Bioinformatic Analysis of Prognostic Value and Immune Cell Infiltration of Chromobox Family Proteins in Esophageal Cancer

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Research

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Abstract

Background

Chromobox (CBX) family proteins (CBXs) are important components of epigenetic regulation complexes. Abnormal expression of CBXs is related to the occurrence and development of various cancers. However, the role and mechanism of CBXs in esophageal cancer (ESCA) need further research.

Methods

The mRNA expression of CBXs, clinicopathological parameters, prognostic values, genetic alteration, enrichment analysis, and immune cell infiltration were analyzed with several databases including Oncomine, UALCAN, Kaplan-Meier plotter, cBioPortal, DAVID, and TIMER2.0.

Results

The expression level of CBX1/2/3/4/8 was significantly upregulated in ESCA while that of CBX7 was down-regulated. Besides, mRNA expressions of CBX1/2/3/4/5/7/8 were correlated with patients' individual cancer stages and the nodal metastatic status. The mRNA expression of CBX3/4/7/8 was significantly correlated with the overall survival (OS) in ESCA. What is more, high mutation rate of CBXs (49%) was also observed in ESCA patients. Then, CBXs were related to the infiltration of a variety of immune cells.

Conclusions

This study may provide new ideas for the selection of prognostic biomarkers in the CBXs in ESCA.

Background

Esophageal cancer has always been a major malignant tumor that threatens human health, and its incidence and mortality are the seventh and sixth of all malignant tumors, respectively[1]. Esophageal cancer is mainly divided into two histological types: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). As a highly aggressive malignant tumor, esophageal cancer is often accompanied by extensive lymph node metastasis, leading to a poor prognosis for patients. Although new treatments for esophageal cancer are emerging,and the molecular mechanism of esophageal cancer has been extensively studied, patient survival rate still needs to be improved[2]. With the research on the development mechanism of esophageal cancer, more prognostic biomarkers will be discovered to guide clinical work and benefit patients. DNA fragments, non-coding RNA, proteins, enzymes, hormones and metabolites can all be used as biomarkers. Hence, exploiting biomarkers with greater potential for prognosis and potential therapeutic targets of ESCA is an emergence.

Chromobox (CBX) family proteins (CBXs) are currently divided into two categories: the polycomb (includes CBX2, CBX4, CBX6, CBX7, and CBX8) and heterochromatin protein 1 (includes CBX1, CBX3 and

CBX5)[3,4]. Moreover, eight members of the CBX protein family are all involved in various biological processes, such as transcriptional repression, cell cycle regulation, lineage-commitment, tumor initiation, progression, development, and chromatin[5]. Abnormal expression of CBX family genes is related to the occurrence and development of various cancers, such as ESCC[6,7],breast cancer[8], lung adenocarcinoma[9], renal cell carcinoma[10], cervical cancer[11], bladder Cancer[12], and colorectal cancer[13].

Investigators reported that CBX3 and CBX8 had essential functions in ESCC [6,7].CBX3 can promote the self-renewal of ESCC stem cells by inhibiting the activation of the P53/P21 pathway[7]. By up-regulating the expression of CD133, CD13, Oct4, Sox2, and Nanog,CBX3 can promote the abilities of spheroid formation, colony formation, proliferation and tumorigenicity[7]. Recent reports have shown that CBX8 can encourage the occurrence of ESCC[14,15]. However, the functions and prognostic roles of other members of the CBX family proteins in ESCA require additional research. Therefore, several large public databases were used to analyze their expression, potential functions, signal pathways, prognostic value and immune cell infiltration in ESCA.

Methods

ONCOMINE.

ONCOMINE (www.oncom ine.org) is a tumor-related online database[16], which has the most complete cancer mutation profile, gene expression data and related clinical data. The expressions of the CBXs in different cancers were analyzed with this database. Student's *t*-test was used to compare the expressions of CBXs in cancer and normal tissues. The threshold settings were follows: a *p*-value < 0.01, a fold change of 1.5, a gene rank in the top 10%, and data type of mRNA.

UALCAN.

UALCAN(http://ualcan.path.uab.edu/)is a website for online analysis and mining of TCGA database[17]. UALCAN can help medical staff to perform biomarker identification, expression profile analysis, survival analysis, etc. of related genes. In our study, the expression of the CBXs, individual cancer stage, and the nodal metastatic status were performed in ESCA patients based on 11 normal and 184 ESCA tissue samples. Student's t-test was used to compare the expression of CBXs with $P < 0.05$ considered as statically significant. The grading standard of the nodal metastatic status was as follows: N0 (no regional lymph node metastasis), N1 (metastases in 1 to 3 axillary lymph nodes), N2 (metastases in 4 to 9 axillary lymph nodes), and N3 (metastases in 10 or more axillary lymph nodes).

Kaplan-Meier Plotter.

Kaplan-Meier plotter (http://www.kmplot.com) is a tool for online analysis of survival prognosis[18]. Using the database, the relationship between the overall survival (OS) of patients and the expression level of

CBXs was analyzed. The parameter settings that come with the tool were used, including log-rank *p*-value, HRs, and 95% CIs and the best cutoff value. $p < 0.05$ was considered as statically significant.

cBioPortal.

cBioPortal (http://www.cbioportal.org) provides visualization tools for research and analysis of cancer genetic data[19,20]. Based on ESCA (TCGA, Firehose Legacy) database, the OS for CBX3/4/8, and CBX7 were explored, while gene mutation rate reached 49% in 184 ESCA patients. cBioPortal was also used to find out the co-expressed genes of the CBXs. $p < 0.05$ was considered to be significant.

DAVID.

DAVID is a biological information database, mainly used for differential gene function and pathway enrichment analysis. Using the database, we obtained the related data pertaining to GO including biological processes (BP), cellular components (CC), and molecular functions (MF), and KEGG for CBXs and their co-expressed genes.

TIMER2.0.

TIMER2.0(http://timer.cistrome.org/) uses RNA-Seq expression profile data to detect the infiltration of immune cells in tumor tissues[21]. The correlations between CBXs members and Tumor-immune infiltrating cells (TIICs) were separately analyzed by TIMER2.0 platform.

Results

The mRNA expression of CBXs in ESCA.

Oncomine, TIMER2.0 and UALCAN databases were used to analyze mRNA expression levels of the eight CBX family proteins in ESCA. Based on the data obtained from Oncomine, the expression of CBXs in many kinds of cancers was significantly different from normal tissues (Fig. 1). As shown in Fig. 2, the expression level of CBX1/2/3/4/8 in tumor tissues was significantly higher than that in normal tissues, while the expression level of CBX7 was lower in the former than the latter with TIMER2.0 (Fig. 2). The expression of CBXs in ESCA was verified by UALCAN databases and the expression level of CBX1/2/3/4/8 was also significantly upregulated in ESCA (Fig. 3). These results were almost consistent with those from TIMER2.0.

Clinicopathological Parameters of CBXs in ESCA Patients.

Using the ESCA database in UALCAN, the correlations between the mRNA expression of CBXs and patients' individual cancer stages, and the nodal metastatic status were separately analyzed. As shown in Fig. 4a, mRNA expressions of CBX1/2/3/4/5/7/8 were correlated with patients' individual cancer stages while the expression of CBX6 had no correlation with that. Moreover, the mRNA expression of CBX7 was down-regulated in all cancer stages. Furthermore, the mRNA expression of CBX1/2/3/4/5/7/8 was

related to the nodal metastatic status while the mRNA expression of CBX6 did not correlate with the nodal metastatic status (Fig. 4b).

Prognostic Value of CBXs in ESCA Patients.

In order to evaluate the value of differentially expressed CBXs in the progression of ESCA, Kaplan-Meier Plotter was used to analyze the correlation between different CBXs and clinical outcomes. As shown in Fig. 5a, the expression of CBX3 (HR = 3.12, $p = 0.00028$) and CBX8 (HR = 2.27, $p = 0.035$) was negatively correlated with the OS of the patient in EAC (Fig. 5a). However, the expression level of CBX7 (HR = 0.48, $p = 0.039$) was positively correlated with the OS. In ESCC, high mRNA expression of CBX4 (HR = 2.93, $p = 0.008$) was significantly correlated with poor OS (Fig. 5b).

