LTBP2 Serves as a Prognostic Biomarker and Correlated with the Immune Infiltration in Stomach and Colon Cancer

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Abstract

Latent transforming growth factor β binding protein 2 (LTBP2) involved in the TGF pathway to induce immunosuppression and immune response. However, the association between the outcome of patients, the infiltrating immune cell and LTBP2 expression is still unclear in human cancers. The LTBP2 expression was analyzed by TIIMER and Oncomine database. Based on the Prognoscan database, the GEPIA database, and the Kaplan-Meier plotter database, the prognostic value was assessed. The immune and stromal score of tumors was calculated through ESTIMATE. We additionally explore the relationship among the LTBP2 expression, the infiltrating immune cells, and its gene markers in the TIMER, TISIDB, and GEPIA database, the enriched KEGG pathways of LTBP2 were evaluated by GSEA. The result showed that LTBP2 expressed differently among the normal and tumor tissues in various sorts of cancer involving stomach adenocarcinoma (STAD) and colon adenocarcinoma (COAD), and three cohorts of COAD presented that the LTBP2 high expression was linked with poorer disease-free survival and the elevated LTBP2 expression correlated with progression-free survival and poorer overall survival in STAD. The LTBP2 was correlated with the stromal and immune score in different cancers. The infiltrating immune cells include the CD8+T cells and CD4+T cells, macrophages, neutrophils, and dendritic cells were correlated with the LTBP2 expression. Meanwhile, LTBP2 was related to the infiltrating immune cell's gene markers and enriched immune-related pathways in STAD and COAD. LTBP2 was the potential to be an independent predictor for the prognosis and a new target for immunotherapy in STAD and COAD.

Background

Gastrointestinal (GI) cancer is common. Colorectal cancer (CRC) and gastric cancer rank third and fourth in the world, respectively (1).A variety of therapeutic strategies have been applied to digestive tract tumors, including surgery, chemotherapy, radiotherapy and molecular targeted therapy. However, the survival time of patients with advanced gastrointestinal tumors is still very poor (2,3). Recent studies have reflected that the strategy of immunotherapy acts as a significant role in the treatment of gastrointestinal patients and has become a new direction that researchers focus on (4). Mainly specific study had presented that anti-cytotoxic T lymphocyte-associated antigen 4 had restricted therapeutic effect in the metastatic gastrointestinal cancer (5,6) and the results of advanced gastrointestinal cancer patients who were treated with the immune inhibitors named anti-PD-1 was not reasonable for us (7–9). Recent researches had suggested that gastroesophageal cancers have three key strategies for therapies containing the checkpoints of the cell cycle, tumor-associated microenvironment, and the regulation of T cells, the inhibition of any target spot may give an effective treatment to the patients (10). Immunotherapy has become essential in the treatment methods for malignant tumors on the basis of above research. Therefore, it is necessary to find effective immunotherapy targets for patients with digestive tract tumors to improve their survival rate.

Latent transforming growth factor β binding protein 2 (LTBP2) is a protein of the LTBP family which includes LTBP1-4 and fibrillin 1, 2, and 3, its functions on secreting extracellular matrix protein added on the stability of the microenvironment (11). Various studies suggest that it has an essential part in the appearance and growth of several malignant tumors, containing thyroid carcinoma (12), pancreatic cancer (13), and colorectal cancer (14). As per the reports, we can summarize that LTBP2 may play in the biological process by activating the TGF-β pathways (15,16). TGF-β was secreted by immune and tumor cells, and the signal pathway of TGF-β mediated immunosuppression added into the tumor microenvironment (17,18), TGF-β can also constrain the immune cell responsiveness (19,20) and excite the angiogenesis (21). Specific tumors were stimulated the naive peripheral CD4+ T cells differentiation into CD4+, CD25+ and regulatory T cells through TGF-β secretion (22–24), so we inferred that LTBP2 was strongly linked to the immune regulation in the tumor microenvironment and lead to the poor survival by activating the TGF beta pathways. While, the specific relationship between the expression of LTBP2 and the infiltration of the immune cells in the tumor microenvironment remains unknown.

As we all know, more evidences are required to confirm that the tumor microenvironment has a strong correlation with the occurrence of human cancers. The extracellular matrix (ECM) was also a member of the stromal microenvironment, and the stability of the stromal microenvironment added on the stable structure and formed the biochemical and physical signals to assure the normal function of cells (25). In the past, LTBP2 was considered as a protein belongs to ECM proteins, deriving from the fibrillin/LTBP ECM glycoprotein family. Meanwhile, the tumor growth and development were mostly impacted by the components of microenvironment, like infiltrated immune cells, fibroblasts, vascular cells, noncellular components comprises of solid ECM and soluble cytokines (26). Therefore, we need to understand the relationship between LTBP2 expression and tumor microenvironment.

In this research, we examined the LTBP2 expression in several types of cancer and explored the correlation between the LTBP2 expression and the patient's prognosis in different tumors, containing STAD and COAD by using TIMER, ONCOMINE and Kaplan Meier plotter. Ultimately, we focus on the association among LTBP2 expression and the immune infiltrating cells on the basis of TIMER database, the result reflected that LTBP2 could affect immune cells infiltration in various human cancers. Mainly in COAD and STAD, and it could be a new target for the immunotherapy and new biomarker for prognostic guidance in gastrointestinal cancer.

Results

The different expression in tumor tissues in comparison with the corresponding normal tissues.

