Role of STX6 as a prognostic factor associated with immune infiltration in hepatocellular carcinoma

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Received April 13, 2022; Accepted August 11, 2022

DOI: 10.3892/ol.2022.13491

Abstract. Syntaxin 6 (STX6), a soluble N-ethylmaleimide-sensitive factor-activating receptor protein, has formed an increasing part of cancer research. However, to the best of our knowledge, the role of STX6 in hepatocellular carcinoma (HCC) is still unclear. In the present study, data from multiple bioinformatics databases, including The Cancer Genome Atlas, Gene Expression Omnibus, Kaplan-Meier plotter, Tumor Immune Estimation Resource (TIMER) and Gene Expression Profiling Integrative Analysis (GEPIA2), and immunohistochemistry (IHC) were utilized to assess the role of STX6 in HCC. The results demonstrated that STX6 expression was upregulated in HCC tissues compared with normal tissues. STX6 expression was significantly associated with tumor size, Edmondson grade and α-fetoprotein (AFP) level. Furthermore, survival analysis demonstrated that high STX6 expression was significantly associated with poor prognosis in patients with HCC. Furthermore, assessment of the immune infiltrates demonstrated that CD163 expression was positively correlated with the STX6 level when analyzed using the TIMER and GEPIA2 databases. IHC results further demonstrated this association. Furthermore, compared with the typically used AFP, STX6 could have an improved diagnostic value in the diagnosis of HCC. In conclusion, STX6 expression was not only positively associated with poor prognosis but may also be involved in the immune inflammatory reaction in HCC. STX6 may become a potential therapeutic and diagnosis maker for patients with HCC.

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Key words: syntaxin 6, prognosis, immune infiltrates, hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancy with the highest morbidity and mortality in numerous countries across the world (1). A number of treatments are used to treat HCC; however, the treatment effects are often limited, especially for advanced stage carcinomas (1,2). Therefore, it is urgent to further explore the carcinogenic targeting molecules of HCC to enhance prognosis and individualized treatments.

Syntaxin 6 (STX6) is a sensitive factor in the soluble n-ethylmaleimide receptor protein and has been reported to serve a role in Parkinson's disease (3,4). It has been reported to be expressed in brain, lung and kidney (5). Furthermore, STX6 is a vesicle transporter, which serves a vital role in intracellular protein transport and membrane structure changes (6). STX6 has been reported to be involved in tumorigenesis in multiple malignant tumors, including esophageal cancer (7), osteosarcoma (8) and renal cell carcinoma (9). Notably, a recent study reported that STX6 is a key factor in macrophages during the immune response in lipopolysaccharide-activated cells (10).

Macrophages are a major component of the inflammatory infiltrate in tumor (11,12). The high levels of tumor-associated macrophages (TAMs) are associated with poor prognosis in a range of tumors, including breast, gastric and colorectal carcinoma, and HCC (13-17). The macrophages are classified as M1 phenotype or M2 phenotype (18). It is now widely accepted that the M2 phenotype supports tumor growth (13-15,17,19). CD163 is widely reported as a scavenger receptor and is a highly specific marker of M2 macrophages (20,21). CD163 has been reported to be an anti-inflammatory molecule as it is mainly expressed by M2 macrophages at sites of inflammation (22). Interestingly, one study reported that STX6 is associated with increased cytokine secretion in activated macrophages (23). However, the mechanisms of STX6 immune infiltration in HCC remain unclear.

The present study analyzed the association between STX6 expression and clinical characteristics and prognosis of patients with HCC. Furthermore, the functions of STX6 in HCC and potential immune infiltration-related molecular mechanisms were assessed.

Materials and methods

Tissue sections. The tumor tissues and para-carcinoma tissue sections were collected between January 2014 and December 2016 and stored in the Human Resources Specimen Bank of the First Affiliated Hospital of Nanchang University (Nanchang, China). Samples were obtained from the Human Genetic Resources Center and Department of Pathology of The First Affiliated Hospital of Nanchang University (Nanchang, China) and examined independently by two pathologists. The patients included 76 males and 14 females between 27 and 81 years old (median 53). None of the patients had previously received other tumor surgery, chemotherapy, radiation therapy, or any other anticancer therapy. As this was a retrospective study, the requirement for informed consent was waived by the ethics committee. The present study was approved by the Clinical Medical Research Ethics Committee of the First Affiliated Hospital of Nanchang University (approval no. 202112020; Nanchang, China).

Immunohistochemistry (IHC). To examine STX6 and CD163 expression in tumor tissues, paired paraffin-embedded tumor slices were obtained from the specimen bank. The sections were then incubated with anti-STX6 (1:100; cat. no. ab140607; Abcam) and anti-CD163 (1:500, ab182422; Abcam) antibodies overnight at 4˚C as previously reported (24). For statistical analysis, the percentage coverage of the protein was scored manually as follows: i) 1 (0-25%); ii) 2 (26-50%); iii) 3 (51-75%); and iv) 4 (76-100%) (24). The intensity of positive staining was also scored as follows: i) 0 (negative); ii) 1 (weak); iii) 2 (moderate); and iv) 3 (strong). The final scores were calculated by multiplying the aforementioned scores. The final scores of the percentage and staining scores were defined as the overall IHC scores (0-12). Scores <6 were considered to be low expression (STX6-Low) and scores ≥6 were considered to be high expression (STX6-High).

