GBP1 Is a Prognostic Biomarker and Correlates With Immune Infiltrates in Gastric Cancer

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Abstract

Background: The guanylate-binding protein 1 (GBP1) belongs to the member of GTPase dynamin superfamily and plays a major role as tumor suppressor or tumorigen in cancers, such as colon cancer, ovarian cancer, melanoma, and head and neck squamous cell carcinoma. However, the relationship between GBP1 and gastric cancer (GC) remains scanty.

Methods: GBP1 mRNA expression in different types of tumors and their corresponding adjacent normal tissues were evaluated via exploring Tumor Immune Estimation Resource (TIMER) and Gene Expression Profiling Interactive Analysis (GEPIA) database, while the protein expression was detected by The Human Protein Atlas (HPA). The relationship between the clinical characteristics and outcome of gastric cancer patients was examined through Kaplan–Meier plotter tool. The correlations between GBP1 and tumor immune infiltrates were evaluated via TIMER and TISIDB. Additionally, the relationship between GBP1 expression and gene marker sets of immune infiltrates were analyzed by TIMER and GEPIA.

Results: GBP1 expression was significantly higher in GC compared with corresponding normal tissues. High GBP1 expression in GC associated with better overall survival (OS HR=0.53, $P=5.2e-09$) and progression-free survival (PFS HR=0.49, $P=1.90e-06$). Moreover, its expression level was positively correlated with different clinical characteristics, such as sex, TNM stage, and Lauren classification. With a comprehensive analysis of three immune-related databases, TIMER, GEPIA, and TISIDB, we found GBP1 not only showed a strong correlation with tumor-infiltrating CD8⁺ T cells, Th1 cells, but also with Tregs, exhausted T cells, M2 macrophage, monocytes. Furthermore, GBP1 was significantly associated with IFN- γ , granzyme B, perforin, FasL and CXCL9, CXCL10, and CXCL11.

Conclusion: This study provides the first evidence that GBP1 is a key gene that correlates with the prognosis and associated with various tumor-infiltrated immune cells in gastric cancer patients. In conclusion, GBP1 may act as a potent prognostic biomarker for predicting cancer progression and a sign of tumor-immune infiltration in GC.

Background

Gastric cancer (GC) is one of the most common malignancy worldwide, which is characterized by a high incidence rate and mortality and gives rise to more than 1,000,000 new cases and 783,000 deaths per year. It is ranked the fifth most frequently diagnosed cancer and the third leading cause of cancer death¹. The major risks, which influence the occurrence and progression of gastric cancer, include *H. pylori* infection, genetic factors, and environmental factors². The environment of solid tumors is a complicated system that is mainly composed of malignant cells, extracellular matrix, non-tumor stromal cells, infiltrating immune cells, and microvessels. Among these, infiltrating immune cells play an important role in determining the progression of tumor. CD8⁺ T cells are responsible for killing malignant cells. Meanwhile, existent components such as Myeloid-derived suppressor cells (MDSC), regulatory T cells, and exhausted T cell may suppress the effects of anti-tumor capacity³. The using of immune therapy in

cancer is promising. The antibodies, such as anti-CTLA-4 Ab and anti-PD-1/PD-L1 Ab which could blockade coinhibitory receptor binding to the ligand, have been shown to be encouraging for treating melanoma, bladder cancer, non-small cell lung cancer, and other tumors⁴⁻⁶. However, there was only a partial patient who suffered from gastric cancer responded to the treatment by the immunotherapy mentioned above⁷. Therefore, further understanding of the molecular mechanisms involved in the GC immune microenvironment is essential for developing new immune therapy.

Guanylate-binding protein 1 (GBP1) belongs to the superfamily of GTPase, 65kDa, which hydrolyzes GTP to GMP instead of GDP and is regarded as the most representative GBPs so far. The molecular structure and the mechanism of action have also been well understood⁸. GBP1 has been reported that it was induced by IFN- γ and involved in mechanisms of gastrointestinal inflammation, and host against bacteria, viruses, and autonomous defense of pathogenic microorganisms such as parasites⁹⁻¹². Besides, several lines of evidence showed that GBP1 correlated with oncogenic process and highly dependent on their context, as its upregulation was linked with better prognosis in some cancer types, such as breast and colorectal cancer¹³⁻¹⁵, but associated with radioresistance and chemotherapy resistance, metastasis, and progress in different types of cancer, such as glioblastoma, ovarian cancer, prostate cancer, and breast cancer¹⁶⁻¹⁹. Yet, the function and mechanism of GBP1 in GC progression and tumor immune environment remains unknown, which is needed additional investigation urgently.

Therefore, in this study, we comprehensively analyzed GBP1 expression, its correlation with the prognosis in GC, and the status of different tumor-infiltrating immune cells via multi-omic analysis tools, mainly including Kaplan-Meier plotter, Gene Expression Profiling Interactive Analysis (GEPIA), Tumor Immune Estimation Resource (TIMER), TISIDB, and The human protein atlas (HPA) databases. In conclusion, our results provide the first evidence that GBP1 can be a potent biomarker for gastric cancer and the underlying mechanism for regulating anti-tumor effects is probably modulating the infiltration of immune cells.

