

Chromobox4/5 Serve as Potential Prognostic Biomarkers and Targets for Breast Invasive Carcinoma

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Abstract

Background: Chromobox (CBX) is a family of epigenetic regulation complexes. Many shreds of evidence suggest that CBXs play a vital role in the tumorigenesis and progression of breast invasive carcinoma (BRCA). However, little is known about the alteration and exact roles of each CBX in BRCA.

Methods: In this study, we explored the exact roles of CBXs in the prognosis of BRCA by analyzing ONCOMINE, UALCAN, Kaplan-Meier Plotter, cBioProtal, STRING, and Metascape databases.

Results: The mRNA expression of CBX1/2/3/4/6/7/8 was significantly altered and associated with the tumor stage. The protein expression level of 8 CBXs was significantly changed and associated with the tumor stage. Moreover, aberrant expression of CBX3/5/7/8 was significantly associated with clinical outcome for BRCA. Multivariate analysis indicated that genetic alteration of CBX4/5 was an independent prognostic factor for the OS in BRCA patients.

Conclusions: These results imply that CBX4/5 might serve as prognostic biomarkers for survival in BRCA patients.

Background

Breast invasive carcinoma (BRCA), follows by lung cancer, is the second most common cancer worldwide, ranking as the first leading cause of cancer-related death among women[1]. With the help of improved therapy, death rates for BRCA have been decreasing during the past decade. However, the incidence of breast cancer has increased rapidly over the years, and the mortality rate is unfavorable. For a long time in the past, most patients wasted an optimal treatment opportunity because of no significant biomarkers at the early stage of BRCA. Scientists have made many efforts to study the mechanisms of the occurrence, development, and metastasis of BRCA. However, the molecular characteristics of breast cancer are still unclear so far.

Considerable evidence has confirmed that a selection of functional genes, such as BRCA1, BRCA2, EGFR, Cyclin D1, Laminin, and Calponin, are taking part in the carcinogenesis and development of BRCA[2]. Although these biomarkers mentioned above have gained the attention of scholars worldwide and further in-depth research has been conducted. Unfortunately, most of these biomarkers were investigated separately and not as a portion of the entire oncogenesis process of the BRCA. Furthermore, a considerable part of the research is still in the stage of preliminary investigation or clinical verification, which cannot provide much help in improving the screening, diagnosis, prognosis, prevention, and treatment of BRCA.

It is well known that the development and progression of breast cancer are the result of independent or combined genetic and epigenetic events[2]. Polycomb group (PcG) complexes are one of the critical complexes of epigenetic regulation, and their dysregulation is closely related to the pathological process of various cancers[3]. Chromobox (CBX) family proteins are one of the main components of PcG

complexes and play an essential role in inhibiting cell differentiation and self-renewal of tumor stem cells, which can regulate tumorigenesis and progression of many cancers, including BRCA[4]. A comprehensive study of the role of CBXs family members in the pathological process of BRCA will have a positive impact on revealing the etiology of BRCA and provide new potential targets for the prevention and treatment of BRCA.

As far as current knowledge, there are eight CBXs family proteins in the human genome, including CBX1-8. They play essential roles in the regulation of gene expression, heterochromatin, and developmental programs. In terms of molecular structure, CBXs can be divided into two groups. One is the HP1 group represented by CBX1, CBX3, and CBX5, which is mainly characterized by having an N-terminal chromosome domain and a C-terminal chromosome shadow domain; the other group is the Pc group represented by CBX2, CBX4, CBX6, CBX7, and CBX8, which contains only a conserved N-terminal chromosome domain. Unlike other family proteins, CBX family protein members localize to different regions of the chromatin depending on the function and do not overlap in the embryonic stem cells. A growing number of findings demonstrated that CBX protein plays a vital role in the development of multiple tumors[4]. Recently, Piqué DG and colleagues reported that CBX2 was a critical regulator in promoting the growth of breast cancer cells[5]. A study by Zeng JS and colleagues indicated that CBX4 could affect the carcinogenic activity of breast cancer by regulating the activity of the Notch1 signaling pathway[6]. Deng H's finding suggested that CBX6 is a negative regulator in breast cancer[7]. However, the roles of the majority of CBXs family members in the carcinogenesis, metastasis, invasion, and recurrence of BRCA remained mostly unknown.

Over the past few years, microarray technology and bioinformatics analysis are utilized widely, making it one of the essential research methods in modern medical biology. In the present study, we conducted a comprehensive analysis of the expression of 8 CBXs subtypes and their relations with clinical parameters in patients with BRCA.

Methods

ONCOMINE Analysis

The ONCOMINE database is an online cancer microarray platform, which is open and robust and provides a powerful data mining tool for cancer researchers[8]. In this study, we retrieved the transcriptional expression of CBXs family members in different cancer tissues and the corresponding normal tissues adjacent to them from the ONCOMINE database. The students' t-test was used to check the difference in the transcriptional expression. Moreover, the thresholds were defined as follows: Cut-off of the p-value: 0.01, fold change: 1.5, gene rank:10%, and data type: mRNA.

UALCAN Analysis

UALCAN—an authoritative, comprehensive, user-friendly, and interactive web resource—could be used to analyze the difference of target genes between tumors and normal tissues and the relationship between

target gene expression and clinical-pathological parameters[9]. In our study, the gene and protein levels of CBXs family members in primary BRCA tissues and their relationship with clinical-pathologic parameters were analyzed by UALCAN. $p \leq 0.05$ was considered statistically significant.

The Kaplan–Meier Plotter Analysis

Kaplan-Meier plotter is a web resource containing microarray gene expression data and survival information is based on databases including TCGA, Gene Expression Omnibus, and Cancer Biomedical Information Grid[10]. With the help of the Kaplan-Meier plotter, we can analyze the expression of target genes' prognostic value in corresponding cancer. In this study, we used the Kaplan-Meier plotter to explore the prognostic value of CBXs family members in the patients with BRCA. According to the mRNA expression of CBXs family members, patients with BRCA were divided into two groups (high vs. low expression). The K-M survival curves were plot based on the mRNA expression level and survival information of 1090 patients with BRCA. Only genes with p -value < 0.05 were considered as significant.

