Decreased Expression of CBX7 is an Independent Predictor of Poor Survival in Cervical Cancer

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Research

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Abstract

Background: CBX7, a component of the PRC1, has been investigated as a potential biomarker in human malignant neoplasias. In present study, the value of CBX7 expression in the diagnostic and prognosis of cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) as examined via bioinformatics analysis of data obtained from Genotype-Tissue Expression (GTEx) database and The Cancer Genome Atlas (TCGA) database.

Methods: Relationships between clinical factors and CBX7 were explored. The Kaplan-Meier method and Cox regression were used to identify the associations between clinicopathological characteristics and overall survival (OS) in CESC. Gene set enrichment analysis (GSEA) was performed using TCGA dataset.

Results: Our results indicated the decreased expression of CBX7 in CESC, and difference in CBX7 expression was also identified according to age subgroups. CESC patients with decreased CBX7 expression had worse prognosis than those with high CBX7 expression. Multivariate analysis showed that CBX7 was an independent risk factor for OS. GSEA demonstrated pathways involved in the biosynthesis of unsaturated fatty acids, glycosaminoglycan biosynthesis-chondroitin sulfate, glyoxylate and dicarboxylate metabolism, nod-like receptor signaling pathway, O-glycan biosynthesis, one carbon pool by folate and protein export as differentially enriched in CESC with decreased CBX7 expression.

Conclusion: We demonstrated that decreased CBX7 expression may be a potential independent biomarker for poor prognosis in CESC.

Introduction

Cervical cancer is an increasing global burden, both in developing and industrialized nations. In 2018, cervical cancer is the fourth leading cause of cancer death and the fourth diagnosed cancer in women globally, with approximately 570,000 cases and 311,000 deaths [1]. Numerous efforts, including implementation of human papillomavirus vaccine (HPV) and cervical screening, have been made to reduce incidence of cervical cancer [2]. But, the reduction the incidence is limited in many underdeveloped nations because of low- or middle-incomes [3]. Most women in early-stage tumors can be cured by surgery, however, women with advanced stage or recurrent disease need combination therapy such as radiotherapy or chemotherapy, the OS remains poor [4]. Therefore, it is an urgency to find a novel and reliable biomarker for cervical cancer diagnosis and prognosis.

Polycomb group (PcG) proteins are a large family of proteins [5], which is structurally diverse but functionally correlated with chromosome X-inactivation, morphogenesis, cellular proliferation, senescence, stem-cell self-renewal and tumorigenesis [6-8]. PcG proteins function as part of the multiprotein complexes Polycomb repressive complexes 1 and 2 (PRC1, PRC2) that involved in gene repression by repressing transcription initiation [9].

In mammals, the Chromobox protein homolog (CBX) protein family including eight members (CBX1-8). CBX7, a component of the PRC1, contains a conserved N-terminal PcG box and a CBX domain which binds methylated lysine residues [10]. Several studies showed that the reduced expression of CBX7 is involved in different human malignant neoplasias, such as gliomas and thyroid, breast, lung, pancreatic and and bladder carcinomas, which indicated that CBX7 may be a tumor suppressor [11-16]. In contrast, CBX7 mainly plays the role of oncogene in gastric cancer [17]. These results reveal the fact that the precise role of CBX7 in cancer progression may vary among different cancer types. However, the roles of CBX7 expression in the diagnostic and prognostic evaluation of patients with cervical cancer have not been well explored.

In this study, the expression of CBX7 mRNA in cervical cancer patients and healthy individuals was compared. In order to analyzed the diagnostic and prognostic value of CBX7, patients were divided into two groups according to the high and low CBX7 expression. We explored the relationship between the expression of CBX7 and clinical characteristics and OS of cervical cancer patients. Using GSEA to identified the signaling pathways which is related to the regulatory mechanisms of CBX7.