Gene expression profiling interactive analysis (GEPIA2) database analysis. GEPIA2 (http://gepia2.cancer-pku.cn/#index) is an interactive online platform, which contains information from >9,000 tumor samples from The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/) and The Genotype-Tissue Expression (GTEx) (https://xenabrowser.net/datapages/) databases, and information from >8,000 control samples. The correlation between STX6 and CD163 was determined by Pearson correlation coefficient analysis in GEPIA2 (25). The iCluster 1 and iCluster 3 datasets (Cutoff-High-75% and Cutoff-Low-25%) were used to analyze the association between prognosis and STX6 expression in patients with HCC (25).

Kaplan-Meier plotter (KM plotter) survival analysis of STX6. Kaplan-Meier plotter (http://kmplot.com/analysis/) can perform survival analysis on >54,000 genes (mRNA, microRNA and protein) in 21 types of tumors (including breast, ovarian, lung, gastric and liver cancer). The data mainly come from the Gene Expression Omnibus and TCGA databases (26). The results were assessed based on the log rank P-value and hazard ratio (HR) with 95% confidence intervals. The RNAseq ID: 10228 (STX6) and Cutoff value used in analysis: 1046 were used. A follow-up threshold of 60 months was used to analyze the association between prognosis and STX6 expression in patients with HCC based on the log rank P-value.

Tumor immune estimation resource (TIMER) database. The TIMER database (https://cistrome.shinyapps.io/timer/) (27) is mainly divided into seven sections: Gene, Survival, Mutation, somatic copy number amplifications (sCNA), differential expression (Diffexp), Correlation and Estimation. Among them, the Gene and Correlation were used in this study. To facilitate the study of tumor immunity and genomic data, the TIMER database applies a deconvolution method (28) to infer the abundance of tumor immune-infiltrating cells (TIICs) from gene expression profiles, reanalyzes the gene expression data of 10,897 samples of 32 cancer types from TCGA and estimates the abundance of 6 TIIC subgroups [B cells, $CD4^+$ T cells, $CD8^+$ T cells, macrophages, neutrophils and dendritic cells (DCs)]. Deconvolution methods define the problem as mathematical equations that model the gene expression of a tissue sample as the weighted sum of the expression profiles from the cells in the population mix (29). These methods are further detailed in previous studies (27,30). The statistical method used by the TIMER database in the present study was the Spearman correlation coefficient analysis. $P<0.05$ was considered to be statistically significant.

University of California Santa Cruz (UCSC) Xena. RNAseq data were extracted from the UCSC Xena portal (https://xenabrowser.net/datapages/) (31), including data from TCGA Liver HCC (LIHC; n=371) and GTEx for corresponding normal tissue (n=160). These data were used to assess the diagnostic effect of STX6 and α-fetoprotein (AFP) in liver cancer using a receiver operating characteristic (ROC) curve as previously reported (32).

Statistical analysis. All bioinformatics analyses were performed using the corresponding database websites. Other statistical analyses of data were performed using GraphPad Prism 6 (GraphPad Software, Inc.) and SPSS 18 (SPSS, Inc.). Data are presented as the median. The Wilcoxon signed-rank test was used to compare the difference between tumor tissues and normal tissues. A Mann-Whitney U test was used to compare two distinct groups of patients (STX6-Low vs. STX6-High). The association of clinicopathological factors with STX6 expression was analyzed using the χ^2 test. A log-rank test was used to compare the survival curves by Kaplan-Meier. The correlation between the IHC score of STX6 and the IHC score of CD163 was analyzed using Pearson correlation coefficient analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

STX6 is highly expressed in HCC. IHC was used to assess the protein expression levels of STX6 in patients with HCC (Fig. 1A). The results demonstrated that the IHC scores of 12 pairs of HCC samples stained for STX6 were significantly

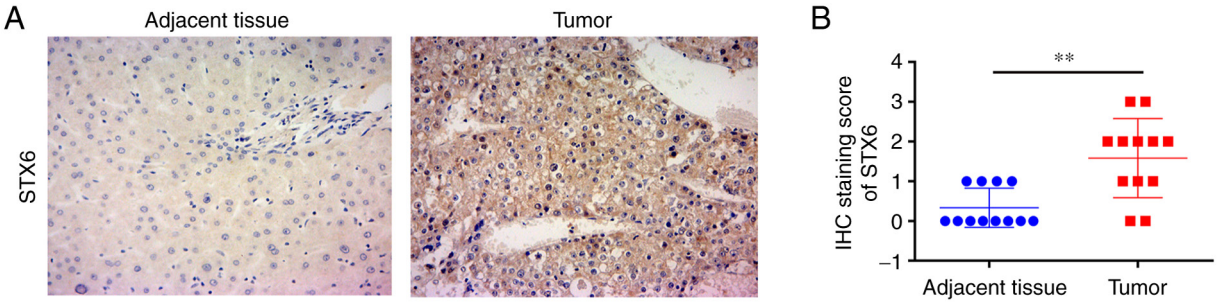

Figure 1. STX6 protein expression in human HCC and paired adjacent tissues. (A) IHC staining of STX6 in HCC and paired adjacent tissues (magnification, x100). (B) STX6 protein expression presented as the IHC staining score in 12 HCC and paired adjacent tissues. Data are presented as the mean ± SD. **P<0.01. HCC, hepatocellular carcinoma; IHC, immunohistochemistry; STX6, syntaxin 6.

higher than those of paired adjacent tissues (Fig. 1B). These results demonstrated that STX6 protein was highly expressed in HCC tissues.