To analyze LTBP2 expression levels in several sorts of cancer, we conducted the data from the ONCOMINE database. The result showed LTBP2 expression was greater in several sorts of cancer, involving the brain and CNS cancer, brain cancer, liver cancer, breast cancer, pancreatic cancer, gastric cancer, colorectal cancer, leukemia, esophageal cancer, lymphoma, and tertoma, and lower expression of LTBP2 was detected in cervical cancer, breast cancer, kidney cancer, lung cancer, melanoma, prostate cancer, sarcoma and vulvar intraepithelial neoplasia (Fig.1a and Supplementary table1). To further determine the expression in different cancers, we verify it by utilizing the TIMER database (Fig.1b), we found that the expression of LTBP2 was

elevated in several sorts of cancer involving colon adenocarcinoma (COAD), cholangiocarcinoma (CHOL), head and neck squamous cell carcinoma (HNSC), esophageal carcinoma (ESCA), liver hepatocellular carcinoma (LIHC), stomach adenocarcinoma (STAD), rectum adenocarcinoma (READ). Interestingly, the LTBP2 expression was lower in comparison to the corresponding normal tissues in uterine corpus endometrial carcinoma (UCEC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), kidney renal papillary cell carcinoma (KIRP), prostate adenocarcinoma (PRAD), breast invasive carcinoma (BRCA), and thyroid carcinoma (THCA).

The relationship among the expression of LTBP2 and the prognosis in various human cancers

It is concluded that LTBP2 was expressed differently in different human cancers, so we determined to understand the relationship between LTBP2 expression and the survival of patients in various human cancers, using the PrognoScan database. LTBP2 expression mainly affected the patient prognosis in 8 types of tumors, containing colorectal, bladder, blood, brain, breast, lung, ovarian, and renal cell carcinoma cancers. Three cohorts (GSE17536, GSE14333, GSE17537) contained 145 samples, 226 samples, and 55 samples at different colorectal cancer stages (36,37). The outcome illustrated that the LTBP2 high expression was related to the poor prognosis in colorectal cancer (DFS HR=3.06, *P*<0.001; DFS HR=1.51, *P*=0.011; DFS HR=2.07, *P*=0.019) (Fig. 2a-c), brain cancer (OS HR=1.98, *P*=0.004) (Fig. 2f), bladder cancer (DSS HR=2.02, *P*=0.002) (Fig. 2d), ovarian cancer (OS HR=1.20, *P*=0.043) (Fig. 2k) and renal cell carcinoma cancer (OS HR=7.83, *P*=0.01) (Fig. 2l). The higher expression of LTBP2 was linked to the better result in lung cancer (OS HR=0.56, *P*=0.006; OS HR= 0.57, *P*=0.023; RFS HR=0.48, *P*<0.001) (Fig. 2h-j), blood cancer (OS HR=0.33, *P*=0,007) (Fig. 2e) breast cancer (DSS HR=0.55, *P*=0.041) (Fig. 2g).

We intended to further investigate the correlation among the expression of LTBP2 and the patient's outcome suffering from various cancers. We analyzed the Affymetrix microarrrys from Kaplan-Meier plotter database to find LTBP2 higher expression was closely linked to the poorer survival of gastric cancer patients (OS HR=1.89, 95%CI=1.52-2.36, *P*=9.5e-09, Fig. 2m; PFS HR=1.4, 95%CI=1.1-1.78, *P*= 0.0062, Fig. 2n) and poorer PFS in ovarian cancer (HR=1.41, 95%CI =1.17-1.7, *P*=0.00035, Fig. 2t). On the contrary, LTBP2 higher expression was linked to the better survival in lung cancer (OS HR=0.53, 95%CI=0.44-0.62, *P*=6.2e-14, Fig. 2q; PFS HR=0.54, 95%CI=0.41-0.71, *P*=9.3e-06, Fig. 2r) and better RFS in breast cancer (HR=0.83, 95%CI=0.71-0.97, *P*=0.016, Fig. 2p). However, there was no correlation between the expression of LTBP2 and OS in breast cancer (Fig. 2o) and ovarian cancer (Fig. 2s).

As a supplement to the result analyzed by the Kaplan-Meier database and Prognoscan database, we examined the association between the expression of LTBP2 and the prognosis based on the GEPIA database, Supplementary Fig. 1 illustrated that the higher LTBP2 expression was linked to the poorer DFS and OS in adrenocortical carcinoma (ACC), brain lower grade glioma (LGG) and stomach adenocarcinoma (STAD); OS in lung squamous cell carcinoma (LUSC), glioblastoma multiforme (GBM), bladder urothelial carcinoma (BLCA) and kidney renal papillary cell carcinoma (KIRP); DFS in esophageal carcinoma (ESCA). All these outcomes showed LTBP2 is a potential to be an independent prognostic predictor in specific cancers, and the prognostic value of LTBP2 varies based on the type of human cancers.

The LTBP2 expression was related to the clinical prognosis of patients with STAD.

We further examined the correlation among the expression of LTBP2 and the clinical characteristic, the outcome reflected that LTBP2 was closely correlated with stage T (*P*=4.043E-05), stage N (*P*=0.044), grade (*P*=1.51E-04), and stage TNM (*P*=3.588E-04) (Fig. 3a-d). The outcome showed that LTBP2 can affect the metastasis of lymph nodes and endorse the progression of tumors in STAD.

We desired to further make clear the correlation between the expression of LTBP2 and the patient's clinical prognosis, Kaplan-Meier plotter database was used to analyzed the result which showed the expression of LTBP2 was linked to the OS and PFS in female and male patients, the expression of LTBP2 was linked with the OS and PFS in the stage T2, stage N0, stage N1+2+3, and stage M0. While, LTBP2 has no relationship with the OS and PFS in stage 1, 3 and 4. It can affect the two types of Lauren classification and have a solid connection with the prognosis in the two kinds of HER2, it was both important in HER2+ and HER2- (Table 1).