CBioPortal and STRING Analysis

CBioPortal and STRING are comprehensive, authoritative, and user-friendly analysis tools[11, 12]. In the cBioPortal, we screened out the 20 frequently neighbor genes associated with each CBX family member according to the operation instruction of the cBioPortal. Then we merged these genes into a gene list and submitted it to the STRING database to conduct a PPI network. Besides, clinicopathological parameters and mRNA expression of CBXs family members from 1084 BRCA patients were downloaded from the CBioPortal database for further analysis. 193 of 1084 BRCA patients were excluded because of the absence of complete follow-up data. Clinical data, including gender, age, race category, pathologic stage, and subtype of BRCA, are summarized in Table 2.

Cox regression analysis

We used Cox regression analysis to assess the association of mRNA expression of CBXs family members with patient survival by the SPSS 24.0 software (IBM) based on the data downloaded from the CBioPortal database. Firstly, we assessed the effect of clinical parameters and mRNA expression of CBXs family members on the survival in patients with BRCA by univariate Cox regression. We retained factors that $p \leq 0.1$ for subsequent analysis. Then, we further analyzed the association of mRNA expression of CBXs family members with patients with BRCA by multivariate Cox regression and adjusted it for other factors, such as age, race category, pathologic stage, and subtype of BRCA, with the method similar to the method of Gang N[13]. The difference was considered statistically significant when $p < 0.05$.

Functional Enrichment Analysis

Metascape is an automated and user-friendly bioinformatic analysis tool for gene annotation and analysis. It can help researchers to understand the biological process (BP), cellular component (CC), molecular function (MF), and pathways of Specific gene[14]. To explore the function of CBXs family

members and their neighbor genes, Metascape analysis was applied. The cut-off p -value and enrichment factors were as follows: p -value:0.05, minimum count of 3, and enrichment factor of >1.5.

Results

Transcription levels of CBXs family members in patients with BRCA

To investigate the potential prognosis and therapeutic value of in patients with BRCA, we explored the mRNA and protein expression of CBXs family members in the ONCOMINE database (www.oncomine.org) and UALCAN (<http://ualcan.path.uab.edu>).

ONCOMINE analysis showed that the mRNA expression of CBX1/2/3/4/5/8 was upregulated and CBX6/7 was downregulated in BRCA tissues when compared to normal tissues(Fig. 1 and Table 1).In the Curtis Breast dataset, CBX1 was over-expressed in BRCA tissues compared with normal tissue,with a fold change of 1.202 and a p -valueof 1.70E-02. In the Gluck Breast dataset, CBX1 was over-expressed in patients with BRCA, with a fold change of 1.365 and a p -value of 4.00E-02. The transcription level of CBX3 was also significantly upregulated in BRCA tissues.In the Curtis Breast datasetand Gluck Breast dataset, CBX3 was significantly upregulated in BRCA with fold changes of 1.597 (p -value= 1.80E-06) and 2.523 (p -value= 1.00E-03), respectively. The results from the Curtis Breast dataset displayed that there were 1.541-fold (p -value= 2.01E-04) and 1.393-fold (p -value= 8.93E-06) increased in CBX4 and CBX5 mRNA expression in BRCA tissues, respectively. There was a similar trend in the Gluck Breast dataset: mRNA expression levels of CBX4 (fold change = 1.326, and p -value = 3.80E-02) and CBX5(fold change = 1.530, and p -value = 1.00E-02) were significantly higher than the normal samples. In the Gluck Breast dataset, CBX8 was over-expressed in patients with BRCA, with a fold change of 1.813 and a p -value of 1.00E-02.

Table 1
Significant changes of CBXs expression in transcription level between BRCA and normal breast tissues (ONCOMINE)

	Types of BRCA VS. Normal	p-value	t-test	Fold Change	Ref	PMID
CBX1	Breast invasive carcinoma	1.70E-02	2.248	1.202	Curtis Breast	22522925
	Breast invasive carcinoma	4.00E-02	2.469	1.365	Gluck Breast	21373875
CBX3	Breast invasive carcinoma	1.80E-06	6.083	1.597	Curtis Breast	22522925
	Breast invasive carcinoma	1.00E-03	7.871	2.523	Gluck Breast	21373875
CBX4	Breast invasive carcinoma	2.01E-04	4.127	1.541	Curtis Breast	22522925
	Breast invasive carcinoma	3.80E-02	2.518	1.326	Gluck Breast	21373875
CBX5	Breast invasive carcinoma	8.93E-06	5.472	1.393	Curtis Breast	22522925
	Breast invasive carcinoma	1.00E-02	4.221	1.53	Gluck Breast	21373875
CBX8	Breast invasive carcinoma	1.00E-02	4.338	1.813	Gluck Breast	21373875

Association of gene and protein levels of CBXs family members with clinical-pathological parameters in patients with BRCA

Next, we conducted a more in-depth analysis of the mRNA expression patterns of the eight CBXs family members using the UALCAN database (Fig. 2A). Unlike the ONCOMINE database, resources of UALCAN are based on level 3 RNA-seq and clinical data from 31 cancer types from the TCGA database. As the result shown in Fig. 2A, mRNA expression of CBX1/2/3/4/6/7/8 was significantly different in BRCA tissues than that in normal tissues, whereas the transcription expression level of CBX5 was not significantly different between BRCA and normal tissues. In addition to examining the mRNA expression pattern of CBXs in BRCA, we also attempted to explore the protein expression pattern of CBXs in BRCA by CPTAC (Clinical Proteomic Tumor Analysis Consortium). As shown in Fig. 2B, significant differences in protein expression levels of all 8 CBXs family members were found in BRCA compared to normal tissues (all $p < 0.05$). The protein expression levels of CBX1/2/3/4/5/7/8 were upregulated, and CBX6 was down-regulated in BRCA tissues when compared to normal tissues. As for the inconsistent results of CBX5 gene and protein expression, the possible reasons are that CBX5 is regulated at the translational level during the development of BRCA.

