Prognostic And Immunological Implications of BTK Expression In Pan-Cancer

Tao Yang

Beijing Shijitan Hospital <https://orcid.org/0000-0003-3127-3944>

Lizheng Hao

Beijing University of Chinese Medicine

Jian Chen

Beijing University of Chinese Medicine

Xueying Zhu

Beijing University of Chinese Medicine

Keyi Sun

Beijing University of Chinese Medicine

Yu Zeng

Beijing University of Chinese Medicine

Yayue Zhang (✉ yayuezhang@126.com)

Beijing University of Chinese Medicine

Zhong Li

Beijing University of Chinese Medicine

Research

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Abstract

Background: Cancer poses a serious threat to human health. Clarifying the potential significance of Bruton's tyrosine kinase (BTK) in cancer has potential clinical value. This study examined the prognostic and immunological value of BTK gene expression in pan-cancer.

Methods: We evaluated the gene expression of BTK in tumor tissues and normal tissues in different cancers. Survival analysis, including Kaplan–Meier analysis and Cox analysis, were performed to explore the prognostic value of BTK for pan-cancer based on survival data from The Cancer Genome Atlas (TCGA) database. Spearman's method was conducted to analyze the interrelation between BTK gene expression and tumor mutational burden (TMB) and microsatellite instability (MSI). We explored the association of BTK expression with the tumor microenvironment based on Estimation of STromal and Immune cells in MAlignant Tumour tissues using Expression data (ESTIMATE) algorithm. Co-expression analysis of BTK expression and immune-related genes was performed. We used Gene Set Enrichment Analysis (GSEA) to examine the molecular mechanisms and pathways of BTK in pan-cancer.

Results: High *BTK* expression in cervical squamous cell carcinoma and endocervical adenocarcinoma (CSEC), head and neck squamous cell carcinoma (HNSC), lung adenocarcinoma (LUAD) and skin cutaneous melanoma (SKCM) was positively correlated with patient prognosis, while high expression of *BTK* in lymphoid neoplasm diffuse large B cell lymphoma (DLBC), brain lower grade glioma (LGG) and esophageal carcinoma (ESCA) corresponded with a worse prognosis. Cox analysis showed that *BTK* was closely associated to the prognosis of HNSC, LGG, SKCM and LUAD. *BTK* expression was correlated with clinical stage, TMB and MSI of 10 types of tumors. In HNSC, LGG, LUAD and SKCM, the expression of BTK was positive correlated with immune and stromal scores.

Conclusion: *BTK* expression can act as a prognostic factor in various cancers, especially in HNSC, LGG, LUAD and SKCM, and this may be from its close association with TMB, MSI and immune cell infiltration.

Introduction

Cancer is a common disease and the leading cause of death in most countries[1]. An estimated 19.3 million newly diagnosed cases of cancer and approximately 10.0 million cancer-related deaths were reported in 2021 May [1]. Studies have revealed that many types of cancer exhibit common genomic characteristics and treatment respond well to corresponding inhibitors[2]. These results have led to the increase in research involving pan-cancer. With the development of next-generation sequencing technology, common mutated genes and other abnormal gene phenotypes in cancers can be identified through pan-cancer model systems and projects, which provide new directions for cancer treatment and prognosis[3].

BTK is a cytoplasmic protein encoded by the *BTK* gene that plays a very important role in the development of B cells. *BTK* mutations cause X-linked agammaglobulinemia, an immunodeficiency

characterized by the inability to produce mature B lymphocytes that is associated with failure of rearrangement of Ig heavy chains[4].

Over the last decade, BTK inhibitors have demonstrated efficacy and tolerability in the treatment of B cell malignancies (especially in relapsed or refractory cases[5]), including in chronic lymphocytic leukemia [6, 7], diffuse large B cell lymphoma [8], mantle cell lymphoma[9], multiple myeloma[10], and follicular lymphoma[11]. Ibrutinib is the first covalent irreversible inhibitor of BTK to be used in the treatment of B-cell cancers[12], and it showed anticancer activity in relapsed/refractory multiple myeloma patients when combined with carfilzomib/dexamethasone[13–16]. Three additional agents targeting BTK have been developed: zanubrutinib (BGB-3111)[17], acalabrutinib (ACP-196)[18], and tirabrutinib (ONO/GS-4059) [19]. In addition to the demonstrated effectiveness of BTK inhibitors in hematological malignancies, several studies have revealed positive therapeutic results in solid tumors[20, 21], such as in non-small cell lung cancer (NSCLC)[22], breast cancer[23], as well as pancreas ductal adenocarcinoma (PDAC)[24]. The potential mechanisms of BTK inhibitors in solid tumors may be related to the dual spectrum of activity of these inhibitors on additional binding enzymes (such as the ERBB family)[25] and the importance of *BTK* in the tumor microenvironment (TME). Several studies showed that BTK is highly expressed on tumor-associated macrophages, the main component of the TME, and promotes tumor progression, angiogenesis and immunosuppression[26, 27]. Therefore, investigating *BTK* expression in malignant tumors and exploring the connection between BTK and TME in pan-cancer may provide significant findings for the development of clinical therapeutics and predicting the prognosis of tumors.