The relationship among the LTBP2 expression and the tumor microenvironment

Some studies have suggested that the tumor microenvironment interrelated with the tumor epithelium and functioned in the malignant process of tumor development. Moreover, the stromal score and the immune score could predict the prognosis of patients in various cancers (38). We used the ESTIMATE algorithm to investigate the correlation among LTBP2 expression and the immune score, the stromal score in 33 kinds of human cancers (Supplementary Table 3 and Fig. 4a), we determined that LTBP2 linked with the stromal score in 31 types of cancers and immune score in 22 kinds of human cancers, mainly the outcome reflected that the LTBP2 was correlated with a stromal score in COAD (r=0.85, *p*<2.2E-16, Fig. 4b) and STAD (r=0.76, *p*<2.2E-16, Fig. 4c), the further study reflected that the immune score has a connection with an expression of LTBP2 in COAD (r=0.6, *p*<2.2E-16, Fig. 4e) and STAD (r=0.37, *p*<5.2E-12, Fig. 4f), we took over the control of KICH, the result reflected that the LTBP2 does not correlate with the immune score (r=-0.21, *p*=0.097, Fig. 4g) and the stromal score (r=-0.081, *p*=0.52, Fig. 4d) in KICH, above results, showed LTBP2 was potential to form an independent predicator factor for patients survival in COAD and STAD.

It is determined that tumor-infiltrating lymphocytes (TILs) are capable of being a prognostic factor for patient's outcome suffering cancer in various human cancers (39). We intended to Fig. out the correlation between LTBP2 expression and the immune infiltrating cells on the basis of the TIMER database, the TISIDB database, and the GEPIA database in different human cancers. The analyzed immune infiltrating cells included B cells, CD8+ T cell, CD4+ T cells, macrophages, neutrophils and dendritic cells. The outcome illustrated that the LTBP2 expression was associated with the tumor

purity in 27 types of human cancers and the B cells infiltration in 21 kinds of cancers, LTBP2 had a strong correlation with the neutrophils in 26 sorts of cancers; CD8+ T cells in 22 sorts of cancers; macrophages in 28 sorts of cancers; the CD4+ T cells in 31 sorts of cancers and dendritic cells in 30 sorts of cancers (Supplementary Fig. 2 and Fig. 4h-j). Especially the LTBP2 expression had a strong correlation with the immune infiltration in COAD (Fig. 4h, B cells: cor=0.141, *p*=4.54E-03; CD8+ T cells: cor=0.191, *p*=1.05E-04; CD4+ T cells: cor=0.65, *p*=1.29E-49; Macrophage: cor=0.574, *p*=9.57E-37; neutrophil cor=0.456, *p*=1.05E-21; dendritic cells: cor=0.551; *p*=2.37E-33) and STAD (Fig. 4j, CD8+ T cells: cor=0.278, *p*=5.62E-08; CD4+ T cells: cor=0.444, *p*=3.73E-19; Macrophage: cor=0.562, *p*=3.11E-32; neutrophil: cor=0.289, *p*=1.41E-08; dendritic cells: cor=0.45; *p*=6.29E-20) adjusting by tumor purity, We also determined that the tumor purity was negatively linked with an expression of LTBP2 in COAD (tumor purity: cor=-0.382, *p*=1.41E-15) and STAD (tumor purity: cor=-0.138, *p*=6.94E-03), but there was no relationship with the LTBP2 expression in KICH (Fig. 4i).

The relationship among the expression of LTBP2 and the gene markers of infiltrating immune cells in STAD, COAD, and KICH

We used KICH as a control for understanding the underlying mechanism of immune infiltrating cells in STAD and COAD, we devoted ourselves to investigate the correlation among LTBP2 expression and the gene markers of immune infiltrating cells. The analyzed infiltrating immune cells included CD8+ T cells, T cells (general), B cells, monocytes, tumor-associated macrophages (TAMs), M1 macrophages, M2 macrophages, neutrophils, natural killer (NK) cells, dendritic cells (DCs), T-helper 1 (Th1) cells, T-helper 2 (Th2) cells, follicular helper T (Tfh) cells, T-helper 17 (Th17) cells, Tregs, and exhausted T cells. The result showed the LTBP2 expression was associated with the different kinds of gene markers of immune infiltrating cells in STAD and COAD, at the same time we considered the expression of LTBP2 has little relation to the gene markers of immune infiltrating cells in KICH (Table 2).

We aimed to further evaluate the degree of association among the expression of LTBP2 and the gene markers of immune infiltrating cells in STAD and COAD. Surprisingly we found that the CD86, CD115 of Monocytes, CCL2, CD68, IL10 of TAMs, INOS, IRF5, COX2 of M1 macrophages and CD163, VSIG4, MS4A4A of M2 macrophages were all positively correlated with the expression of LTBP2 in COAD (Fig. 5a-d) and STAD (Fig. 5g-j), but there was no major evidence that showed the expression of LTBP2 has a connection with the similar gene markers of immune infiltrating cells in KICH (Fig. m-p). All these outcomes were checked on the GEPIA database, and we found a similar conclusion from the GEPIA database (Table 3). The above outcome recommended that LTBP2 may involve in the macrophage polarization in STAD and COAD.

As we all known, the DC cells had an effe ct of the tumor metastasis by elevating Tregs and overwhelming the CD8+T cell cytotoxicity (40). In this study, we were pleasantly surprised to determined that the gene markers of DC cells including HLA-DQB1, HLA-DPB1, HLA-DPB1, HLA-DRA, CD11c, BDCA-4, and BDCA-1 were positively linked to the LTBP2 expression (Table 2), and the elevated levels of LTBP2 expression usually have poorer survival in COAD and STAD, we can summarize that LTBP2 may affect the survival by mediating an involvement of DC cells.

