CBX3 Is a Prognostic Biomarker That Is Correlated With Lymphocyte Infiltration in Hepatocellular Carcinoma

Jia-Ning Zhang
Eastern Hepatobiliary Surgery Hospital
Qi fei TAO
Eastern Hepatobiliary Surgery Hospital
Dong yang Ding
Eastern Hepatobiliary Surgery Hospital
YUAN YANG
Eastern Hepatobiliary Surgery Hospital
Wei-Ping Zhou (ehphwp@126.com)
Eastern Hepatobiliary Surgery Hospital

Research

Keywords: Liver cancer, CBX3, Prognostic marker, Bioinformatic analysis, Immune checkpoint, HAVCR2, Immune cells infiltration

Posted Date: September 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-835590/v1

Abstract

Background: CBX3 is a key gene that is involved in immune cell regulation, however, its prognostic values and its correlation with infiltrating lymphocytes in various cancers have not been clearly established. This study aims to investigate the role CBX3 in hepatocellular carcinoma (HCC).

Methods: We first reviewed the expression of CBX3 in different cancers and adjacent tissues using oncomine database. Next, the authors focus on the expression of CBX3 in hepatocellular carcinoma. Therefore, the expression of CBX3 in hepatocellular carcinoma were analyzed through UALCAN online analysis website and the Human Protein Atlas (www.proteinatlas.org) website. In addition, we further found that CBX3 can be identified as an effective marker for the prognostic guidance of hepatocellular carcinoma according to the Kaplan-Meier plotter database and the Bioinformatics analysis online websites (www.aclbi.com). Next, we used the Bioinformatics analysis online websites to explore whether the expression level of CBX3 in liver cancer is related to the infiltration of certain immune cells. In addition, we also predicted the correlation between immune checkpoint and CBX3 in liver cancer.

Results: The analysis results preliminarily show that CBX3 be expressed abnormally in many cancers, and CBX3 was significantly up-regulated in HCC. The high expression of CBX3 indicated survival outcomes and it showed a huge potential as a effective marker for the prognostic guidance of hepatocellular carcinoma. Furthermore, we found that CBX3 in liver cancer is related to the infiltration of certain immune cells, including CD4+ T cells, macrophages and B cells. In addition, the results showed HAVCR2 is most likely to become an effective immune checkpoint for HCC patients immunotherapy with high CBX3 expression.

Conclusions: CBX3 is a potential diagnostic and prognostic marker in HCC and related to the infiltration of certain immune cells. It is expected to become a breakthrough point in immunotherapy in the future.

Introduction

Among malignant tumors, liver cancer is associated with high levels of morbidity and mortality (1–3). Due to the metastatic characteristics of liver cancer cells, many patients are not suitable for surgical resection at the time of diagnosis (4–6). For these patients, immunotherapy is highly recommended as a treatment option (7, 8). Immune related genes play important roles in immunotherapy. However, the roles of immune responses in tumor progression and disease outcomes have not been clearly established. Therefore, in this study, we investigated immune related genes in hepatocellular carcinoma with the aim of providing new perspectives for tumor diagnosis, treatment and prognosis.

CBX3,as a member of chromobox family proteins, is present in many cancers, including colon cancer (9), osteosarcoma (10), glioma (11) and liver cancer (15). It is correlated with tumor progression. CBX3 affects cancer progression by causing an immune response (12–14). Therefore, CBX3 is an important immune related gene of which immune response mechanisms in various cancer cells, including liver

cancer cells, should be investigated. In this study, we evaluated the correlation between immune cell infiltration levels and CBX3 levels in liver cancer cells.

Materials And Methods

Validation of the mRNA expression levels of CBX3

We used the oncomine database (https://www.oncomine.org/resource/login.html) to validate mRNA expression levels of the CBX3 gene in various tumor types. Expression thresholds were set as: p = 0.01 and fold change = 2.

Analysis of expression levels of CBX3 in hepatocellular carcinoma and normal tissues

To further clarify the expression levels of CBX3 in liver cancer cells. We evaluated the expression levels of CBX3 at the transcriptome and protein levels. Using the TCGA data, mRNA expression levels of CBX3 in liver cancer and normal tissues were assessed through the UALCAN online analysis website (http://ualcan.path.uab.edu/). Protein expression levels were determined through the Human Protein Atlas (www.proteinatlas.org) website.

Diagnostic and prognostic values of CBX3

In this analysis, we used different online websites to analyze the prognostic values of CBX3 in liver cancer. Comparisons between CBX3 expression levels and survival outcomes were performed using the Kaplan-Meier plotter database, GEPIA database, and the www.aclbi.com Bioinformatics analysis online websites. To establish the ROC curve of the diagnostic value of CBX3 on liver cancer, we analyzed and calculated the results through www.aclbi.com online websites based on TCGA Database.