Materials And Methods

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Data Collection

Level 3 expression data and mRNA expression profiles (309 cases, including 3 normal cases, Workflow Type: HTseq-FPKM) were downloaded from TCGA database. Clinical characteristics regarding to survival time for cervical cancer patients was also obtained from TCGA database. Using the GTEx database, we obtained expression data for 10 normal cervical tissues (Table S1). Using R software (version 3.6.3) or Practical Extraction and Report Language (Perl) scripts on JAVA software to processed all data [18].

GSEA

To explore the potential correlations underlying the effection of CBX7 expression on cervical cancer prognosis, we applied GSEA to identified biological pathways pertaining to cervical cancer pathogenesis-associated CBX7 regulatory networks [19, 20]. CBX7-high and CBX7-low expression were the phenotype labels. Each analysis of gene set permutations were conducted 1000 times.

Statistical Analysis

The correlations between expression of CBX7 and clinical features were evaluated by the logistic regression and Wilcoxon signed-rank test. The CBX7 expression of CESC cohort was obtained using box plots. Clinical factors associated with OS in CESC were identified using the Cox regression and Kaplan-Meier method. Univariate Cox analysis was applied to search for potential prognostic factors, and the correlations between CBX7 expression and survival along with clinical characteristics (age, grade, stage, lymph node status, and distant metastasis status) were examined using multivariate Cox analysis. The

two groups of CBX7 expression was identified by the median values. Statistical significance was defined as P value<0.05.

Results

Characteristics of the Study Population

The clinical data pertaining to CESC were obtained from the TCGA database (Table S2), including patients' age, sex, clinical stage, histologic grade, tumor-node-metastasis (TNM) classification and survival status (Table 1).

Decreased Expression of CBX7 in CESC

CBX7 expression in CESC tissues and normal cervical tissues was compared, as shown in Fig. 1. The results showed that expression of CBX7 was decreased in CESC (p = 4.07e-09). Moreover, difference in CBX7 expression was also obtained according to age (p = 0.044). The expression of CBX7 mRNA in various human tissues and different cancer types from Gene Expression Profiling Interactive Analysis (GEPIA) was shown in Fig. 2, which computed the data from TCGA in the form of transcripts per million.

Decreased CBX7 Expression is an Independent Risk Factor for OS in CESC

Kaplan-Meier curves indicated that decreased expression of CBX7 was correlated with poor OS (p = 6.06e-04) (Table S3). Subgroup analyses showed that decreased CBX7 expression critically affected the OS of CESC with histological grades G1/G2 (p = 0.022) and G3/G4 (p = 0.043), clinical stage I/II (p = 0.002), tumor stage T1 (p = 0.003) and T4 (p = 0.032), stage N0 (p = 0.002), and stage M0 (p=0.004) (Fig. 3). The univariate and multivariate Cox analyses were used to explore whether CBX7 is an independent predictor of worse OS in CESC after excluding patients without complete clinical data. In this study, Cox regression analysis including 246 patients. The univariate Cox regression analysis showed that CBX7 was related to OS (hazard ratio (HR) = 0.416, 95% confidence interval (CI) = 0.251-0.689; p <0.001). In addition, we also obtained clinicopathological variables, including tumor stage (HR = 1.566, 95% CI = 1.214-2.020, p <0.001), T classification (HR = 1.564, 95% CI = 1.291-1.895, p <0.001), M classification (HR = 1.334, 95% CI = 1.005-1.771, p = 0.046), N classification (HR = 2.028, 95% CI = 1.451-2.834, p<0.001), were closely correlated with OS. In the multivariate Cox regression analysis, the tumor N classification (HR = 1.637, 95% CI = 1.031-2.597, p = 0.036) and CBX7 (HR = 0.351, 95% CI = 0.206-0.596, p<0.001) were significantly correlated with OS. These data indicated that CBX7 expression was an independent risk factor for predicting worse OS in CESC (Table 2).