As a whole, the outcome reflected that the LTBP2 expression was correlated with gene markers of exhausted T cells and Tregs involving CCR8, FOXP3, TGFB1, STAT5B, CTLA-4, PD-1, TIM-3, and LAG3 in COAD (Fig. 5e-f and Table 2) and STAD (Fig. 5k-l and Table 2), but no relationship in KICH (Fig. 5q-r). The main impact of cytotoxic T cells killing tumor cells was minimized by Tregs that was mediated by FOXP3 (41), The related results show that LTBP2 affects the killing effect of tumor by affecting the expression of FOXP3 in tumor microenvironment. Furthermore, there is a strong correlation between TIM-3 and the expression of LTBP2 in COAD and STAD. LTBP2 may also affect immune escape through TIM-3, which acts on T cell exhaustion. The above results show that the expression of LTBP2 is closely related to immune infiltration in the microenvironment of COAD and STAD, and LTBP2 may play an important role in immune escape.

We further employed the TISIDB database to ensure the relationship among the LTBP2 expression and the abundance of TILs in various human cancers, the outcomes were alike to the TIMER database, LTBP2 expression was closely linked to the infiltrating immune cells in different human cancers including COAD and STAD (Fig. 6a), but no association with KICH. Additionally, we observed that LTBP2 expression was correlated to the macrophages (rho=0.615, *p*<2.26E-16) and Treg (rho=0.62, *p*<2.26E-16) in COAD (Fig. 6b, c). The macrophages (rho=0.531, *p*<2.26E-16) and Treg (rho=0.47, *p*<2.26E-16) were linked with the expression of LTBP2 in STAD (Fig. 6d, e).

The Enriched Pathways of LTBP2 in COAD and STAD

We evaluated the enriched pathways of LTBP2 in COAD and STAD as per the GSEA software 4.0.3, the outcome illustrated that the LTBP2 expression was linked to the ECM receptor interaction, the leukocyte trans-endothelial migration, the TGF beta signaling pathways, the hedgehog signaling pathway, the MAPK signaling pathway, the cytokine-cytokine receptor interaction, the chemokine signaling pathway, the B cells receptor pathway, the WNT signaling pathway, the T cell receptor signaling pathway, the Toll-like receptor signaling pathways, the VEGF signaling pathways, the NOTCH signaling pathways, the intestinal immune network for IgA production, the ABC transporters, the MTOR signaling pathway and the natural killer cell mediated cytotoxicity in COAD (Fig. 7a and Supplementary Table 3). The LTBP2 participate in the hedgehog signaling pathway, the ECM receptor interaction, the cytokine-cytokine receptor interaction, the JAK-STAT signaling pathway, the MAPK signaling pathway, the leukocyte trans-endothelial migration, the TGF beta signaling pathway, the Toll-like receptor signaling pathways, the WNT signaling pathway, the chemokine signaling pathway, the endocytosis, the T cell receptor signaling pathway, the MTOR signaling pathway, the VEGF signaling pathways, the natural killer cell mediated cytotoxicity, the NOTCH signaling pathways and the B cell receptor signaling pathway in STAD (Fig. 7b and Supplementary Table 4). We can discuss that the co-expressed pathways of LTBP2 were linked with the immune function and the tumor microenvironment in COAD and STAD.

Discussion

Owing to an increase in morbidity and lethality of malignant tumors and lack of suitable detection for early examination, we must find some effective and sensitive biomarker for directing prognosis and therapy, mainly the immunotherapy for malignancy tend to form the hot pot. In this research, we determine the potential underlying mechanism for the LTBP2, and we would seem to search for an effective biomarker for indicating the survival of patients and its relationship linked with the immune infiltration in tumor microenvironment.

LTBP2 was showed in the tumor tissues of different human cancers, and the levels of expression in tumor tissues were much different in comparison to the normal tissues in serval types of cancer, the elevated variance can be reflected in the brain and CNS cancer, the breast cancer, the colorectal cancer, the esophageal cancer, the gastric cancer, the leukemia, the liver cancer, the lymphoma, the pancreatic cancer and tertoma, and lower expression of LTBP2 was detected in cervical cancer, breast cancer, lung cancer, kidney cancer, prostate cancer, melanoma, sarcoma, and vulvar intraepithelial neoplasia. From the ONCOMINE database, and a similar result can be seen in the TIMER database, it was exhibited that LTBP2 expression was elevated in COAD, CHOL, STAD, HNSC, ESCA, READ, and LIHC, but the lower expression of LTBP2 was seen in UCEC, KIRP, BRCA, LUSC, LUAD, THCA, and PRAD. We focus on the LTBP2 expression and its prognostic value in STAD and COAD in this research. The result showed that the higher LTBP2 expression was linked to the poorer PFS and OS in STAD and poorer DFS in COAD, it was also showed that LTBP2 can direct the survival in other human cancers including renal cell carcinoma cancer, breast cancer, lung cancer, bladder cancer, blood cancer, ovarian cancer, and brain cancer. The result showed that LTBP2 was the potential to be a prognostic biomarker in COAD and STAD. We further evaluate the LTBP2 prognostic value in STAD based on the Kaplan-Meier plotter database, it was showed that LTBP2 was closely related to the PFS and OS in female, male, stage N0, stage N3, stage T2, stage 1+2+3, two types of Lauren classification, stage M0, and HER2 status. These results indicated that LTBP2 has huge prognostic values in STAD. The relation among LTBP2 expression and the stage T, stage N, stage TNM and the grade was strong in STAD, the past report has suggested a view that the LTBP2 expression was correlated with the lymph node metastasis and the progression of TNM stages in pancreatic carcinoma(13). It also stated that the LTBP2 can act on the metastasis of lymph node in head and neck squamous cell carcinoma by activating the TGFB1 and the elevated expression of LTBP2 was linked with the higher TNM stages(42), it is reflected that the LTBP2 has a significant part in tumor progression and lymph node metastasis in STAD.