Immune cell infiltrating levels and immune checkpoint inhibitor analysis

We used the www.aclbi.com online website to analyze the HCC patients in TCGA database. Through different scoring systems (TIMER and QUANTISEQ), we used this tool to evaluate the abundance of tumor infiltrating immune cells (TIICs) that may be associated with CBX3 gene expression levels in HCC tissues, and to obtain the Spearman correlation heat map. Moreover, we also used the online tool to analyze the immune checkpoints associated with expression levels of CBX3 in liver cancer tissues.

Results

mRNA expression levels of CBX3 in various human cancer types

To evaluate the expression levels of CBX3 in different types of cancer and normal tissues, we first used the Oncomine database for preliminary exploration. Of the 20 evaluated tumor types, CBX3 was found to be highly expressed in 16 cancer tissues (Fig. 1A). Compared to normal tissues, expression levels of CBX3 in gastric cancer, bowel cancer, breast cancer and other tumor types were elevated. Therefore, CBX3

may be involved in the oncogenesis of many cancer types. However, this database does not show the expression levels of CBX3 in normal liver and liver cancer tissues.

Expression levels of CBX3 in HCC

We used the UALCAN database (http://ualcan.path.uab.edu/) to analyze the expression levels of CBX3 at the transcriptome level. We found that, compared to adjacent normal tissues, expression levels of CBX3 in HCC tissues were significantly elevated (Fig. 2A). Simultaneously, representative data for CBX3 protein level analysis (Fig. 2B) were obtained from the Human Protein Atlas database. The degree of immunohistochemical staining in HCC tissues (Patient id: 983) was stronger than in the normal liver tissue. These findings suggest that CBX3 is highly expressed in liver cancer.

Correlation between CBX3 expression levels and survival outcomes

Using the GEPIA database, it was found that, compared to patients with suppressed expression levels of CBX3, patients with elevated expression levels of CBX3 exhibited significantly reduced overall survival outcomes ($p = 0.00046$; Fig. 3A). Disease-free survival outcome analysis revealed comparable results to those of overall survival outcomes (Fig. 3B).

In addition, we verified the correlation between expression levels of CBX3 and patient's survival outcomes using other databases. Using the Kaplan-Meier Plotter database, it was found that survival time for patients with elevated CBX3 expression levels was significantly low than that of patients with suppressed CBX3 expression levels ($p = 6.9e - 07$; Fig. 3C). Disease-free survival time (Fig. 3D) for patients with elevated CBX3 expression levels was significantly low compared to that of patients with suppressed CBX3 expression levels ($p = 2e - 05$).

Diagnostic values of CBX3 in HCC

Diagnostic values of CBX3 were evaluated through the TCGA database. First, we used dichotomy to allocate patients into two groups; the CBX3 high expression group and the CBX3 low expression group (Fig. 4A). Figure 4B shows that the median survival time for patients in the CBX3 low-expression CBX3 group (5.8 years) was significantly more than that of the CBX3 high-expression group (3.1 years; $p = 0.000142$). The predicted diagnostic efficacy of CBX3 is shown in Fig. 4C. With 1-year survival time as the cut-off point, the AUC value of CBX3 as a diagnostic marker was 0.724 (95% CI, 0.665 – 0.783); With 3-year survival time as the cut-off point, the AUC value of CBX3 as a diagnostic marker was 0.658 (95% CI, 0.593 – 0.723); With 5-year survival time as the cut-off point, the AUC value of CBX3 as a diagnostic marker was 0.648 (95%CI, 0.571 – 0.725). These findings show that CBX3 is a potential good prognostic marker for liver cancer.

CBX3 expression is correlated with immune infiltration levels in HCC

We further evaluated whether the expression level of CBX3 is correlated with prognosis and immune infiltration. Using the QUANTISEQ algorithm, the results (Fig. 5A) revealed that immune infiltrating cells

that are significantly correlated with CBX3 expression levels are T regulatory cells (Tregs), CD8 + T cell, CD4 + T cells, monocytes, macrophage M2, macrophage M1 and B cells. Through the TIMER algorithm (Fig. 5B), the associated immune infiltrating cells were found to be CD4 + T cells, neutrophils, myeloid dendritic cells, macrophages and B cells. Based on these findings, we postulated that infiltration levels of CD4 + T cells, macrophages and B cells are positively correlated with expression levels of CBX3 in HCC.

Correlation between CBX3 and immune checkpoints

Immune checkpoint inhibitors play an increasingly important role in cancer treatment. To evaluate the correlation between expression levels of CBX3 and immune checkpoints, we made an online prediction using onlin website The results showed Immune checkpoints, including CD274, CTLA4, HAVCR2, LAG3, PDCD1, SIGLEC15, and TIGIT, which were associated with CBX3, were differentially expressed (Fig. 6).

