Mathematical Biosciences and Engineering

MBE, 20(5): 9511–9529.
DOI: 10.3934/mbe.2023418
Received: 25 January 2023
Revised: 05 March 2023
Accepted: 12 March 2023
Published: 20 March 2023

http://www.aimspress.com/journal/MBE

Research article

The function of guanylate binding protein 3 (GBP3) in human cancers by pan-cancer bioinformatics

Tongmeng Jiang[1,2,*,†], Pan Jin[3,4,†], Guoxiu Huang[5] and Shi-Cheng Li[6,*]

[1] Key Laboratory of Hainan Trauma and Disaster Rescue, The First Affiliated Hospital of Hainan Medical University, Hainan Medical University, Haikou 571199, China
[2] Engineering Research Center for Hainan Bio-Smart Materials and Bio-Medical Devices, Key Laboratory of Hainan Functional Materials and Molecular Imaging, Key Laboratory of Emergency and Trauma, Ministry of Education, College of Emergency and Trauma, Hainan Medical University, Haikou 571199, China
[3] Health Science Center, Yangtze University, Jingzhou 434023, China
[4] Collaborative Innovation Centre of Regenerative Medicine and Medical BioResource Development and Application Co-constructed by the Province and Ministry, Guangxi Medical University, Nanning 530021, China
[5] Health Management Center, The People's Hospital of Guangxi Zhuang Autonomous Region; Guangxi Health Examination Center, Nanning 530021, China
[6] Department of Cardiology, The People's Hospital of Guangxi Zhuang Autonomous Region; Institute of Cardiovascular Sciences, Guangxi Academy of Medical Sciences, Nanning 530021, China

* **Correspondence:** Email: jiangtongmeng@hainmc.edu.cn, leesc_gx@foxmail.com.

† These two authors contributed equally.

Abstract: As a guanylate binding protein (GBPs) member, GBP3 is immune-associated and may participate in oncogenesis and cancer therapy. Since little has been reported on GBP3 in this field, we provide pan-cancer bioinformatics to investigate the role of GBP3 in human cancers. The GBP3 expression, related clinical outcomes, immune infiltrates, potential mechanisms and mutations were conducted using tools including TIMER2.0, GEPIA2.0, SRING, DAVID and cBioPortal. Results showed an increased risk of high GBP3 in Brain Lower Grade Glioma (LGG) and Lung Squamous Cell Carcinoma (LUSC) and a decreased risk of GBP3 in Sarcoma (SARC) and Skin Cutaneous Melanoma (SKCM) ($p \leq 0.05$). GBP3 was negatively correlated with CAFs in Esophageal

Adenocarcinoma (ESCA) and positively correlated with CAFs in LGG, LUSC and TGCG ($p \leq 0.05$). In addition, GBP3 was positively correlated with CD8+ T cells in Bladder Urothelial Carcinoma (BLCA), Cervical Squamous Cell Carcinoma (CESC), Kidney Renal Clear Cell Carcinoma (KIRC), SARC, SKCM, SKCM-Metastasis and Uveal Melanoma (UVM) ($p \leq 0.05$). Potentially, GBP3 may participate in the homeostasis between immune and adaptive immunity in cancers. Moreover, the most frequent mutation sites of GBP3 in cancers are R151Q/* and K380N. This study would provide new insight into cancer prognosis and therapy.

Keywords: GBP3; cancer; prognosis; immune infiltrates; mutation

1. Introduction

Guanylate binding proteins (GBPs) are the dominant factors fighting against pathogens invading the organic body, thus regulating innate immunity [1]. GBPs constitute many interferon (IFN) inducible GTPases in mammals and present seven subtypes (GBP1-7) in the human body [2,3]. Recently, GBP orthologues have been reported in numerous cancer studies, including GBP1, GBP2 and GBP3 [4−6]. Indeed, the roles and mechanisms of GBP1 and GBP2 in many tumors have been well understood. Briefly, the oncogenic role of GBP1 has been reported in cervical cancer [7], lung cancer [8,9], glioblastoma [10] and prostate cancer [11]. Likewise, GBP2 plays critical roles in glioblastoma [12], breast cancer [5,13,14], pancreatic cancer [15] and colorectal cancer [16]. Xu et al. [17] demonstrated the potential targeting treatment of GBP3 for glioblastoma. However, little research has been established to discover the function and molecular mechanism of GBP3 enrolled in different cancers, especially the expression, mutation, prognostic outcome and immune effects.

For decades, many databases have been launched to identify the characteristics and oncogenic roles of genes and proteins in different cancers, where The Cancer Genome Atlas (TCGA) is the most popular one [18]. In addition, the Genotype-Tissue Expression (GTEx) project helps screen the genetic variation of normal tissue in humans [19]. Under the convenience of these cancer research projects, pan-cancer analysis is available to generate a specific observation of an exciting gene in the tumor microenvironment, cancer progression and outcome. In the tumor microenvironment, many factors influence the progress of the tumor and the potential therapeutic methods, which include PH, inflammation, microorganisms and immune infiltration [20−22]. Immune infiltration often relates to the immune responses or checkpoints in tumor immunotherapy [23−25]. Therefore, understanding the relationship between a particular gene with immune infiltration would help find novel therapy or prognosis for cancer.

In this research, we analyzed the expression and function of GBP3 in multiple cancers based on TCGA data. The clinical outcome, immune infiltration correlation, mutation and related molecular mechanism of GBP3 were also calculated. Our study may give a snapshot of understanding the oncogenic and immune role of GBP3, in especially, the mutation sites, e.g., R151Q/* and K380N are listed.

2. Materials and methods

2.1. Gene expression analysis

The expressions of GBP3 in different cancer and normal tissue in TGCA and GTEx were analyzed using TIMER2.0 (http://timer.cistrome.org/) [26,27] and GIPIA2 (http://gepia2.cancer-pku.cn/#index) [28,29] separately. First, GBP3 was submitted to TIMER2.0 using the feature "Gene_DE" to obtain its expression using log_2TPM by Wilcoxon test in TCGA. Also, GBP3 was submitted to GEPIA2.0 using the pane "Expression DIY" with the feature "Gene expression Profile" under the algorithm of ANOVA methods. Both TCGA normal and GTEx data were matched. Parameters included $|Log_2FC|$ cutoff at 1.5, q-value cutoff at 0.05. Then, a Venn plot was performed to show the interaction results between TIMER2.0 and GIPIA2.

2.2. Clinical outcome analysis

The clinical outcome analysis was also conducted using TIMER2.0 (http://timer.cistrome.org/) [26,27] and GIPIA2 (http://gepia2.cancer-pku.cn/#index) [28,29] separately. Briefly, GBP3 was submitted to GEPIA2.0 using the pane "Survival Analysis" under the algorithm of Overall Survival (OS) and Disease Free Survival (DFS) methods with the group cutoff at 50%. The survival map feature was also obtained with the significant level at 0.05 and group cutoff at 50% but without P-Value Adjustment. Moreover, the OS analysis was also generated using the pane "Gene_Outcome" in TIMER2.0.

2.3. Immune infiltration analysis

The relationships of GBP3 and immune infiltrates of cancer-associated fibroblasts (CAFs), CD8 positive T cells and macrophages were obtained using TIMER2.0 (http://timer.cistrome.org/) [26,27] with the feature "Immune". In addition, multiple covariates of GBP3 and aforementioned immune infiltrates for cancer outcome (Overall Survival) were also analyzed using a multivariable Cox proportional hazard model. Afterward, a heatmap showing the normalized coefficients of infiltrate will be displayed across multiple cancer types.

2.4. GBP3-related factor analysis

Protein-Protein Interaction (PPI) was performed using STRING 11.5 (https://string-db.org/cgi/input.pl) [30]. Briefly, GBP3 was submitted to STRING websites, and the parameters were 100 max interactions in Homo Sapiens. Then, all factors related to GBP3 were enriched by DAVID (https://david.abcc.ncifcrf.gov/) [31] with GO_term BP (biological process) and KEGG pathway.

2.5. Gene mutation analysis

The mutation of GBPs in multiple cancers was analyzed using cBioPortal (http://cbioportal.org) [32]. The 3D structure of GBP3 was also presented.