Association analysis between STX6 expression and clinical features of patients with HCC. The relationship between STX6 protein expression and the clinicopathological characteristics of 90 patients with HCC was evaluated. STX6 protein expression was significantly associated with HCC tumor size (P=0.003), Edmondson grade (P=0.020) and AFP level (P=0.019) (Table I).

Association between STX6 expression and patient survival in HCC. Survival analysis demonstrated that patients with high STX6 expression had worse prognosis (Fig. 2A). Furthermore, analysis using KM plotter (Fig. 2B) demonstrated that high expression levels of STX6 were associated with worse overall survival (OS; P=0.00013; HR, 2.01; 95% CI, 1.4-2.89) in patients with HCC. Additionally, in the GEPIA2 database analysis, high STX6 expression was associated with worse disease-free survival [P=0.0043; HR(high), 1.8; p(HR), 0.0048] and OS [P=0.000086; HR, 6.4; p(HR)=0.00044] in patients with HCC (Fig. 2C and D). These data demonstrated that dysregulated expression of STX6 affected the clinical outcomes of patients with HCC.

Analysis of immune infiltration. STX6 expression was significantly positively correlated with infiltration by B cells ($r=0.389$; $P=6.93\times10^{-14}$), $CD4^+$ T cells ($r=0.541$; $P=1.50\times10^{-27}$), macrophages ($r=0.535$; $P=1.17\times10^{-26}$), neutrophils ($r=0.457$; $P=3.11\times10^{-19}$) and DCs ($r=0.416$; $P=1.15\times10^{-15}$), and significantly positively associated with $CD8^+$ T cell infiltration ($r=0.247$; $P=3.77\times10^{-6}$; Fig. 3A), which demonstrated that STX6 serves a crucial role in the immune infiltration of HCC. STX6 expression was also significantly positively associated with macrophage TAMs: CCL2 ($r=0.271$; $P=1.28\times10^{-7}$), CD68 ($r=0.298$; $P=5.38\times10^{-9}$), IL10 ($r=0.31$; $P=1.11\times10^{-9}$), MS4A4A ($r=0.202$; $P=9.06\times10^{-5}$), MSR1 ($r=0.371$; $P=1.62\times10^{-13}$) and VSIG4 ($r=0.222$; $P=1.65\times10^{-5}$) were also analyzed (Fig. 3B). The results demonstrated that STX6 mRNA expression was significantly correlated with TAMs [CC motif chemokine ligand 2 (CCL2), CD68, IL10, V-set and immunoglobulin domain containing 4, macrophage scavenger receptors-type 1 and membrane spanning 4-domains A4A], DCs, $CD4^+$ T cells, CD163, etc. (Table SI). To further assess the association of STX6 with CD163, STX6 and CD163 expression was analyzed using the GEPIA2 and TIMER databases. These results demonstrated a small association between STX6 and CD163 (cor=0.173 in Fig. 3C and R=0.18 in Fig. 3D). The results of the present study demonstrated that STX6 expression was positively associated with B and T-cell receptor signaling pathways during HCC pathogenesis, indicating that STX6 was related to the immune response. These findings suggested that STX6 expression may be associated with the infiltration of immune cells in HCC.

STX6 is positively associated with CD163 according to IHC. STX6 and CD163 expression in HCC and adjacent noncancerous tissues was assessed using IHC (Figs. 1A and 4A). The results demonstrated that the protein expression levels of STX6 and CD163 in cancer tissues were significantly increased compared with those in adjacent tissues (Figs. 1B and 4B). To further examine the association between STX6 and CD163, IHC was performed (Fig. 4C). The results demonstrated that CD163 levels were significantly positively associated with the levels of STX6 in paired paraffin-embedded tumor slices (Fig. 4D and E).

Diagnostic values of STX6 and AFP as assessed using ROC curve analyses. The ROC curve analyses of two markers (STX6 and AFP) in HCC and normal tissues are presented (Fig. 5). ROC curve analysis indicated that the area under the curve (AUC) values for STX6 and AFP were 0.942 (CI, 0.916-0.967) and 0.720 (CI, 0.668-0.773), respectively, using TCGA data (tumor, n=374; normal, n=50). AUC of STX6 (sensitivity, 0.940; specificity, 0.850) was higher than that of AFP (sensitivity, 0.940; specificity, 0.516) in TCGA (Table II). Furthermore, the ROC curve analysis demonstrated that the AUC values of STX6 and AFP were 0.844 and 0.746, respectively, according to the combined TCGA (tumor, n=371) and GTEx (normal, n=160) datasets. The sensitivity of the ROC curve for STX6 (0.827) was higher than that for AFP (0.563). The specificity of the ROC curve of STX6 (0.762) was lower than that of AFP (0.869). Compared with the aforementioned datasets, STX6 had a higher AUC value (0.947) and specificity (0.917) in the our HCC data of the present study. The sensitivity of ROC curve of STX6 is 0.856 in the present HCC data. These results demonstrated that STX6 may be a diagnostic marker for HCC.