We aimed to further explore the potentiality of LTBP2 directing the prognosis in COAD and STAD, the stromal score and immune score had a close relation to the LTBP2 expression in STAD and COAD, it showed that LTBP2 was associated with the state of tumor microenvironment and effect on the patient's survival in STAD and COAD.

Immune cell infiltration is an important part of tumor microenvironment (43). The expression of LTBP2 was correlated with the infiltrating levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils and dendritic cells adjusting by tumor purity, and the tumor purity was negatively related to the LTBP2 expression. The TAMs, M2 macrophages, Monocytes, Tregs, exhausted T cells, DCs gene markers had a similar correlation with LTBP2 expression. Especially it has a strong correlation with the TAMs, the monocytes, the M1 macrophages, and M2 macrophages, the outcome suggests that LTBP2 could mediate the tumor-associated macrophage polarization.

The expression of LTBP2 also correlated with the various gene markers of general T cells including CD3D, CD2, and CD3E, it indicated that LTBP2 impacted the response of T cells. The gene markers of dendritic cells including HLA-DQB1, HLA-DPB1, HLA-DPA1, HLA-DRA, CD11c, BDCA-4, BDC1-1 were related to the LTBP2 expression and DCs can influence the metastasis of tumor (40), it showed that the LTBP2 can lower down the cytotoxicity of CD8+ T cells and affect the tumor metastasis mediated by DCs in COAD and STAD. Meanwhile, the strong correlation between the gene markers of Tregs and the expression of LTBP2 can be reflected, showing that the LTBP2 could support the tumor development by suppressing the cytotoxicity of CD8+ T cells in COAD and STAD as the past study pointed that FOXP3 has an important part in the inhibition of the cytotoxicity of CD8+ T cells(41). The TISIDB database verified the LTBP2 has a relation to the mass of immune infiltrating cells in different types of cancer, mainly linked with the macrophages and the Tregs, it showed that the LTBP2 certainly has a significant part in macrophages polarization and the suppression of CD8+ T cells cytotoxicity.

At last, we evaluated the co-expressed pathways of LTBP2 expression in the COAD and STAD by using the GSEA, the outcome reflected that LTBP2 expression was linked with the cytokine-cytokine receptor interaction and the TGF beta pathway contributed to the tumor microenvironment, so it reflected that the LTBP2 has a significant part in tumor microenvironment in COAD and STAD. LTBP2 expression also had a strong correlation with the leukocyte trans-endothelial migration, and it provides evidence that LTBP2 may have contacted with immune infiltration of lymphocytes. The strong relationship between the Toll-like receptor signaling pathways and LTBP2 expression also revealed that LTBP2 was possibly associated with the tumor immunity in the tumor microenvironment.

The LTBP2, is a component of fibrillin- LTBP superfamily, related to the extracellular matrix proteins. It was consisted of LTBP1,2,3 and 4, along with participating in fibrillin microfibrils and the activation of TGF B1(44). An article presented a view that LTBP can perform the secretion and activation of TGF B1 by binding SL-TGFB, and it was important for TGF B1 storage by requisite fibrillin microfibrils in the extracellular matrix protein (45). TGF B was secreted by immune and tumor cells and persuaded the immunosuppression, it also added to the tumor microenvironment by combining other cells (17,18). Some papers determined that the TGF B pathways were capable of creating the metastasis and progression in the advanced tumors (46,47). Moreover, the TGF B itself can affect the response of immune cells (19,20) and support the angiogenesis (21). TGF B was important for inducing the epithelial-mesenchymal transition in different tumors, causing the elevated tumor progression (48), and it added on the regulation of the tumor microenvironment as it can communicated with the tumor cells. It is determined that TGF B was able to stimulate the differentiation of naive peripheral CD4+ T cells into CD4+ CD25+ regulatory T cells in some tumors contain the colon cancer in which the TNF-α, interleukin (IL)-1β, and IFN-γ was

improved (49). As per these outcomes, we can discuss why the LTBP2 expression related to poor survival and immune infiltration, it was strongly possible that LTBP2 increase the secretion of chemokines from tumor cells based on the activated TGF B pathways, causing the recruitment of the immune cells into the tumor microenvironment. Therefore, the LTBP2 interacted with the immune infiltration and increase in the poor survival of patients was declared in this study.

Conclusion

As a whole, the expression of LTBP2 has a strong correlation with the immune infiltration in CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and DCs of different human carcinoma including the COAD and STAD, for this reason, elevated LTBP2 expression lead to the poorer prognosis in STAD and COAD, also, the infiltrating immune cell's gene markers indicated LTBP2 take part in the regulation of TAMs, Tregs, T cell exhaustion, and DCs. We concluded that LTBP2 was the potential to be an independent predictor for the prognosis and a new target for immunotherapy in STAD and COAD.

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Material And Methods

The ONCOMINE Database

The ONCOMINE database is a platform used for online analysis of tumor data (https://www. oncomine.org/resource/login.html) (27), we obtained different LTBP2 expression in various types of cancer-based on ONCOMINE database 4.5. The threshold parameters were set up in the following way: fold change of 2, gene rank of top 10%, and p-value of 0.0001. Results were shown by rank (%), *p*-value, and fold changes.

The GEPIA Database

The GEPIA is an online database (http://gepia.cancer-pku.cn/) which was utilized to analyze gene expression and the relationship between the expression of gene and survival. In this database, the variance of gene expressed in various cancers was evaluated, and the relationship between the prognosis of patients and the expressed gene was added, the survival included overall survival (OS) and disease-free survival (DFS), the database contains tumors of total 9736 in number and normal tissues in 8587 number based on GTEx and TCGA (28). We utilized the value of LTBP2 expression to be a cutoff that can differentiate the degree of LTBP2 expression.