3. Results

3.1. GBP3 expressed differently in cancers

To best reveal the expression of GBP3 in different cancers, and normal tissues, TCGA and GETx data were included in this study. Figure 1A shows GBP3 significantly upregulated in BRCA, CHOL, ESCA, GBM, HNSC, KIRC, STAD and THCA, whereas down-regulated in COAD, KICH, LUSC, PCPG, PRAD and PEAD when to compare with normal tissues in TCGA data. Furthermore, GBP3 was down-regulated in SKCM compared with SKCM-metastasis in TCGA data.

In TCGA and GTEx data, GBP3 was significantly higher in DLBC, GBM, LAML, LGG, PAAD and STAD than in normal tissues and significantly lower in KICH and UCS than in normal tissues (Figure 1B).

Thus, it reveals that GBP3 was significantly upregulated in two cancer types and down-regulated in one in both TCGA and GTEx databases (Figure 1C). The two cancer types that presented upregulated GBP3 were GBM and STAD and the one cancer that presented downregulated GBP3 was KICH (Figure 1D).

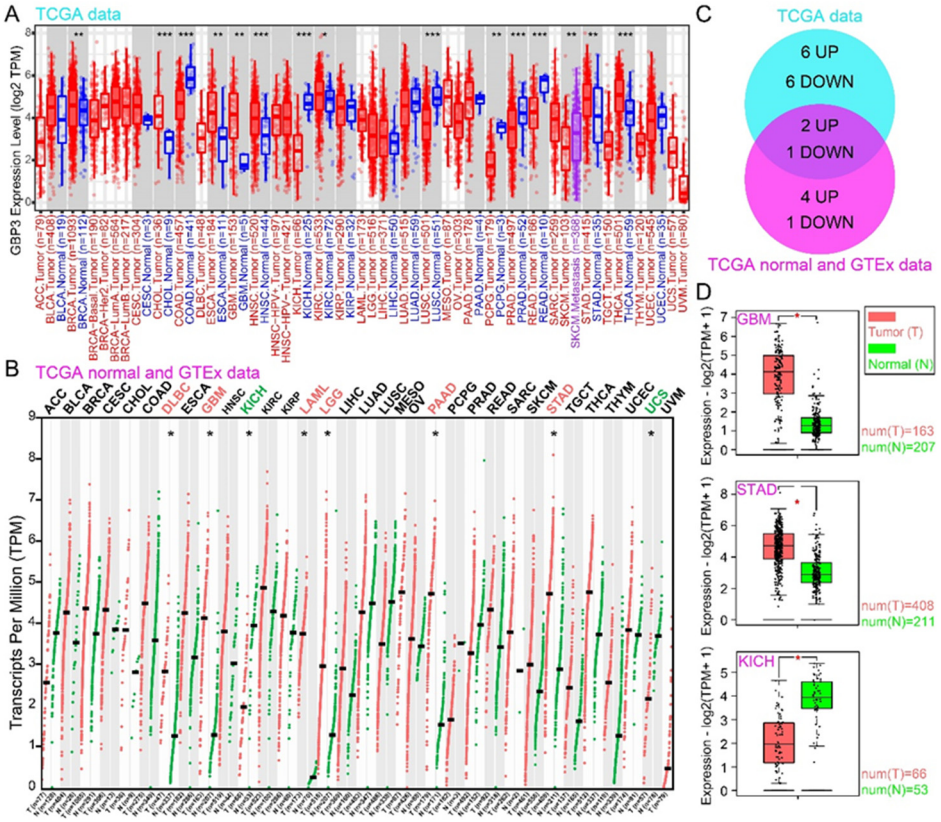

Figure 1. A pan-cancer analysis for GBP3 expression. (A) GBP3 levels in TCGA database. Red indicates tumor, blue indicates normal and purple indicates metastasis. (B) GBP3 level in TCGA and GTEx databases. Red indicates the tumor, and green indicates the normal. (C) Venn plot shows the GBP3 expressed significantly in (A) and (B). (D) GBM, STAD and KICH are the intersection cancer types with significantly expressed GBP3 (A) and (B). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.2. The level of GBPs correlates with different outcomes in cancers

The clinical outcomes were shown separately using a survival map and Kaplan-Meier plots from GEPIA2.0 (Figure 2A and B) and TIMER 2.0 (Figure 2C). Figure 2A shows that GBP3 is the increasing risk in LGG and LUSC and is the decreasing risk in KIRC, SARC and SKCM in overall survival (OS). Also, GBP3 is the increasing risk in LGG in disease-free survival (DFS), as revealed in GEPIA2.0 (Figure 2B). To better affirm it, the OS results of GBP3 in cancers were also submitted to TIMER2.0. As Figure 2C shows, GBP3 is the increasing risk in LGG, LUSC, PAAD and UVM, while is the decreasing risk in BLCA, MESO, SARC, SKCM and SKCM-Metastasis in TIMER2.0. TIMER2.0 and GEPIA2.0 revealed that patients with low GBP3 levels have better OS rates in LGG and LUSC, while low GBP3 level patients have worse OS rates in SARC and SKCM.

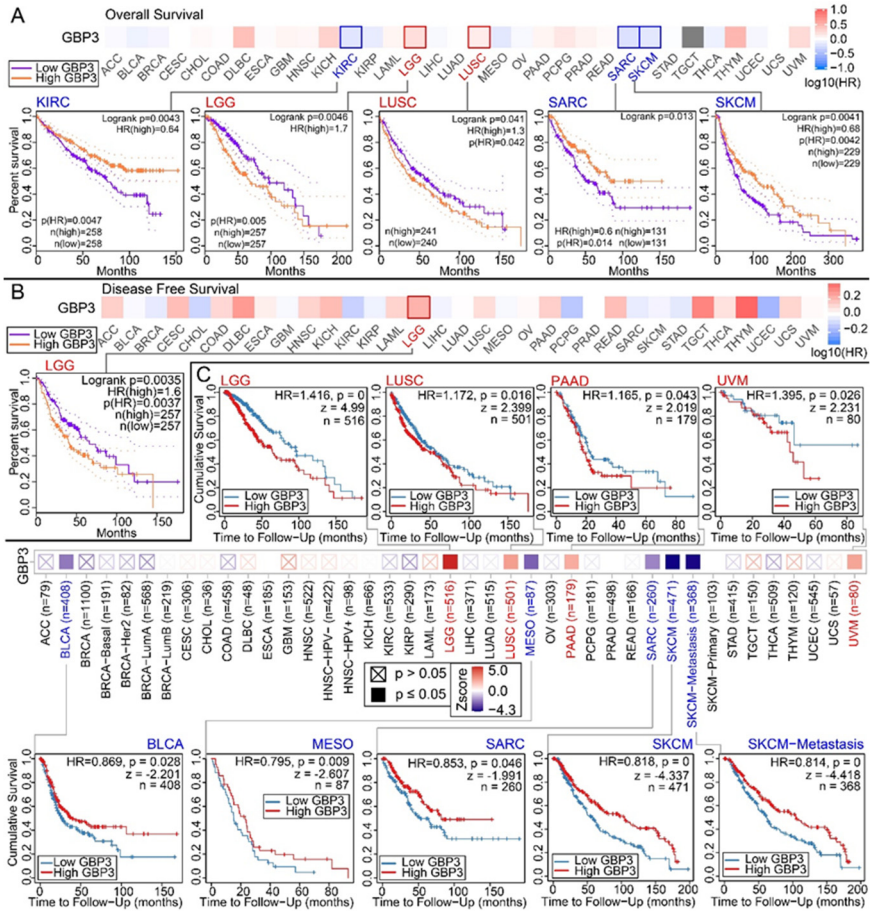

Figure 2. GBP3 related clinical outcome in cancers. (A) Overall survival of GBP3 in GEPIA2.0. (B) Disease-free survival of GBP3 in GEPIA2.0. (C) Overall survival of GBP3 in TIMER2.0. Red indicates increasing risk, and blue indicates decreasing risk.