Identification of CBX7-Related Signaling Pathways by GSEA

To identify the signaling pathways that are differentially enriched in cervical cancer, GSEA of gene expression profiles was performed to compare differentially enriched signaling pathways between CESC patients with high and low levels of CBX7 expression. GSEA revealed significant differences (NOM: normal; FDR: false discovery rate; FDR < 0.25 and NOM P-Value < 0.05) in the enrichment of the

Molecular Signatures Database (MSigDB) Collection (c2.cp.kegg.v7.1.symbols.gmt), and the results were showed in Table 3. Gene sets related protein export, glycosaminoglycan biosynthesis-chondroitin sulfate, focal adhesion, proteasome, biosynthesis of unsaturated fatty acids, nod-like receptor signaling pathway, O-glycan biosynthesis and glyoxylate and dicarboxylate metabolism were various enriched with the decreased expression of CBX7 phenotype (Fig. 4).

Discussion

Currently, emerging evidence demonstrates that a number of cancer suppressors and oncogenes participates in CESC, some of which, including cyclin dependent kinase inhibitor 2A, DNA topoisomerase II alpha and minichromosome maintenance complex component 2 [21]. Nevertheless, novel biomarkers still needed for the diagnosis and prognosis of CESC. In present research, a gene expression profile was identified based on bioinformatics analysis. CBX7 is first reported to extend the lifespan of many normal human cells via the downregulation of Ink4a/Arf locus expression [22]. In the field of oncology, CBX7 is rarely explored in cervical cancer, as yet. We exhaustively analyzed CBX7 expression and patients' clinical characteristics and identified its potential prognostic value in CESC.

In our study, we found that the decreased CBX7 expression was related to worse survival in CESC. Kaplan-Meier curves also indicated that the decreased expression of CBX7 was related to poor outcomes in CESC. Cox analyses showed that the CBX7 expression may be a potential biological marker for CESC prognosis.

CBX7 as a chromobox family protein, regulating several genes that are important for malignant neoplasias development and progression, such as drug resistance and epithelial-mesenchymal transition (EMT) [23, 24]. It has been demonstrated that the loss of CBX7 enhances tumorigenicity and metastatic potential in epithelial ovarian cancer [25]. Accumulating evidence indicates that decreased CBX7 may also serve as an adverse prognostic biomarker for colon carcinoma patients [26]. The latest study reveals that CBX7 inhibits cell motility and cell growth and induces apoptosis in cervical cancer [27]. The present study showed that decreased CBX7 expression in CESC, this result is consistent with findings for CBX7 expression in other types of tumors. We also found that age was associated with CBX7 expression.

Many studies have reported that CBX7 closely correlated with malignant transformation. Federico et al. found that CBX7 and Protein Arginine Methyltransferase 1 (PRMT1) complex is critical for PRMT1 and for the expression of E-cadherin, a crucial hallmark of EMT [28]. Jung et al. demonstrated that CBX7 act as a crucial regulator that induces human malignant hematopoietic stem and progenitor cells by non-canonical and canonical interactions and higher expression of CBX7 can also inhibit proliferation and promoted differentiation in acute myeloid leukemia, revealing therapeutic possibilities for leukemia [29]. Nawaz et al reported that CBX7 inhibit glioma cell migration through YAP/TAZ-CTGF-JNK signaling axis and identified the critical role of CBX7 in gliomagenesis [30]. Based on above reports, the decreased CBX7 expression indicates the progression of cell proliferation, which might promote poor outcomes in CESC. Pallante et al. explored CBX7 represents a critical prognostic factor in a wide range of cancer types [31].

Consistently, the present study revealed that CBX7 expression was related to OS in CESC, and the potential mechanism may be correlated with the biosynthesis of unsaturated fatty acids and O-glycan, protein export, glyoxylate and dicarboxylate metabolism, glycosaminoglycan biosynthesis-chondroitin sulfate, one carbon pool by folate and the nod-like receptor signaling pathway, as identified by GSEA. Moreover, we also identified the prognostic value of CBX7 expression in age subgroup of CESC and also explored that CBX7 expression correlated with G1/G2, G3/G4, stage I/II, T1, T4, N0 and M0 patients.