Furthermore, cBioPortal was used to verify the relationships between CBX3/4/8, CBX7 and the OS of patients with ESCA. High mRNA expression of CBX3/4/8 was significantly correlated with poor OS (Fig. 6a), but CBX7 did not seem to have a significant effect on OS (Fig. 6b).

Genetic Alteration and Enrichment Analysis of CBXs.

We analyzed the alterations in CBXs with cBioPortal. As shown in Fig. 7a and Fig. 7b, the CBXs were altered in 90 out of 184 patients with ESCA (49%) and the percentage of genetic alterations in CBX1/2/3/4/5/6/7/8 was 11, 14, 22, 8, 7, 10, 2.2, and 9% (Fig. 7a and Fig. 7b). Then, cBioPortal was used to find out the top 50 co-expressed genes of eight CBXs members (Additional file 1.) and we performed GO and KEGG analysis on CBXs and these 392 co-expressed genes in DAVID. As shown in Fig. 7c, biological processes(BPs) such as DNA replication (GO:0006260), mitotic nuclear division (GO:0007067), sister chromatid cohesion (GO:0007062), axon extension (GO:0048675), chromosome segregation (GO:0007059), mitotic spindle organization (GO:0007052), somatic hypermutation of immunoglobulin genes (GO:0016446), mismatch repair (GO:0006298), DNA damage response and detection of DNA damage (GO:0042769), positive regulation of helicase activity (GO:0051096) were remarkably regulated by the CBXs (Fig. 7c). As were shown in Fig. 7d, CBXs were mainly involved in cellular components (CCs) including nucleoplasm (GO:0005654), nuclear chromosome and telomeric region (GO:0000784), intracellular ribonucleoprotein complex (GO:0030529), chromosome and centromeric region (GO:0000775), condensed chromosome kinetochore (GO:0000777), PcG protein complex (GO:0031519), nucleus (GO:0005634), PRC1 complex (GO:0035102), Ndc80 complex (GO:0031262), nuclear envelope (GO:0005635) (Fig. 7d). Moreover, CBXs also affected the molecular functions (MF), such as chromatin binding (GO:0003682), identical protein binding (GO:0042802), methylated histone binding (GO:0035064), protein binding (GO:0005515), protein homodimerization activity (GO:0042803), actin binding (GO:0003779), single-stranded RNA binding (GO:0003727), ribosomal large subunit binding (GO:0043023), mismatched DNA binding (GO:0030983), S100 protein binding (GO:0044548) (Fig. 7e). KEGG analysis showed that CBXs and these 392 co-expressed genes were mostly participated in Mismatch repair (hsa03430), DNA replication (hsa03030) and Fatty acid metabolism (hsa01212) (Fig. 7f). GO result of CBXs including BP (Table 1), CC (Table 2) and MF (Table 3) were sorted in descending order based on the gene counts.

Table 1
Go analysis revealing biological processes

Category	Count	*p*-Value	Related genes
negative regulation of transcription, DNA-templated	16	4.73E-02	CBX5, CBX4, CBX3, CBX1, FHL2, PBXIP1, BRCA1, PA2G4, PPM1F, ELK3, CENPF, SBNO2, DDIT3, TIMELESS, NOSTRIN, TRIM11;
protein sumoylation	8	6.33E-03	CBX8, NUP133, CBX4, PARP1, CBX2, BRCA1, SMC1A, SENP1;
covalent chromatin modification	7	1.92E-02	CBX7, CBX6, CBX4, CBX3, CBX2, ANP32E, TET1;

Table 2
Go analysis revealing cellular components

Category	Count	p-Value	Related genes
nucleus	127	1.55E-03	BCL2L15, IP6K2, CBX8, CBX7, CBX6, CBX5, CBX4, CBX3, CBX2, CBX1, MICAL2, SMAD9, ZBTB12, PTK6, PA2G4, LSM5, NDC80, CLK3, CLK2, HNRNPL, POLA1, EHD2, EIF6, LHX2, ANP32E…
cytoplasm	115	2.28E-02	CBX7, APOBEC3F, PNPT1, COA1, UBE2C, EIF2AK1, RASSF7, CCZ1, SMAD9, PTK6, PA2G4, PAFAH2, HNRNPL, CCT6A, POLA1, EIF6, CDK1, UBE2O, BLMH, DZIP1, CPEB2, ITGB1, ACADVL, SCHIP1, AKAP8L…
nucleoplasm	82	1.63E-05	CBX8, CBX7, CMTM8, CBX6, CHTOP, CBX5, CBX4, UBE2C, GINS3, CPSF3, CBX2, CBX1, ATAD2, SMAD9, PTK6, PA2G4, SORBS1, LSM5, CLK3, CLK2…
nucleolus	24	4.89E-02	ACADVL, CHTOP, CBX5, PARP1, DDX56, ACSL5, HNRNPR, PA2G4, MRM2, TSEN54, ORC4, POLA1, TCOF1, EIF6, KLHL7, TWISTNB, UBTF, TIMELESS, MPHOSPH10, KAT7, NEK2, EZR, DTL, ATF3;
nuclear chromosome, telomeric region	11	1.57E-04	SUN2, ORC4, SSB, CBX5, MSH2, PARP1, CBX3, CBX1, CDK1, MCM6, THOC5;
nuclear envelope	10	2.91E-03	SUN2, POLA1, CENPF, CLIP1, CBX5, NUP133, PARP1, CBX3, S100A6, NEMP1;
chromosome, centromeric region	7	6.37E-04	CENPF, CBX3, DSCC1, NUF2, CBX1, SMC1A, NDC80;
PcG protein complex	5	1.45E-03	CBX8, CBX7, CBX6, CBX4, CBX2;
PRC1 complex	4	1.57E-03	CBX8, CBX7, CBX4, CBX2;
heterochromatin	4	6.56E-03	CBX8, CBX7, CBX6, CBX2;

Table 3
Go analysis revealing molecular functions

Category	Count	p-Value	Related genes
protein binding	188	4.12E-03	PRDM8, DSCC1, CEP19, HNRNPU, LOXL4, HNRNPR, ANTXR2, ZFYVE1, CBX8, CBX7, CBX6, CHTOP, APOBEC3F, PNPT1, CBX5, PPP1R27, CBX4, UBE2C, CBX3, CPSF3, CBX2, CBX1, RASSF7, CCZ1, PYCR1…
identical protein binding	27	1.73E-03	SCHIP1, MVP, FHL2, FBLN1, PKD2, DVL2, SUN2, PARP1, CBX3, PYCR1, PTK6, LYZ, SDCBP2, NDC80, CLK3, CLK2, NUDT21, SDK1, KLHL7, TRIP10, BLMH, TCF4, MCM6, RBMX, PAFAH1B3, ATF3, MCU;
protein homodimerization activity	25	5.11E-03	PKD2, CTSE, AOC3, CBX5, IZUMO1, EIF2AK1, CBX1, CEP131, CACYBP, SDCBP2, MSH6, GGCT, NUDT21, CENPF, CLIP1, MSH2, KLHL7, DDIT3, DPYD, S100A6, CASQ2, TIMELESS, TCF4, NBL1, ATF3;
chromatin binding	20	1.41E-04	CBX7, CBX5, CBX4, CBX2, ATAD2, CBX1, NUCKS1, HOXD13, SMC1A, HOXD10, PRIMPOL, MSH6, POLA1, CENPF, EXO1, LHX2, UBTF, CDK1, TCF4, RBMX;
methylated histone binding	6	2.98E-03	CBX8, MSH6, CBX7, CBX5, CBX4, CBX2;
single-stranded RNA binding	5	8.19E-03	CBX8, CBX7, CBX6, CBX4, RBMX;

Immune Cell Infiltration of CBXs in ESCA Patients.