The TIMER Database

The TIMER database (https://cistrome. shinyapps.io/timer/) (29) was utilized to analyze the relationship among immune cells infiltrating and gene expression in several human cancers, infiltrating immune cells included B cells, CD4+ T cells, CD8+ T cells, macrophages, neutrophils and dendritic cells. The tumor purity was a significant factor to analyse the immune infiltrating of tumor samples on the basis of genomics method (30). The resource obtains from the statistics previously reported in The Cancer Genome Atlas (TCGA).

Kaplan-Meier plotterDatabase

The database (http://kmplot.com/analysis/) (31) was utilized to examine the correlation between the LTBP2 and the patient's prognosis in different sorts of cancer. All outcomes were conducted with HR (hazard ratio) and *p* values or log-rank *p* values, the quartile of LTBP2 expression obtained the high or low expression by setting median as the cutoff in samples.

Prognoscan Database

The PrognoScan database (http://www.abren.net/PrognoScan/) (32) revealed the correlation between LTBP2 expression and the patient's prognosis in different tumors, the database was utilized to assess the connection between specific gene expression and patients prognosis, the data were gathered from the public tumor microarray datasets. The threshold parameter was formed as Cox *p*-value <0.05.

GSEA Analyse

The co-expressed biological function and pathways, which are associated with the expression of LTBP2, were analyzed by the GSEA software 4.0.3. As, the GSEA software is an analytical technique performed through the computer. It is mainly used to find out the variance between the various biological state and the statistical significance of priori defined genes (33). We determined it to be statistically important based on the false discovery rate < 25% and threshold nominal *P*-value < 0.05. All these profiles of gene RNA-seq expression were belonged to the TCGA database and administered by R software.

TISIDB Database

The TISIDB database was an online website that contains many heterogeneous data types, and the relationship between the different tumor and the immune system can be evaluated in the web (34). In the study, we utilized the website to examine the association between infiltrating immune cells and LTBP2 expression.

Statistical Analysis

The GEPIA database, the Kaplan-Meier plotter database, the Prognoscan database, and the TISIDB database were evaluated by using *P*-values and hazard ratio. The *P*-values was computed by log-rank test, and the correlation among gene expression, and the gene markers of immune cells were assessed by Spearman's correlation. We form the standard to explain the association, as it was defined as 0.00–0.29 (weak), 0.30–0.59 (moderate), 0.60–0.79 (strong), 0.80–1.00 (very strong) (35). We defined it mainly through P values < 0.05, and the GEPIA database and the Kaplan-Meier plotter database were all evaluated by the R (version 3.5.2). We calculated the stromal score and the immune score by analyzing the TCGA database based on the ESTIMATE.

Declarations

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Disclosure statement

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Availability of data and materials

All data in the article can be obtained by contacting the corresponding author.

Authors' contributions

SC and ZhW drafted the manuscript. SC, YG and LmT provided the design idea of this study. HyL, YG, and HjY processed the data and supplemented the ideas. YZ corrected the manuscript and data. All authors read and approved the final manuscript.

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Tables

Table 1. Correlation of LTBP2 expression level and clinical prognosis in gastric cancer

	Overall survival (n=875)			Progression-free survival (n=640)		
	N	HR	*P*-Value	N	HR	*P*-Value
Sex						
Female	236	2.05(1.32-3.17)	0.0011	201	1.81(1.23-2.66)	0.0022
Male	544	1.41(1.05-1.89)	0.0237	437	1.39(1.09-1.77)	0.0073
Stage						
1	67	2.96(0.79-11.02)	0.0907	60	1.45(0.47-4.53)	0.5192
2	140	1.57(0.82-3.01)	0.1716	131	1.68(0.89-3.16)	0.1039
3	305	1.63(1.11-2.38)	0.0115	186	1.16(0.8-1.69)	0.425
4	148	1.46(0.98-2.16)	0.0614	141	1.3(0.88-1.91)	0.1881
Stage T						
1	14	-	-	14	-	-
2	241	1.57(1.02-2.41)	0.0407	239	2.11(1.37-3.26)	0.0006
3	204	1.37(0.97-1.94)	0.0715	204	1.1(0.79-1.53)	0.5759
4	38	1.49(0.65-3.43)	0.3452	39	1.62(0.75-3.53)	0.22
Stage N						
0	76	2.52(1.03-6.16)	0.0365	72	2.48(1.01-6.06)	0.0396
1	225	1.43(0.95-2.17)	0.0856	222	1.89(1.26-2.84)	0.0018
2	121	1.50(0.95-2.36)	0.0767	125	1.41(0.91-2.19)	0.1174
3	76	1.90(1.00-3.26)	0.0189	76	1.48(0.87-2.53)	0.148
1+2+3	422	1.58(1.21-2.06)	0.0007	423	1.67(1.29-2.17)	8.2e-05
Stage M						
0	444	1.50(1.13-1.98)	0.0046	443	1.71(1.3-2.24)	0.0001
1	56	1.52(0.85-2.7)	0.1523	56	1.28(0.71-2.28)	0.409
Lauren classification						
Intestinal	320	1.86(1.28-2.70)	0.0009	263	1.89(1.32-2.72)	0.0004
Diffuse	241	1.47(1.04-2.07)	0.0028	231	1.47(1.04-2.08)	0.03
Mixed	32	-	-	28	-	-
Differentiation						
Poorly differentiated	165	1.82(1.10-3.01)	0.0173	121	1.28(0.81-2.04)	0.289
Moderately differentiated	67	1.75(0.91-3.37)	0.088	67	1.33(0.72-2.48)	0.3642
Well differentiated	32	-	-	5	-	-
HER2 status						
HER2 negative	532	1.70(1.30-2.23)	9.4e-05	408	1.35(1.04-1.75)	0.0233
HER2 positive	343	2.41(1.64-3.54)	3.7e-06	232	1.59(1.15-2.2)	0.0051

N, number; HR, hazard ratio.