After discovering that the mRNA and protein levels of CBXs family members in BRCA were different from normal tissues, we next analyzed the relationship between the mRNA and protein levels of different CBXs family members and the patients' individual cancer stages by UALCAN (<http://ualcan.path.uab.edu>). As shown in Fig. 2C1-8, mRNA expressions of CBX1/2/3/4/6/7/8 were remarkably correlated with patients' individual cancer stages. The mRNA expressions of CBX1/2/3/4/8 were positively related to the patients' individual cancer stages, while CBX6/7 were inversely related to it. Moreover, the protein expression levels of CBXs were also correlated with cancer stages in patients with BRCA (Fig. 2D1-8). However, gene and mRNA expression levels of CBXs seemed to be not absolutely positively or negatively correlated with cancer stage, and the reason may be due to the small sample size (only 4 BRCA patients were at stage 2).

In general, the results above indicated that gene or protein levels of CBXs family members were significantly associated with the clinicopathological parameters of BRCA patients.

Prognostic value of mRNA expression of CBXs family members in patients with BRCA

Furthermore, we conducted a Kaplan-Meier plotter analysis to reveal the prognostic significance of mRNA expression of CBXs family members in patients with BRCA. As Kaplan-Meier survival curves shown in Fig. 3, higher mRNA expressions of CBX3 (HR = 1.48, 95% CI: 1.07–2.05, and log-rank $p = 0.018$), CBX5 (HR = 1.50, 95% CI: 1.08–2.10, and log-rank $p = 0.016$) and CBX8 (HR = 0.66, 95% CI: 0.48–0.91, and log-rank $p = 0.012$) were closely associated with poor OS (over survival), while lower mRNA expression of CBX7 (HR = 0.58, 95% CI: 0.42–0.79, and log-rank $p = 0.000064$) was significantly associated with favorable OS in patients with BRCA. The alteration of mRNA expression of CBX1 (HR = 1.33, 95% CI: 0.97–1.84, and log-rank $p = 0.077$), CBX2 (HR = 1.36, 95% CI: 0.98–1.89, and log-rank $p = 0.065$), CBX4 (HR = 1.18, 95% CI: 0.84–1.67, and log-rank $p = 0.340$) and CBX6 (HR = 0.75, 95% CI: 0.53–1.05, and log-rank $p = 0.096$) has no relationship with the OS in patients with BRCA. In summary, the results above suggested that the increased CBX3/5/8 and decreased CBX7 mRNA expressions were associated with poor OS in patients with BRCA.

Independent prognostic value of mRNA expression of CBXs family members in terms of OS in patients with BRCA

As we had found that some of CBXs family members are closely associated with the prognosis of patients with BRCA, we explored the independent prognostic value of mRNA expression of CBXs family members in terms of OS (over survival) in BRCA patients. For the need of Cox survival regression analysis, we downloaded clinical data and mRNA expression of CBXs family members of 894 BRCA patients in the TCGA database from the cBioPortal website (Table 2).

Table 2

Basic characteristics of 891 BRCA patients.

Variables	BRCA patients(N=891)
Gender (Male/Female)	0/891
Age (years, Mean±SD)	57.72±13.10
Race Category	
White	673
Black or African American	158
Asian	59
American Indian or Alaska Native	1
Pathologic stage	
1	160
2	515
3	195
4	13
5	8
Subtype	
BRCA_Basal	164
BRCA_Her2	66
BRCA_LumA	458
BRCA_LumB	168
BRCA_Normal	35

Univariate analysis showed that high age (HR = 1.034,95% CI = 1.020–1.048, p-value = 0.000), advanced pathologic stage (HR = 1.656,95% CI = 1.399–1.959, p-value = 0.000),high mRNA expression of CBX5(HR = 1.247,95% CI = 1.071–1.452, p-value = 0.004), and low mRNA expression of CBX4(HR = 0.753,95% CI = 0.579–0.979, p-value = 0.034)were related to poor OS of patients with BRCA(Table 3).

Table 3

Univariate analysis of overall survival in 891 BRCA specimens.

Variables	Univariate analysis		
	Hazard ratio	95%CI	p-value
Age(years)	1.034	1.020–1.048	0.000*
Race Category	1.033	0.831–1.283	0.773
Pathologic stage	1.656	1.399–1.959	0.000*
Subtype	1.046	0.888–1.231	0.876
CBX1	1.156	0.968–1.380	0.110
CBX2	0.985	0.811–1.196	0.878
CBX3	1.034	0.872–1.226	0.700
CBX4	0.753	0.579–0.979	0.034*
CBX5	1.247	1.071–1.452	0.004*
CBX6	0.961	0.799–1.157	0.676
CBX7	0.882	0.724–1.074	0.211
CBX8	0.841	0.638–1.109	0.220

In multivariate analysis, we found that advanced pathologic stage(HR = 1.734,95% CI = 1.456–2.063, p-value = 0.000), high mRNA expression of CBX4(HR = 0.678,95% CI = 0.462–0.994, p-value = 0.047) and CBX5(HR = 1.224,95% CI = 1.019–1.470, p-value = 0.030) were independently associated with shorter OS in patients with BRCA(Table 4). In shorter, these results implied that the alteration of mRNA expression of CBX4 and CBX5 was an independent prognostic factor for the OS in patients with BRCA.

Table 4
Multivariate analysis of overall survival in 891 BRCA specimens.

Variables	Multivariate analysis		
	Hazard ratio	95%CI	p-value
Age(years)	1.037	1.023–1.051	0.000*
Race Category	0.930	0.728–1.186	0.561
Pathologic stage	1.734	1.456–2.063	0.000*
Subtype	1.017	0.821–1.258	0.876
CBX1	1.183	0.962–1.453	0.110
CBX2	0.953	0.710–1.278	0.749
CBX3	0.881	0.678–1.144	0.343
CBX4	0.678	0.462–0.994	0.047*
CBX5	1.224	1.019–1.470	0.030*
CBX6	0.933	0.725–1.200	0.589
CBX7	0.896	0.698–1.150	0.390
CBX8	1.070	0.740–1.547	0.717

Protein-protein interaction (PPI) network construction

We further analyzed the 20 neighbor genes significantly associated with each CBXs family member via cBioPortal and crossed these genes with CBXs family members to obtain a collection containing 163 genes. Then, this gene collection was submitted to the STRING database to identify the interactions of them at the protein expression level. Furthermore, the PPI network is shown in Fig. 4A.