In the proliferation and progression of malignant tumors, the role of immune cells cannot be ignored. In our study, we used TIMER2.0 to analyze the relationship between eight members of CBXs and immune cell infiltration (Fig. 8). The expression of CBX1 was mainly involved in the infiltration of B cells, myeloid dendritic cells, macrophages and neutrophils in ESCA. CBX2 was in connection with the infiltration of B cells. Additionally, CBX3 was correlated with the infiltration of B cells and $CD4^+$T cells. CBX4 was correlated with the infiltration of macrophages, and neutrophils, while CBX5 was associated with the infiltration of $CD4^+$T cells, $CD8^+$T cells, myeloid dendritic cells, macrophages and neutrophils. Moreover, CBX6 was correlated with the infiltration of myeloid dendritic cells, macrophages and neutrophils, and CBX7 was connected with the infiltration of B cells, $CD4^+$T cells, $CD8^+$T cells, myeloid dendritic cells, macrophages and neutrophils. With regard to CBX8, the infiltration of $CD4^+$T cells, $CD8^+$T cells and myeloid dendritic cells was kept in with the expression.

Discussion

So far, many studies have reported that CBXs are related to the occurrence and development of a variety of tumors. However, the relationship between CBXs and the occurrence and development of ESCC urgently needs further elucidation. In this study, we analyzed the multiple levels of CBXs in ESCC

including mRNA expression, mutation, prognostic value and Immune Cell Infiltration through a variety of online databases.

High expression of CBX1 was related to poor differentiation of breast cancer and led to poor OS[22]. The expression of CBX1 was closely related to the staging of gastric cancer and lymph node metastasis[23]. In addition, CBX1 interacted with the transcription factor HMGA2, activates the Wnt/βCatenin signaling pathway and affected the prognosis of patients with liver cancer[24]. In this study, the expression of CBX1 in the tissues of patients with ESCA was significantly higher than that in normal tissues, which was basically consistent with the results of other researchers. However, our research showed that the expression of CBX1 was not significantly correlated with the OS of patients with ESCA. This might be because the sample size was too small and need further research with a larger sample size to confirm. Further, the mRNA expression of CBX1 was significantly correlated with individual cancer stage and nodal metastatic status.

Recent studies have shown that CBX2, a member of the CBXs family, is overexpressed in several tumors. Loss of CBX2 impaired the proliferation of leukemia cells[25]. In ovarian, breast and lung tumors, the total rate of CBX2 amplification exceeded 30% and CBX2 was significantly associated with HER-2 positive status in breast cancer[26]. In addition, CBX2 was a critical regulator of the spread of ovarian cancer and chemoresistance[27]. In this study, the expression of CBX2 in esophageal cancer tissues was significantly increased, while it was also significantly correlated with individual cancer stage and nodal metastatic status. Although patients with high expression of CBX2 had lower OS in esophageal adenocarcinoma, the difference was not significant.

Up-regulation of CBX3 has been found in a variety of cancers, such as LUAD, colorectal cancer (CRC), gastric cancer and ESCC[6,28–30]. CBX3 directly inhibited the expression of transcription repressors NCOR2 and ZBTB7A, thereby affecting the proliferation, colony formation and migration of LUAD cells[28]. In addition, CBX3 controlled the development of CRC by directly regulating CDKN1A (p21Waf1/Cip1)[29]. CBX3 affected the prognosis of gastric cancer by regulating the cell cycle, mismatch repair and immune-related pathways[30]. Down-regulation of miR-377 could promote the self-renewal of ESCC stem cells by promoting the expression of CBX3 and inhibiting the activation of the P53/P21 pathway[6]. In the present study, CBX3 was highly expressed in esophageal cancer tissues. The highly expressed CBX3 was significantly related to the poor OS of esophageal adenocarcinoma patients, which implied that CBX3 as an oncogene might take a significant part in the prognosis of EAC. Furthermore, CBX3 was also significantly correlated with individual cancer stage and nodal metastatic status, which further suggested that CBX3 might serve as a prognostic marker for EAC.

In HCC cells, after knocking out CBX4, proliferating cell nuclear antigen and cyclin E2 were down-regulated, and p16 was up-regulated, resulting in decreased cell proliferation and impaired cell cycle progression[31]. CBX4 promoted the migration and invasion of breast cancer cells[32]. Moreover, CBX4 increased the expression and activity of P53, CDK2, Cyclin E, MMP2, MMP9 and CXCR4 by up-regulating

the expression of BMI-1, and promoted the proliferation and metastasis of lung cancer in vitro[33]. In this study, the expression of CBX4 in the tissues of patients with ESCA was significantly higher than that in normal tissues. Furthermore, the mRNA expression was significantly correlated with individual cancer stage and nodal metastatic status, while higher CBX4 mRNA expression was correlated with worse OS and the differences were significant in ESCC. In summary, CBX4 might be a potential prognostic marker for ESCC.

Although the expression of CBX5 in esophageal cancer tissues had no significant difference compared with normal tissues in our study, the role of CBX5 in other malignant tumors deserved our attention. CBX5 was highly expressed in NSCLC and affected the growth of tumor cells[34]. The high expression of CBX5 was related to the poor prognosis of breast cancer patients and promoted tumor metastasis[35] ,which was different from our research results. Therefore, whether CBX5 is related to the occurrence and development of cancer still needs further research to confirm.

Similar to CBX5, CBX6 was confirmed to be upregulated in a variety of cancers including HCC[34], glioblastoma multiforme[36]and breast cancer[37]. However, our report did not find a significant correlation between CBX5 and the occurrence and development of ESCA. So the role of CBX5 in ESCA is still vague.

Surprisingly, the role of CBX7 in tumors seemed to be inconsistent with other CBXs members. CBX7 was found to be an important tumor suppressor in pancreatic cancer and inhibited the PTEN/Akt signaling pathway by increasing the level of PTEN transcription[38]. Moreover, CBX7 inhibited breast cancer tumorigenicity through epigenetic induction of DKK-1 mediated[39]. CBX7 expression in CRC tissue was significantly reduced or absent compared with normal colonic mucosa[40]. In our study, the expression of CBX7 in esophageal cancer tissues was significantly decreased. In EAC, patients with high expression of CBX7 had a higher OS, while patients with high expression of CBX7 had lower cancer stages and better lymph node metastasis status. Therefore, the data reported here suggested that the expression of CBX7 might reflect the prognosis of EAC and CBX7 might be an important tumor suppressor.

As an important member in the CBX family, CBX8 was defined as a cancer-promoting factor in a variety of malignant tumors, such as colorectal cancer[13], metastatic prostate cancer[41], ESCC[42].High CBX8 expression was associated with a low distant metastasis rate and good prognosis in patients with colorectal cancer[13] .CBX8 knockout inhibited cell proliferation, colony- forming ability, DNA repair and promoted apoptosis in ESCC[42].In this study, the expression of CBX8 was significantly increased in ESCA and the mRNA expression was significantly correlated with individual cancer stage and nodal metastatic status. Moreover, high CBX8 expression was associated with poor OS in ESCC. The above results all indicated that CBX8 might have prognostic value in ESCC.

Then, GO analysis of CBXs and their co-expressed genes that may have prognostic value showed that the functions of these genes mainly involved DNA replication, methylation and mitosis. Changes in KEGG were mainly enriched in mismatch repair, DNA replication, pyrimidine metabolism, cell adhesion

molecules (CAMs). These functions and pathways were all related to the occurrence and development of cancer.

Many studies had confirmed that immune cells in the tumor microenvironment played a great role in the occurrence and development of tumors[43–45]. Our research showed that CBXs were related to the infiltration of a variety of immune cells including B cells, $CD4^+$T cells, $CD8^+$T cells, myeloid dendritic cells, macrophages and neutrophils. This suggested that CBXs might become a new target for immunotherapy.