Methods

Kaplan-Meier survival curve analysis

The Kaplan Meier Plotter (<http://kmplot.com/analysis/>) is an effective tool for meta-analysis based discovery and validation of survival biomarkers²⁰. The system includes gene chip and RNA-seq data-sources for the databases include GEO (Gene Expression Omnibus), EGA (European Genome-phenome Archive), and TCGA (The Cancer Genome Atlas). It is adequate to analyze the effect of 54k genes on survival in 21 cancer types. Kaplan-Meier plots were used to analyze the relationship between GBP1 gene expression and survival rates in liver, gastric, breast, ovarian, and lung cancers based on the hazard ratios (HR) and log-rank P-values.

TIMER database analysis

Tumor Immune Estimation Resource (TIMER) is a comprehensive resource for systematical analysis of immune infiltrates across diverse cancer types (<https://cistrome.shinyapps.io/timer/>)²¹. The database collects 10,897 samples across 32 cancer types from the TCGA database to determine the abundance of immune infiltrates by a statistical method of deconvolution from gene expression profiles²². TIMER was used to analyze the expression level of GBP1 in tumor and adjacent normal tissues, and the correlation of GBP1 expression with the abundance of 6 types of infiltrating immune cells, including B cells, CD4⁺ T cells, CD8⁺ T cells, neutrophils, macrophages, and dendritic cells in gastric cancer patients via gene modules. The marker genes used for the analysis of tumor-infiltrating immune cells including CD8⁺ T cells, T cells (general), B cells, TAMs (Tumor-associated macrophages), monocytes, M1 macrophages, M2 macrophages, natural killer (NK) cells, neutrophils, 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. were based on data from previous studies²³⁻²⁵. GBP1 gene was on the x-axis and related marker genes are on the y-axis, and the levels of gene expression were shown by log2 RSEM (RNA-Seq by Expectation-Maximization).

GEPIA database analysis

The Gene Expression Profiling Interactive Analysis (GEPIA) database (<http://gepia.cancer-pku.cn/>) is an interactive web server for analyzing the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and Genotype Tissue Expression (GTEx) based on the UCSC (University of California, Santa Cruz) Xena project²⁶. GEPIA was utilized for investigating the RNA expression level of GBP1 in cancer and their corresponding normal tissues. In addition, we used GEPIA to confirm the marker sets of immune cells that are significantly correlated genes from the TIMER analysis.

TISIDB database analysis

TISIDB is a web portal for tumor and immune system interaction, which integrates multiple heterogeneous data types (<http://cis.hku.hk/TISIDB/>)²⁷. The database enables users to perform analysis of relevance between selected genes and immunological data like lymphocytes, immunomodulators (immunoinhibitor, immunostimulator and MHC molecules), and chemokines. We applied the TISIDB database to analyze the correlation between the GBP1 expression and lymphocyte chemokines.

The Human Protein Atlas analysis

The Human Protein Atlas (HPA) (<https://www.proteinatlas.org/>) is a Swedish-based program initiated in 2003 with the aim to map all the human proteins in cells, tissues, and organs using an integration of various omics technologies, including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics, and systems biology²⁸. Additionally, the HPA database provides measurements of RNA levels. In our study, the HPA database was used to analyze protein expression of GBP1 between normal and gastric cancer tissues. where the immunohistochemical staining was performed using the anti-GBP1 antibody CAB015450.

Results

GBP1 is overexpressed in gastric cancer

Expression of GBP1 in various types of cancer and normal tissues visualized as dot plots based on the RNA-seq data from TCGA and GTEx datasets through GEPIA. The database showed that the mRNA level of GBP1 was significantly higher in STAD (Stomach adenocarcinoma), DLBC (Lymphoid Neoplasm Diffuse Large B-cell Lymphoma), ESCA (Esophageal carcinoma), GBM (Glioblastoma multiforme), HNSC (Head and Neck squamous cell carcinoma), KIRC (Kidney renal clear cell carcinoma), LGG (Brain Lower Grade Glioma), PAAD (Pancreatic adenocarcinoma), TGCT (Testicular Germ Cell Tumors), but it was significantly lower in KICH (Kidney Chromophobe), PRAD (Prostate adenocarcinoma), UCEC (Uterine Corpus Endometrial Carcinoma) and UCS (Uterine Carcinosarcoma) (**Figure 1A**). Meanwhile, the analysis of TCGA RNA-seq data using the TIMER database showed that GBP1 mRNA was significantly overexpressed in STAD, ESCA (Esophageal carcinoma), HNSC and KIRC, while it was significantly decreased in KICH, LUAD (Lung adenocarcinoma), LIHC (Liver hepatocellular carcinoma), LUSC (Lung squamous cell carcinoma), PRAD, SKCM (Skin Cutaneous Melanoma), THCA and UCEC (**Figure 1B**). To verify the protein profile of GBP1 expression level between tumor and normal tissue in gastric cancer, we used HPA for searching evidence of pathology immunohistochemistry (IHC), whose results showed that GBP1 was not found in the normal gastric tissue, but detected in the malignant tissue and mainly located on the cytoplasmic and membranous of cells that distributed in tumor stroma (**Figure 1C**). Collectively, the results consistently showed that GBP1 expression was varied in different tumor backgrounds, among which it was elevated in gastric cancer tissue compared to the corresponding normal tissue.