Functional Enrichment Analysis of CBXs family members in Patients With BRCA

In order to predict the functions and pathways of CBXs family members and their neighbor genes, we conducted functional enrichment analysis by analyzing GO(Gene Ontology) and KEGG(Kyoto Encyclopedia of Genes and Genomes) in Metascape.

The top 20 GO enrichment items included three functional groups: biological processes (10 items), cellular component (8 items), and molecular function (2 items), as shown in Fig. 4. Biological processes such as GO:0007059(chromosome segregation), GO:0010564(regulation of cell cycle process), GO:0051304(chromosome separation), GO:0033044(regulation of chromosome organization), GO:0045132(meiotic chromosome segregation), GO:0051052(regulation of DNA metabolic process),

GO:0051307(meiotic chromosome separation),GO:0016570(histone modification), GO:0070507(regulation of microtubule cytoskeleton organization),GO:0022411(cellular component disassembly) were significantly regulated by the CBXs and their neighbor genes alteration in patients with BRCA. Cellular components, including GO:0098687(chromosomal region), GO:0005819(spindle), GO:1990234(transferase complex), GO:0000792(heterochromatin), GO:0005815(microtubule organizing center), GO:0000307(cyclin-dependent protein kinase holoenzyme complex), GO:0045171(intercellular bridge) and GO:0010369(chromocenter) were significantly associated with the mutation of CBXs and their neighbor genes. In addition, the alteration of CBXs and their neighbor genes also conspicuously affected the molecular functions, such as GO:0003682(chromatin binding) and GO:0042826(histone deacetylase binding).

In KEGG analysis,the top 8 pathways for the CBXs family members and their neighbor genes, including hsa04110(Cell cycle), hsa04120(Ubiquitin mediated proteolysis), hsa04115(p53 signaling pathway), hsa03460(Fanconi anemia pathway), hsa05168(Herpes simplex infection), hsa04540(Gap junction), hsa04530(Tight junction) and hsa05202(Transcriptional misregulation in cancer) were identified(Fig. 4).Among these pathways, the cell cycle signaling pathway, p53 signaling pathway and Transcriptional misregulation in cancer were reported to be closely related to the tumorigenesis and pathogenesis of multiple cancer. Furthermore, we also carried out a Metascape protein-protein interaction enrichment analysis to further understand the relationship between CBXs family members and BRCA (Fig. 4). There are four MOCDE components drawn out from the PPI network. As the results are shown in Fig. 4, the biological function of the most significant MOCDE component was mainly associated with cell cycle, Oocyte meiosis, and Progesterone-mediated oocyte maturation.

Discussion

Abnormal epigenetic regulation is one of the vital pathogenesis mechanisms in many tumors, including BRCA. Numerous studies have shown that CBXs family members participate in the regulation of proliferation, differentiation, and apoptosis, which are essential factors for epigenetic regulation in the development and progression of BRCA[4]. Although the role of CBXs family members in BRCA has been partially confirmed, a comprehensive elucidation of the different roles of distinct CBXs members in BRCA is still necessary. In the present study, we sought to comprehensively explore the expression patterns, association with clinical parameters, and the prognostic value of CBXs family members in BRCA.

Results from our research indicated that almost all CBXs family members in BRCA were aberrantly regulated at the gene or protein level compared to normal controls, suggesting that they have an essential role in the tumorigenesis of BRCA. Although the gene and protein expression of CBX5 is inconsistent, it may be explained by the small sample size, and it may also be related to that CBX5 is regulated during the translation process.Studies by several groups have implied that CBX5 is over-expressed in breast cancer cells[15, 16].

In the present study, due to the small sample size of some cancer stage, the relationship between CBXs and cancer stage is not an absolute correlation. Nevertheless, mRNA or protein expression of CBXs family members is associated with the clinicopathological parameters of BRCA patients. Besides, the results of the Kaplan-Meier plotter analysis revealed that higher mRNA expressions of CBX3/5/8 were closely associated with poor OS. In contrast, lower CBX7 was significantly associated with favorable OS patients with BRCA. Multivariate analysis demonstrated that upregulation in the mRNA of CBX4/5 was an independent risk factor for poor OS in BRCA patients.

Finally, we investigated the potential biological functions and involved pathways of the genetic alteration in CBXs and their neighbor genes in BRCA patients. Our results implied that biological processes such as GO:0007059(chromosome segregation), cellular components, including GO:0098687(chromosomal region), molecular functions, such as GO:0003682(chromatin binding), and pathways such as hsa04110(Cell cycle) were significantly associated with the alteration of CBXs and their neighbor genes in BRCA.

CBX1(also called HP1 β), a classic member of the CBXs family, plays pivotal roles in the epigenetic control of chromatin structure and gene expression. The mobilization of CBX1 could promote chromatin changes, which will initiate the DNA damage response[17]. Over-expression of CBX1 had been found in hepatocellular carcinoma tissues and cell lines at both protein and mRNA levels. In HCC tissues, higher CBX1 expression correlated with larger tumor size, poor tumor differentiation, and tumor metastasis[18]. Similarly, the study by M Shiota and colleagues indicated that the overexpression of CBX1 exerts oncogenic activity in patients with prostate cancer, which implied poor clinical outcomes[19]. Recently, CBX1 has been found to be overexpressed in BRCA. The up-regulation of CBX1 was associated with unfavorable overall survival and disease-free survival in patients with BRCA. Young-Ho Lee and colleagues found that CBX1 expression was upregulated in nearly 60% of BRCA samples, and CBX1 expression level was positively correlated with the differentiation of the BRCA and the expression of Ki-67, a well-known BRCA biomarker. Therefore, they considered CBX1 as one of the potential biomarkers for breast cancer prognosis[20]. Our study results suggested that the mRNA and protein expression levels of CBX1 in BRCA tissues were significantly higher than that in the normal tissues. Furthermore, the mRNA and protein expression of CBX1 was closely associated with patients' pathological stage. Inconsistent with the previous study, there was no significant association between CBX1 and clinical outcomes in our study. Moreover, it may attribute to the different pathological types of patients included in the two studies.