Table I. Association between STX6 expression and clinical characteristics of patients with hepatocellular carcinoma.

		STX6 expression (IHC score)				
Variables	No. (%)	Low (<6), n	High (≥6), n	Df	χ^2	P-value
Sex				1	0.017	0.897
Male	76 (84.4)	42	34			
Female	14 (15.6)	8	6			
Age, years				1	0.057	0.810
<55	37 (41.1)	20	17			
≥55	53 (58.9)	30	23			
Size, cm				1	9.085	0.003
<5	56 (62.2)	38	18			
≥5	34 (37.8)	12	22			
Diolame complete				1	0.188	
Yes	54	31	23			0.665
No	36	19	17			
Number of tumors				1	1.125	0.289
Single	72 (80)	38	34			
Multiple	18 (20)	12	6			
TNM staging				1	1.798	0.180
I+II	77 (85.6)	45	32			
III+IV	13 (14.4)	5	8			
Microvascular invasion				1	1.309	0.253
No	64 (71.1)	38	26			
Yes	26 (28.9)	12	14			
Edmondson grade				1	5.399	0.020
I+II	67 (74.4)	42	25			
III	23 (25.6)	8	15			
Cirrhosis				1	0.243	0.622
Negative	16 (17.8)	8	8			
Positive	74 (82.2)	42	32			
HBV				1	0.800	0.371
Absent	25 (27.8)	12	13			
Present	65 (72.2)	38	27			
ALT, U/l				1	0.458	0.499
<45	55 (61.1)	29	26			
≥45	35 (38.9)	21	14			
AFP, ng/ml				1	5.478	0.019
<400	69 (76.7)	43	26			
≥400	21 (23.3)	7	14			

AFP, α-fetoprotein; ALT, alanine aminotransferase; HBV, hepatitis B virus; IHC, immunohistochemistry; STX6, syntaxin 6.

Discussion

The progression of liver HCC is rapid and numerous patients present with advanced HCC (33,34). Regulation of immune infiltration is increasingly recognized as being important in tumor development (35). Finding breakthroughs in immunotherapy has become the focus of current research. Furthermore, an effective early detection method is still lacking in the current treatment of HCC. AFP has been recognized as a tumor marker for HCC but it has poor sensitivity and specificity (36,37). Exploring novel therapeutic and diagnostic markers is still the top priority of scientific research in this field.

In the present study, the role of the STX6 gene in the development of HCC was analyzed using IHC. STX6 protein expression in HCC was analyzed and the results demonstrated that STX6 expression was upregulated in HCC tissues compared with normal tissues. The association