Table 2. Correlation analysis between LTBP2 and relate genes and markers of immune cells in COAD, STAD and KICH based on TIMER database.

Description	Gene markers	STAD				COAD				KICH			
		None		Purity		None		Purity		None		Purity	
		Cor	*P*	Cor	P	Cor	*P*	Cor	*P*	Cor	*P*	Cor	*P*
CD8+ T cell	CD8A	0.315	***	0.315	***	0.357	***	0.257	***	-0.025	0.844	0.067	0.595
	CD8B	0.185	**	0.193	**	0.187	***	0.12	0.015	0.035	0.777	0.124	0.326
T cell (general)	CD3D	0.252	***	0.253	***	0.393	***	0.285	***	-0.043	0.73	0.029	0.819
	CD3E	0.308	***	0.318	***	0.504	***	0.418	***	-0.034	0.785	0.037	0.77
	CD2	0.324	***	0.334	***	0.442	***	0.35	***	-0.003	0.982	0.086	0.497
B cell	CD19	0.336	***	-0.218	***	0.398	***	0.309	***	0.18	0.148	0.226	0.069
	CD79A	0.371	***	0.364	***	0.539	***	0.455	***	0.255	0.039	0.312	0.011
Monocyte	CD86	0.422	***	0.419	***	0.609	***	0.542	***	-0.094	0.453	-0.027	0.831
	CD115(CSF1R)	0.597	***	0.584	***	0.746	***	0.707	***	-0.093	0.457	-0.019	0.88
TAM	CCL2	0.43	***	0.419	***	0.611	***	0.538	***	-0.141	0.259	-0.095	0.452
	CD68	0.364	***	0.357	***	0.554	***	0.491	***	0.027	0.827	0.125	0.319
	IL10	0.45	***	0.449	***	0.429	***	0.374	***	0.06	0.631	0.161	0.2
M1 Macrophage	INOS(NOS2)	0.02	0.692	0.02	0.705	-0.084	0.074	-0.128	0.01	0.043	0.731	0.104	0.409
	IRF5	0.398	***	0.4	***	0.343	***	0.346	***	-0.177	0.155	-0.135	0.282
	COX2(PTGS2)	0.204	***	0.193	**	0.212	***	0.141	*	-0.073	0.561	-0.079	0.533
M2 Macrophage	CD163	0.483	***	0.496	***	0.641	***	0.584	***	0.097	0.438	0.195	0.119
	VSIG4	0.445	***	0.445	***	0.584	***	0.512	***	0.131	0.294	0.217	0.082
	MS4A4A	0.489	***	0.485	***	0.566	***	0.495	***	-0.025	0.843	0.066	0.602
Neutrophils	CD66b(CEACAM8)	0.037	0.449	0.085	0.261	-0.131	*	-0.109	0.028	0.068	0.589	0.068	0.588
	CD11b(ITGAM)	0.517	***	0.518	***	0.681	***	0.638	***	-0.039	0.757	0.052	0.68
	CCR7	0.445	***	0.45	***	0.578	***	0.508	***	0.018	0.889	0.09	0.475
Natural killer cell	KIR2DL1	0.081	0.098	0.092	0.072	0.092	0.049	0.034	0.491	-0.122	0.331	-0.093	0.462
	KIR2DL3	0.016	0.747	0.002	0.975	0.116	0.013	0.075	0.132	-0.296	0.016	-0.279	0.024
	KIR2DL4	-0.036	0.468	-0.045	0.386	0.129	*	0.046	0.36	-0.408	**	-0.399	*
	KIR3DL1	0.057	0.244	0.052	0.308	0.18	**	0.12	0.016	-0.112	0.372	-0.091	0.469
	KIR3DL2	0.103	0.036	0.109	0.034	0.232	***	0.168	**	-0.115	0.357	-0.082	0.516
	KIR3DL3	-0.05	0.312	-0.034	0.513	0.042	0.364	0.038	0.446	-0.265	0.031	-0.265	0.033
	KIR2DS4	0.025	0.605	0.023	0.655	0.123	*	0.08	0.105	-0.23	0.063	-0.206	0.099
Dendritic cell	HLA-DPB1	0.339	***	0.339	***	0.579	***	0.497	***	-0.102	0.416	-0.034	0.791
	HLA-DQB1	0.203	***	0.191	**	0.346	***	0.257	***	-0.124	0.321	-0.08	0.524
	HLA-DRA	0.234	***	0.234	***	0.471	***	0.373	***	-0.07	0.578	-0.001	0.991
	HLA-DPA1	0.294	***	0.293	***	0.537	***	0.454	***	-0.125	0.316	-0.068	0.592
	BDCA-1(CD1C)	0.477	***	0.482	***	0.546	***	0.493	***	-0.144	0.247	-0.092	0.468
	BDCA-4(NRP1)	0.64	***	0.622	***	0.701	***	0.649	***	0.075	0.549	0.146	0.244
	CD11c(ITGAX)	0.507	***	0.506	***	0.675	***	0.624	***	-0.065	0.602	-0.002	0.99
Th1	T-bet(TBX21)	0.306	***	0.323	***	0.427	***	0.345	***	-0.164	0.188	-0.12	0.342