CBX2 participates in the regulation of many cellular processes, such as cell cycle, proliferation, apoptosis, and tumorigenesis. In recent years, research on CBX2 as a tumor gene has been widely reported. Mao J et al. revealed that mRNA expression of CBX2 was upregulated in HCC cell, and knockdown of CBX2 significantly increased the apoptosis of HCC cell by inhibiting the expression of WTIP, a vital regulator of the Hippo pathway[21]. A study by Wheeler LJ demonstrated that CBX2 acts as an essential promoter of high grade serous ovarian cancer[22]. However, the connection between CBX2 and BRCA has not been studied in depth. In the present study, mRNA and protein expression was significantly increased and

associated with clinical-pathological parameters of BRCA patients, which was following the findings of Zheng S's study. However, results in our study found that the relationship between mRNA expression of CBX2 and clinical outcome of BRCA patients was not significant, and further studies are needed.

CBX3(also encoded by HP1 γ) is upregulated in various types of cancers, including pancreatic cancer, hepatocellular carcinoma, osteosarcoma, and lung adenocarcinoma[3, 23]. Alam H et al. revealed that CBX3 is one of the most frequently upregulated proteins in human lung adenocarcinoma (LUAD), and high HP1 γ mRNA levels are associated with poor prognosis in LUAD patients[24]. Zhao SP et al. reported that CBX3 stimulated the growth of glioma by regulating the activity of CDKN1A(Cyclin dependent kinase inhibitor 1), suggesting that CBX3 is a protumorigenic gene in glioma[25]. The results of our study found that the mRNA and protein expression in BRCA was higher than that in normal tissues. Additionally, the correlation between high CBX3 expression and the clinicopathological characteristics of patients with BRCA was also confirmed. Furthermore, the outcomes of survival analysis indicated that high CBX3 expression is associated with poor prognosis in BRCA patients, implying that CBX3 participated in the tumorigenesis of BRCA.

CBX4 is involved in the proliferation and migration of cancer cells. Similar to CBX2, CBX4 exhibits oncogenic activities in a series of malignancies, such as osteosarcoma, lung cancer, and hepatocellular carcinoma[4]. A study by Hu C et al. showed that CBX4 stimulates the proliferation and metastasis of lung cancer by regulating the expression level of BMI-1(B cell specific insertion site-1 of molotovirus)[26]. Previously, several teams have reported that CBX4 was overexpressed in BRCA tissue, and higher mRNA expression of CBX4 has a close relationship with clinical parameters such as tumor size, individual cancer stage, and shorter OS[6, 27]. Results from our study revealed that the gene and protein expression of CBX4 was upregulated in BRCA and was significantly correlated to the individual cancer stage. Moreover, an elevated level of CBX4 showed no significant association with reduced OS in patients with BRCA. These results mentioned above from our study implied that CBX4 might play a unique role in the development and progression of BRCA.

CBX5(also called HP1 α) is essential for the proliferation and metastasis of aberrant cells. The repressive growth characteristics of CBX5 have been reported in several tumors, such as gastric tumor, and lung cancer[28, 29]. However, a study by Vad-Nielsen J and colleagues suggested that CBX5 was down-regulated at mRNA and protein level in metastatic BRCA compared to the non-metastatic one[3]. Moreover, this result implied that CBX5 is a tumor suppressor in BRCA. Similar to the study by Vad-Nielsen J et al., our results showed that CBX5 was increased in the BRCA and was dramatically related to patients' individual cancer stages. Besides, results in our study found that high mRNA expression of CBX5 predicts the poor prognosis for BRCA patients. Consistent with the role of CBX4 as an oncogene, high expression of CBX5 is an independent risk factor for shorter OS of BRCA patients, indicating that CBX5 may become a potential prognostic biomarker for BRCA patients.

CBX6 is an essential member of the CBXs family. Mounting evidence has demonstrated that CBX6 has growth-inhibitive characteristics and serves as a tumor suppressor in several human cancers[7, 30].

However, few studies focus on the prognostic value of CBX6 in BRCA. In our study, the mRNA and protein expression of CBX6 was decreased in BRCA tissues compared to normal tissues. And a lower expression of CBX6 was markedly inversely correlated with patients' individual stage. Besides, survival analysis results demonstrated that the prognostic role of CBX6 in BRCA is not yet affirmatory. In a word, further verifications are needed to figure out the exact role of CBX1/2/4/6 in BRCA patients.

CBX7, one of the classical CBXs family members, had been reported conflicting roles in different types of tumors. Some studies suggested that CBX7 was increased in ovarian cancer and prostate cancer[31, 32]. Compared with patients expressing down-regulated CBX7, patients with upregulated CBX7 had lower overall survival and progression-free survival[32]. On the other hand, few studies had found that the expression of CBX7 was decreased in some malignant carcinomas, such as lung cancer, thyroid cancers, and bladder cancers[33, 34]. Moreover, lower expression of CBX7 exhibits poor prognosis, indicating that CBX7 serves as an oncosuppressor in these cancers. Regarding BRCA, the transcription level of CBX7 was decreased in BRCA tissues and was markedly and negatively correlated with the OS of BRCA patients. In the present study, the mRNA and protein expression of CBX7 in BRCA was slightly higher than those in normal tissues and was dramatically positively correlated with patients' individual cancer stages in BRCA patients. In addition, alteration in CBX7 expression was associated with OS in BRCA patients, suggesting a unique role for CBX7 in BRCA.