Our research has certain limitations. First, our data are all derived from online databases, lacking further verification by cell experiments, animal experiments and clinical experiments. Second, the number of samples in the online database was small, so a larger sample size is needed to verify our results. Finally, the specific molecular mechanism of CBXs needs further research to verify our research and findings.

Conclusion

In conclusion, we comprehensively analyzed the expression and prognostic value of the eight members of CBXs. The expression level of CBX1/2/3/4/8 in tumor tissues was significantly higher than that in normal tissues and the expression level of CBX7 was lower, while the expression level of CBX5/6 was different but not significant. The expression of CBX3/4/8 was negatively correlated with the OS of the patients, while high expression of CBX7 had a higher OS in EAC. Moreover, mRNA expressions of CBX1/2/3/4/5/7/8 were correlated with patients' individual cancer stages and the nodal metastatic status. These results indicated that CBX3/7/8 in EAC and CBX4 in EACC might be potential prognostic biomarkers. The functions of CBXs mainly involved DNA replication, methylation and mitosis. In addition, CBXs were also significantly related to the infiltration of six types of immune cells. In summary, this study may provide new ideas for the selection of prognostic biomarkers in the CBXs in ESCA.

Abbreviations

CBXs：Chromobox family proteins；ESCA：esophageal cancer；

OS：overall survival；ESCC：esophageal squamous cell carcinoma

EAC：esophageal adenocarcinoma；GO: Gene ontology;

KEGG: Kyoto Encyclopedia of Gene and Genome; BP：biological processes；

CC：cellular components；MF：molecular functions；

TIICs：Tumor-immune infiltrating cells；CRC：colorectal cancer；

CAMs：cell adhesion molecules；

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated from the analysis process of this study are available from the corresponding author on reasonable request.

Competing interests

All of the authors approved the publication of the paper and declared no conflicts of interests.

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Authors' contributions

Liangjiang Xia ,Wei Jiang analyzed and interpreted data , contributed equally to the whole study.. Liangbin Pan collect data and performed GO/KEGG analysis. Jingkang Yuan were major contributors in the manuscript writing. Haitao Ma, Yu Feng designed the study and revised the manuscript. All listed authors read and approved the final version of manuscript for publication.

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References

1. Sung H, Ferlay J, Siegel R, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians.* 2021.
2. Kelly R. Emerging Multimodality Approaches to Treat Localized Esophageal Cancer. *Journal of the National Comprehensive Cancer Network : JNCCN.* 2019;17(8):1009-1014.
3. Ma R, Zhang Y, Sun T, Cheng B. Epigenetic regulation by polycomb group complexes: focus on roles of CBX proteins. *Journal of Zhejiang University. Science. B.* 2014;15(5):412-428.
4. Kaustov L, Ouyang H, Amaya M, et al. Recognition and specificity determinants of the human cbx chromodomains. *The Journal of biological chemistry.* 2011;286(1):521-529.

5. van Wijnen A, Bagheri L, Badreldin A, et al. Biological functions of chromobox (CBX) proteins in stem cell self-renewal, lineage-commitment, cancer and development. *Bone.* 2021;143:115659.
6. He Z, Chen J, Chen X, Wang H, Tang L, Han C. microRNA-377 acts as a suppressor in esophageal squamous cell carcinoma through CBX3-dependent P53/P21 pathway. *Journal of cellular physiology.* 2021;236(1):107-120.
7. Wang G, Tang J, Zhan W, et al. CBX8 Suppresses Tumor Metastasis via Repressing Snail in Esophageal Squamous Cell Carcinoma. *Theranostics.* 2017;7(14):3478-3488.
8. Iqbal M, Siddiqui S, Ur Rehman A, et al. Multiomics integrative analysis reveals antagonistic roles of CBX2 and CBX7 in metabolic reprogramming of breast cancer. *Molecular oncology.* 2021.
9. Wang Z, Fang Z, Chen G, et al. Chromobox 4 facilitates tumorigenesis of lung adenocarcinoma through the Wnt/β-catenin pathway. *Neoplasia (New York, N.Y.).* 2021;23(2):222-233.
10. Jiang N, Niu G, Pan Y, et al. CBX4 transcriptionally suppresses KLF6 via interaction with HDAC1 to exert oncogenic activities in clear cell renal cell carcinoma. *EBioMedicine.* 2020;53:102692.
11. Li R, Yan Q, Tian P, et al. CBX7 Inhibits Cell Growth and Motility and Induces Apoptosis in Cervical Cancer Cells. *Molecular therapy oncolytics.* 2019;15:108-116.
12. Hoffmann M, Dehn J, Droop J, et al. Truncated Isoforms of lncRNA ANRIL Are Overexpressed in Bladder Cancer, But Do Not Contribute to Repression of INK4 Tumor Suppressors. *Non-coding RNA.* 2015;1(3):266-284.
13. Tang J, Wang G, Zhang M, et al. Paradoxical role of CBX8 in proliferation and metastasis of colorectal cancer. *Oncotarget.* 2014;5(21):10778-10790.
14. Zhang L, Zhou Y, Cheng C, et al. Genomic Analyses Reveal Mutational Signatures and Frequently Altered Genes in Esophageal Squamous Cell Carcinoma. *American journal of human genetics.* 2020;107(3):579.
15. Zhang Y, Chen H, Zhu H, Sun X. CBX8 promotes tumorigenesis and confers radioresistance in esophageal squamous cell carcinoma cells through targeting APAF1. *Gene.* 2019;711:143949.
16. Rhodes D, Yu J, Shanker K, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. *Neoplasia (New York, N.Y.).* 2004;6(1):1-6.
17. Chandrashekar D, Bashel B, Balasubramanya S, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia (New York, N.Y.).* 2017;19(8):649-658.
18. Nagy Á, Lánczky A, Menyhárt O, Győrffy B. Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets. *Scientific reports.* 2018;8(1):9227.
19. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer discovery.* 2012;2(5):401-404.
20. Gao J, Aksoy B, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Science signaling.* 2013;6(269):pl1.
21. Li T, Fu J, Zeng Z, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic acids research.* 2020;48:W509-W514.

22. Lee Y, Liu X, Qiu F, O'Connor T, Yen Y, Ann D. HP1β is a biomarker for breast cancer prognosis and PARP inhibitor therapy. *PloS one.* 2015;10(3):e0121207.
23. Chen Z, Sun S, Zhu S, Bu J. Identification of the Roles of Chromobox Family Members in Gastric Cancer: A Study Based on Multiple Datasets. *BioMed research international.* 2020;2020:5306509.
24. Yang Y, Pan Y, Tian Q, Wu D, Su S. CBX1 Indicates Poor Outcomes and Exerts Oncogenic Activity in Hepatocellular Carcinoma. *Translational oncology.* 2018;11(5):1110-1118.
25. Di Costanzo A, Del Gaudio N, Conte L, et al. The HDAC inhibitor SAHA regulates CBX2 stability via a SUMO-triggered ubiquitin-mediated pathway in leukemia. *Oncogene.* 2018;37(19):2559-2572.
26. Clermont P, Sun L, Crea F, et al. Genotranscriptomic meta-analysis of the Polycomb gene CBX2 in human cancers: initial evidence of an oncogenic role. *British journal of cancer.* 2014;111(8):1663-1672.
27. Wheeler L, Watson Z, Qamar L, et al. CBX2 identified as driver of anoikis escape and dissemination in high grade serous ovarian cancer. *Oncogenesis.* 2018;7(11):92.
28. Alam H, Li N, Dhar S, et al. HP1γ Promotes Lung Adenocarcinoma by Downregulating the Transcription-Repressive Regulators NCOR2 and ZBTB7A. *Cancer research.* 2018;78(14):3834-3848.
29. Liu M, Huang F, Zhang D, et al. Heterochromatin protein HP1γ promotes colorectal cancer progression and is regulated by miR-30a. *Cancer research.* 2015;75(21):4593-4604.
30. Lin H, Lian J, Xia L, Guan G, You J. CBX3 Promotes Gastric Cancer Progression and Affects Factors Related to Immunotherapeutic Responses. *Cancer management and research.* 2020;12:10113-10125.
31. Wang B, Tang J, Liao D, et al. Chromobox homolog 4 is correlated with prognosis and tumor cell growth in hepatocellular carcinoma. *Annals of surgical oncology.* 2013:S684-692.
32. Sanyal S, Mondal P, Sen S, Sengupta Bandyopadhyay S, Das C. SUMO E3 ligase CBX4 regulates hTERT-mediated transcription of CDH1 and promotes breast cancer cell migration and invasion. *The Biochemical journal.* 2020;477(19):3803-3818.
33. Hu C, Zhang Q, Tang Q, et al. CBX4 promotes the proliferation and metastasis via regulating BMI-1 in lung cancer. *Journal of cellular and molecular medicine.* 2020;24(1):618-631.
34. Zhang K, Wang J, Yang L, et al. Targeting histone methyltransferase G9a inhibits growth and Wnt signaling pathway by epigenetically regulating HP1α and APC2 gene expression in non-small cell lung cancer. *Molecular cancer.* 2018;17(1):153.
35. De Koning L, Savignoni A, Boumendil C, et al. Heterochromatin protein 1alpha: a hallmark of cell proliferation relevant to clinical oncology. *EMBO molecular medicine.* 2009;1(3):178-191.
36. Li G, Warden C, Zou Z, et al. Altered expression of polycomb group genes in glioblastoma multiforme. *PloS one.* 2013;8(11):e80970.
37. Deng H, Guan X, Gong L, et al. CBX6 is negatively regulated by EZH2 and plays a potential tumor suppressor role in breast cancer. *Scientific reports.* 2019;9(1):197.

