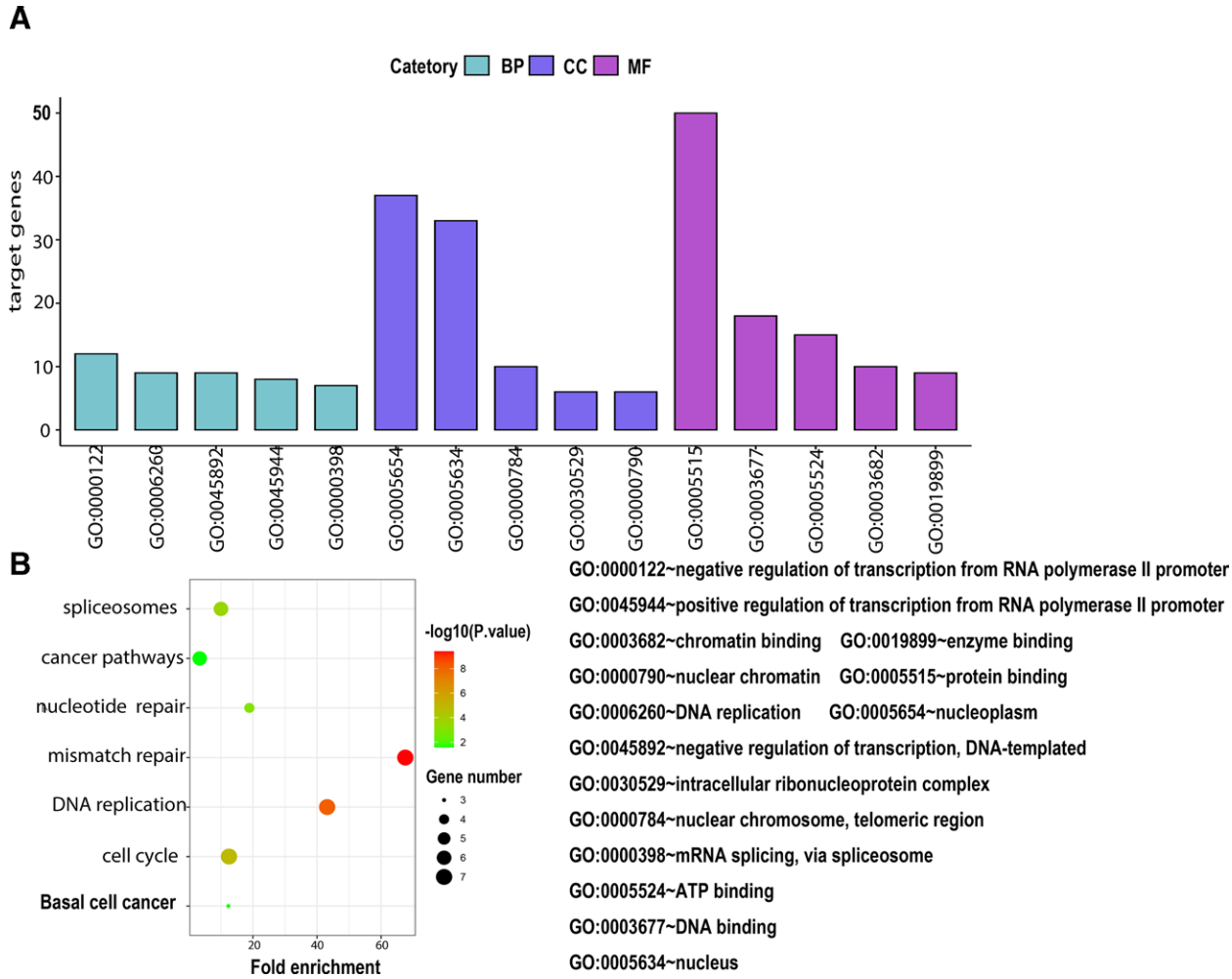

Figure 10. Enrichment analysis of CBXs in ESCA (DAVID). (A) BP, CC, and MF of GO analysis. (B) KEGG analysis. BP = biological process, CBXs = chromobox family members, CC = cellular component, ESCA = esophageal cancer, GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function.

be considered as a prognostic biomarker for the survivals and play an important role in the tumorigenesis and progression of ESCA. Additionally, Tian et al[25] found that CBX7 is related to the clinical staging and vascular invasion of cervical cancer. And they also found that compared with patients with low or high CBX7 expression, cervical cancer patients with negative CBX7 expression had a lower OS rate. Actually, some of members of CBXs protein were also suggested as biomarkers for other types of cancer. For example, in CRC, CBX3 served as prognostic biomarkers for survivals.[6] And CBX5/7 may act as a biomarker for the prognosis of skin cutaneous melanoma.[26]