Currently, surgery is the major treatment for CESC. The decreased CBX7 expression also adversely affected OS in patients with early stage I/II cancers, but not in those with advanced stage III/IV cancers, which further indicates the potential prognostic role of CBX7 expression in subgroup analyses and its application to precision therapy for CESC. However, the number of patients with advanced stage is decreased, which is a limitation in present research, future works need to increase the number of the samples to verify this result.

Conclusions

This study demonstrates that CBX7 is downregulated in CESC, and decreased CBX7 expression is associated with clinical progression and is an independent risk factor for OS in CESC. The finding suggests that CBX7 may be a potential biomarker in CESC.

Abbreviations

CESC: cervical squamous cell carcinoma and endocervical adenocarcinoma; GTEx: Genotype-Tissue Expression database; TCGA: The Cancer Genome Atlas; OS: Overall survival; GSEA: Gene set enrichment analysis; HPV: human papillomavirus vaccine; PcG: Polycomb group; PRC: Polycomb repressive complexes; CBX: Chromobox protein homolog; GEPIA: Gene Expression Profiling Interactive Analysis. HR: hazard ratio; CI: confidence interval; MSigDB: Molecular Signatures Database; EMT: epithelial-mesenchymal transition.

Declarations

Acknowledgments

The results of this study are based on data generated by the TCGA and GTEx database. We would like to thank “TCGA Research Network” for generating, curating, and providing the bladder cancer clinical data.

Authors’ contributions

LA analyzed the data and was the major contributor to performing the experiments and writing the manuscript. LA and JZ were involved in critically revising the manuscript for important intellectual content. ZJ, XY and PW made substantial contributions to conceiving the research and designing the draft of the research process. WO and HW contributed to writing the manuscript. All authors read and

approved the final manuscript. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The data for this research were downloaded from the TCGA and GTEx database, a public website. Our study did not require ethical board approval because it did not involve human or animal trials.

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Availability of data and materials

The following information was supplied regarding data availability: The raw measurements are available in Supplemental Files. Table S1 showed CBX7 expression in cervical cancer tissues and normal cervical tissues. Table S2 showed the clinical data of cervical cancer patients. Table S3 showed the overall survival of cervical cancer.

Ethical statement and consent to participate

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This research does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

Competing interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Tables

Tables 1-3 are available in the Supplementary Files.