3.3. GBP3 is associated with CAFs in cancers

The relationships between GBP3 and immune infiltrates, including cancer-associated fibroblasts (CAFs), CD8+ T cells and macrophages, are shown in Figures 3 and 4. Indeed, GBP3 is negatively correlated with CAFs in ESCA ($p \leq 0.05$) with all CAF algorithms (EPIC, MCPCOUNTER and

XCELL) and Tumor Immune Dysfunction and Exclusion (TIDE) algorithm. On the contrary, GBP3 is positively correlated with CAFs in LGG, LUSC and TGCG ($p \leq 0.05$) with the four algorithms above (Figure 3). Differently, GBP3 positively correlates with $CD8^+$ T cells in BLCA, ESEC and UVM in all algorithms, including TIMER, EPIC, MCPCUNTER, CIBERSORT, CIBERSORT-ABS, QUNTISEQ and XCELL. Moreover, GBP3 positively correlates with CD8+ T cells in SARC, SKCM and SKCM-Metastasis not only in the seven algorithms above but also in T cell $CD8^+$ naïve_XCELL, T cell $CD8^+$ central memory_XCELL and T cell $CD8^+$ effector memory_XCELL (Figure 4A). In contrast, the situation of GBP3 with macrophage in cancers was complicated. Figure 4B shows GBP3 positively correlates with macrophage in BRCA, GBM, KICH, KIRC, LGG, LUAD and PRAD in EPIC, TIMER and XCELL algorithms. Moreover, GBP3 negatively correlates with M0 macrophage in CESC, GBM and KIRC in CIBERSORT and CIBERSORT-ABS algorithms. The relationship between GBP3 and M1 macrophage is positively relative in BRCA-Basal, CESC, ESCA, GBM, HNSC, HNSC-HPV^-, KIRC, LGG, LUSC, LUAD, PCPG, SARC, SKCM-Metastasis, STAD and THYM within the algorithms of CIBRSORT, CIBERSORT-ABS, QUANTISEQ and XCELL. In addition, GBP3 is also positively associated with M2 macrophage in LGG and LIHC within the algorithms of CIBRSORT, CIBERSORT-ABS, QUANTISEQ and XCELL.

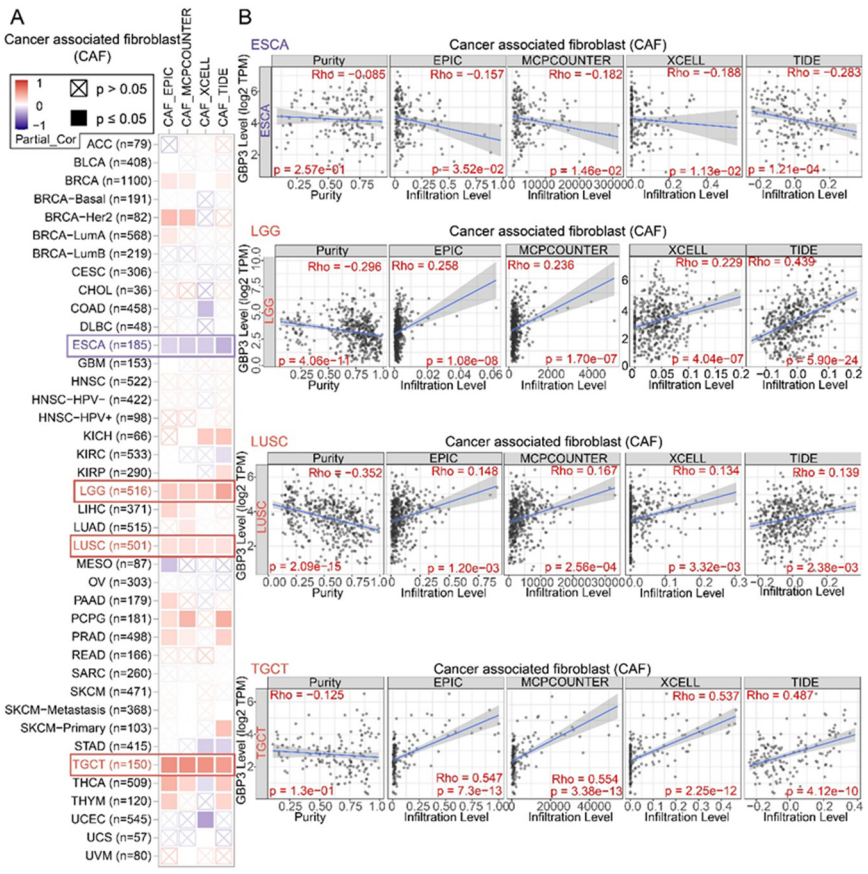

Figure 3. The correlation between GBP3 and CAFs in TCGA cancer types. (A) Heatmap shows GBP3 is associated with CAFs in ESCA, LGG, LUSC and TGCT. Blue indicated a negative correlation, and red indicated a positive correlation. (B) Gene plots show the correlation between GBP3 and CAFs in ESCA, LGG, LUSC and TGCG, respectively.

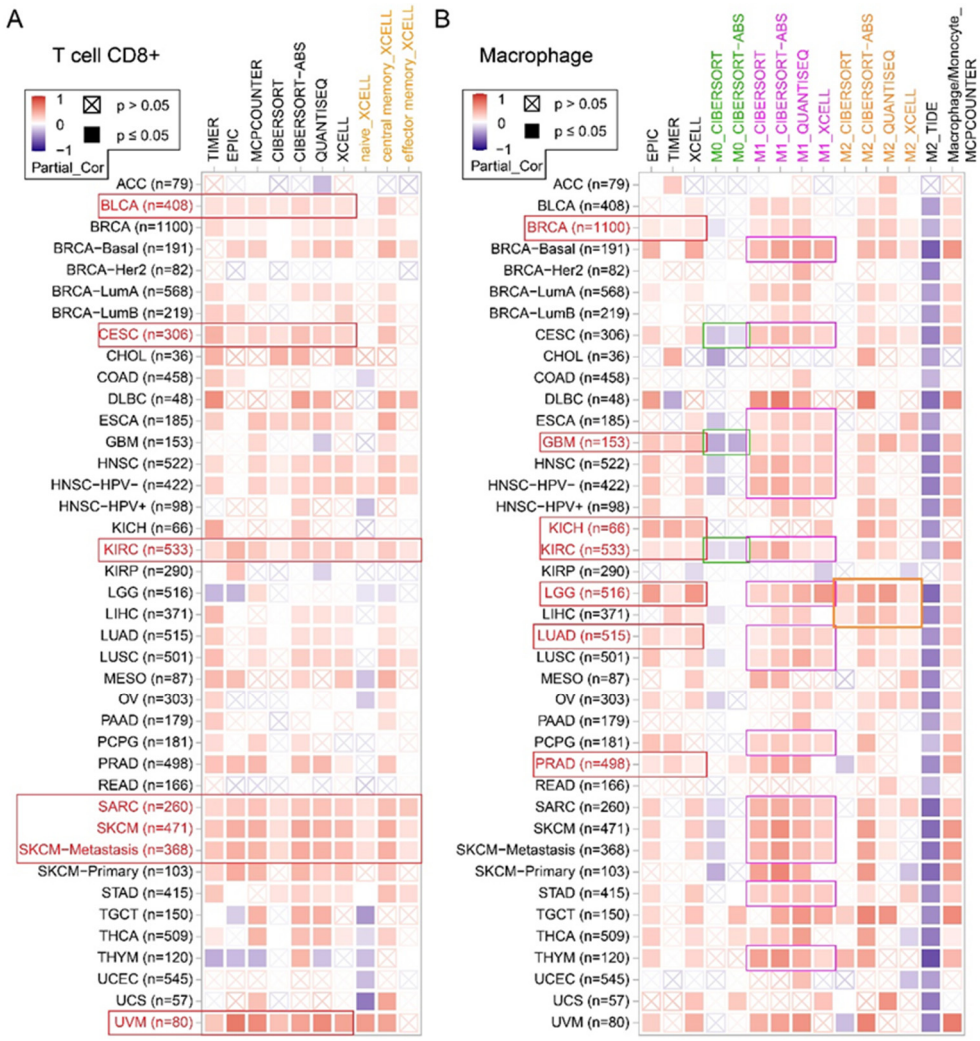

Figure 4. The correlation between GBP3 and (A) CD8+ T cells and (B) macrophage in TCGA cancer types.