Among these immune checkpoints, the most closely related and statistically significant is HAVCR2. Therefore, it could be a good therapeutic target for HCC patients. More studiesshould determine whether CBX3 can be used as a marker for the application of immune checkpoint inhibitors in cancer treatment.

Discussion

Among gastrointestinal tumors, HCC is associated with high fatality rates (16, 17). Currently, the therapeutic options for unresectable advanced liver cancer are not efficacious. Traditional non-surgical therapeutic methods, including chemotherapy and radiotherapy do not have satisfactory outcomes (18–20). New therapeutic options for liver cancer are constantly emerging, immunotherapy is among them (21). Immunotherapy has been proven to play an important role in cancer treatment, with good outcomes. Among breast cancers, triple-negative breast cancer is one of the most difficult subtype to treat. However, immunotherapy was shown to achieve good results in the treatment of triple-negative breast cancer (22). Immune dysfunction plays a pivotal role in the progression of multiple myeloma. Immunotherapy has shown great promise in the treatment of multiple myeloma (23). In HCC, immunotherapy has also shown positive outcomes,for example, the immune checkpoint (PD-L1 or PD-1) inhibitors have shown good therapeutic values in the treatment of liver cancer (24, 25).

Various studies are evaluating immune-related genes with potential in the treatment of liver cancer. CBX3 promotes the progression of many cancer types and can affect immunotherapeutic responses. However, the prognostic values of CBX3 for HCC and whether it is involved in corresponding immune responses have not been determined. Therefore, we aimed at determining whether CBX3 is involved in related immune responses in HCC and if it can be used as a tumor marker to predict the prognosis of cancer patients. We found that CBX3 is highly expressed in various tumor tissues, including breast cancer, colorectal cancer, cholangiocarcinoma and many other tumors. In HCC tissues, expression levels of CBX3 were found to be significantly elevated when compared to normal tissues. The survival time for HCC patients with elevated CBX3 expression levels was significantly low than that of patients with suppressed CBX3 expression levels. These findings show that CBX3 is potential tumor marker for HCC. Further analysis revealed that CBX3 has good prognostic values.

Moreover, CBX3 expression levels were found to be correlated with immune infiltration levels, including B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils and DCs.

For the treatment of HCC, we analyzed the correlation between CBX3 expression and immune checkpoints involved in liver cancer. CBX3 and A were found to be significantly correlated. More studies should be performed in future to verify the relationship between the two.

Conclusion

In summary, this study provides a comprehensive analysis of the CBX3 in HCC. The results offer an insight into the potential utility of CBX3 as a diagnostic and prognostic marker for HCC. Further analysis regarding the lymphocyte infiltration of CBX3 in HCC may provide clues as to whether they can serve as potential Immunotherapy targets. Taken together, the present findings have demonstrated the importance of CBX3 expression in HCC and that CBX3 could be a novel candidate prognostic indicator and therapeutic target for HCC patients. These findings provide a basis for further studies on CBX3 in liver cancer diagnosis and treatment.

Declarations

Acknowledgements

Not applicable

Authors' contributions

Jia-ning zhang mainly designed the experimeand writing the manuscript, Qi fei TAO analyzed the data, Dong yang DING achieved the result. Yuan YANG and Wei ping Zhou helped to acquire data. All authors read and approved the final manuscript.

Funding

No funding was received for the creation of this article.

Availability of data and materials

The raw data of this study are derived from the TCGA database, which are publicly available databases.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