CBX8 has been established as a pivotal protein in regulating cell cycle progression, cell proliferation, and cell differentiation in numerous tumors, such as hepatocellular carcinoma, esophageal squamous cell carcinoma, and colon cancer. Emerging evidence suggests that patients with tumors mentioned above exhibited high expression of CBX8 and poor OS[35–37]. Some research teams have made efforts to reveal the prognostic significance of CBX8 in different cancers. However, the results are quite different. Some studies implied that the high expression of CBX8 was one of the poor factors for the prognosis of colorectal cancer[38]. In contrast, others suggested that high expression of CBX8 was significantly associated with better clinical outcomes in patients with esophageal squamous cell carcinoma[35]. In our study, higher mRNA and protein expression of CBX8 was observed in BRCA tissues. Moreover, BRCA patients with high CBX8 expression had advanced cancer stages compared to patients with low CBX8 expression. However, survival analysis in our study suggested that mRNA expression of CBX8 showed a significant correlation with prognosis in BRCA patients.

The limitations of our research are as follows: Firstly, all data applied in this research came from free and open databases on the Internet. In other words, further study is needed to confirm the prognostic roles of CBXs. Secondly, the study did not address the diagnosis and treatment of CBX in BRCA, so the role of CBXs in the diagnosis and treatment of BRCA will need to be investigated subsequently. Finally, the results of our study lack further experimental validation. Therefore, further study is needed to verify the role of distant CBXs in the occurrence and development of BRCA.

Conclusion

The mRNA or protein expression of CBXs family members was significantly changed and dramatically associated with tumor stage in patients with BRCA. An aberrant expression of CBX was found to be significantly associated with the clinical outcomes of BRCA patients. Moreover, multivariate analysis implied that genetic alteration in CBX4/5 was an independent risk factor for poor OS in patients with BRCA. The results above will contribute to a better understanding of the molecular biology mechanism of BRCA and help to provide potential targets and develop strategies for more accurate targeting for the clinical prediction and treatment for BRCA patients.

Abbreviations

CBX:Chromobox;CBX1/2/3/4/6/7/8:Chromobox1/2/3/4/6/7/8; BRCA: breast invasive carcinoma; BRCA1/2: breast invasive carcinoma1/2; EGFR: epidermal growth factor receptor; PcG: Polycomb group; BP: biological process; CC: cellular component; MF: molecular function;HR: Hazard ratio; CI: confidence interval;OS: over survival; PPI: Protein-protein interaction; KEGG:Kyoto Encyclopedia of Genes and Genomes;GO: Gene Ontology; LUAD: human lung adenocarcinoma; CDKN1A:Cyclin dependent kinase inhibitor 1; BMI-1:B cell specific insertion site-1 of molotovirus;

Declarations

Ethics approval and consent to participate

Our research protocol has been approved by the Ethics Committee of the Guangzhou Red Cross Hospital(2020.347). Since the source data of the study were retrieved from public online databases, it could be confirmed that written informed consent has been obtained.

Consent for publication

Not applicable.

Competing interests

All the authors declared that there was no conflict of interest with the contents of this article.

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Authors' contributions

LB carried out the data search, participated in the study design. YY carried out the statistics analysis. DP carried out the Functional Enrichment Analysis. All authors read and approved the final manuscript.

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Availability of data and materials

Further information and requests for resources should be directed to Dongping Ye (yedongping927@126.com).

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Figures

Analysis Type by Cancer	Cancer vs. Normal															
	CBX1	CBX2	CBX3	CBX4	CBX5	CBX6	CBX7	CBX8	CBX1	CBX2	CBX3	CBX4	CBX5	CBX6	CBX7	CBX8
Bladder Cancer	2	2	2	1		1	4									
Brain and CNS Cancer	2	3	1	12	2	5	1	12	1	11	1					
Breast Cancer	1	6	1	20	8	2		2	1	19	4					
Cervical Cancer	1		1	5		4				1						
Colorectal Cancer	6	10		24	18	10		4		12	6					
Esophageal Cancer	2	1		4				1		1						
Gastric Cancer	6	5		4	6			1		1						
Head and Neck Cancer	5	1	2	13	2	3		1		3						
Kidney Cancer	1	1	1	7	2	1	2	1		1	1					
Leukemia	1	3	5	1	1	2		5	4		3	1	7			
Liver Cancer	4			2		1				1						
Lung Cancer	12	3		12	2	8			1	7						
Lymphoma	1	1		4	3		5	6	2	7	1		1			
Melanoma				3		1	1						1			
Myeloma				1												1
Other Cancer	3	1	3	8	2	6	2		2	5	1					
Ovarian Cancer		1		2					1	5						
Pancreatic Cancer	2		1	1		3					1					
Prostate Cancer				4	5	1	3		2	4						
Sarcoma	10			11	2	10	1	2		9	2					
Significant Unique Analyses	58	8	41	4	139	3	49	9	67	13	12	28	3	92	16	1
Total Unique Analyses	348		273		361		327		357		298		257		243	

Figure 1

The transcription levels of CBXs family members in different types of cancer diseases (ONCOMINE). The numbers in the figure represent statistically significant data sets, where red represents high expression and blue represents target gene down-regulation. The threshold was as following: p-value: 0.01, fold change: 1.5, gene rank: 10%, data type: mRNA.