To better uncover the function of GBP3 and its related immune infiltrates for clinical outcomes in cancers, the OS rates between GBP3 and CAFs (Figure 5), CD8+ T cells (Figure S1) and macrophages (Figure S2) were observed. GBP3 and CAFs positively correlate with KIRP's OS rate with all algorithms, including TIDE, XCELL, MCPCOUNTER and EPIC. Besides, for the CAF TIDE algorithm, GBP3 presents a positive correlation in ACC, BLCA, BRCA-LumB, CESC, HNSC-HPV+, KICH, LGG, MESO, STAD and THCA. For the CAF XCELL algorithm, GBP3 presents a positive correlation in KICH, OV, READ and THCA, whereas GBP3 presents a negative correlation in HNSC-HPV+ and SARC. For the MCPCOUNTER algorithm, GBP3 shows a positive correlation in ACC, BLCA, BRCA-LumB, GBM, KIRC, LGG, OV, READ and UVM. For the EPIC algorithm, GBP3 presents a positive correlation in ACC, BLCA, BRCA-LumB, GBM, KIRC, LGG, MESO and OV (Figure 5A). In the meantime, low levels of CAFs with a low level of GBP3 showed better OS rates than high levels of CAFs with a low level of GBP3 in KIRP cancer in all CAF algorithms (EPIC, MCPCOUNTER, XCELL and TIDE) (Figure 5B). However, there is no apparent rhythm between GBP3 and CD8+ T cells/macrophage by OS rate.

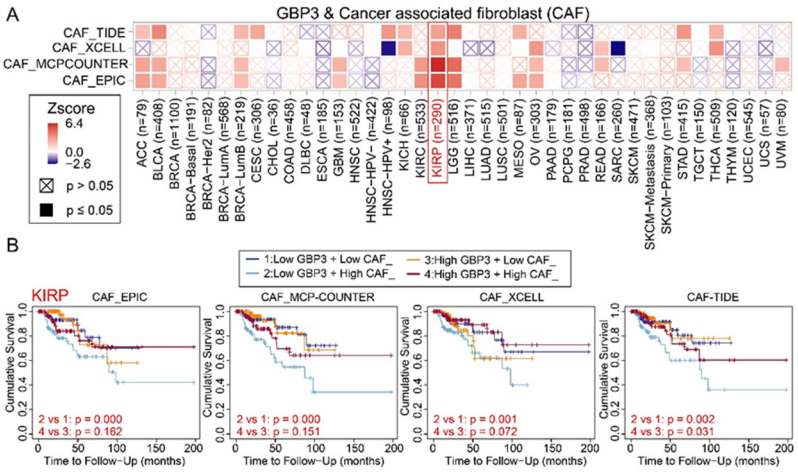

Figure 5. The overall survival (OS) of GBP3 & CAFs in TCGA cancer types. (A) OS map. (B) Kaplan-Meier plots of significant cancer types in (A).

3.4. GBP3 and its related molecules in oncogenic and anti-cancerous mechanism

We used the STRING web tool to find the related molecules to GBP3 and limited the max number to 100. As shown by the PPI (Figure 6A), there were only 31 proteins related to GBP3. The top 10 biological processes GO terms ranked by -Log10FDR, include type I interferon signaling pathway, defense response to the virus, response to virus, interferon-gamma-mediated signaling pathway, negative regulation of viral genome replication, positive regulation of calcidiol 1-monooxygenase activity, interleukin-1 beta production, immune response, innate immune response and positive regulation of chemokine biosynthetic process (Table 1, Figure 6B). Moreover, the top 10 enriched KEGG pathways were Influenza A, Herpes simplex infection, Measles, Hepatitis C, RIG-I-like receptor signaling pathway, Pertussis, Toll-like receptor signaling pathway, Osteoclast differentiation, NOD-like receptor signaling pathway, Cytosolic DNA-sensing pathway (Table 2, Figure 6C).

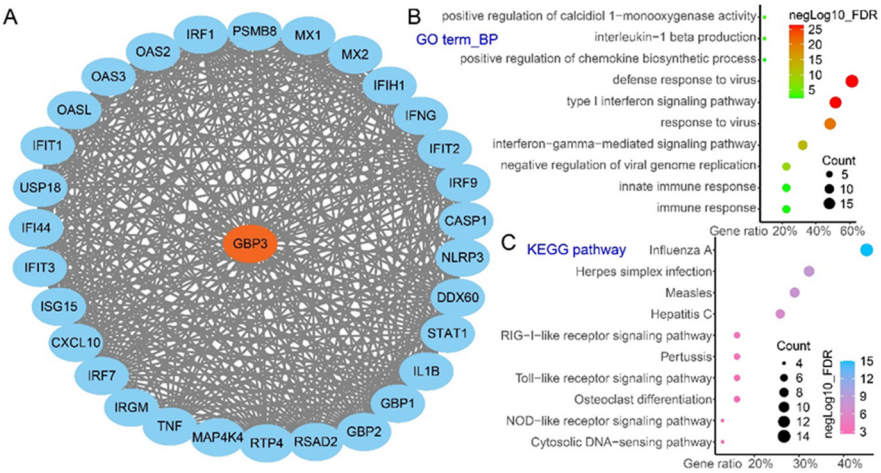

Figure 6. The molecules related to GBP3 and potential mechanism in oncogenesis. (A) PPI network. (B) Top 10 enrichments of GO term_BP. (C) Top 10 enrichments of KEGG pathway.

Table 1. GBP3-related molecules enriched biological process GO terms.

Category	Term	Count	%	PValue	Genes	Fold Enrichment	Bonferroni	Benjamini	FDR
GOTERM_BP_DIRECT	GO:0060337~type I interferon signaling pathway	16	51.6129	1.31×10^{-29}	RSAD2, STAT1, MX2, MX1, ISG15, IFIT1, PSMB8, IFIT3, IFIT2, OASL, OAS2, IRF1, OAS3, IRF7, GBP2, IRF9	135.4194	5.26×10^{-27}	4.40×10^{-27}	4.06×10^{-27}
GOTERM_BP_DIRECT	GO:0051607~defense response to virus	19	61.29032	2.19×10^{-29}	RSAD2, STAT1, MX2, MX1, ISG15, IFIT1, DDX60, IFIT3, IFIT2, OASL, CXCL10, IFNG, OAS2, IRF1, OAS3, NLRP3, GBP1, IRF9, GBP3	62.37498	8.80×10^{-27}	4.40×10^{-27}	4.06×10^{-27}
GOTERM_BP_DIRECT	GO:0009615~response to virus	15	48.3871	1.51×10^{-23}	RSAD2, MX2, MX1, IFI44, IFIT1, DDX60, TNF, IFIT3, IFIT2, OASL, IFIH1, IFNG, OAS2, OAS3, IRF7	73.8651	6.06×10^{-21}	2.02×10^{-21}	1.86×10^{-21}
GOTERM_BP_DIRECT	GO:0060333~interferon-gamma-mediated signaling pathway	10	32.25806	3.38×10^{-15}	IFNG, STAT1, OAS2, IRF1, OAS3, IRF7, GBP2, GBP1, IRF9, OASL	76.29259	1.34×10^{-12}	3.39×10^{-13}	3.13×10^{-13}
GOTERM_BP_DIRECT	GO:0045071~negative regulation of viral genome replication	7	22.58065	7.03×10^{-11}	RSAD2, OAS3, MX1, ISG15, IFIT1, TNF, OASL	94.79355	2.82×10^{-08}	5.65×10^{-09}	5.21×10^{-09}
GOTERM_BP_DIRECT	GO:0060559~positive regulation of calcidiol 1-monooxygenase activity	3	9.677419	9.25×10^{-06}	IFNG, IL1B, TNF	541.6774	3.71×10^{-03}	6.20×10^{-04}	5.72×10^{-04}
GOTERM_BP_DIRECT	GO:0032611~interleukin-1 beta production	3	9.677419	3.08×10^{-05}	IL1B, CASP1, NLRP3	325.0065	0.012287	0.001766	0.00163
GOTERM_BP_DIRECT	GO:0006955~immune response	7	22.58065	8.54×10^{-05}	CXCL10, IFNG, OAS2, IL1B, OAS3, GBP2, TNF	9.006513	0.033743	0.004285	0.003955
GOTERM_BP_DIRECT	GO:0045087~innate immune response	7	22.58065	9.59×10^{-05}	IFIH1, MX2, MX1, IRF7, NLRP3, DDX60, IRGM	8.818005	0.037833	0.004285	0.003955
GOTERM_BP_DIRECT	GO:0045080~positive regulation of chemokine biosynthetic process	3	9.677419	1.38×10^{-04}	IFNG, IL1B, TNF	162.5032	0.053825	0.005532	0.005106

Table 2. GBP3-related molecules enriched KEGG pathways.