In this work, we studied the expression of *BTK* in 33 different cancers and performed survival analysis of the prognostic power of *BTK* in various cancer types using TCGA database. On the strength of the relationship between the expression of *BTK* and the degree of immune cell infiltration, as well as the immune score, we found that *BTK* may be a potential prognostic factor related to TME status in pan-cancer.

Results

BTK mRNA expression levels in Pan-cancer

The workflow for the analyses performed in this study is shown in Fig. 1. To explore the expression of BTK in pan-cancer, we first evaluated the expression level of BTK mRNA in different cancer types using the ONCOMINE database. BTK mRNA showed higher expression in breast cancer compared with normal tissues, but lower expression in lung cancer, colorectal cancer and sarcoma compared with normal tissues (Fig. 2A). The results in kidney cancer and leukemia were contradictory. One dataset demonstrated that BTK mRNA expression was higher in kidney cancer compared with normal tissues, while the other two datasets showed that BTK mRNA was expressed at lower levels in kidney cancer compared with the normal group. For leukemia, the results from two datasets indicated that BTK expression was higher in leukemia than in normal tissues, while three datasets indicated that BTK expression was lower in leukemia than in normal tissues.

We further analyzed the RNA sequencing data in TCGA database and obtained 11057 mRNA expression profiles from 33 types of cancer. The mRNA expression profiles consisted of 10327 tumor samples and 730 normal samples. BTK mRNA was expressed higher in breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), glioblastoma multiforme (GBM), HNSC, kidney chromophobe (KICH), renal transparent cell carcinoma (KIRC), renal papillary cell carcinoma (KIRP) and rectum adenocarcinoma (READ) than the corresponding normal tissues (Fig. 2B). Lower BTK mRNA expression was found in bladder urothelial carcinoma (BLCA), colon adenocarcinoma (COAD), LUAD, lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma (PAAD) and READ compared with corresponding normal tissues. We further explored changes in BTK gene expression based on the cBio-Portal database. Among the 10953 patients of 32 cancer types in the TCGA, 218 patients had altered BTK gene expression (about 2%). The highest rate of change was related to gene mutation, followed by gene amplification. Of all cancers, uterine cancer showed the most frequent changes (Fig. 2C).

Correlation analysis of BTK expression and clinical stage of Pan-cancer

The expression of BTK was significantly related with the clinical stage in ten types of cancers. Higher BTK expression level was observed in stage I adrenocortical carcinoma (ACC) patients than stage III patients (Fig. 3A). In BLCA, the BTK expression of stage III–IV patients was higher than in stage II patients (Fig. 3B). In ESCA, BTK was expressed at lower levels in patients with stage I but highly expressed in patients with stage III (Fig. 3C). In KICH, BTK expression was higher in patients with stage IV compared with stage II (Fig. 3D). In KIRP, BTK expression in stage I patients was the highest and markedly higher than that in patients with stage III–IV (Fig. 3E). A similar trend was observed with LUAD (Fig. 3F). In SKCM, stage II patients showed the lowest expression of BTK, and this was significantly lower than that in stage I and stage III patients (Fig. 3G). In stomach adenocarcinoma (STAD) patients, BTK was the lowest in stage I, and this was significantly lower than that in stage II–IV patients (Fig. 3H). In testicular germ cell tumors (TGCT), BTK expression was higher in patients with stage I compared with patients with stage III (Fig. 3I). In THCA, BTK was supremely expressed in the stage I and stage III but less in stage II patients (Fig. 3J).