Figures

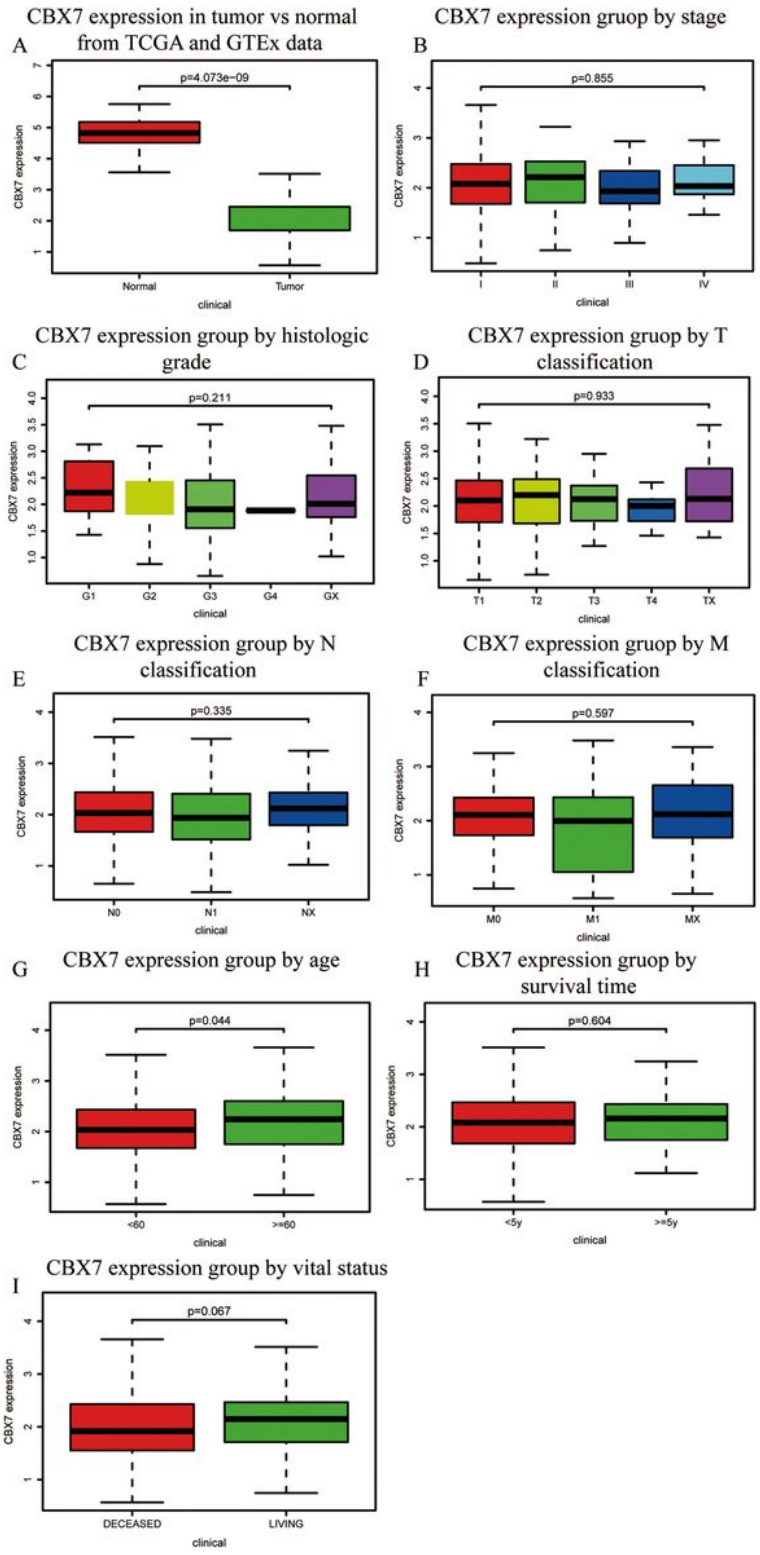

Figure 1

CBX7 expression in CESC. (A) The expression of CBX7 was compared between normal individuals and cervical cancer patients and assessed according to (B) clinical stage, (C)histologic grade, (D-F) TNM classification, (G) patient age, (H) survival time and (I) vital status.