38. Ni S, Wang H, Zhu X, et al. CBX7 suppresses cell proliferation, migration, and invasion through the inhibition of PTEN/Akt signaling in pancreatic cancer. *Oncotarget.* 2017;8(5):8010-8021.
39. Kim H, Park J, Won H, Lee J, Kong G. CBX7 inhibits breast tumorigenicity through DKK-1-mediated suppression of the Wnt/β-catenin pathway. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology.* 2015;29(1):300-313.
40. Pallante P, Terracciano L, Carafa V, et al. The loss of the CBX7 gene expression represents an adverse prognostic marker for survival of colon carcinoma patients. *European journal of cancer (Oxford, England : 1990).* 2010;46(12):2304-2313.
41. Emadi Baygi M, Soheili Z, Schmitz I, Sameie S, Schulz W. Snail regulates cell survival and inhibits cellular senescence in human metastatic prostate cancer cell lines. *Cell biology and toxicology.* 2010;26(6):553-567.
42. Zhang Y, Chen H, Zhu H, Sun X. CBX8 promotes tumorigenesis and confers radioresistance in esophageal squamous cell carcinoma cells through targeting APAF1. *Gene.* 2019;711:143949-.
43. Tanaka A, Sakaguchi S. Regulatory T cells in cancer immunotherapy. *Cell Res.* Jan 2017;27(1):109-118.
44. Ostuni R, Kratochvill F, Murray PJ, Natoli G. Macrophages and cancer: from mechanisms to therapeutic implications. *Trends Immunol.* Apr 2015;36(4):229-239.
45. Janco JMT, Lamichhane P, Karyampudi L, Knutson KL. Tumor-Infiltrating Dendritic Cells in Cancer Pathogenesis. *J. Immunol.* Apr 2015;194(7):2985-2991.

Figures

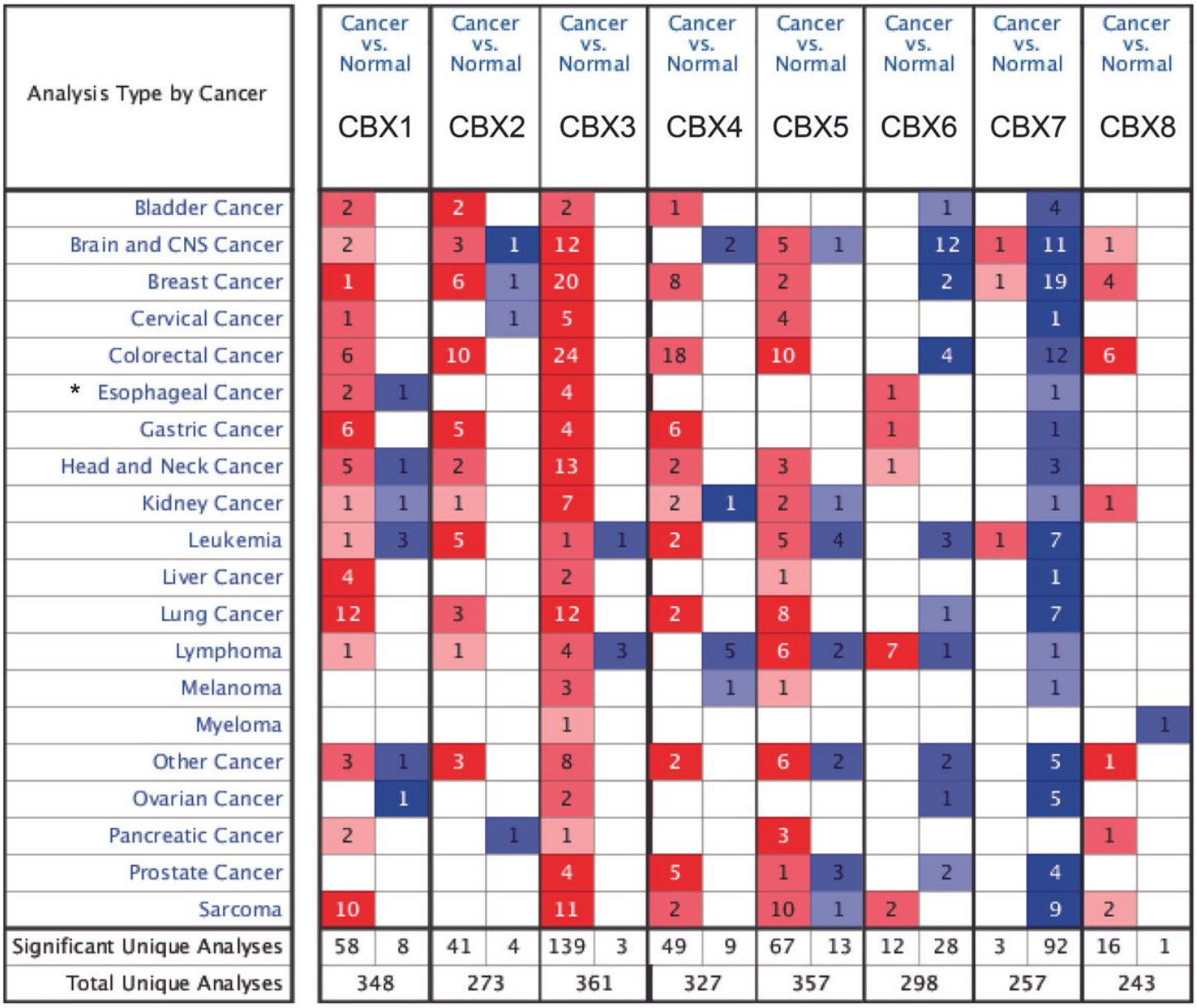

Figure 1

Transcriptional levels of the CBXs in different cancers (Oncomine, p value: 0.001 and fold −change: 1:5, gene rank: 10%, data type: mRNA , upregulated : red and downregulated :blue)