Category	Term	Count	%	PValue	Genes	Fold Enrichment	Bonferroni	Benjamini	FDR
KEGG_PATHWAY	hsa05164:Influenza A	14	45.16129	2.61×10^{-17}	RSAD2, STAT1, MX1, TNF, IFIH1, CXCL10, IFNG, OAS2, IL1B, OAS3, CASP1, IRF7, NLRP3, IRF9	27.67414	1.59×10^{-15}	1.59×10^{-15}	9.91×10^{-16}
KEGG_PATHWAY	hsa05168:Herpes simplex infection	10	32.25806	4.03×10^{-10}	IFIH1, IFNG, STAT1, OAS2, IL1B, OAS3, IRF7, IFIT1, TNF, IRF9	18.79508	2.46×10^{-08}	1.23×10^{-08}	7.66×10^{-09}
KEGG_PATHWAY	hsa05162:Measles	9	29.03226	9.99×10^{-10}	IFIH1, IFNG, STAT1, OAS2, IL1B, OAS3, MX1, IRF7, IRF9	23.27481	6.10×10^{-08}	2.03×10^{-08}	1.27×10^{-08}
KEGG_PATHWAY	hsa05160:Hepatitis C	8	25.80645	3.58×10^{-08}	STAT1, OAS2, IRF1, OAS3, IRF7, IFIT1, TNF, IRF9	20.68872	2.18×10^{-06}	5.46×10^{-07}	3.40×10^{-07}
KEGG_PATHWAY	hsa04622:RIG-I-like receptor signaling pathway	5	16.12903	3.40×10^{-05}	IFIH1, CXCL10, IRF7, ISG15, TNF	24.56786	0.00207	4.14×10^{-04}	2.58×10^{-04}
KEGG_PATHWAY	hsa05133:Pertussis	5	16.12903	4.46×10^{-05}	IL1B, IRF1, CASP1, NLRP3, TNF	22.93	0.002719	4.54×10^{-04}	2.83×10^{-04}
KEGG_PATHWAY	hsa04620:Toll-like receptor signaling pathway	5	16.12903	1.73×10^{-04}	CXCL10, STAT1, IL1B, IRF7, TNF	16.22406	0.010484	0.001506	9.38×10^{-04}
KEGG_PATHWAY	hsa04380:Osteoclast differentiation	5	16.12903	3.90×10^{-04}	IFNG, STAT1, IL1B, TNF, IRF9	13.12786	0.023512	0.002974	0.001852
KEGG_PATHWAY	hsa04621:NOD-like receptor signaling pathway	4	12.90323	4.52×10^{-04}	IL1B, CASP1, NLRP3, TNF	24.56786	0.027173	0.00306	0.001906
KEGG_PATHWAY	hsa04623:Cytosolic DNA-sensing pathway	4	12.90323	6.69×10^{-04}	CXCL10, IL1B, IRF7, CASP1	21.49688	0.040014	0.003711	0.002312

3.5. GBP3 mutation in cancers

The mutation frequency of GBP3 in TCGA cancer types was conducted by cBioPoral. Figure 7A indicates that GBP3 mutation mostly happened in UCEC. Among these mutations, missense is the major type. Moreover, the R151Q/* site in GBP3 was the most common, with one R151Q site and one R151* site in UCEC, one R151Q site in GBM and one R151Q site in COAD. Furthermore, the K380N site in GBP3_C was the most frequent, with two cases in UCEC and one in COAD (Figure 7B). In addition, the R151Q/* site and K380N site in the GBP3 structure are shown in Figure 7C.

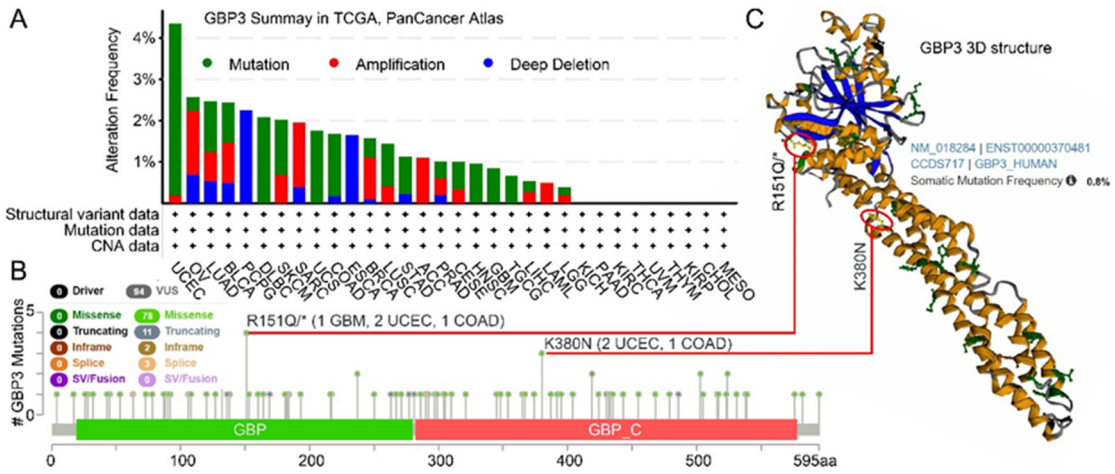

Figure 7. Mutation frequency of GBP3 in TCGA. (A) GBP3 mutation in cancer types. (B) GBP3 mutation types. (C) GBP3 3D structure and the mutation sites.

4. Discussion

GBP3 is one of the GBP families acknowledged as innate immunity modulators[1]. Since GBPs have raised their oncogenic roles in cancers, many reports have tried to find how they work. Moreover, GBP1 and GBP2 are mostly investigated [4,5,11,12]. GBP3 is highly related to GBP1 and GBP2 in PPI and, thus, may present a similar function. Recently, some researchers observed the molecular mechanisms of GBP3 in oncogenesis and cancer outcomes. For instance, GBP3 modulates the SQSTM1-ERK1/2 pathway, enhancing the proliferation of glioma cells [6]. Also, GBP3 exhibits its prognostic effect in SKCM cancer [33]. However, little has been discovered, and pan-cancer bioinformatic research may better help reveal how GBP3 works in different cancers.

In this work, GBP3 varies in many cancer types in different databases. However, the intersection results between TCGA and GTEx reveal that GBP3 is highly expressed in GBM and STAD while lowly expressed in KICH. Thus, GBP3 may be a novel monitor to indicate whether a person easily suffers from GBM, STAD or KICH. Meanwhile, if somebody expressed a high level of GBP3, they may have GBM or STAD. Likewise, if someone expressed a deficient level of GBP3, KICH cancer may be a possible reason.

We also combined the overall survival results of TIMER2.0 and GEPIA2.0 and observed that GBP3 is an increasing risk of LGG and LUSC, whereas it is a decreasing risk of SARC and SKCM. It means that LGG or LUSC patients with lower GBP3 would have better a survival rate, and SARC or SKCM patients with higher GBP3 would have a better survival rate. Intriguingly, GBP3 is an oncogenic factor in glioma[6], which may support our results in the increasing risk possibility of GBP3 in LGG. On the contrary, the high level of GBP3 in SKCM would favor the OS results has been demonstrated [33].

Due to the activation effects on innate immunity by GBPs mediating pathogen-selective inflammasome activation [34], they may also confer to the tumor microenvironment (TME) [35] because microorganisms have also participated [22]. In TME, CAFs, CD8+ T cells and macrophages are three major types of immune cells [36–38]. Therefore, untangling the relationship between GPB3 and CAFs, CD8+ T cells or macrophages may help generate targeted immune therapy.

CAFs are fibroblasts in cancerous stomas contributing to cancer progression[36]. For decades, the function of CAFs has been concluded as expressing and secreting active factors that influent cancerous cells and immune cells, and, thus, CAFs have become a promising therapeutic target in immunotherapy [39,40]. Since GBP3 is one of GBPs and is immune-related, unveiling the relationship between GBP3 and CAFs in TME is essential. Based on this, the negative correlation of GBP3 of CAFs in ESCA and the positive correlation of GBP3 of CAFs in LGG, LUSC and TGCT suggest that increasing GBPs in ESCA or suppressing GBP3 in LGG/LUSC/TGCT may be a potential cancer immunotherapy. In addition, the accordance between outcome and CAF-correlation results of GBP3 in LGG and LUSC further indicates the therapeutic targeting possibility of GBP3 in LGG and LUSC. However, after we investigated the overall survival of both GBP3 and CAFs in TGCA cancers, we found that a low level of GBP3 together with a low level of CAFs present better survival rates than a low level of GBP3 together with a high level of CAFs only in KIRP ($p < 0.05$) with all CAF algorithms (EPIC, MCPCOUNTER, XCELL and TIDE). Moreover, this phenomenon also happened in LGG ($p < 0.05$) only with three CAF algorithms (EPIC, MCPCOUNTER and TIDE). Therefore, more investigation should be done to ensure the function of GBP3 in LGG. In contrast, no significant relationships between GBP3 and CAFs in LUSC.