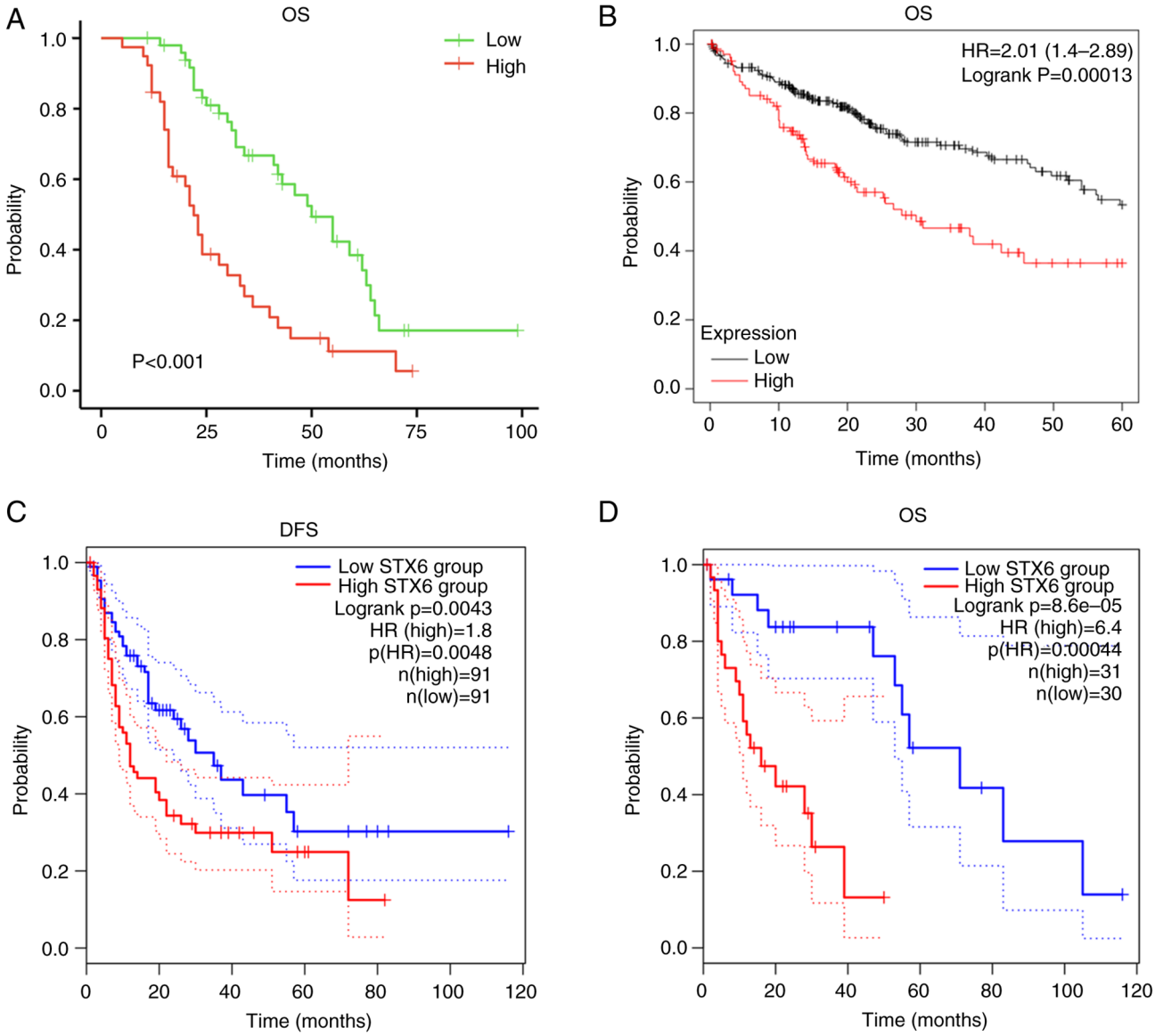

Figure 2. Predictive value of STX6 for prognosis in HCC. (A) OS in patients with the present HCC data. (B) OS analyzed using Kaplan-Meier plotter. (C) DFS and (D) OS analyzed using Gene Expression Profiling Integrative Analysis 2. DFS, disease-free survival; HCC, hepatocellular carcinoma; HR, hazard ratio; OS, overall survival; STX6, syntaxin 6.

between STX6 and clinical characteristics of patients was further considered. These results demonstrated that high protein expression levels of STX6 were significantly associated with tumor size, Edmondson grade and the AFP level in patients with HCC. Furthermore, these results demonstrated that patients with high STX6 expression had a worse prognosis as demonstrated by analysis of the survival data in the KM plotter and GEPIA2 databases, which are based on the results of transcriptome sequencing data analysis. Furthermore, patients with high STX6 protein expression also had a worse prognosis as demonstrated by the survival data of patients with HCC in the present study. A previous study reported that STX6 could be a prognostic biomarker for patients with renal cell carcinoma based on TCGA transcriptome sequencing data (9). This is supported by the present study which combined analysis of two different datasets, which demonstrated that STX6 could promote the process of HCC malignancy.

The present study demonstrated that STX6 was associated with infiltration of immune cells based on analysis using the TIMER database. The results demonstrated that high STX6 mRNA expression in HCC was positively associated with high immune infiltration. STX6 expression was significantly positively correlated with the levels of immune infiltration, including B cell, $CD4^+$ T cell, DC, macrophage and neutrophil infiltration, in HCC, and significantly positively associated with $CD8^+$ T cells. Furthermore, the gene markers of M2 macrophages (CD163 and CD115) and TAMs (CCL2 and IL10) were significantly positively correlated with STX6 expression. Association between STX6 and CD163 was assessed using IHC and the results confirmed the aforementioned findings. It could be concluded that the M2 macrophage CD163 expression was also enhanced by increased levels of STX6. It has previously been reported that high rates of infiltration of M2 macrophages into the tumor stroma could inhibit T cell proliferation and downregulate antitumor immune responses (38,39). STX6