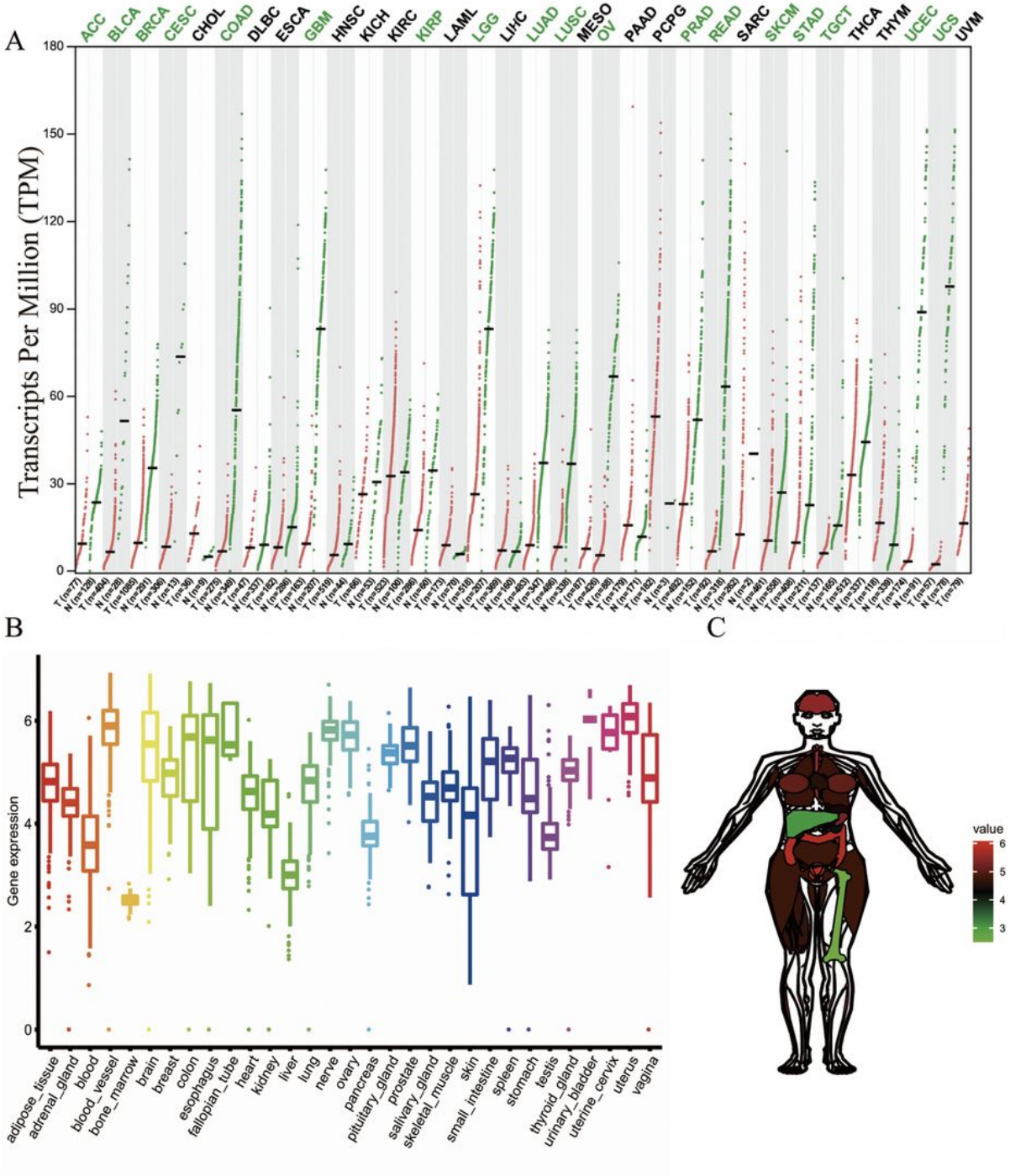

Figure 2

Expression of CBX7 in different human tissues. (A) Expression of CBX7 in 33 cancer types and paired normal samples. (B) and (C) Boxplot and anatomical map show the CBX7 expression in various tissues.