CD8+ T cells are bodyguards when a person has cancer because they are critical factors in the adaptive immune system to fight against cancer [41]. Moreover, the homeostasis and interaction between innate and adaptive immunity, as well as its related immune infiltration, are essential for cancer therapy [21,37]. Since GBP3 is one of the GBPs, which participate in innate immunity[1], the relationship between GPB3 and CD8+ T cells for cancer outcome should be observed. Our results indicated that GBP3 was positively correlated with CD8+ T cells in BLCA, ECSC, KIRC, SKRC, SKCM, SKCM-Metastasis and UVM. This means a high level of GBP3 with a high level of CD8+ T cells has better overall survival than a high level of GBP3 with a low level of CD8+ T cells in BLCA, ECSC, KIRC, SKRC, SKCM, SKCM-Metastasis and UVM. This also aligns with the OS results of GBP3 in SKRC and SKCM. In addition, GBP3 may cooperate with CD8+ T cells to treat these cancers. Recently, many kinds of literature listed macrophages, capacities in innate and adaptive immunity[38]. Furthermore, the complicated relationship between GBP3 and macrophage and its subtypes in different cancers suggests that more specific studies should be done in this area.

To better investigate the potential molecular mechanism of GBP3 in oncogenesis and cancer therapy, we input the GBP3-related proteins into biological process GO and KEGG pathway enrichments. In line with public results, some biological processes and pathways associated with GBP3 participate in cancer progression and therapy, which include type I interferon signaling pathway [42], interferon-gamma-mediated signaling pathway [43], immune response [44,45], innate immune response [37,46], positive regulation of chemokine biosynthetic process [47], RIG-I-like receptor signaling pathway [48,49], Toll-like receptor signaling pathway [49,50], NOD-like receptor signaling pathway [51] and Cytosolic DNA-sensing pathway [52]. For instance, GBP3 could be regulated by interferon-γ [53], which is upregulated by immune checkpoint blockade therapy for cancer [43]. Anticancer immune responses by RIG-I-like receptor signaling pathway and Toll-like receptor signaling pathway are agonists for innate immune system receptors [49], in which GBP3 plays a vital role [1]. Therefore, GBP3 may act as a target therapeutic gene for different cancers via the abovementioned pathways. In addition, we also conducted the mutation frequency and types of GBP3 in TCGA cancers. Moreover, we found that R151Q/* and K380N were mostly common mutation sites and frequently happened in UCEC, which suggests that GBP3 may be a possible precise target for

UCEC therapy.

5. Conclusions

This work investigates the expression, outcome, immune infiltrates and mutation of GBP3 in multiple cancer types. Our results may give a snapshot of GBP3 in cancer progression and therapy, especially in LGG, LUSC, SKRC and SKCM. Since only third-party tools and TCGA data, more data from other sources is needed for verification in the future.

Acknowledgments

We would like to thank the Scientific Research Startup Foundation for Scholars of Hainan Medical University (XRC2022019), Guangxi Natural Science Foundation (2020GXNSFBA238016, 2022JJA141262), Jingzhou Medical Health Science and Technology Project (2022HC36), Natural Science Research Talent Project of Hainan Medical University (Grant JBGS202101), Project for Functional Materials and Molecular Imaging Science Innovation Group of Hainan Medical University and Nanhai Young-Talent Program of Hainan (20202007) for supporting this study.

Conflict of interest

The authors declare no conflict of interest in this study.

Abbreviations

ACC: Adrenocortical Carcinoma; BLCA: Bladder Urothelial Carcinoma; BRCA: Breast Invasive Carcinoma; CAFs: Cancer-associated fibroblasts; CESE: Cervical Squamous Cell Carcinoma; CHOL: Cholangiocarcinoma; COAD: Colorectal Adenocarcinoma; DLBC: Diffuse Large B-Cell Lymphoma; ESCA: Esophageal Adenocarcinoma; GBM: Glioblastoma Multiforme; HNSC: Head and Neck Squamous Cell Carcinoma; KICH: Kidney Chromophobe; KIRC: Kidney Renal Clear Cell Carcinoma; KIRP: Kidney Renal Papillary Cell Carcinoma; LAML: Acute Myeloid Leukemia; LGG: Brain Lower Grade Glioma; LIHC: Liver Hepatocellular Carcinoma; LUAD: Lung Adenocarcinoma; LUSC: Lung Squamous Cell Carcinoma; MESO: Mesothelioma; OV: Ovarian Serous Cystadenocarcinoma; PAAD: Pancreatic Adenocarcinoma; PCPG: Pheochromocytoma and Paraganglioma; PRAD: Prostate Adenocarcinoma; SARC: Sarcoma; SKCM: Skin Cutaneous Melanoma; STAD: Stomach Adenocarcinoma; TGCG: Testicular Germ Cell Tumors; THCA: Thyroid Carcinoma; THYM: Thymoma; UCEC: Uterine Corpus Endometrial Carcinoma; UCS: Uterine Carcinosarcoma; UVM: Uveal Melanoma.

References

1. J. V. Corte-Real, H. M. Baldauf, J. Abrantes, P. J. Esteves, Evolution of the guanylate binding protein (GBP) genes: Emergence of GBP7 genes in primates and further acquisition of a unique GBP3 gene in simians, *Mol. Immunol.*, **132** (2021), 79–81. https://doi.org/10.1016/j.molimm.2021.01.025
2. S. Huang, Q. Meng, A. Maminska, J. D. MacMicking, Cell-autonomous immunity by IFN-induced GBPs in animals and plants, *Curr. Opin. Immunol.*, **60** (2019), 71–80. https://doi.org/10.1016/j.coi.2019.04.017
3. K. Tretina, E. S. Park, A. Maminska, J. D. MacMicking, Interferon-induced guanylate-binding proteins: Guardians of host defense in health and disease, *J. Exp. Med.*, **216** (2019), 482–500. https://doi.org/10.1084/jem.20182031
4. K. Lipnik, E. Naschberger, N. Gonin-Laurent, P. Kodajova, H. Petznek, S. Rungaldier, et al., Interferon gamma-induced human guanylate binding protein 1 inhibits mammary tumor growth in mice, *Mol. Med.*, **16** (2010), 177–187. https://doi.org/10.2119/molmed.2009.00172
5. P. Godoy, C. Cadenas, B. Hellwig, R. Marchan, J. Stewart, R. Reif, et al., Interferon-inducible guanylate binding protein (GBP2) is associated with better prognosis in breast cancer and indicates an efficient T cell response, *Breast Cancer*, **21** (2014), 491–499. https://doi.org/10.1007/s12282-012-0404-8
6. H. Xu, L. Sun, Y. Zheng, S. Yu, J. Ou-Yang, H. Han, et al., GBP3 promotes glioma cell proliferation via SQSTM1/p62-ERK1/2 axis, *Biochem. Biophys. Res. Commun.*, **495** (2018), 446–453. https://doi.org/10.1016/j.bbrc.2017.11.050
7. W. J. Chen, L. Xiong, L. Yang, L. J. Yang, L. Li, L. Huang, et al., Long Non-Coding RNA LINC01783 Promotes the Progression of Cervical Cancer by Sponging miR-199b-5p to Mediate GBP1 Expression, *Cancer Manag. Res.*, **12** (2020), 363–373. https://doi.org/10.2147/CMAR.S230171
8. L. Cheng, L. Gou, T. Wei, J. Zhang, GBP1 promotes erlotinib resistance via PGK1activated EMT signaling in nonsmall cell lung cancer, *Int. J. Oncol.*, **57** (2020), 858–870. https://doi.org/10.3892/ijo.2020.5086
9. J. Song, Q. Y. Wei, GBP1 promotes non-small cell lung carcinoma malignancy and chemoresistance via activating the Wnt/beta-catenin signaling pathway, *Eur. Rev. Med. Pharmacol. Sci.*, **24** (2020), 5465–5472. https://doi.org/10.26355/eurrev_202005_21331
10. X. Ji, H. Zhu, X. Dai, Y. Xi, Y. Sheng, C. Gao, et al., Overexpression of GBP1 predicts poor prognosis and promotes tumor growth in human glioblastoma multiforme, *Cancer Biomark.*, **25** (2019), 275–290. https://doi.org/10.3233/CBM-171177
11. J. Zhao, X. Li, L. Liu, J. Cao, M. A. Goscinski, H. Fan, et al., Oncogenic Role of Guanylate Binding Protein 1 in Human Prostate Cancer, *Front. Oncol.*, **9** (2019), 1494. https://doi.org/10.3389/fonc.2019.01494
12. S. Yu, X. Yu, L. Sun, Y. Zheng, L. Chen, H. Xu, et al., GBP2 enhances glioblastoma invasion through Stat3/fibronectin pathway, *Oncogene*, **39** (2020), 5042–5055. https://doi.org/10.1038/s41388-020-1348-7
13. F. Rahvar, M. Salimi, H. Mozdarani, Plasma GBP2 promoter methylation is associated with advanced stages in breast cancer, *Genet. Mol. Biol.*, **43** (2020), 20190230. https://doi.org/10.1590/1678-4685-GMB-2019-0230