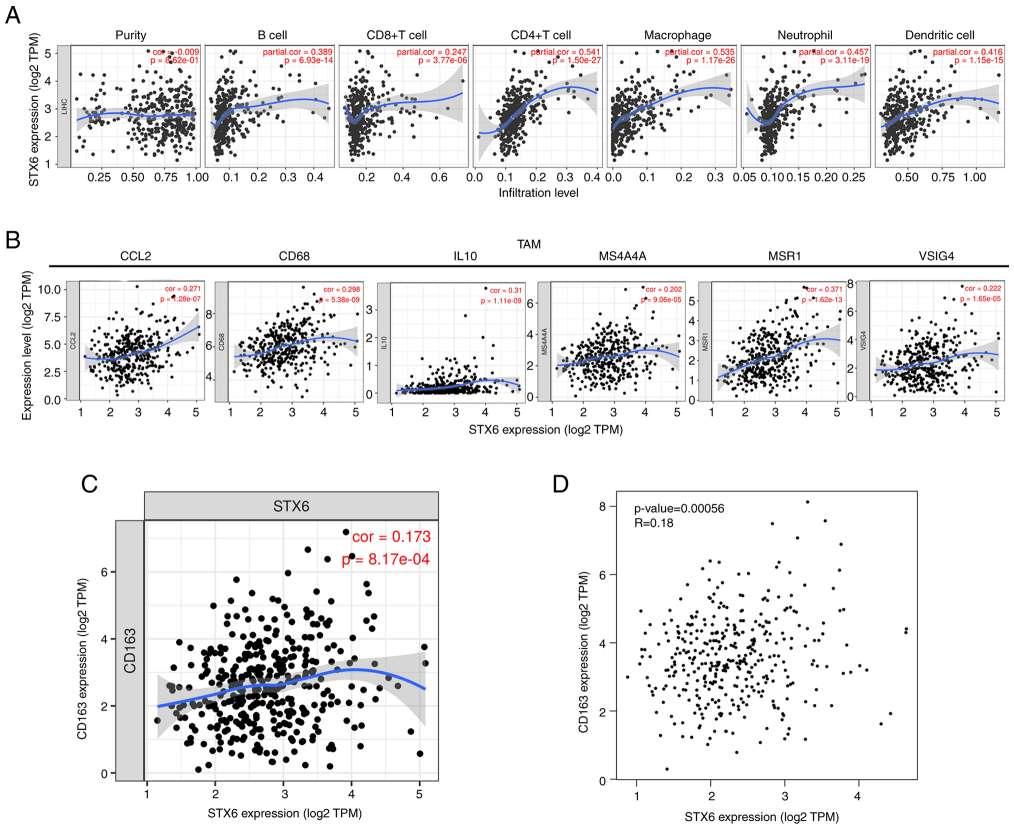

Figure 3. Relationship between STX6 expression and immune cell infiltration levels in hepatocellular carcinoma. (A) Correlation of STX6 expression with tumor-infiltrating immune cells in LIHC (n=371). Scatter plots presenting the correlations between STX6 expression and marker molecules, including (B) TAMs (CCL2, CD68, IL10, VSIG4, MSR1, MS4A4A), and (C and D) CD163. The blue curve and gray area in the figure represent the general trend direction. LIHC, liver hepatocellular carcinoma; STX6, syntaxin 6; CCL2, CC motif chemokine ligand 2; MS4A4A, membrane spanning 4-domains A4A; MSR1, macrophage scavenger receptor 1; TAMs, tumor-associated macrophages; TPM, transcripts per million; VSIG4, V-set and immunoglobulin domain containing 4.

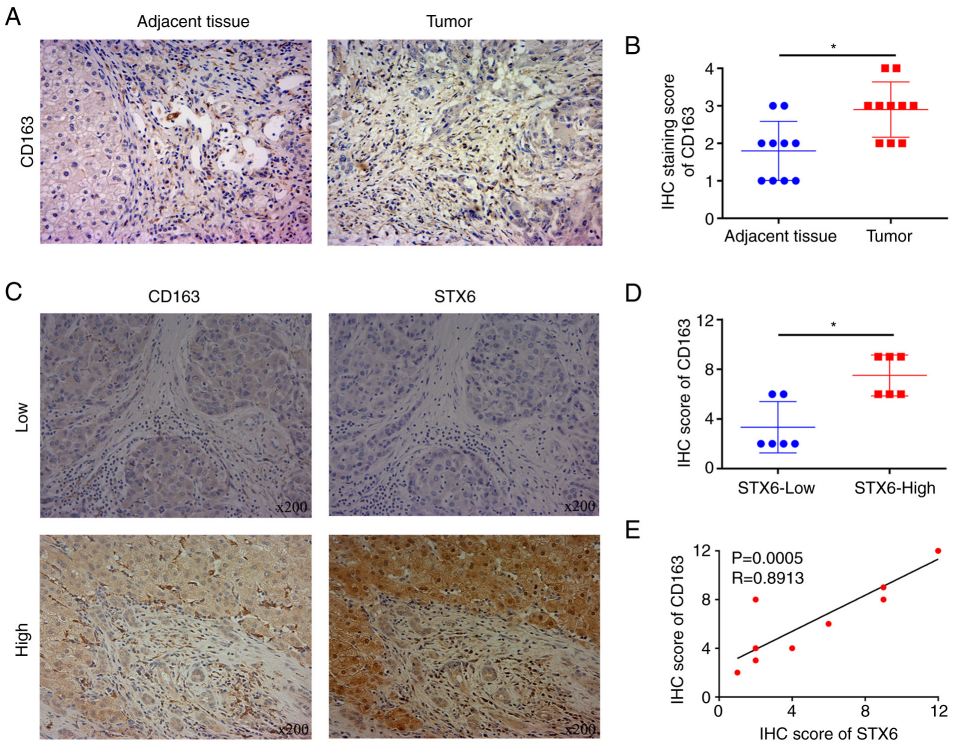

Figure 4. Expression levels of STX6 and CD163 in HCC. (A) CD163 expression in HCC and adjacent noncancerous tissues (magnification, x100). (B) IHC staining score of CD163 in HCC and adjacent noncancerous tissues. (C) STX6 and CD163 expression in paired paraffin-embedded tumor slices (magnification, x100). (D) IHC score of CD163 in tumor tissues from patients in the high and low STX6 groups. (E) Correlation between the IHC score of STX6 and the IHC score of CD163. *P<0.05. HCC, hepatocellular carcinoma; IHC, immunohistochemistry; STX6, syntaxin 6.