Prognostic significance of GBP1 expression in human cancers

In order to investigate whether the cancer patient survival rate was correlated with GBP1 expression level in various types of cancers, the Kaplan-Meier plotter database was used. Higher GBP1 expression was correlated with more favourable prognosis in gastric cancer (Overall Survival (OS) HR=0.53, 95%CI=0.43 to 0.66, $P=5.2e-09$; Progression-Free Survival (PFS) HR=0.49; 95%CI=0.36 to 0.66, $P=1.90e-06$) and HCC (OS HR=0.6, 95%CI=0.4 to 0.92, $P=0.018$; PFS HR=0.69, 95%CI=0.49 to 0.98, $P=0.036$), while lower GBP1 expression corresponded with better prognosis in BRCA (PFS HR=1.44, 95%CI=1.12 to 1.83, $P=0.036$) and OV (PFS HR=1.4, 95%CI=1.21 to 1.63, $P=4.7e-06$), and it had no correlation with the survival of lung cancer (**Figure 2**). Additionally, high GBP1 expression associated with better OS in KIRC, KIRP, LUAD, LUSC, PAAD, and PCPG, while low GBP1 expression associated with better OS in ESCA, SARC, THYM, and THCA. Besides, the results showed that high GBP1 was positively correlated with better PFS in ESCA, KIRP, and HNSC (**Additional file 1: Table S1**). These results suggested that GBP1 was a potent prognostic biomarker depending on cancer types.

Correlation between GBP1 expression and clinical characteristics of GC patients

To further understood the relevance and underlying mechanisms of GBP1 expression in cancer, we investigated the relationship between the GBP1 expression and clinical characteristics factors including

Sex, Stage T/N/M, Lauren classification, and differentiation of gastric cancer patients in the Kaplan-Meier plotter databases. Overexpression of GBP1 was correlated with better OS and PFS in female and male patients as well as two types of Lauren classification ($P<0.05$), but no association was found between the characteristic of differentiation and OS or PFS. In addition, high expression of GBP1 was positively correlated with OS/PFS in stages except that it was independent of OS in stage 2 ($P<0.05$). TNM staging is an important basis for judging the treatment and prognosis of gastric cancer patients²⁹, and we found that GBP1 was associated with OS and PFS of patients in stage T2 (OS HR=0.47, $P=0.0059$; PFS HR=0.48, $P=0.0021$) and T3 (OS HR=0.51, $P=0.0021$; PFS HR=0.59, $P=0.01$), but not in advanced stage T4 (OS HR=0.56, $P=0.23$; PFS HR=1.78, $P=0.21$). As for the stage N, which refers to lymph node involvement, GBP1 was positively significant with N1-N3 regional lymph node metastasis and higher GBP1 expression indicated better OS (HR=0.48, $P=1.06e-05$) and PFS (HR=0.42, $P=2.1e-05$); Notably, in patients at stage M0, GBP1 is positively correlated with OS (HR=0.5, $P=7.10e-05$) and PFS (HR=0.53, $P=0.0002$), even if the patients with distant metastasis, high expression of GBP1 still indicates better PFS (HR=0.5, $P=0.0014$) (**Table 1**). Collectively, these results demonstrated that GBP1 as a potent prognostic marker could impact the prognosis in gastric cancer with distinct clinical characteristics.

GBP1 expression positively related to the infiltration of activated T cells in gastric cancer

It has been reported that tumor-infiltrating lymphocytes play an important role in anti-tumor activity, which can be an independent factor of several cancer types³⁰⁻³². To explore the underlying mechanisms of GBP1 and good prognosis in gastric cancer, we focused on infiltrating lymphocytes in gastric tumor environment. To determine the relationship between the GBP1 expression level and infiltrating immune lymphocytes abundance, TIMER was utilized to analyze the correlation between GBP1 expression and the infiltration levels of 6 immune cell types. It was found that GBP1 expression was significantly negatively correlated with tumor purity in STAD (cor=-0.205, $P=5.8e-05$), indicating that GBP1 might be expressed by the non-malignant cells like stromal cells and (or) infiltrating immune cells in tumor tissue. We observed that GBP1 expression was correlated with infiltrating immune cells in different types of cancers whose GBP1 expression was significantly changed in TIMER and GEPIA simultaneously (**Additional file 2: Figure S1A-L**). In gastric cancer, it showed that high GBP1 markedly correlated with the infiltration of CD8⁺ T cells (cor=0.54, $P=2.11e-29$), macrophage (cor=0.204, $P=8.02e-05$), neutrophils (cor=0.595, $P=7.52e-37$), dendritic cells (cor=0.536, $P=5.10e-29$), but negatively correlated with B cells (cor=-0.278, $P=5.44e-08$), and there was no statistical significance with CD4⁺ T cells (cor=0.101, $P=5.34e-02$) (**Figure 3A**). For learning about more details of tumor immune microenvironment in gastric cancer, TISIDB was also used to analyze GBP1 expression and infiltration of different immune cells. We found that GBP1 was positively correlated with a variety of cell subtypes, among which the groups with higher correlation contained mainly CD8⁺ and CD4⁺ T cells. According to the status of cells, these two types of T cells were divided into activated/central memory (cm)/ effector memory (em) subsets. The result showed that activated cells have the highest correlation among CD8⁺ and CD4⁺ T cells ($\rho=0.701$, $P<2.2e-16$; $\rho=0.681$, $P<2.2e-16$, respectively) (**Figure 3B, C**). In addition, GBP1 is also positively associated with anti-angiogenic chemokines CXCL9, CXCL10, and CXCL11 in gastric cancer, it is similar to that in colon cancer

where GBP1 was involved in the angiostatic immune reaction¹⁵(**Additional file 3: Figure S2A, B**). Taken together, these results suggested that GBP1 was an important gene in the tumor immune microenvironment, increasing the infiltration abundance of activated T cells.