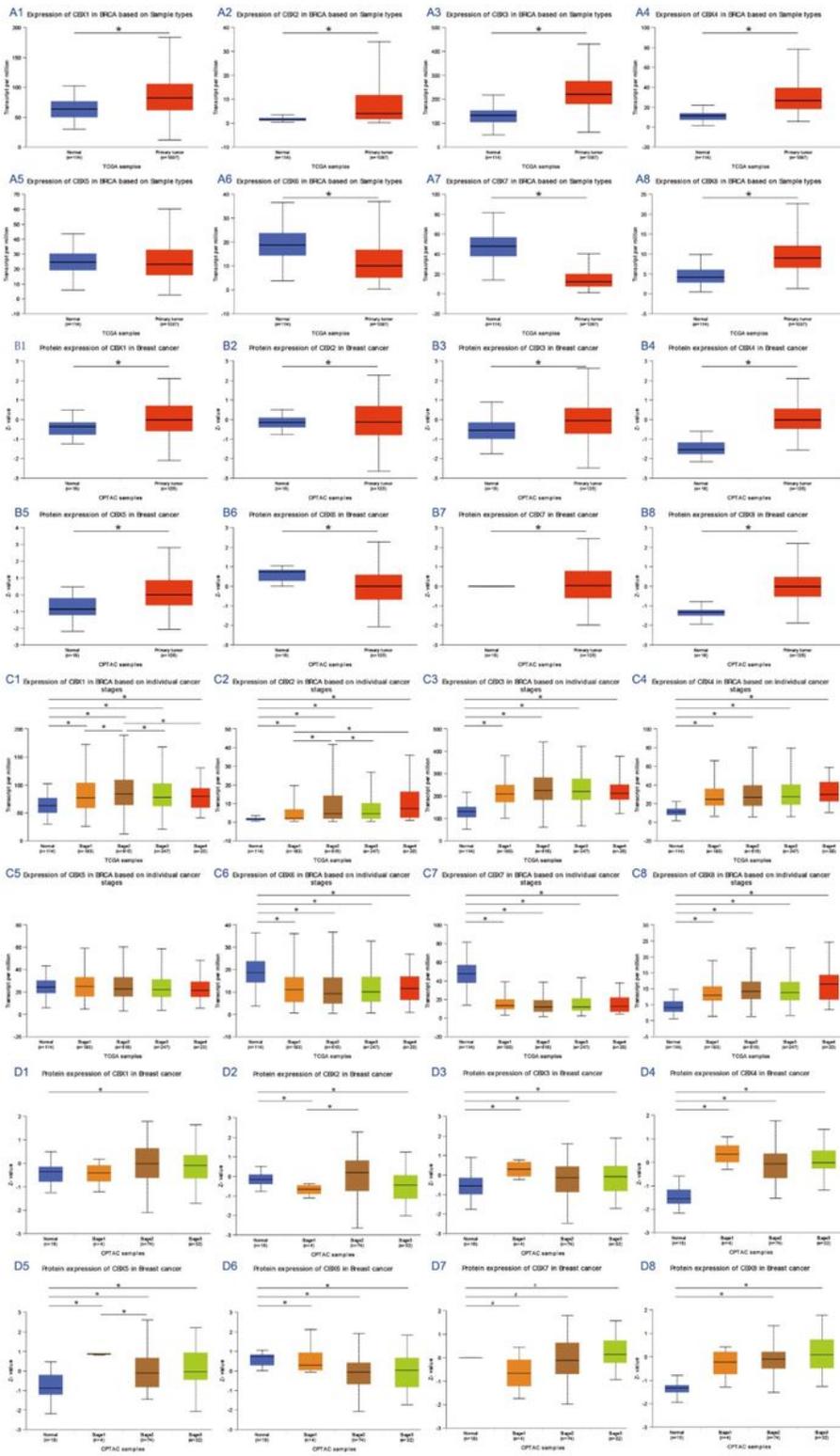


Figure 2

A. The mRNA expression of CBXs family members in BRCA tissues and adjacent normal tissues. B. The protein expression level of CBXs family members in BRCA tissues and adjacent normal tissues. Correlation between mRNA expression level of CBXs family members and individual cancer stages of BRCA patients. D. Correlation between protein expression level of CBXs family members and individual cancer stages of BRCA patients. (UALCAN). *Indicate that the results are statistically significant.