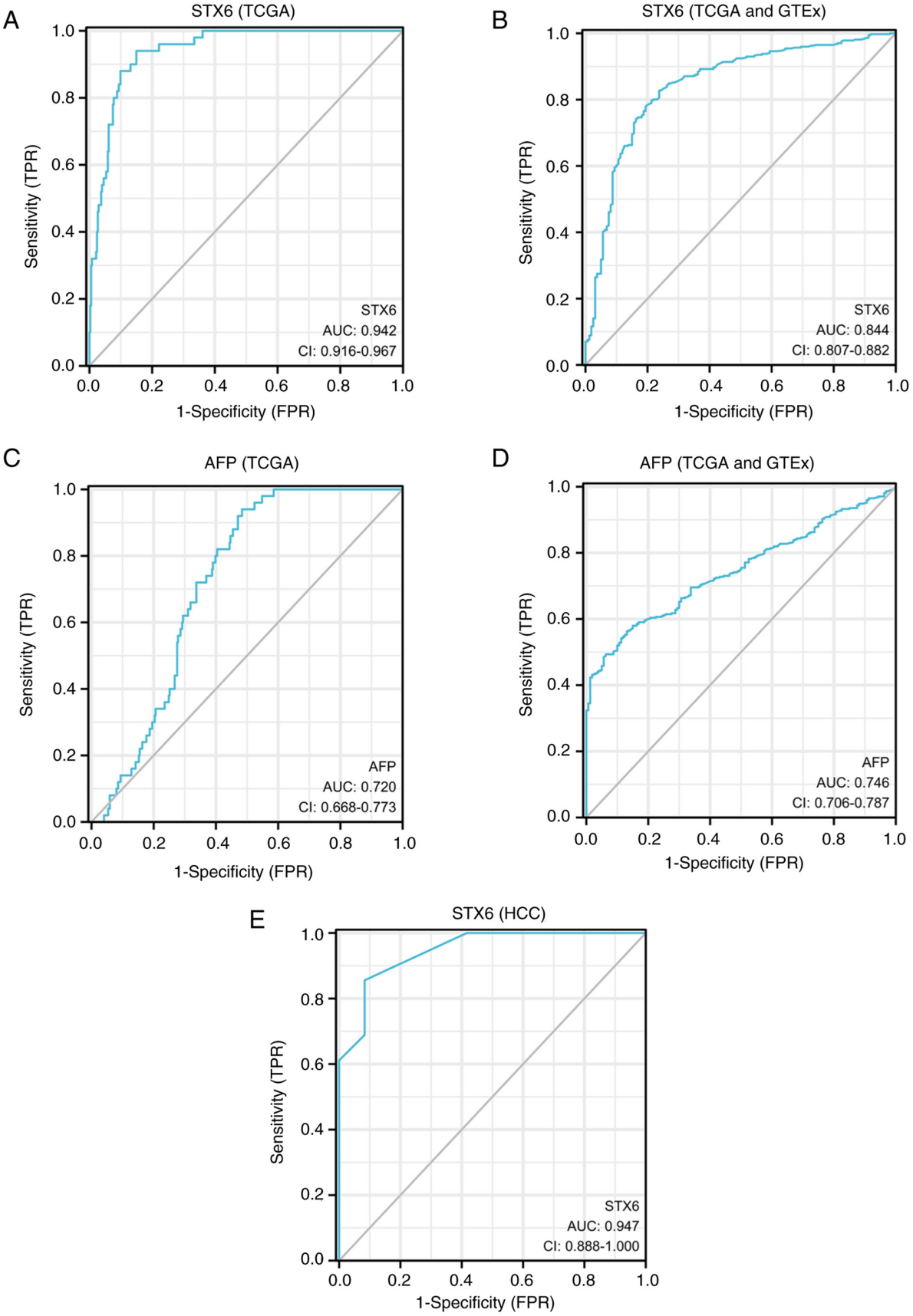

Figure 5. Diagnostic values of STX6 and AFP indicated by ROC curve analyses based on the TCGA and GTEx datasets. (A) Diagnostic value of STX6 assessed using ROC curve analysis of data from TCGA (tumor, n=374; normal, n=50). (B) Diagnostic value of STX6 assessed using ROC curve analysis of data from TCGA (tumor, n=371) and GTEx (normal, n=160) databases. (C) Diagnostic value of AFP assessed using ROC curve analysis of data from TCGA (tumor, n=374; normal, n=50). (D) Diagnostic value of AFP assessed using ROC curve analysis of data from TCGA (tumor, n=371) and GTEx (normal, n=160) databases. (E) Diagnostic value of STX6 assessed using ROC curve analysis of data from the present study (tumor, n=90; normal, n=12). AFP, α-fetoprotein; AUC, area under the curve; FPR, false positive rate; GTEx, Genotype-Tissue Expression; HCC, hepatocellular carcinoma; ROC, receiver operating characteristic; STX6, syntaxin 6; TCGA, The Cancer Genome Atlas; TPR, true positive rate.