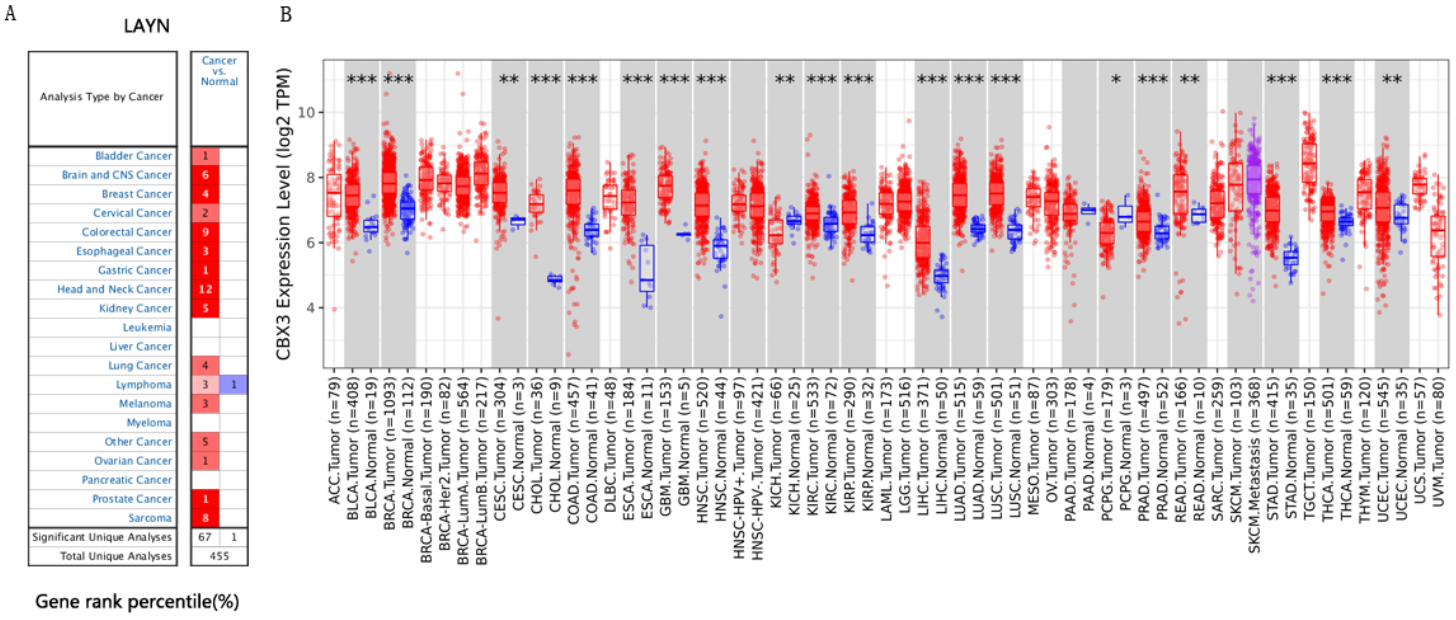

Figure 1

CBX3 expression levels in different human cancer types. A, Elevated or suppressed CBX3 expression levels in different cancers compared to normal tissues in the Oncomine database. B, Human CBX3 expression levels in different tumor types from the TCGA database were determined by TIMER (*p<0.05, **p<0.01, ***p<0.001).