14. J. Zhang, Y. Zhang, W. Wu, F. Wang, X. Liu, G. Shui, et al., Guanylate-binding protein 2 regulates Drp1-mediated mitochondrial fission to suppress breast cancer cell invasion, *Cell Death Dis.*, **8** (2017), 3151. https://doi.org/10.1038/cddis.2017.559
15. B. Liu, R. Huang, T. Fu, P. He, C. Du, W. Zhou, et al., GBP2 as a potential prognostic biomarker in pancreatic adenocarcinoma, *PeerJ*, **9** (2021), 11423. https://doi.org/10.7717/peerj.11423
16. J. Wang, H. Min, B. Hu, X. Xue, Y. Liu, Guanylate-binding protein-2 inhibits colorectal cancer cell growth and increases the sensitivity to paclitaxel of paclitaxel-resistant colorectal cancer cells by interfering Wnt signaling, *J. Cell. Biochem.*, **121** (2020), 1250–1259. https://doi.org/10.1002/jcb.29358
17. H. Xu, J. Jin, Y. Chen, G. Wu, H. Zhu, Q. Wang, et al., GBP3 promotes glioblastoma resistance to temozolomide by enhancing DNA damage repair, *Oncogene*, **41** (2022), 3876–3885. https://doi.org/10.1038/s41388-022-02397-5
18. A. Blum, P. Wang, J. C. Zenklusen, SnapShot: TCGA-analyzed tumors, *Cell*, **173** (2018), 530. https://doi.org/10.1016/j.cell.2018.03.059
19. G. T. Consortium, Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans, *Science*, **348** (2015), 648–660. https://doi.org/10.1126/science.1262110
20. T. Wu, Y. Dai, Tumor microenvironment and therapeutic response, *Cancer Lett.*, **387** (2017), 61–68. https://doi.org/10.1016/j.canlet.2016.01.043
21. Z. Wang, L. Fu, J. Zhang, Y. Ge, C. Guo, R. Wang, et al., A comprehensive analysis of potential gastric cancer prognostic biomarker ITGBL1 associated with immune infiltration and epithelial-mesenchymal transition, *Biomed. Eng. Online*, **21** (2022), 30. https://doi.org/10.1186/s12938-022-00998-5
22. T. Jiang, T. Yang, Y. Chen, Y. Miao, Y. Xu, H. Jiang, et al., Emulating interactions between microorganisms and tumor microenvironment to develop cancer theranostics, *Theranostics*, **12** (2022), 2833–2859. https://doi.org/10.7150/thno.70719
23. K. Li, Z. Zhang, Y. Mei, M. Li, Q. Yang, Q. Wu, et al., Targeting the innate immune system with nanoparticles for cancer immunotherapy, *J. Mater. Chem. B*, **10** (2022), 1709–1733. https://doi.org/10.1039/d1tb02818a
24. F. Petitprez, M. Meylan, A. de Reynies, C. Sautes-Fridman, W. H. Fridman, The tumor microenvironment in the response to immune checkpoint blockade therapies, *Front. Immunol.*, **11** (2020), 784. https://doi.org/10.3389/fimmu.2020.00784
25. C. Guo, Z. Luo, D. Ismtula, X. Bi, H. Kong, Y. Wang, et al., TIGIT as a novel prognostic marker for immune infiltration in invasive breast cancer, *Comb. Chem. High Throughput. Screen.*, **26** (2023), 639–651. https://doi.org/10.2174/1386207325666220629162823
26. T. Li, J. Fan, B. Wang, N. Traugh, Q. Chen, J. S. Liu, et al., TIMER: A web server for comprehensive analysis of tumor-infiltrating immune cells, *Cancer Res.*, **77** (2017), 108–110. https://doi.org/10.1158/0008-5472.CAN-17-0307
27. T. Li, J. Fu, Z. Zeng, D. Cohen, J. Li, Q. Chen, et al., TIMER2.0 for analysis of tumor-infiltrating immune cells, *Nucleic Acids Res.*, **48** (2020), 509–514. https://doi.org/10.1093/nar/gkaa407
28. Z. Tang, B. Kang, C. Li, T. Chen, Z. Zhang, GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis, *Nucleic Acids Res.*, **47** (2019), 556–560. https://doi.org/10.1093/nar/gkz430

29. Z. Tang, C. Li, B. Kang, G. Gao, C. Li, Z. Zhang, GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses, *Nucleic Acids Res.*, **45** (2017), 98–102. https://doi.org/10.1093/nar/gkx247
30. D. Szklarczyk, A. L. Gable, K. C. Nastou, D. Lyon, R. Kirsch, S. Pyysalo, et al., The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets, *Nucleic Acids Res.*, **49** (2021), 605–612. https://doi.org/10.1093/nar/gkaa1074
31. G. D. Jr, B.T. Sherman, D. A. Hosack, J. Yang, W. Gao, H. C. Lane, et al., DAVID: database for annotation, visualization, and integrated discovery, *Genome Biol.*, **4** (2003), 3. https://doi.org/10.1186/gb-2003-4-5-p3
32. E. Cerami, J. Gao, U. Dogrusoz, B. E. Gross, S. O. Sumer, B. A. Aksoy, et al., The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data, *Cancer Discov.*, **2** (2012), 401–404. https://doi.org/10.1158/2159-8290.CD-12-0095
33. Q. Wang, X. Wang, Q. Liang, S. Wang, L. Xiwen, F. Pan, et al., Distinct prognostic value of mRNA expression of guanylate-binding protein genes in skin cutaneous melanoma, *Oncol. Lett.*, **15** (2018), 7914–7922. https://doi.org/10.3892/ol.2018.8306
34. S. Feng, D. E. Tuipulotu, A. Pandey, W. Jing, C. Shen, C. Ngo, et al., Pathogen-selective killing by guanylate-binding proteins as a molecular mechanism leading to inflammasome signaling, *Nat. Commun.*, **13** (2022), 4395. https://doi.org/10.1038/s41467-022-32127-0
35. X. Li, D. Song, S. Su, X. He, F. Cao, C. Yang, et al., Critical role of guanylate binding protein 5 in tumor immune microenvironment and predictive value of immunotherapy response, *Front. Genet.*, **13** (2022), 984615. https://doi.org/10.3389/fgene.2022.984615
36. R. Kalluri, The biology and function of fibroblasts in cancer, *Nat. Rev. Cancer*, **16** (2016), 582–98. https://doi.org/10.1038/nrc.2016.73
37. H. Raskov, A. Orhan, J. P. Christensen, I. Gogenur, Cytotoxic CD8(+) T cells in cancer and cancer immunotherapy, *Br. J. Cancer*, **124** (2021), 359–367. https://doi.org/10.1038/s41416-020-01048-4.
38. H. Rodriguez, R. Prados-Rosales, J. L. Lavin, M. Mazzone, J. Anguita, Editorial: Macrophage metabolism and immune responses, *Front. Immunol.*, **11** (2020), 1078. https://doi.org/10.3389/fimmu.2020.01078
39. T. Liu, C. Han, S. Wang, P. Fang, Z. Ma, L. Xu, et al., Cancer-associated fibroblasts: an emerging target of anti-cancer immunotherapy, *J. Hematol. Oncol.*, **12** (2019), 86. https://doi.org/10.1186/s13045-019-0770-1
40. W. Shen, P. A. Yao, W. Li, C. Gu, T. Gao, Y. Cao, et al., Cancer-associated fibroblast-targeted nanodrugs reshape colorectal tumor microenvironments to suppress tumor proliferation, metastasis and improve drug penetration, *J. Mater. Chem. B*, (2022). https://doi.org/10.1039/d2tb02253b
41. B. Farhood, M. Najafi, K. Mortezaee, CD8(+) cytotoxic T lymphocytes in cancer immunotherapy: A review, *J. Cell. Physiol.*, **234** (2019), 8509–8521. https://doi.org/10.1002/jcp.27782
42. A. Mantovani, I. Barajon, C. Garlanda, IL-1 and IL-1 regulatory pathways in cancer progression and therapy, *Immunol. Rev.*, **281** (2018), 57–61. https://doi.org/10.1111/imr.12614
43. L. Ni, J. Lu, Interferon gamma in cancer immunotherapy, *Cancer Med.*, **7** (2018), 4509–4516. https://doi.org/10.1002/cam4.1700