Correlation analysis between mRNA levels of GBP1 and markers of immune cells in tumor microenvironment

The correlation between GBP1 expression and the status of tumor infiltrating immune cells according to the levels of different subsets of immune cells marker gene expression in STAD tissues were identified using TIMER and GEPIA databases. The immune cells analyzed in gastric cancer tissues included CD8⁺ T cells, T cells (general), B cells, tumor-associated macrophages (TAMs), monocytes, M1 and M2 macrophages, neutrophils, DCs, and natural killer (NK) cells. Moreover, different subsets of functional T cells, namely, T helper 1 (Th1), Th2, follicular helper T (Tfh), Th17, regulatory T (Tregs), and exhausted T cells were also analyzed. Due to the tumor purity of clinical samples affects the analysis of immune infiltration, the correlation analysis was adjusted for purity. After adjustments, the expression level of GBP1 showed a positively correlated with most immune markers in divergent immune cell types and various functional T cells in STAD (**Table 2**).

Interestingly, we found that the expression levels of most marker sets of CD8⁺ T cells, T cells(general), dendritic cells, M2 macrophage, monocytes, Th1, Tregs and exhausted T cells have strong correlations with GBP1 expression in STAD using TIMER and GEPIA databases (**Table 2, Table 3**). Within these immune cells, CD8⁺ T cells as major cytotoxic lymphocytes could inhibit tumor progression through directly induce cell death, comprising an important part of TILs in tumor immune microenvironment. Thus, the abundance of CD8⁺ T cells in the tumor immune microenvironment might be associated with a favorable prognosis³³. As shown in **Figure 4A** and **Table 3**, GBP1 expression level strongly correlated with the markers of CD8⁺ T cells, CD8A ($r=0.708$, $P=0.00e+00$) and CD8B ($r=0.538$, $P=0.00e+00$). And the cytotoxic molecules granzyme B (GZMB), perforin (PRF1) and FasL (FASLG) which usually equipped with CD8⁺ T cells were also included in this study and were shown that higher expression of GBP1 markedly increased the level of GZMB ($r=0.788$, $P=1.46e-81$), PRF1($r=0.739$, $P=9.64e-67$) and FASLG ($r=0.731$, $P=1.33e-64$) (**Figure 4B**). These findings indicate that GBP1 may result in a higher survival rate via increasing the infiltration abundance of CD8⁺ T cells and their effect of direct killing in STAD.

It suggested that GBP1 in colorectal cancer was mainly involved in the activities of Th1 cells for anti-tumor¹⁵. Herein, in this study, we investigated the relationship between sets of markers of Th1 cells including T-bet, STAT4, STAT1, and IFN- γ , and we found that there was a strong correlation between GBP1 and markers of Th1 cells (**Table2, Figure 4C**). In addition, the expression of GBP1 correlated significantly with the expression of the marker genes of different subsets of T cells in GC, namely Tregs or exhausted T cells, such as FOXP3, CCR8, PD1, TIGIT, Tim3 and LAG3($P<0.0001$; **Table 2**). High expression of GBP1 may also activate Tregs while the FOXP3⁺ Tregs is a major factor that could inhibit cytotoxic T cells from killing tumor cells. Interestingly, LAG3, as a pivotal gene that participates in T cell exhaustion, has a

strong positive correlation with GBP1 expression, suggesting that high GBP1 expression plays an important role in LAG3 mediating T cell exhaustion.

We observed that the levels of expression in the majority of monocytes and M2 macrophage immunomarker genes were strongly correlated with GBP1 expression in STAD (**Table 2**). CD86 and CD115 of monocytes, and CD163, VSIG4, and MS4A4A of M2 phenotype greatly correlated with GBP1 expression level in GC ($P < 0.0001$; **Table 2**). High GBP1 expression related to high infiltration level of DCs in STAD, while DC markers such as HLA-DPB1, HLA-DRA, HLA-DPA1, and CD11c also show significant correlations with GBP1 expression ($P < 0.0001$; **Table 2**), it may reveal that GBP1 regulate macrophage polarization and DCs infiltration in GC. Furthermore, we assessed the interrelationship among GBP1 level and immune markers in GC in the GEPIA database, whose results were similar to those in TIMER. Strikingly, a significant correlation between GBP1 expression and markers of immune cells occurred in the tumor but barely found in the adjacent normal tissue (**Table 3**). Collectively, these findings suggested that GBP1 expression strongly correlated with infiltration of immune cells with different phenotypes which indicated it may involve in different immunological pathways in tumor microenvironment, but the underlying mechanisms remained to be further explored.

Discussion

Here, we executed a comprehensive study demonstrating the role of GBP1 in GC. GBP1 mRNA expression was significantly higher in gastric carcinoma tissue compared with adjacent normal stomach tissue, and high GBP1 expression level correlated with a favorable prognosis. Upregulation of GBP1 associated with better prognosis in patients with different clinical characteristics such as Lauren classification, lymph node metastasis, or distant metastasis. Furthermore, GBP1 mRNA levels correlated with the abundance of tumor-infiltrated immune cells according to the levels of markers for different immune cell types in GC. To our knowledge, it is the first report that GBP1 is a potential effective prognostic biomarker for GC that involved in tumor immune regulation.