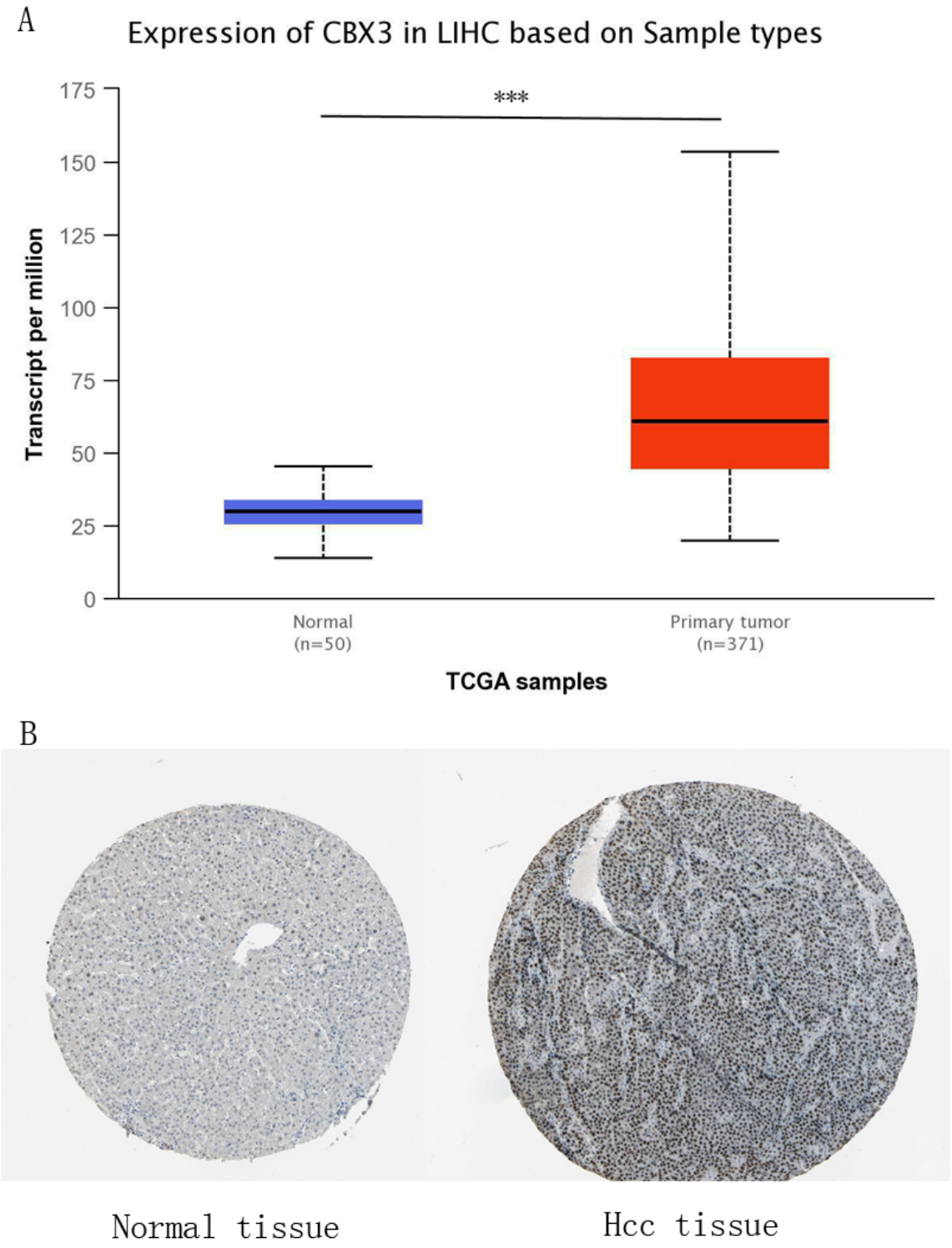

Figure 2

Expression levels of CBX3 in normal liver and cancer tissues. A, Expression levels of CBX3 in normal liver and liver cancer tissues at the transcriptome level. B, Expression levels of CBX3 in normal liver and cancer tissues at the protein level.