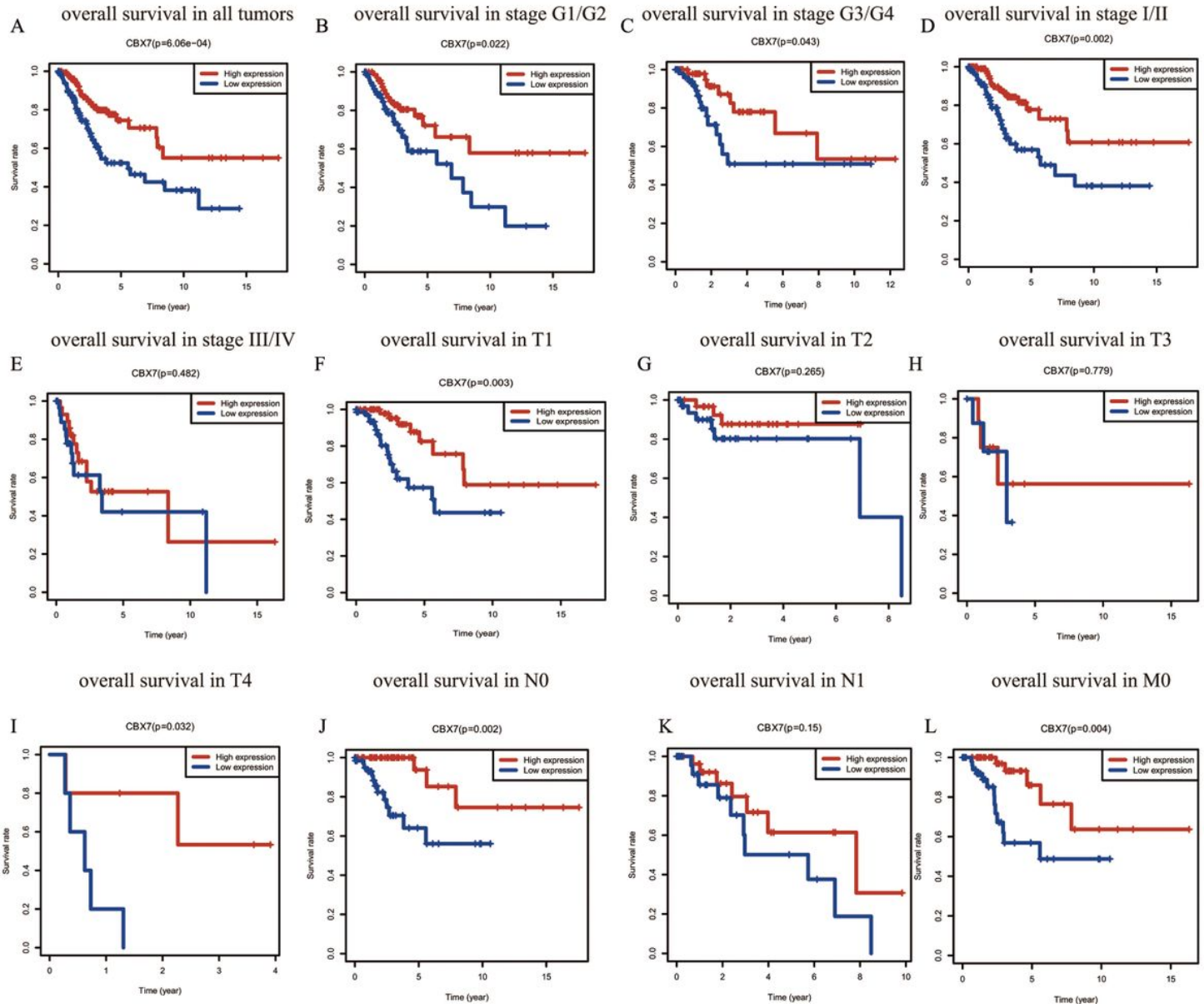

Figure 3

Kaplan-Meier curves for OS in cervical cancer. (A) Kaplan-Meier survival curves for OS in all patients and subgroups of (B) histologic grade G1/G2 and (C) G3/G4; (D) cancer stage I/II, and (E) III/IV; and (F-L) TNM classification T1, T2, T3, T4, N0, N1, and M0.