	STAT4	0.385	***	0.391	***	0.418	***	0.327	***	-0.137	0.272	-0.079	0.531
	STAT1	0.144	*	0.151	*	0.385	***	0.335	***	0.183	0.14	0.212	0.089
	IFN-γ(IFNG)	0.021	0.67	0.151	*	0.185	***	0.122	0.013	-0.018	0.884	0.052	0.679
	TNF-α(TNF)	0.159	*	0.145	*	0.35	***	0.29	***	-0.29	0.018	-0.264	0.033
Th2	GATA3	0.379	***	0.396	***	0.559	***	0.518	***	-0.089	0.478	-0.085	0.502
	STAT6	0.292	***	0.294	***	0.243	***	0.245	***	0.063	0.615	0.053	0.674
	STAT5A	0.459	***	0.471	***	0.407	***	0.394	***	0.005	0.966	0.066	0.602
	IL13	0.159	*	0.183	**	0.264	***	0.216	***	0.026	0.833	0.025	0.842
Tfh	BCL6	0.469	***	0.449	***	0.534	***	0.475	***	0.15	0.288	0.13	0.301
	IL21	0.109	0.026	0.122	0.017	0.208	***	0.161	**	-	-	-	-
Th17	STAT3	0.489	***	0.477	***	0.429	***	0.402	***	-0.01	0.936	0.029	0.819
	IL17A	-0.086	0.08	-0.009	0.078	-0.069	0.141	-0.066	0.183	-	-	-	-
Treg	FOXP3	0.421	***	0.431	***	0.698	***	0.651	***	0.168	0.178	0.219	0.079
	CCR8	0.484	***	0.495	***	0.653	***	0.617	***	0.26	0.035	0.305	0.013
	STAT5B	0.595	***	0.588	***	0.489	***	0.523	***	-0.108	0.385	-0.093	0.464
	TGFβ(TGFB1)	0.648	***	0.643	***	0.672	***	0.598	***	0.14	0.262	0.181	0.149
T cell exhaustion	PD-1(PDCD1)	0.267	***	0.279	***	0.418	***	0.33	***	-0.111	0.372	-0.058	0.647
	CTLA4	0.197	***	0.204	***	0.464	***	0.391	***	0.036	0.772	0.109	0.389
	LAG3	0.152	*	0.151	*	0.369	***	0.28	***	-0.017	0.893	0.028	0.824
	TIM-3(HAVCR2)	0.455	***	0.458	***	0.597	***	0.539	***	0.571	***	0.492	***
	GZMB	0.057	0.246	0.037	0.475	0.075	0.107	0.038	0.447	0.723	***	0.683	***

*P < 0.01; **P < 0.001; ***P < 0.0001

Table 3. Correlation analysis between LTBP2 and relate genes and markers of monocyte, TAM, M1 macrophages, M2 macrophages, Treg and T cell exhaustion in GEPIA.

Description	Gene markers	COAD				STAD				KICH			
		Tumor		Normal		Tumor		Normal		Tumor		Normal	
		R	*P*	R	*P*	R	*P*	R	*P*	R	*P*	R	*P*
Monocyte	CD86	0.72	***	-0.09	0.57	0.41	***	-0.27	0.11	-0.054	0.66	0.48	0.015
	CD115(CSF1R)	0.82	***	0.3	0.06	0.6	***	0.12	0.48	-0.039	0.75	0.76	***
TAM	CCL2	0.74	***	-0.14	0.39	0.43	***	0.37	0.028	-0.12	0.33	-0.096	0.65
	CD68	0.61	***	0.05	0.76	0.35	***	-0.4	0.015	0.08	0.52	0.51	0.011
	IL10	0.57	***	-0.095	0.56	0.46	***	-0.12	0.49	0.027	0.83	0.015	0.94
M1 Macrophage	INOS (NOS2)	-0.036	0.55	-0.09	0.58	0.025	0.61	0.039	0.82	0.097	0.44	0.38	0.059
	IRF5	0.36	***	0.14	0.4	0.4	***	-0.11	0.54	-0.14	0.27	-0.22	0.29
	COX2 (PTGS2)	0.33	***	0.005	0.98	0.24	***	0.55	**	-0.05	0.68	-0.058	0.78
M2 Macrophage	CD163	0.72	***	0.34	0.03	0.35	***	0.48	*	0.11	0.37	0.59	*
	VSIG4	0.71	***	0.051	0.75	0.43	***	0.16	0.35	0.15	0.23	0.5	0.012
	MS4A4A	0.71	***	0.046	0.77	0.49	***	0.2	0.25	0.013	0.92	0.2	0.25
Treg	FOXP3	0.73	***	0.095	0.55	0.4	***	-0.24	0.16	-0.24	0.16	0.29	0.15
	CCR8	0.7	***	-0.041	0.8	0.45	***	-0.2	0.25	0.27	0.03	0.32	0.12
	STAT5B	0.53	***	0.43	*	0.58	***	0.75	***	-0.007	0.96	0.78	***
	TGFβ (TGFB1)	0.76	***	0.5	**	0.64	***	0.5	*	0.14	0.26	0.27	0.19
T cell exhaustion	PD-1(PDCD1)	0.45	***	0.22	0.16	0.1	0.04	-0.17	0.33	0.078	0.13	0.33	*
	CTLA4	0.41	***	-0.016	0.92	-0.011	0.82	-0.22	0.19	0.34	*	0.59	*
	LAG3	0.14	0.024	0.16	0.31	-0.031	0.54	-0.18	0.3	0.19	0.13	0.15	0.47
	TIM-3(HAVCR2)	0.65	***	0.16	0.32	0.31	***	-0.012	0.94	-0.061	0.62	0.77	***
	GZMB	-0.077	0.21	-0.13	0.4	-0.075	0.13	-0.37	0.025	-0.11	0.37	0.82	***

*P < 0.01; **P < 0.001; ***P < 0.0001

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1a.png
- SupplementaryFigure1b.png
- SupplementaryFigure1c.png
- SupplementaryFigure2a.png
- SupplementaryFigure2b.png
- SupplementaryFigure2c.png
- SupplementaryFigure2d.png
- Supplementarytable1.docx
- Supplementarytable2.docx
- Supplementarytable3.docx
- Supplementarytable4.docx
- Supplementarytable5.docx