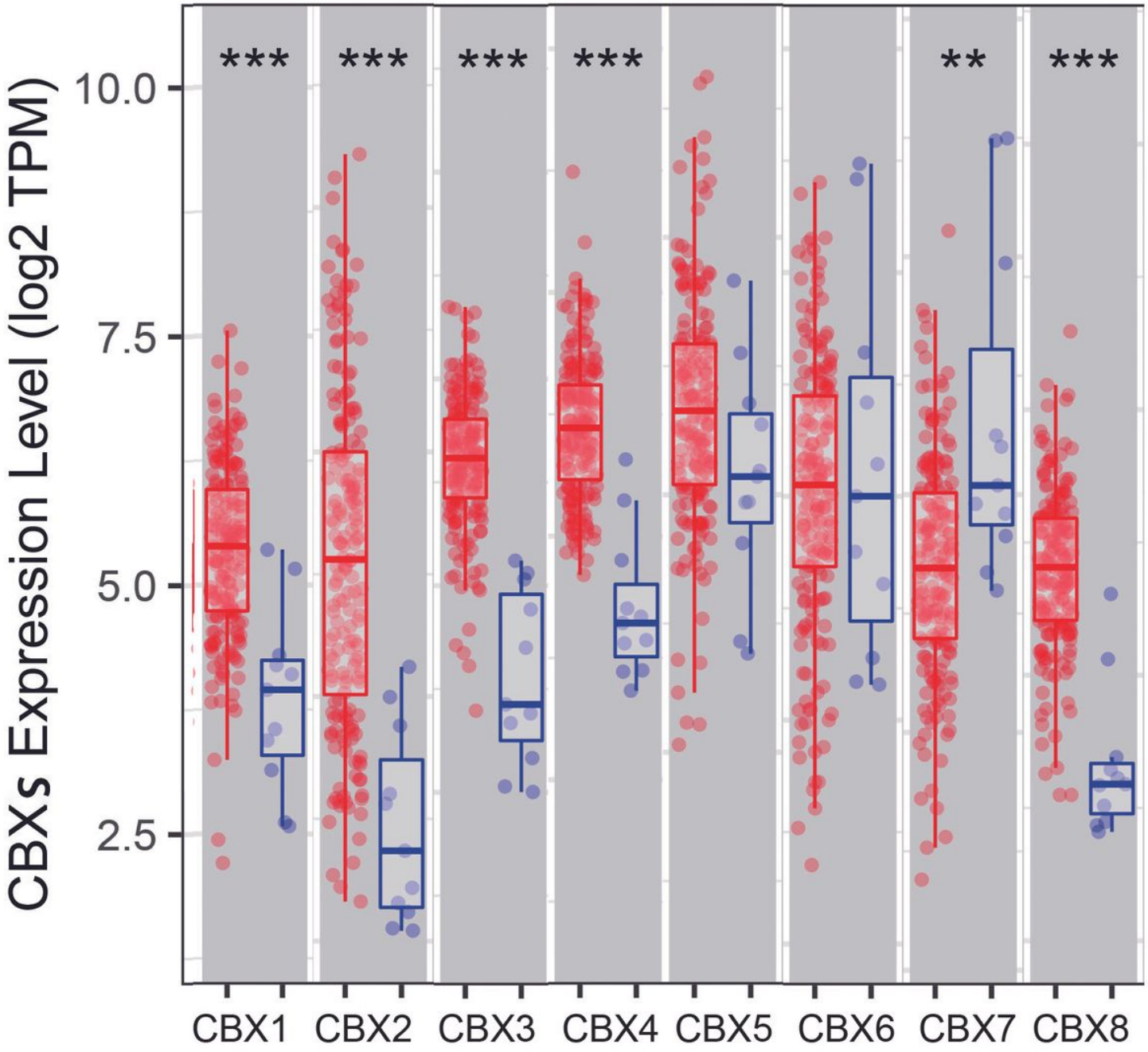

Figure 2

The mRNA expression level of CBXs in ESCA (TIMER2.0, *p<0.05, **p<0.01, ***p<0.001.). The expression level of CBX1/2/3/4/8 in tumor tissues was significantly higher than that in normal tissues, while the expression level of CBX7 was lower in the former than the latter

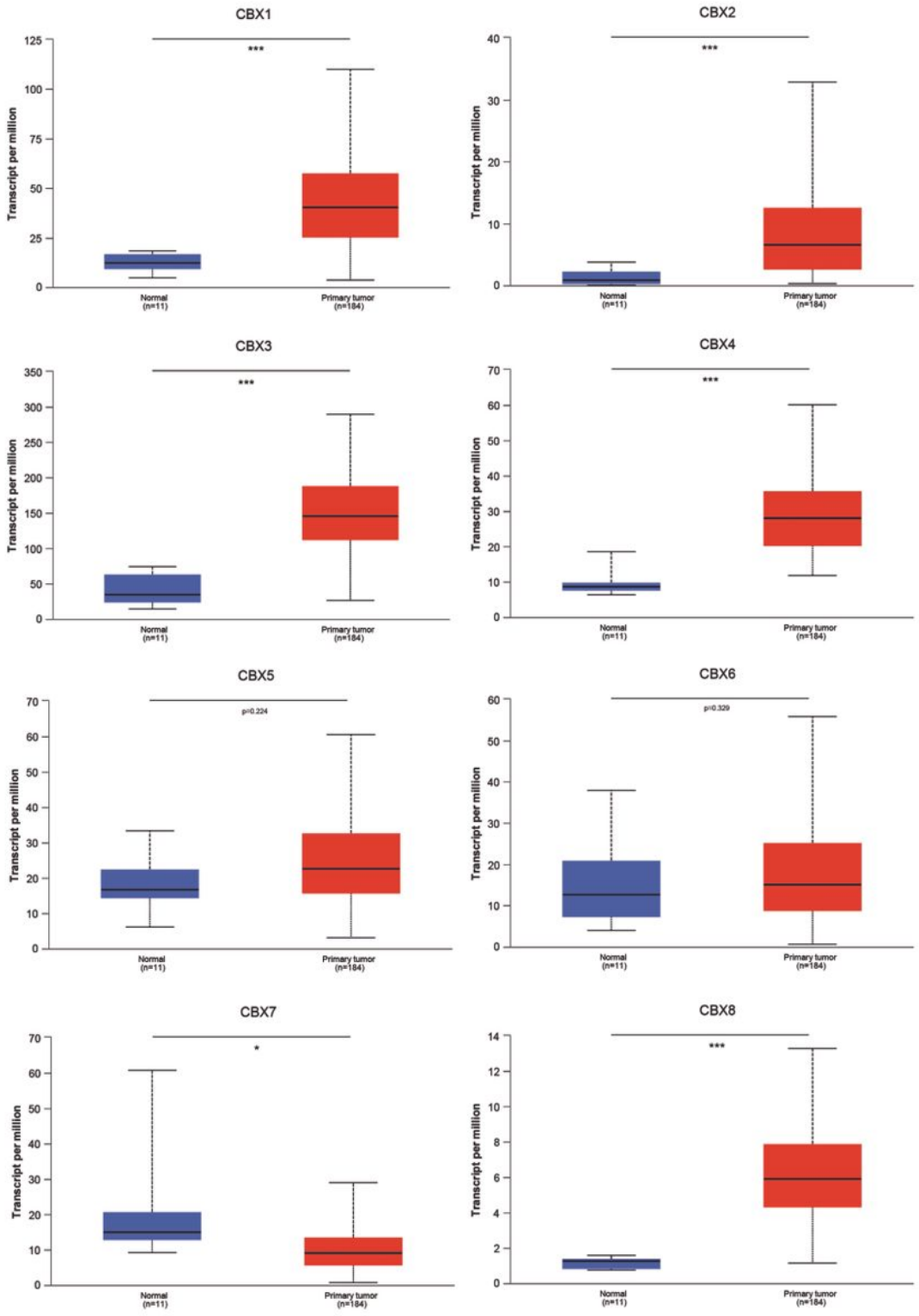

Figure 3

The mRNA expression of CBXs in ESCA tissues and normal tissues (UALCAN, *p<0.05, **p<0.01, ***p<0.001.). The expression level of CBX1/2/3/4/8 was significantly upregulated in ESCA.