GBP1 is found to be expressed by various cell types located on cytoplasm and membrane, such as T cells³⁴, monocytes³⁵, and endothelial cells³⁶, which is upregulated not only by proinflammatory cytokines IFN- γ , but also IL-1 α , IL-1 β , and TNF- α ^{37,38}. It has been reported that GBP1 participates in the activities of host defense virus and microbial, autoimmune diseases, and cancer³⁹. Recently, conflicting evidences suggested that GBP1 was a double-edged sword in different tumor contexts. In colon cancer, GBP1 acts as a tumor suppressor and controls the invasive and angiogenic capability of endothelial cells through decreasing the expression of MMP-1^{14,40}, but it was driven by EGFR for further invasion in glioblastoma and a potential therapeutic target for triple-negative breast cancer and prostate cancer^{41,42}. It also contributes to chemoresistance and radioresistance^{18,43}. It has never been reported the relationship between GBP1 expression and GC. Herein, we used different databases to investigate the mRNA, and protein expression level of GBP1 in STAD. We found that GBP1 mRNA (from TIMER database) and RNA (from GEPIA database) expression level was higher in gastric cancer tissue than it in adjacent normal

tissue via two different databases (**Figure 1A, B**). Moreover, we collected IHC evidences from HPA which revealed that the protein of GBP1 was higher in cancer compared with normal tissue and mainly expressed on cytoplasmic and membranous of non-malignant cells those in tumor stroma (**Figure 1C**), it likely express in endothelial cells and immune cells and further tests are needed. Concerning survival analysis, GBP1 was positively correlated with better overall survival and progression-free survival (**Figure 2**). In addition, we found that GBP1 was strongly associated with other types of tumors. High level of GBP1 indicated better overall survival in KIRC, KIRP, LUAD, LUSC, PAAD, and PCPG, while low GBP1 expression associates with better overall survival in ESCA, SARC, THYM, and THCA (**Table1, Additional file 1: Table S1**). Furthermore, in GC patients, high GBP1 expression correlated with better prognosis of female and male patients, two types of Lauren classification, and lymph-node metastasis (stageN1-N3) (**Table1**). These results strongly suggested that GBP1 was a potentially favorable prognostic biomarker for GC patients.

The tumor immune microenvironment plays an important role in cancer progression. Immunotherapy, especially immune checkpoint inhibitors, is an effective manner to improve the environment of tumor immunity, which is a hot topic in the treatment of cancer for significantly prolonging survival of patients in many cancers. However, there are only partial patients suffering from STAD respond to the checkpoint inhibitors. It is pressing to elucidate the immunophenotypes of tumor-immune interactions and identify novel immune-related therapeutic targets in gastric cancers. As several lines of studies have been reported that GBP1 was a necessary molecule in the immunological activities such as antiviral, autoimmune diseases, and cancer via different types of immune cells^{15,35,44}. We hypothesized that GBP1 may correlate with the tumor-infiltrated immune cells. By data analysis from TIMER, we found that there were significant positive correlations between GBP1 expression and CD8⁺ T cells, macrophages, neutrophils, and DCs, but correlated negatively with the abundance of B cells. Besides, the association was not found between GBP1 expression and CD4⁺ T cells infiltration (**Figure 3A**). Moreover, we found that the correlations between GBP1 and various immune cell types in different tumors are obviously different, which may be the cause for the discrepancy between GBP1 and different tumor prognosis (**Additional file 2: Figure S1A-L**). It suggested that GBP1 may play an important role in regulating tumor immunity.

T lymphocytes are the major part of antitumor. In order to make enduring and efficient immunity of restraining cancer, they are needed to be primed and activated toward effector phenotype, and it is crucial to understand the status of T cells in the tumor immune microenvironment. Moreover, Forster et, al. reported that silencing of GBP1 induced enhanced activation of early T cell Ag receptor/CD3 signaling molecules via regulating cytoskeleton of T cells, indicating that GBP1 was involved in the functions of T cells³⁴. Thus, TISIDB, a new published public database was used to analyze the subsets of infiltrated immune cells. The results showed that GBP1 was positively correlated with various cell subtypes and had the highest correlation with activated CD8⁺ and CD4⁺ T lymphocytes subsets (**Figure 3B, C**). Next, we investigated the correlation of GBP1 expression and sets of immune cells marker and their functional molecules via TIMER and GEPIA database. It displayed that GBP1 was positively with markers of CD8⁺ T