Association of BTK expression with TMB and MSI in Pan-cancer

TMB, the number of somatic mutations per megabase of sequenced DNA, has emerged as a biomarker for cancer patients receiving immune checkpoint inhibitor (ICI) treatment. TMB is closely related to the response rate of ICIs and survival across multiple tumor types; it may bring supplementary guidance in filtering patients for ICI-based therapies [28]. We next explored the association between TMB and the expression of BTK in pan-cancer. We found that the expression of BTK was significantly correlated with TMB in 13 cancer types. TMB was positively associated with the expression of BTK in two types of cancer: COAD and uterine corpus endometrial carcinoma (UCEC). In contrast, the expression of BTK was

negatively associated with TMB in 11 cancer types: CHOL, PAAD, ACC, STAD, LUAD, TGCT, liver hepatocellular carcinoma (LIHC), HNSC, THCA, LUSC and BRCA (Fig. 4A, Table 1).

MSI is a high hypermutable phenotype in cancer caused by the deficiency of DNA mismatch repair activity [29]. In colorectal cancer, approximately 15% of patients are classified as MSI-high, and ICI treatment showed optimal efficacy in this patient group [30]. We next evaluated whether BTK expression was related to MSI of various cancer types. Our results showed that the expression of BTK was significantly correlated with MSI in 12 cancers. BTK expression showed a negative correlation with MSI in 11 cancer types, including LUSC, STAD, HNSC, TGCT, SKCM, OV, ESCA, LIHC, KIRP, DLBC and PAAD (Fig. 4B, Table 1). We observed a positive association between BTK expression and MSI only in COAD.

Table 1
Correlations between TMB, MSI and BTK gene expression in pan-cancer.

TMB			MSI		
Cancer Type	coefficient	p-Value	Cancer Type	coefficient	p-Value
LUAD	-0.233559024	1.1669E-07	LUSC	-0.295996743	1.99525E-11
STAD	-0.252360773	9.37839E-07	STAD	-0.304975186	1.71996E-09
PAAD	-0.343149701	1.6079E-05	HNSC	-0.20923057	2.5997E-06
HNSC	-0.191362068	1.92537E-05	TGCT	-0.370937589	2.9706E-06
THCA	-0.171055275	0.000160954	COAD	0.184501972	0.000125904
LIHC	-0.196693708	0.000176469	SKCM	-0.157158335	0.000644909
LUSC	-0.156413239	0.000524762	OV	-0.175548247	0.003678745
TGCT	-0.207066818	0.012455148	ESCA	-0.210899257	0.007429581
CHOL	-0.377302743	0.023298983	LIHC	-0.138457869	0.007733121
ACC	-0.252537009	0.024745746	KIRP	-0.129546164	0.028771627
BRCA	-0.070932671	0.026850665	DLBC	-0.313312526	0.030128526
COAD	0.104770378	0.03715542	PAAD	-0.156641944	0.038440666
UCEC	0.08932575	0.040763955			

Multifaceted prognostic value of BTK in Pan-cancer

To access the prognostic value of BTK for pan-cancer, the Kaplan–Meier method was used to evaluate the impact of BTK expression on overall survival (OS), disease-specific survival (DSS), progression-free

interval (PFI) and disease-free interval (DFI) of 33 cancer types. BTK expression was correlated with seven types of cancer, including CESC, DLBC, ESCA, HNSC, LGG, LUAD and SKCM (Fig. 5). Among these seven cancer types, BTK played an unfavorable role in three cancer types, including DLBC ($n = 47$, OS: $P = 0.007$; PFI: $P = 0.041$), LGG ($n = 524$, OS: $P < 0.001$; DSS: $P < 0.001$; PFI: $P < 0.001$) and ESCA ($n = 161$, DFI: $P < 0.024$). In contrast, BTK had a positive role in four cancer types, including CESC ($n = 293$, DSS: $P = 0.008$; PFI: $P = 0.013$), HNSC ($n = 524$, OS: $P = 0.044$; DSS: $P = 0.028$), LUAD ($n = 513$, OS: $P < 0.001$; DSS: $P = 0.008$; PFI: $P = 0.029$) and SKCM ($n = 457$, OS: $P = 0.001$; DSS: $P < 0.001$; PFI: $P = 0.018$).