Table II. Diagnostic values of STX6 and AFP in patients with liver hepatocarcinoma according to receiver operating characteristic curve analyses.

Index	AUC	95% CI	Cut-off	Sensitivity	Specificity	Youden index	Data
AFP	0.720	0.668-0.773	2.613	0.940	0.516	1.456	TCGA
STX6	0.942	0.916-0.967	2.334	0.940	0.850	1.790	TCGA
AFP	0.746	0.706-0.787	3.341	0.563	0.869	1.432	TCGA + GTEx
STX6	0.844	0.807-0.882	1.702	0.827	0.762	1.590	TCGA + GTEx
STX6	0.947	0.888-1.000	1.500	0.856	0.917	0.772	HCC

TCGA data (tumor, n=374; normal, n=50). TCGA (Tumor=371) + GTEx (normal=160) data. HCC data (tumor, n=90; normal, n=12). AFP, α-fetoprotein; AUC, area under the curve; CI, confidence interval; GTEx, Genotype-Tissue Expression; HCC, hepatocellular carcinoma; STX6, syntaxin 6; TCGA, The Cancer Genome Atlas.

upregulation could be one of the routes that link immunosuppression and the development of HCC. Overall, these results demonstrated the potential regulatory role of STX6 in immune inflammatory responses in HCC.

AFP has a diagnostic value as a marker for liver cancer in clinical settings (40-42). Abnormal AFP levels in adult plasma have been reported to be a marker of the pathological condition of HCC (43). In the present study, the results demonstrated that the AUC of STX6 was significantly higher than the AUC of AFP based on TCGA data (0.942 vs. 0.720) and the sensitivity of STX6 was also higher than that of AFP based on combined TCGA and GTEx data (0.827 vs. 0.563). Nevertheless, the specificity of the AUC curve of STX6 was lower than the AUC of AFP based on combined TCGA (tumor, n=371) and GTEx (normal, n=160) data (0.762 vs. 0.869). This could have been due to the high STX6 expression in multiple other tumor types (7,8,44), which reduce its diagnostic specificity in HCC. However, compared with the aforementioned datasets, STX6 demonstrated a higher AUC value (0.947) and specificity (0.917) in data from the present study. Collectively, these results demonstrated that STX6 has the potential to be a powerful diagnostic maker in HCC.

The present study demonstrated that STX6 protein expression was significantly associated with HCC tumor size, Edmondson grade, AFP level and prognosis of patients with HCC. Furthermore, STX6 may be involved in the immune-inflammatory response of HCC and may become a novel potential diagnostic marker for patients with HCC. However, there were some limitations in the present study. For the assessment of the association between STX6 and CD163, the absence of double-staining IHC or immunofluorescence staining was a limitation of the present study. Furthermore, the present study was only an initial early-stage experiment and future work is required which should include experiments on larger numbers of clinical samples and more in-depth research on the molecular mechanism of STX6 in HCC. Further studies should analyze fresh tissues and HCC cell lines and explore the effects of STX6 on the phenotype of HCC cells.

In summary, the present study demonstrated that STX6 expression was associated with the clinical characteristics and prognosis of patients with HCC. Furthermore, STX6 may associate with CD163 to participate in the modulation of inflammatory responses in HCC. Compared with AFP, STX6 may become a valuable novel tumor marker for the diagnosis of patients with HCC and combination of STX6 with AFP may have higher diagnostic value. The present study also provided further insight into the molecular mechanism of STX6 in HCC.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant no. 82103165), The Key R & D General Project of Jiangxi Science and Technology Department (grant no. 20203BBGL73187), the Natural Science Foundation of Jiangxi Province (grant no. 20212BAB216036), The Education Department of Jiangxi Province Science and Technology Research Projects (grant no. GJJ160246) and the Young Talent Cultivation Project of the First Affiliated Hospital of Nanchang University (grant no. YFYPY202007).

Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the GEPIA2 repository, https://gepia2.cancer-pku.cn/#index; National Cancer Institute GDC Data Portal repository, https://portal.gdc.cancer.gov/; the Kaplan-Meier Plotter repository, https://kmplot.com/analysis/; TIMER repository, https://cistrome.shinyapps.io/timer/; TCGA repository, https://portal.gdc.cancer.gov/; and UCSC Xena repository [TCGA cohort, GDC TCGA Liver Cancer (LIHC), TCGA-LIHC.htseq_fpkm.tsv; GTEX cohort, GTEX, gtex_RSEM_gene_tpm], https://xenabrowser.net/datapages/. The other datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YZ, LL, ZF and YT analyzed the data and prepared the manuscript. YL and JX designed the study. YL and JX confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The paired paraffin-embedded tumor slices were obtained from Human Genetic Resources Center of the First Affiliated Hospital of Nanchang University (Nanchang, China). The project was approved by the Clinical Medical Research Ethics Committee of the First Affiliated Hospital of Nanchang University (approval no. 202112020; Nanchang, China). As this was a retrospective study, the requirement for informed consent was waived by the ethics committee.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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