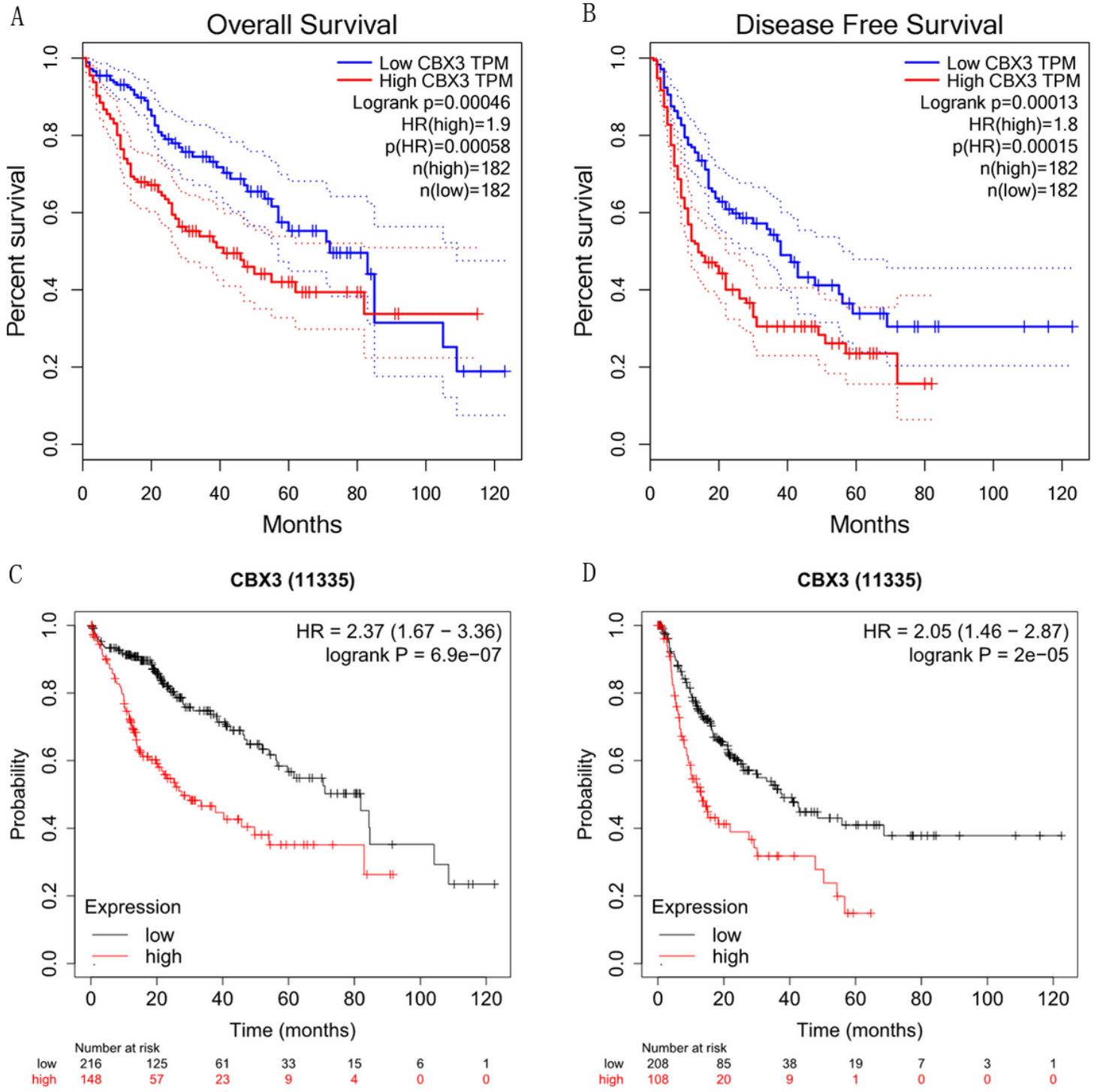

Figure 3

Prognostic values of CBX3. In the GEPIA database, expression levels of CBX3 were correlated with overall survival times (3A) and Disease-free survival time (3B) of liver cancer patients. In the Kaplan-Meier Plotter

database, CBX3 expression levels were correlated with overall survival time (3A) and Disease-free survival time (3B) of liver cancer patients.

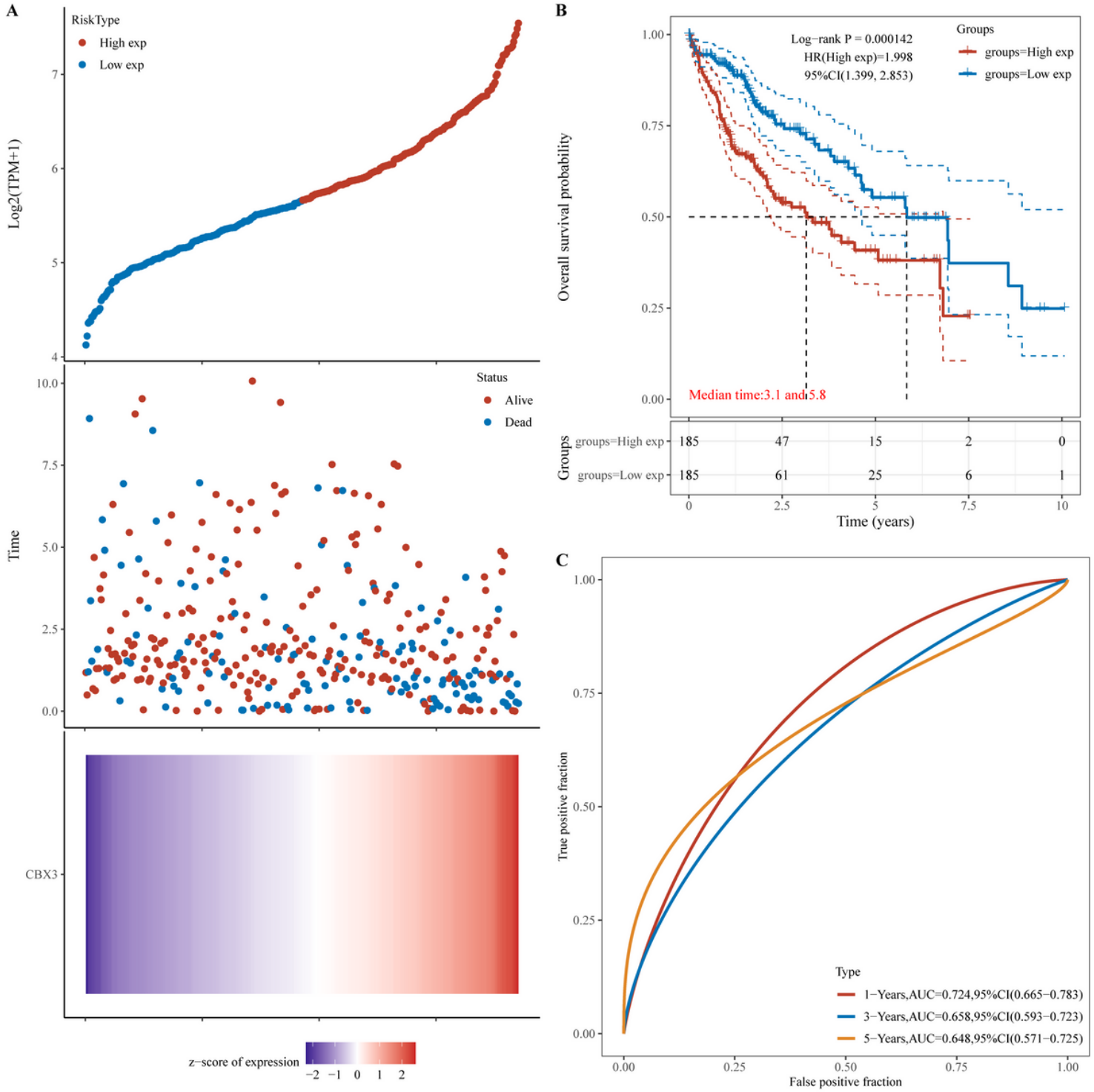

Figure 4

Predictive effects of CBX3 as a tumor marker on the prognosis of patients with liver cancer. A, Correlation between gene expression and survival time as well as survival status in the TCGA data set. The top represents the scatter plot of gene expression from low to high. Different colors represent different expression groups. The middle scatter plot represents survival time and survival status corresponding to