44. D. S. Chen, I. Mellman, Elements of cancer immunity and the cancer-immune set point, *Nature*, **541** (2017), 321–330. https://doi.org/10.1038/nature21349
45. J. Reiser, A. Banerjee, Effector, memory, and dysfunctional CD8(+) T cell fates in the antitumor immune response, *J. Immunol. Res.*, **2016** (2016), 8941260. https://doi.org/10.1155/2016/8941260
46. C. Gerada, K. M. Ryan, Autophagy, the innate immune response and cancer, *Mol. Oncol.*, **14** (2020), 1913–1929. https://doi.org/10.1002/1878-0261.12774
47. N. Karin, Chemokines and cancer: New immune checkpoints for cancer therapy, *Curr. Opin. Immunol.*, **51** (2018), 140–145. https://doi.org/10.1016/j.coi.2018.03.004
48. W. Zheng, D. R. E. Ranoa, X. Huang, Y. Hou, K. Yang, E. C. Poli, et al., RIG-I-like receptor LGP2 is required for tumor control by radiotherapy, *Cancer Res.*, **80** (2020), 5633–5641. https://doi.org/10.1158/0008-5472.CAN-20-2324
49. C. Bourquin, A. Pommier, C. Hotz, Harnessing the immune system to fight cancer with Toll-like receptor and RIG-I-like receptor agonists, *Pharmacol. Res.*, **154** (2020), 104192. https://doi.org/10.1016/j.phrs.2019.03.001
50. T. H. Tran, T. T. P. Tran, D. H. Truong, H. T. Nguyen, T. T. Pham, C. S. Yong, et al., Toll-like receptor-targeted particles: A paradigm to manipulate the tumor microenvironment for cancer immunotherapy, *Acta Biomater.*, **94** (2019), 82–96. https://doi.org/10.1016/j.actbio.2019.05.043
51. P. Liu, Z. Lu, L. Liu, R. Li, Z. Liang, M. Shen, et al., NOD-like receptor signaling in inflammation-associated cancers: From functions to targeted therapies, *Phytomedicine*, **64** (2019), 152925. https://doi.org/10.1016/j.phymed.2019.152925
52. J. Kwon, S. F. Bakhoum, The cytosolic DNA-sensing cGAS-STING pathway in cancer, *Cancer Discov.*, **10** (2020), 26–39. https://doi.org/10.1158/2159-8290.CD-19-0761
53. P. de Ron, S. Dremier, P. Winlow, A. Jenkins, E. Hanon, A. N. da Costa, Correlating behaviour and gene expression endpoints in the dopaminergic system after modafinil administration in mouse, *Eur. Neuropsychopharmacol.*, **26** (2016), 729–740. https://doi.org/10.1016/j.euroneuro.2016.01.010

AIMS Press

©2023 the Author(s), licensee AIMS Press. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0)

Supplementary

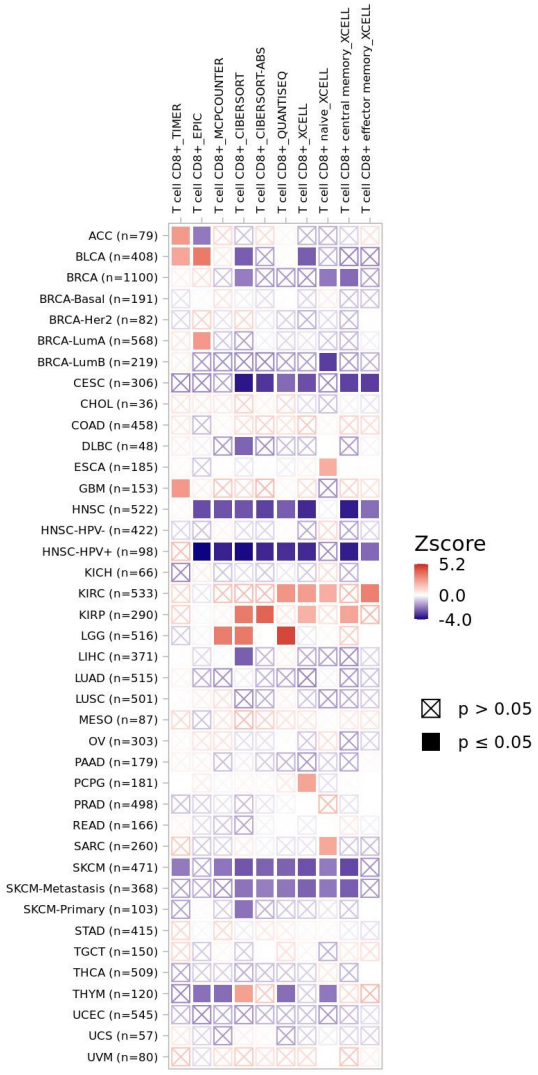

Figure S1. The overall survival (OS) map of GBP3 & CD8+ T cells in TCGA cancer types.

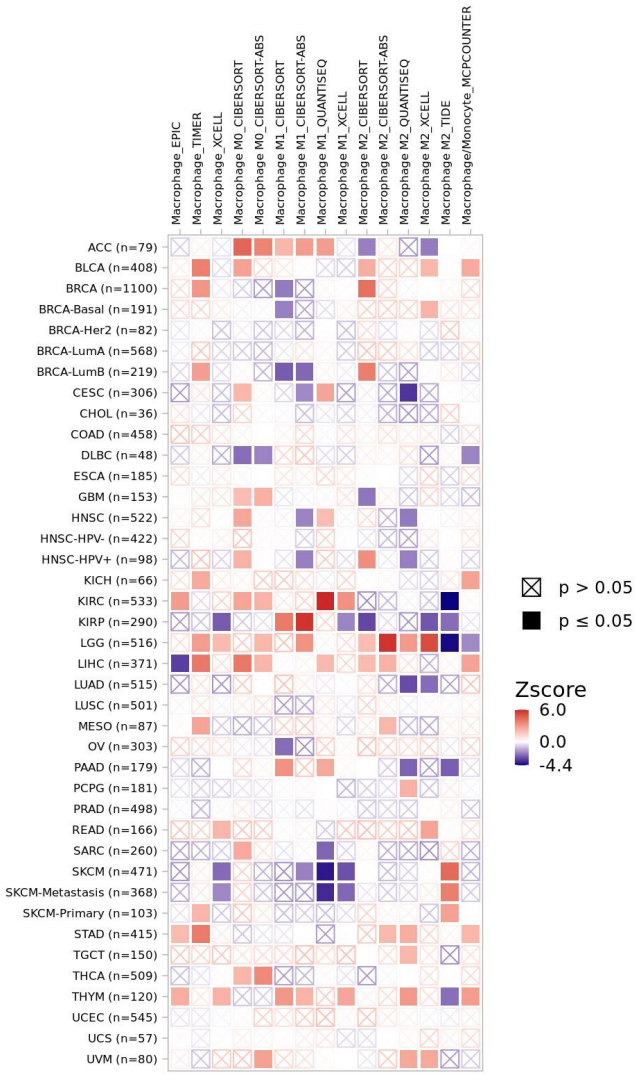

Figure S2. The overall survival (OS) map of GBP3 & macrophage in TCGA cancer types.