cells. Another important finding was that there was a strong correlation between GBP1 and the cytotoxic molecules like FasL, Perforin, and Granzyme B which were responsible for killing malignant cells (**Table 2,3; Figure 4A, B**). As far as we know, it was the first study to find that GBP1 may positively regulate the infiltrated abundance and expression of cytotoxic molecules in CD8⁺ T cells. Another significant counterpart of T cells, CD4⁺ T cells including Th1, Th2, Tfh, Tregs, and Th17 subtypes, were also involved in this study. Interestingly, the result showed that GBP1 was strongly correlated with STAT1 and IFN- γ (IFNG) which were the markers of Th1 cells (**Table 2,3; Figure 4C**). The result was similar to that in colorectal cancer where high expression of GBP1 in stromal was associated with the presence of IFN- γ dominated Th1 cells and increased cancer survival¹⁴. Although the outcomes from TIMER showed that GBP1 expression does not correlate with CD4⁺ T cells infiltration (**Figure 3A**), we found that GBP1 was still strongly associated with various markers of different subtypes of CD4⁺ T cells in GEPIA and TIMER database, this may be related to the tumor purity and bias of sample collection. Meanwhile, we found that GBP1 was correlated with CXCL9, CXCL10, and CXCL11 in GC indicated that GBP1 was associated with the antiangiogenic activities, which were consistent with the previous published study¹⁵ (**Additional file 3: Figure S2A, B**). Collectively, GBP1 may act as a tumor suppressor which contributes to better prognosis of gastric cancer by promoting the anti-tumor effects of tumor infiltrated lymphocytes in tumor tissue, but the underlying mechanism was needed to be further study.

Although it seemed that high expression of GBP1 was related to the positive effect of anti-tumor, the cancer continues to progress, indicating that GBP1 may also be involved in other immunomodulatory pathways. Recently, Chen et al. reported that gastric cancer recruited M2 macrophages promote tumor metastasis, revealed a novel aspect of macrophages with respect to cancer metastasis⁴⁵. In this study, we found that GBP1 expression showed a higher correlation with M2 macrophage markers such as CD163, VSIG4, and MS4A4A than M1 macrophage markers, it revealed that the potential regulating role of GBP1 in polarization of tumor-associated macrophages (**Table 2, Table 3**). Moreover, the increased GBP1 expression was positively associated with the abundance of Tregs markers (FOXP3, CCR8, and STAT5B) and exhausted T cells markers (PD-1, CTLA4, Tim-3, TIGIT, and LAG3) (**Table 2, Table 3**). These results indicated that GBP1 may participating in activating Tregs and inducing T cells dysfunction which could be in favor of promoting tumor escape from immune surveillance. Among the exhausted related markers, LAG3 has the highest correlation with GBP1 expression. It is a novel identified a crucial protein on the surface of dysfunctional T cells, and its targeted inhibitors have great prospects in the treatment of cancer⁴⁶, the pathway by which GBP1 regulates the expression of co-inhibitory receptors on T cells remains to be explored. Our results suggested that GBP1 may play a double-edged sword role in tumor immune microenvironment of STAD, while it is also contributed to the immunosuppressive factors in tumor.

There were some limitations to this study. First of all, we did not collect our own clinical samples to verify these results which were based on the public databases to detect GBP1 expression and its prognostic value in different tumors. Secondly, the sample sizes in databases were limited and continuously updated lead to the deviation of consequences, which means more samples should be enrolled for reliable results.

Lastly, we did not execute in vitro experiments and animal models to verify the relationship between GBP expression and progression of gastric cancer and its mechanism of regulating the function of tumor infiltrating immune cells. Therefore, subsequent studies were needed.

In summary, our study systematically demonstrated that higher GBP1 expression associated with increased survival and infiltration of various immune cells in GC. Especially, the levels of activated T lymphocytes and their related functional molecules, M2 macrophages, Tregs and exhausted T cells markedly increased along with GBP1 expression. Therefore, our study firstly provides basic evidence of the role of GBP1 that may act as a favorable biomarker for the prognosis of gastric cancer patients.

Abbreviations

GC: Gastric cancer; MDSC: Myeloid-derived suppressor cells; GBP1: Guanylate-binding protein 1; GEPIA: Gene Expression Profiling Interactive Analysis; TIMER: Tumor Immune Estimation Resource; HPA: The human protein atlas; GEO: Gene Expression Omnibus; EGA: European Genome-phenome Archive; TCGA: The Cancer Genome Atlas; HR: Hazard ratios; TAMs; Tumor-associated macrophages; NK: Natural killer; DCs: Dendritic cells; Th1: T-helper 1; Th2: T-helper 2; Tfh: Follicular helper T; Th17: T-helper 17; RSEM: RNA-Seq by Expectation-Maximization; GTEx: Genotype Tissue Expression; STAD: Stomach adenocarcinoma; DLBC: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; HNSC: Head and Neck squamous cell carcinoma ; KIRC: Kidney renal clear cell carcinoma; LGG: Brain Lower Grade Glioma; PAAD: Pancreatic adenocarcinoma; TGCT: Testicular Germ Cell Tumors; KICH: Kidney Chromophobe; PRAD: Prostate adenocarcinoma; UCEC: Uterine Corpus Endometrial Carcinoma; UCS: Uterine Carcinosarcoma; LUAD: Lung adenocarcinoma; LIHC: Liver hepatocellular carcinoma; LUSC: Lung squamous cell carcinoma; SKCM: Skin Cutaneous Melanoma; IHC: immunohistochemistry; OS: Overall Survival; PFS: Progression-Free Survival.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the Kaplan Meier Plotter (<http://kmplot.com/analysis/>), the Tumor Immune Estimation Resource (TIMER) (<https://cistrome.shinyapps.io/timer/>), the Gene Expression Profiling Interactive Analysis

(GEPIA) database (<http://gepia.cancer-pku.cn/>), TISIDB (<http://cis.hku.hk/TISIDB/>), the Human Protein Atlas (HPA) (<https://www.proteinatlas.org/>).