gene expression levels of different samples. The bottom image represents expression heat map of the gene. B, Kaplan-Meier survival analysis in the TCGA data set. C, ROC curve of the gene at different time. The higher the AUC value, the stronger the predictive ability of the gene.

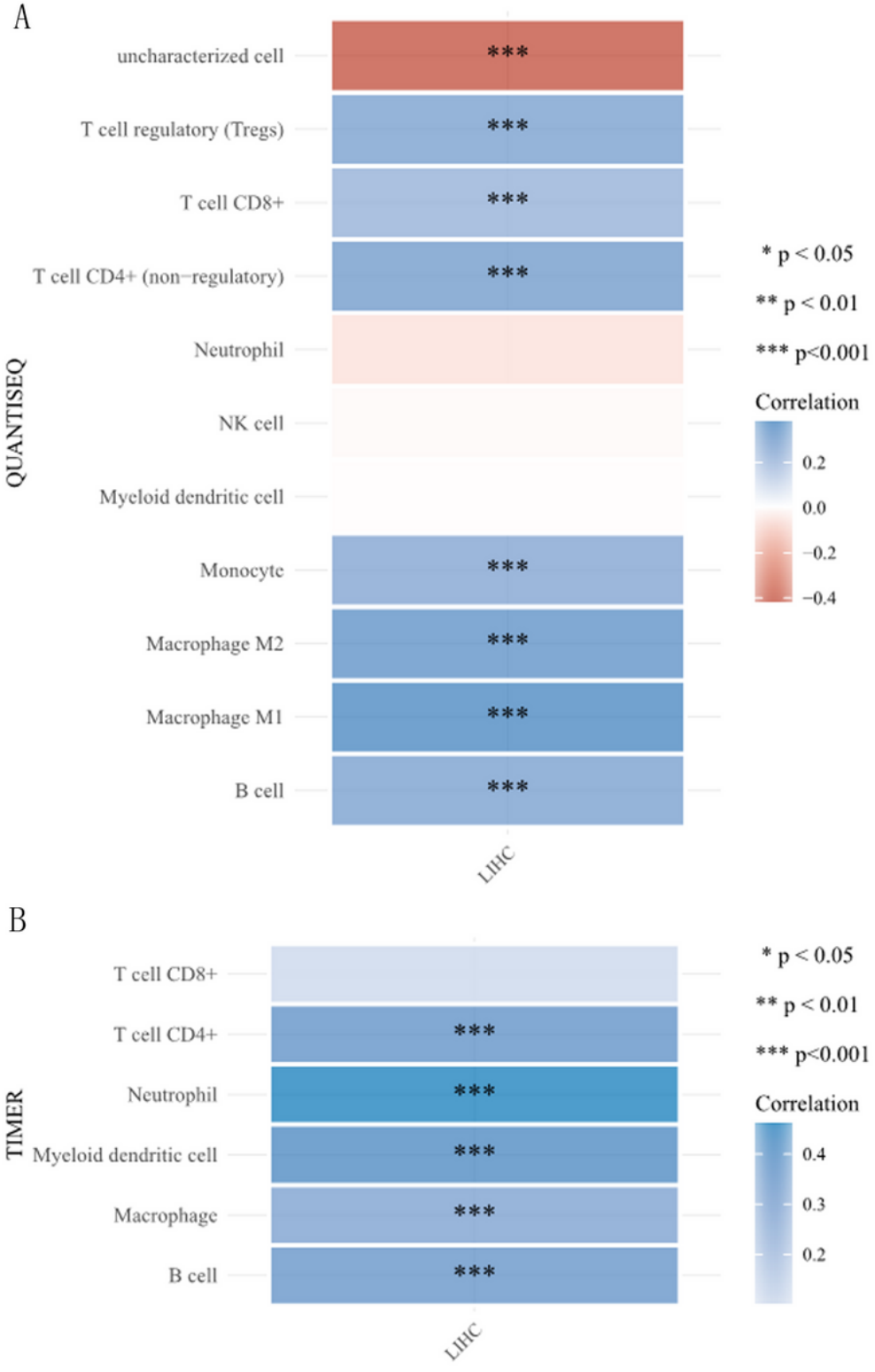

Figure 5

Correlation between immune infiltrating cells and CBX3 expression. Through the A algorithm, immune infiltrating cells that were related to CBX3 expression were predicted. Different colors represent correlation

coefficients. The darker the color, the stronger the correlation, *p < 0.05, **p < 0.01.