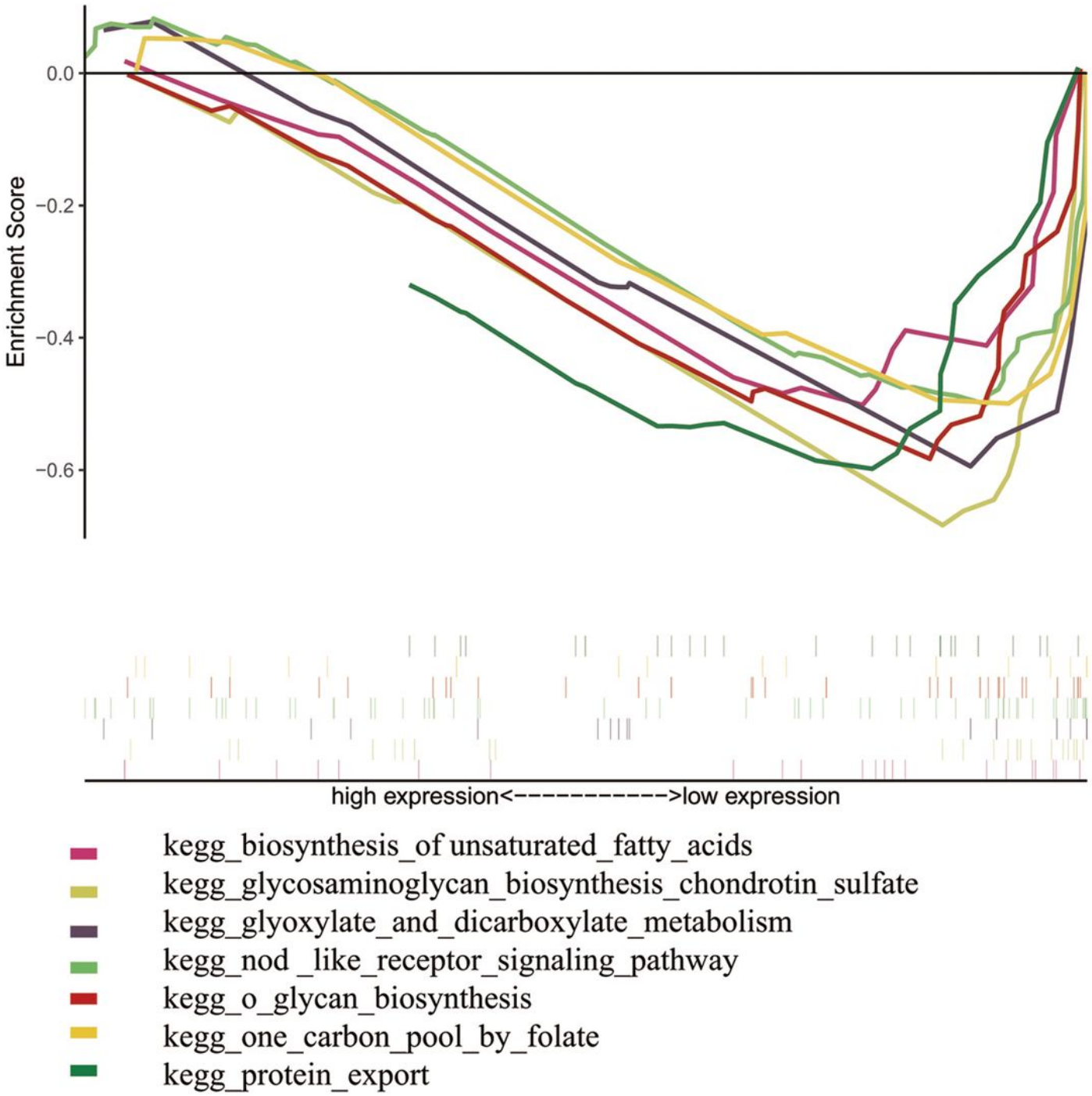

Figure 4

Enrichment plots from GSEA. The GSEA results indicated that genes involved in biosynthesis of unsaturated fatty acids, glycosaminoglycan biosynthesis-chondroitin sulfate, glyoxylate and dicarboxylate metabolism, nod-like receptor signaling pathway, O-glycan biosynthesis, one carbon pool by folate and protein export were differentially enriched in cervical cancer patients with decreased CBX7 expression.

Supplementary Files

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- Tables.docx
- TableS1CBX7expression.docx
- TableS2Clinicalcharacteristicsofpatients.docx
- TableS3Theoverallsurvivalofcervicalcancer.docx