We also investigated the correlations between BTK and survival by Cox analysis; the survival analysis also included OS, DSS, DFI, and PFI. These results showed that BTK played a detrimental role in DLBC (OS: HR = 5.857, [95% CI (1.825–18.800)], $P = 0.003$) (Fig. 6A), acute myeloid leukemia (LAML) (OS: HR = 1.834, [95% CI (1.058–3.180)], $P = 0.031$) (Fig. 6A), LGG (OS: HR = 1.883, [95% CI (1.457–2.435)], $P < 0.001$; DSS: HR = 1.971, [95% CI (1.499–2.592)], $P < 0.001$; PFI: HR = 1.664, [95% CI (1.354–2.045)], $P < 0.001$) (Fig. 6A–C), and prostate adenocarcinoma (PRAD) (PFI: HR = 1.784, [95% CI (1.139–2.793)], $P = 0.011$) (Fig. 6C). BTK expression was a protective prognostic factor in CESC (OS: HR = 0.563, [95% CI (0.352–0.899)], $P = 0.016$; DSS: HR = 0.424, [95% CI (0.239–0.751)], $P = 0.003$; PFI: HR = 0.470, [95% CI (0.289–0.763)], $P = 0.002$; DFI: HR = 0.442 [95% CI (0.202–0.970)], $P = 0.042$) (Fig. 6A–D), HNSC (OS: HR = 0.720, [95% CI (0.567–0.916)], $P = 0.007$; DSS: HR = 0.617, [95% CI (0.450–0.845)], $P = 0.003$; PFI: HR = 0.740, [95% CI (0.575–0.951)], $P = 0.019$) (Fig. 6A–C), LUAD (OS: HR = 0.726, [95% CI (0.602–0.874)], $P < 0.001$; DSS: HR = 0.776, [95% CI (0.633–0.952)], $P = 0.015$; PFI: HR = 0.831, [95% CI (0.712–0.970)], $P = 0.019$; DFI: HR = 0.754 [95% CI (0.592–0.960)], $P = 0.022$) (Fig. 6A–D), sarcoma (SARC) (OS: HR = 0.748, [95% CI (0.580–0.965)], $P = 0.025$) (Fig. 6A), SKCM (OS: HR = 0.780, [95% CI (0.672–0.906)], $P = 0.001$; DSS: HR = 0.754, [95% CI (0.640–0.889)], $P < 0.001$) (Fig. 6A–B), thyroid carcinoma (THCA) (DSS: HR = 0.123, [95% CI (0.018–0.854)], $P = 0.034$) (Fig. 6B), CHOL (PFI: HR = 0.387, [95% CI (0.161–0.929)], $P = 0.034$) (Fig. 6C) and BLCA (DFI: HR = 0.529, [95% CI (0.282–0.989)], $P = 0.046$) (Fig. 6D).

Effect of BTK expression on TME in Pan-cancer

Cancer advancement and progression are closely related to the TME, which also plays an important role in the therapeutic efficiency of ICIs [31, 32]. Given our results demonstrating the prognostic value of BTK in multiple cancers, we further explored the interconnection between the expression of BTK and the TME in pan-cancer. This assessment is especially important for HNSC, LGG, LUAD and SKCM, as BTK gene expression was closely related to both OS and DSS in these four cancer types (comprehensively considered about results of Kaplan-Meier and Cox analysis). We used the ESTIMATE algorithm to estimate stromal cell and immune cell scores in HNSC, LGG, LUAD and SKCM. BTK expression showed a dramatically positive association with both immune and stromal scores in HNSC, LGG, LUAD and SKCM, indicating that with the upregulation of BTK expression, the content of stromal cells and immune cells also increases (Fig. 7).