Then we found that CBXs are differentially expressed in the esophagus and normal tissues in gastric cancer tissues through IHC (Fig. 7). This allows us to reasonably speculate that the CBX family may also have a great influence on gastrointestinal diseases. Indeed, cancer of the digestive system (gastric cancer, ESCA, pancreatic cancer, liver cancer, gallbladder cancer, colon cancer, and rectal cancer, etc.) is one of the most common cancers in the world, characterized by aggressiveness, metastatic potential and adverse outcomes.[27] They account for a large part of global cancer-related deaths, and have high morbidity and mortality.[28] There are many pathogenic factors, among which the most important factors are excessive drinking and heavy smoking.[29] Despite the emergence of many studies and new technologies, they still maintain a low survival rate and lack methods to guide successful treatment. Discovery of biomarkers that improve the characteristics of gastrointestinal cancers may optimize the treatment of gastrointestinal cancers. Therefore, the study of CBX family as biomarkers may have certain significance for the treatment of gastrointestinal cancer. Hua et al[30] used the IHC to detect the expression of CBX7 in 45 cases of gastric cancer patients and matched adjacent tissues, and found that the expression of CBX7 in gastric cancer tissues was significantly down-regulated and associated with TNM staging (stage III and IV). This also indicates that the up-regulation of CBX7 expression can exert anti-cancer effects by inhibiting cell migration and invasion. In addition, Li et al[31] found that the high expression of CBX3 mRNA and the low expression of CBX8 are independent risk factors for the shorter survival of patients with pancreatic cancer. Then Zhu et al[32] found that CBX2 mRNA and protein expression in CRC cells were higher than normal colonic epithelial FHC cells and para-cancerous tissues by real-time fluorescent quantitative PCR and western blot detection (both $P < .05$). Additionally, Lin et al[33] used the methods of colony formation, cell cycle and transwell assays to explore the functions of CBX3 on proliferation, migration and cell cycle. And they found that the expression of CBX3 is up-regulated in human gastric cancer tissues, and the expression level is highly related to adverse physical signs.[33] All the above results indicate that CBXs have a great effect on the diagnosis and treatment of gastrointestinal cancer, which also prompted us to explore the effect of CBXs on ESCA.