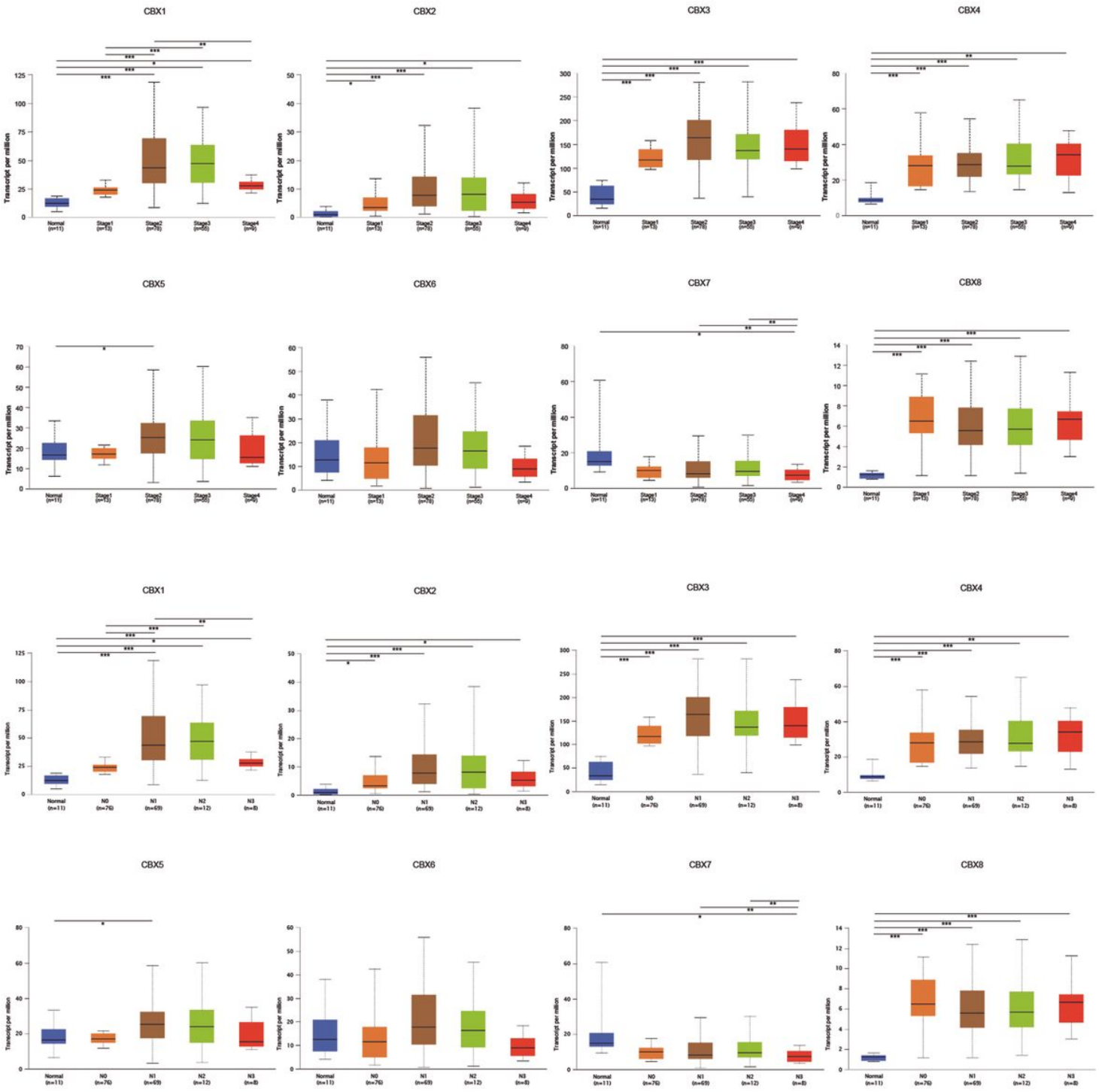

Figure 4

Clinicopathological parameters and CBX mRNA levels in ESCA patients (UALCAN, *p<0.05, **p<0.01, ***p<0.001): (a) relationship between mRNA expression of distinct CBXs and individual cancer stages; (b) relationship between mRNA expression of distinct CBXs and nodal metastatic status. Except for CBX6, other CBXs members are related to individual cancer stages and nodal metastatic status.

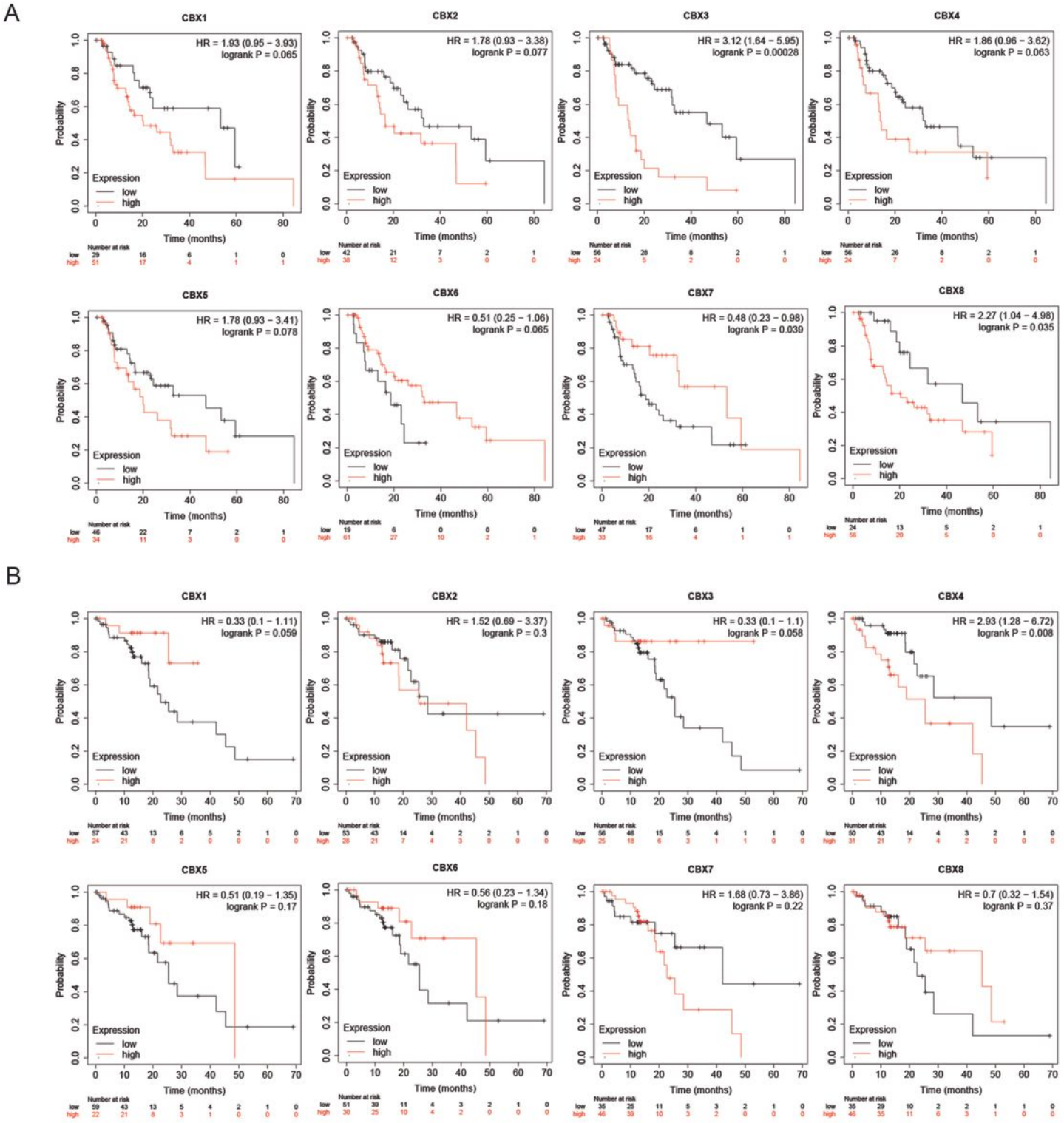

Figure 5

Prognostic value of mRNA level of CBXs in ESCA patients (Kaplan-Meier plotter, p <0:05 was considered statistically significant): (a) relationship between mRNA expression of distinct CBXs and OS in ESCC; (b) relationship between mRNA expression of distinct CBXs and OS in EAC;