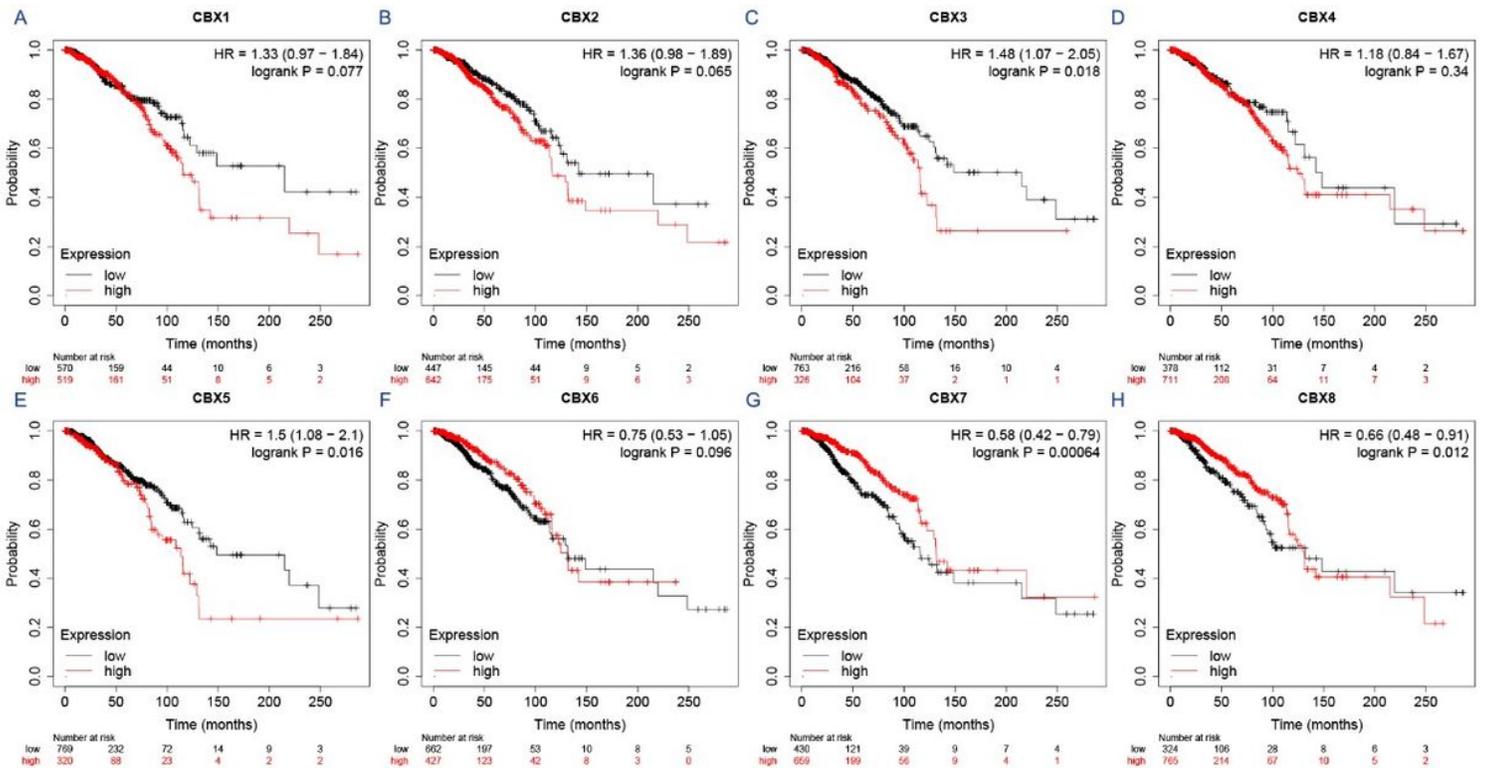


Figure 3

The prognostic value of mRNA expression of CBXs family members in BRCA patients. The OS survival curves comparing patients with high (red) and low (black) CBXs family members expression in BRCA patients were plotted using Kaplan–Meier plotter database at the threshold of p-value of < 0.05. (Kaplan-Meier Plotter).

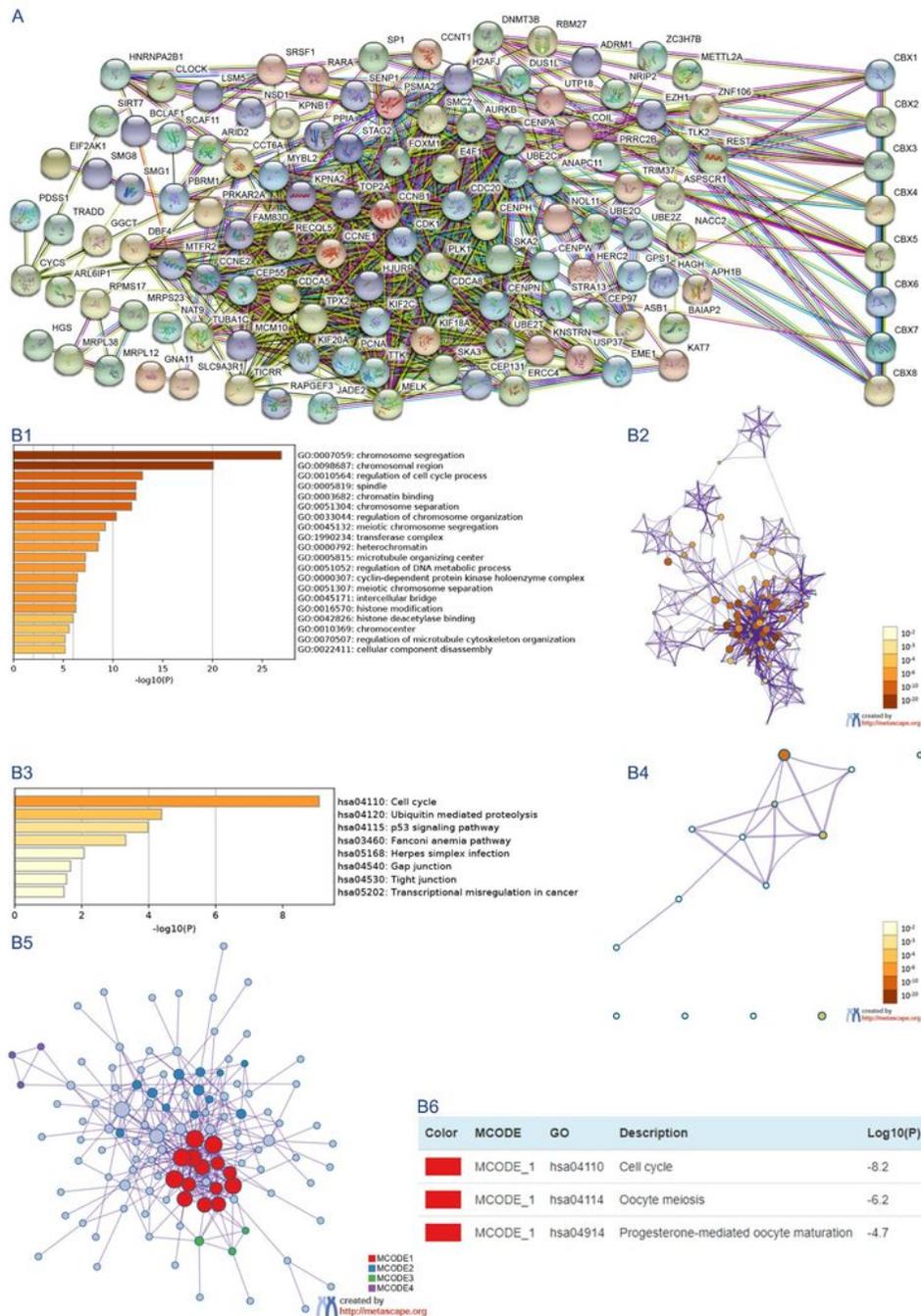


Figure 4

The protein–protein interaction network and enrichment analysis of CBXs family members and neighboring genes in BRCA (STRING and Metascape). A. Network of CBXs family members and 20 frequently altered neighbor genes of each member. B1. Heatmap of Gene Ontology (GO) enriched terms colored by p-values. B2. Network of GO enriched terms colored by p-value, where terms containing more genes tend to have a more significant p-value. B3. Heatmap of Kyoto Encyclopedia of Genes and

Genomes (KEGG) enriched terms colored by p-values. B4. Network of KEGG enriched terms colored by p-value, where terms containing more genes tend to have a more significant p-value. B5. Protein–protein interaction (PPI) network and four most significant MCODE components form the PPI network. B6. Independent functional enrichment analysis of three MCODE components.