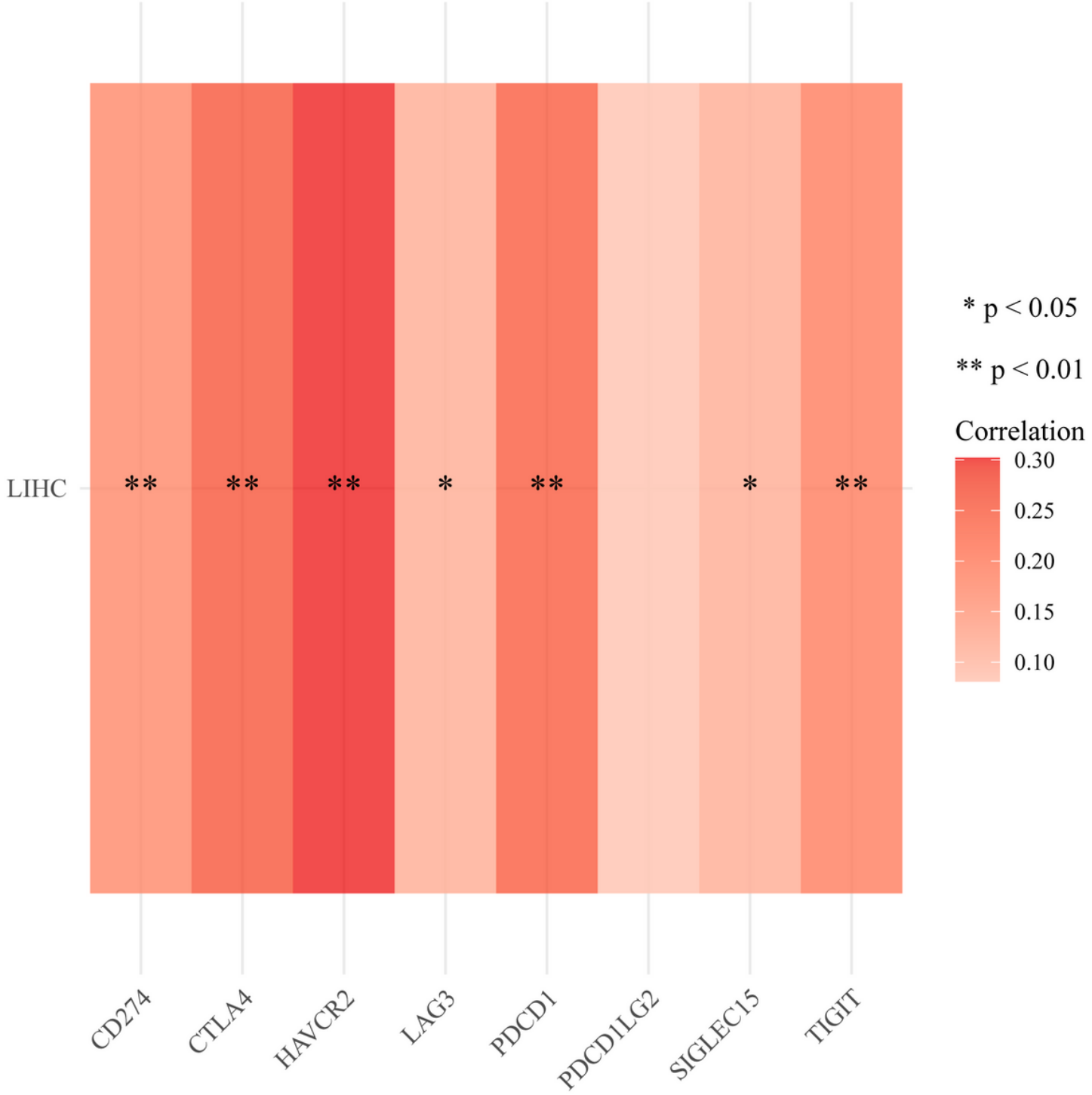

Figure 6

Immune checkpoints correlated with CBX3 expression in liver cancer tissues. Heat maps of expression levels of immune checkpoint-related genes in HCC tissues. The horizontal axis represents different immune checkpoint genes while the vertical axis represents HCC tumor tissues. Each box in the figure represents the correlation between the expression of the CBX3 and the immune checkpoint. Correlation

analysis of gene expression in HCC tumors, *p < 0.05, **p < 0.01, ***p < 0.001. Different colors represent changes in correlation coefficients.