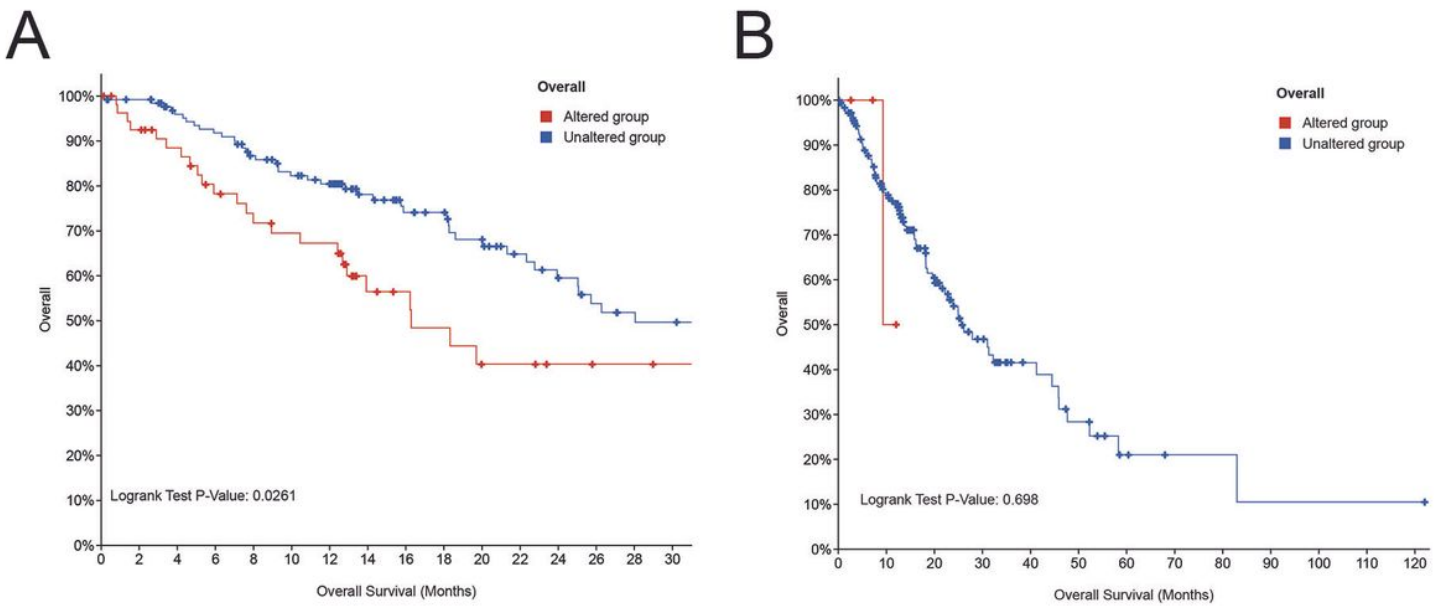

Figure 6

Prognostic value of mRNA level of CBXs in ESCA patients (cBioPortal, p <0:05 was considered statistically significant): (a) high mRNA expression of CBX3/4/8 was significantly correlated with poor OS in ESCA; (b) relationship between mRNA expression of CBX7 and OS in ESCA.

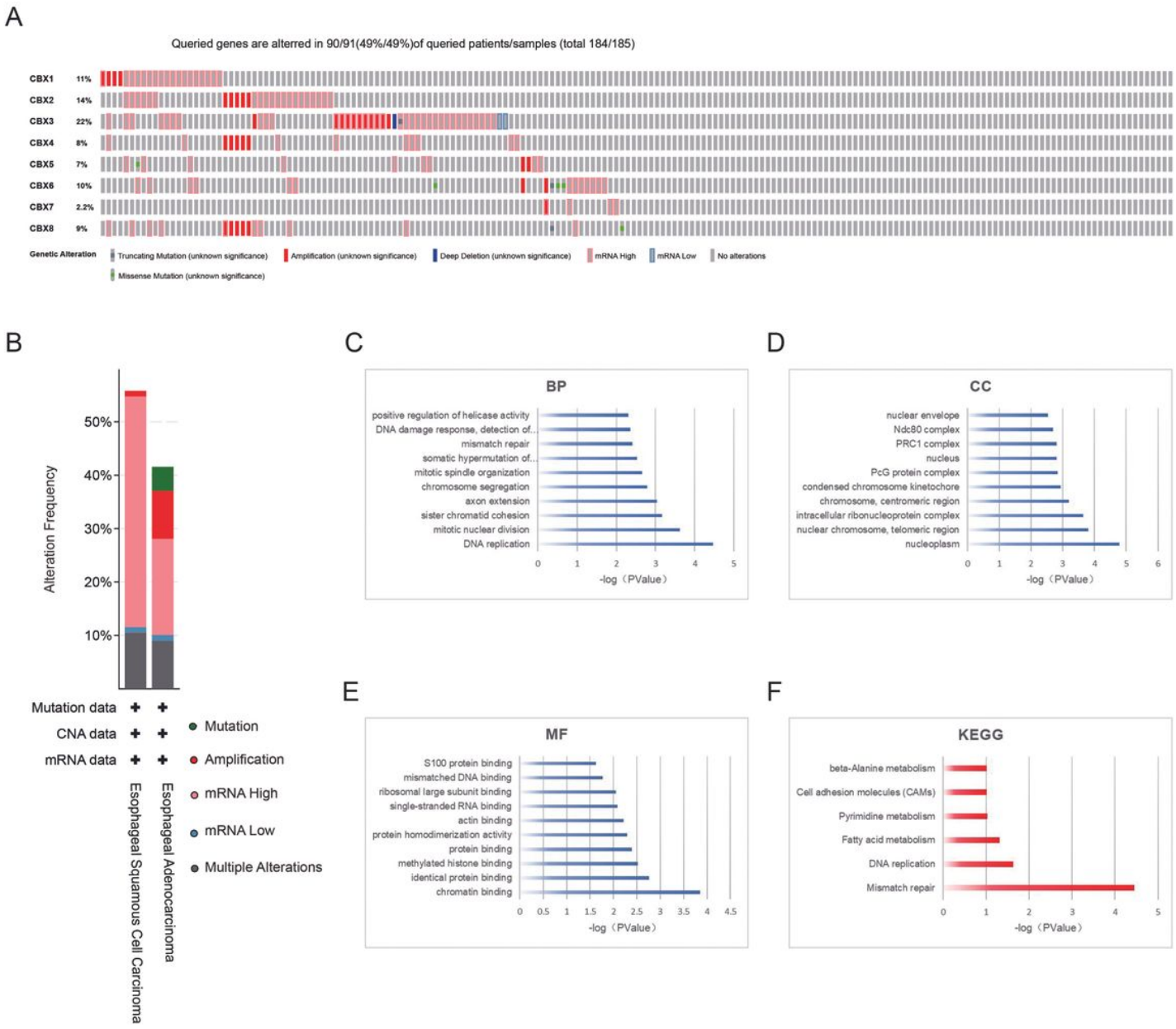

Figure 7

Genetic alterations, and enrichment analysis of CBXs in ESCA (cBioPortal, DAVID): (a) alterations in CBXs in ESCA; (b) summary of CBX alteration in ESCA; (c-f) enrichment analysis of CBXs. BP: biological processes; CC: cellular components; MF: molecular functions.

Figure 8

Correlations between differentially expressed CBXs and immune cell infiltration (TIMER2.0).

Supplementary Files

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- Additionalfile1..xlsx