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

Authors' contributions

X.F. and Z.Z.: designed and organized the study, selected and analyzed data, wrote the manuscript; Q.C., C.Y. and T.T.: interpretation of data; drew the figures; H.C. and Y.T.: reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

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Tables

Table 1. Correlation of GBP1 and Gastric cancer prognosis with different clinicopathological factors by Kaplan-Meier plotter database.						
Clinicopathological characteristics	Overall survival			Progression-free survival		
	N	Hazard ratio	P-value	N	Hazard ratio	P-value
SEX						
Female	236	0.36(0.22-0.6)	3.10E-05	236	0.37(0.21-0.64)	0.00021
Male	544	0.61(0.49-0.77)	1.80E-05	544	0.57(0.43-0.75)	0.00005
Stage						
1	67	0.25(0.09-0.73)	0.0063	67	0.13(0.02-1.03)	0.023
2	140	0.48(0.21-1.09)	0.072	140	0.46(0.23-0.94)	0.03
3	305	0.49(0.36-0.66)	1.10E-06	305	0.51(0.33-0.8)	0.003
4	148	0.57(0.36-0.91)	0.016	148	0.52(0.32-0.83)	0.0055
STAGE T						
1	14	-	-	14	-	-
2	241	0.47(0.27-0.82)	0.0059	241	0.48(0.3-0.78)	0.0021
3	204	0.51(0.33-0.79)	0.0021	204	0.59(0.39-0.89)	0.01
4	38	0.56(0.22-1.45)	0.23	38	1.78(0.72-4.43)	0.21
STAGE N						
0	74	0.18(0.04-0.79)	0.01	72	0.2(0.05-0.84)	0.015
1	225	0.51(0.29-0.88)	0.0014	222	0.45(0.26-0.76)	0.0023
2	121	0.44(0.26-0.74)	1.50E-03	125	0.54(0.33-0.89)	0.014
3	76	0.47(0.25-0.9)	0.02	76	0.49(0.26-0.93)	0.025
1+2+3	422	0.51(0.36-	9.50E-	423	0.55(0.4-	0.00017

		0.72)	05		0.75	
STAGE M						
0	444	0.5(0.35-0.71)	7.10E-05	444	0.57(0.42-0.77)	0.0002
1	56	0.4(0.2-0.82)	0.097	56	0.38(0.18-0.79)	0.0078
LAUREN CLASSIFICATION						
Intestinal	320	0.48(0.35-0.66)	2.90E-06	320	0.53(0.33-0.84)	0.0066
Diffuse	241	0.43(0.27-0.69)	0.00024	241	0.5(0.32-0.77)	0.0014
DIFFERENTIATION						
Poor	165	0.61(0.37-1.01)	0.052	165	0.62(0.35-1.1)	0.1
Moderate	67	0.77(0.4-1.48)	0.43	67	0.71(0.31-1.61)	0.41
Bold values indicate $P < 0.05$						

Table 2. Correlation analysis between GBP1 and relate genes and markers of immune cells in TIMER database.

Description	Gene makers	None		Purity	
		Core	P	Core	P
CD8+ T cell	CD8A	0.708	***	0.69	***
	CD8B	0.538	***	0.52	***
T cell(general)	CD3D	0.671	***	0.656	***
	CD3E	0.646	***	0.634	***
	CD2	0.72	***	0.705	***
B cell	CD19	0.262	***	0.239	***
	CD79A	0.309	***	0.274	***
Monocyte	CD86	0.674	***	0.658	***
	CD115(CSF1R)	0.504	***	0.494	***
TAM	CCL2	0.303	***	0.27	***
	CD68	0.359	***	0.342	***
	IL10	0.47	***	0.454	***
M1 Macrophage	iNOS(NOS2)	0.184	**	0.178	**
	IRF5	0.162	**	0.142	*
	COX2(PTGS2)	0.114	0.0208	0.093	0.0696
M2 Macrophage	CD163	0.58	***	0.562	***
	VSIG4	0.521	***	0.5	***
	MS4A4A	0.562	***	0.545	***
Neutrophils	CD66b(CEACAM8)	0.074	0.134	0.064	0.213
	CD11b(ITGAM)	0.427	***	0.411	***
	CCR7	0.348	***	0.348	***
Natural killer cell	KIR2DL1	0.426	***	0.403	***
	KIR2DL3	0.449	***	0.43	***
	KIR2DL4	0.649	***	0.631	***
	KIR3DL1	0.432	***	0.413	***
	KIR3DL2	0.503	***	0.479	***