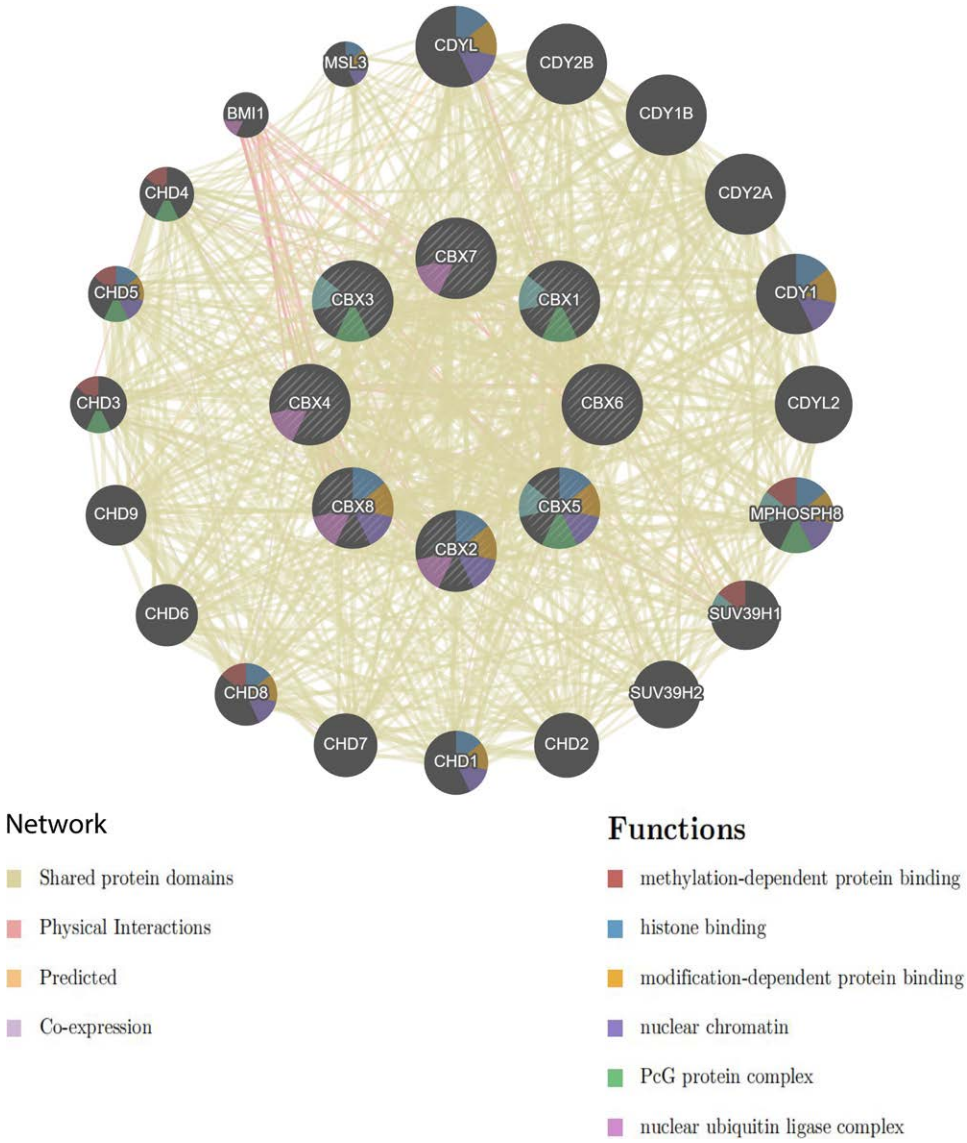

Figure 11. PPI network of CBXs expression. The network shows in shared protein domains, physical interactions, prediction, and co-expression by the network nodes of the different colors. CBXs = chromobox family members, PPI = protein-protein interaction.

In addition, the results of KEGG reveals that the CBXs and related genes are enriched in mismatch repair, DNA replication, cancer pathways, and RNA splicing (Fig. 10B). Among these, DNA mismatch repair is an evolutionarily conserved process that corrects mismatches generated during DNA replication and escape proofreading.[34] Therefore, the CBX family may affect the tumorigenesis and progression of ESCA by regulating RNA splicing and DNA replication. With respect to the results of GSCALite, we found that most members of the CBX family are involved in the activation of DNA damage response, epithelial-mesenchymal transition and hormone AR pathways (Fig. 8C). Among them, CBX6/7 are mainly related to the inhibition of cell cycle and apoptosis pathways. Indeed, Sakai et al[35] transcriptome analysis showed that CBX6 regulates the genome involved in cancer cell migration and metastasis. Moreover, Wu et al[36] found that CBX5 targeted by miR-589-5p can accelerate cell proliferation and migration in renal cell carcinoma. Also, as a transcriptional repressor, overexpression of CBX8 has been found to be associated with tumor development in many cancers. For example, Zhang et al[37] first explored the correlation between CBX8 and radioresistance and found that CBX8 was up-regulated in ESCC tissues and cells, and was used as an indicator of poor prognosis for patients with ESCC. At the same time, Wang et al[38] found that CBX8 inhibited cell proliferation, migration and metastasis in ESCC. Additionally, the findings of Meng et al[39] showed that the down-regulation of miR-30b in ESCC may increase cell proliferation, inhibit cell apoptosis and promote cell migration by targeting CBX3 and activating the JAK2/STAT3 signaling pathway. All these findings indicate that CBXs play a great role in the occurrence and progression of ESCA.

Besides, in recent years, more and more evidences have shown that the tumor immune microenvironment plays a key role in the occurrence and development of ESCA.[40] Inflammation is the part of the body's response to invading pathogens and involves a variety of regulatory mechanisms to limit the damage caused by excessive immune responses to the host.[41] Then, we further explored the relationship between the expression of the CBX family and certain immune cells. Presently, the results of immune infiltration of CBXs in ESCA patients show that the expression of CBXs are highly related to the infiltration of the six immune cell types (Fig. 9), indicating that CBXs are associated with the immune status and the prognosis of ESCA. Therefore, CBXs may play an

important role in immune cell infiltration and serve as a prognostic biomarker for patients with ESCA. All these results may provide some important information for the treatment of ESCA in the future. In recent years, many studies show that countless disease conditions ranging from type 2 diabetes to neurodegenerative diseases and cardiovascular diseases are caused by persistent, unresolved inflammation in metabolic tissues such as fat, liver, and pancreas.[42] Therefore, immune cells have attracted more and more attention from these disease experts and clinicians. Obviously, chronic inflammation plays an important role in the development of various cancers, especially in the digestive organs. Those infectious factors that affect inflammation can not only induce genetic and gene expression changes, including point mutations, deletions, recombination, and methylation of various tumor-related genes through various mechanisms, but also regulate and affect several tumor-related messenger RNAs.[43] It can be seen that immune cells have a certain influence on tumor occurrence and development. Recent clinical investigations and studies have shown that immunotherapy may provide great help in the treatment of patients with ESCA. In fact, cancer cells can functionally shape their microenvironment by secreting various cytokines, chemokines and other factors, and enable them to play a decisive role in tumor survival and progression.[44]

5. Conclusions

In summary, the results obtained from this work indicate the clinical significance and prognostic value of CBXs in ESCA, and reveal the molecular mechanisms involved in the occurrence and progression of ESCA. Additionally, we found that CBXs are closely related to various immune cells such as B cells, CD4+ cells, and macrophages. Therefore, CBX may be a biomarker in the context of ESCA immunotherapy. All these may help provide some help for the treatment of ESCA patients, and is expected to be applied in clinical practice in the future.

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