	KIR3DL3	0.225	***	0.241	***
	KIR2DS4	0.399	***	0.398	***
Dendritic cell	HLA-DPB1	0.601	***	0.576	***
	HLA-DQB1	0.467	***	0.426	***
	HLA-DRA	0.641	***	0.62	***
	HLA-DPA1	0.589	*	0.562	0.0297
	BDCA-1(CD1C)	0.155	***	0.112	***
	BDCA-4(NRP1)	0.352	***	0.335	***
	CD11c(ITGAX)	0.534	***	0.519	***
Th1	T-bet(TBX21)	0.713	***	0.71	***
	STAT4	0.619	***	0.612	***
	STAT1	0.8	***	0.802	***
	IFN- γ (IFNG)	0.793	***	0.787	***
	TNF- α (TNF)	0.305	***	0.263	***
Th2	GATA-3	0.413	**	0.386	**
	STAT6	0.168	***	0.171	***
	STAT5A	0.477	**	0.467	**
	IL13	0.175	**	0.181	*
Tfh	BCL6	0.189	***	0.158	***
	IL21	0.496	***	0.489	***
Th17	STAT3	0.369	0.012	0.369	0.015
	IL-17A	0.123	***	0.125	***
Treg	FOXP3	0.581	***	0.555	***
	CCR8	0.604	***	0.598	***
	STAT5B	0.247	***	0.262	***
	TGF β (TGFB1)	0.302	***	0.295	***
T cell exhaustion	PD-1(PDCD1)	0.598	***	0.574	***
	CTLA4	0.647	***	0.623	***
	TIGIT	0.727	***	0.723	***

TIM-3(HAVCR2)	0.703	***	0.682	***
LAG3	0.775	***	0.753	***
TAM, tumor-associated macrophage; Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; Cor, R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity.* $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$.				

Table 3. Correlation analysis between GBP1 and relate genes and markers of immune cells in GEPIA database.

Description	Gene makers	Tumor		Normal	
		R	P	R	P
CD8	CD8A	0.7	***	0.24	0.17
	CD8B	0.54	***	0.11	0.51
Monocyte	CD86	0.65	***	0.34	0.044
	CD115(CSF1R)	0.5	***	0.42	0.011
M2 Macrophage	CD163	0.57	***	0.32	0.055
	VSIG4	0.5	***	0.31	0.065
	MS4A4A	0.54	***	0.39	0.019
Dendritic cell	HLA-DPB1	0.6	***	0.31	0.071
	HLA-DRA	0.63	***	0.36	0.031
	HLA-DPA1	0.58	***	0.27	0.12
	CD11c(ITGAX)	0.53	***	0.52	**
Th1	T-bet(TBX21)	0.71	***	0.41	0.014
	STAT4	0.64	***	0.59	***
	STAT1	0.81	***	0.91	***
	IFN- γ (IFNG)	0.8	***	0.39	0.018
Treg	FOXP3	0.6	***	0.32	0.058
	CCR8	0.61	***	0.38	0.023
	STAT5B	0.29	***	0.42	0.011
T cell exhaustion	PD-1(PDCD1)	0.62	***	0.31	0.063
	CTLA4	0.64	***	0.28	0.1
	TIGIT	0.73	***	0.31	0.066
	TIM-3(HAVCR2)	0.68	***	0.56	***
	LAG3	0.78	***	0.43	**

Tumor, correlation analysis in tumor tissue of TCGA. Normal, correlation analysis in normal tissue of TCGA. * $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$.

Figures

Figure 1

GBP1 expression in different types of cancers. (A) The level of GBP1 expression in different tumor types from the TCGA database in TIMER. (B) Expression of GBP1 in various types of cancer and normal tissues visualized as dot plots based on the RNA-seq data of TCGA and GTEx through GEPIA online tool. *P < 0.05, **P < 0.01, ***P < 0.001; Red boxes represent tumor tissues; green boxes represent normal tissues. (C) GBP1 expression by IHC evidence was visualized from the HPA database. Red and green dashed lines represent the average value of all tumor and normal tissues, respectively. TCGA, The Cancer Genome Atlas; GTEx, Genotype Tissue Expression; GEPIA, Gene Expression Profiling Interactive Analysis; IHC, immunohistochemistry; HPA, Human Protein Atlas.

Figure 2

Correlation between GBP1 expression and Kaplan-Meier survival curve analysis of the prognostic significance in different types of human cancers using the Kaplan-Meier plotter database. (A-B) High expression of GBP1 correlates with better OS and PFS in gastric cancer (n=875,n=640); (C-D) No correlation was found with OS, low expression of GBP1 indicates better PFS in breast cancer (n=1402, n=414); (E-F) High expression of GBP1 correlates with better OS and PFS in liver cancer (n=192,n=370); (G-H) No correlation was found with OS, low expression of GBP1 indicates better PFS in ovarian cancer(n=1656, n=1435). OS, overall survival, PFS, progression-free survival.

Figure 3

GBP1 expression positively correlated with the infiltration of activated T cells in gastric cancer. (A) Correlation analysis of GBP1 expression and infiltration levels of immune cells in GC tissues using the TIMER database (n=415). GBP1 expression negatively correlated with tumor purity and infiltration of B cells; positively with infiltration of CD8+ T cells, macrophages, neutrophils, and dendritic cells. No correlation was found between GBP1 expression and infiltration with CD4+ T cells. (B-C) GBP1 expression correlated with different status of T lymphocytes in GC tissues using the TISIDB database. GBP1 expression showed a positive correlation with most immune cells in GC (B), while the most correlation was found among activated CD4+ and CD8+ T cells (C). GC, gastric cancer;

Figure 4

Correlation analysis between mRNA levels of GBP1 and markers of immune cells in tumor microenvironment using TIMER database. (A) GBP1 expression positively correlates with markers of CD8+ T cells. (B) GBP1 expression positively correlated with cytotoxic molecules. (C) Correlation analysis showed a positive association between expression levels of GBP1 and markers of Th1 cells.

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