Correlation between BTK expression and immune cell infiltration in Pan-cancer

To explore the association between immune cell infiltration and the expression of BTK in the above four types of cancer (HNSC, LGG, LUAD and SKCM), we used the CIBERSORT algorithm to estimate the content of 22 types of immune cells in tumor tissue. The expression of BTK was markedly related to the levels of some infiltrating immune cells in HNSC (12 cell types), LGG (4 cell types), LUAD (9 cell types) and SKCM (9 cell types). Specifically, BTK expression was positively correlated with the infiltration degree of memory B cells in LUAD (Fig. 8A). We also observed significant correlations between BTK expression and the infiltration degrees of naive B cells in HNSC, LUAD and SKCM, with a positive relation in HNSC and SKCM and a negative relation in LUAD (Fig. 8B). BTK expression was negatively correlated to the levels of infiltrating active dendritic cells in HNSC and LUAD (Fig. 8C). The infiltration degrees of resting dendritic cells increased with the increase of BTK expression in LUAD (Fig. 8D). In LGG, a positive relationship between BTK expression and infiltrating eosinophils was detected (Fig. 8E). The infiltration degree of M0 macrophages was negatively associated with BTK expression in HNSC, LUAD and SKCM (Fig. 8F). For M1 macrophages, the infiltration degrees increased with the increase of BTK expression only in SKCM (Fig. 8G). The infiltration levels of monocytes were positively correlated with the expression of BTK in the four indicated cancer types (Fig. 8H). BTK expression was positively correlated with the infiltration degrees of M1 macrophages in HNSC and LUAD, but negatively related in SKCM (Fig. 8I). The infiltrating levels of activated mast cells were negatively correlated with BTK expression in HNSC, LGG and SKCM (Fig. 8J). BTK expression was positively connected with the degrees of infiltrating resting mast cells in HNSC and LUAD (Fig. 8K). The degrees of infiltrating resting NK cells were adversely related to the expression of BTK in HNSC (Fig. 8L). For plasma cells, the infiltrating levels were positively correlated with BTK expression in SKCM, but negatively correlated with BTK expression in LUAD (Fig. 8M). Moreover, BTK expression was positively correlated with the levels of infiltrating activated CD4 memory T cells in HNSC and SKCM (Fig. 8N). The levels of infiltrating resting CD4 memory T cells were negatively related to BTK expression in HNSC, but positively correlated with BTK expression in LGG (Fig. 8O). The expression of BTK was positively correlated with the infiltration degrees of regulatory T cells in HNSC (Fig. 8P). For CD8 T cells, their infiltration degrees increased with the increase of BTK expression in HNSC and SKCM (Fig. 8Q). Overall, these results suggest that BTK expression is closely related to immune cell infiltration in four different cancer types in various ways, which could partly explain differences in patient survival.

Co-expression of BTK with immune checkpoint genes and GSEA in Pan-cancer

Immune checkpoints play a crucial role in tumor immune escape and the formation of the TME. To understand the correlations of BTK expression and immune checkpoints, we conducted gene co-expression analyses between BTK and immune-related genes. Our results showed that BTK was positively associated with most immune checkpoint genes in HNSC, LGG, LUAD and SKCM, such as BTLA, CD200, TNFRSF14, NRP1, LAIR1 and TNFSF4 genes (Fig. 9A).

Next, we performed GSEA analysis to explore the effects of BTK gene expression on molecular mechanisms and pathways in selected cancers. GO functional annotation results demonstrated that BTK

positively regulated biological process of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains, antigen receptor mediated signaling pathway, B cell activation, B cell mediated immunity, humoral immune response, immune response regulating cell surface receptor signaling pathway, leukocyte migration, lymphocyte differentiation, negative regulation of immune system process, positive regulation of cytokine production, positive regulation of establishment of protein localization, regulation of immune effector process and others (Fig. 9B). KEGG pathway analysis showed that BTK could up-regulate several essential tumor immune related pathways, including cytokine-cytokine receptor interaction, chemokine signaling pathway, T cell receptor signaling pathway, primary immunodeficiency, natural killer cell mediated cytotoxicity, JAK-STAT signaling pathway, systemic lupus erythematosus, cell adhesion molecules cams and others (Fig. 9C).

Discussion

BTK is an important kinase that plays a crucial role in B cell proliferation and differentiation[33, 34]. Studies have demonstrated critical functions for BTK in the development of many B cell malignancies and it also plays a major part in the TME [35, 36]. While *BTK* has been identified as an oncogene in B-cell malignancies, some reports have suggested *BTK* as a tumor suppressor gene in some epithelial tumors and an important member of the p53 and p73 injury response[37, 38]. Preclinical data have shown that *BTK* is present in the subtypes of some tumors as well as cells that contribute to TCM, such as macrophages[39, 40], dendritic cells[41, 42], and endothelial cells[20, 43]. The expression of *BTK* in B cells and macrophages can be used as a target to regulate the immunosuppressive phenotypes[44]. Ibrutinib, the main *BTK* inhibitor, was approved by the FDA for the treatment of hematological diseases in adults, including mantle cell lymphoma, small lymphocytic lymphoma, chronic lymphocytic leukemia, marginal zone lymphoma, Waldenstrom's macroglobulinemia, and chronic graft versus host disease[45], and it has shown initial indication for use in solid tumors. Therefore, further exploration of the role of *BTK* in cancer is of great importance.

In this study, we analyzed *BTK* mRNA expression in 33 different cancers in ONCOMINE and found that *BTK* was expressed at higher levels in breast cancer compared with normal tissue, which is consistent with the study by Kennedy et al.[46], while low *BTK* mRNA expressions were found in colorectal cancer, lung cancer and sarcoma. One study showed that *p65BTK* is highly expressed in CRC, and high expression represented an adverse prognostic factor[47]. We also observed contradictory results in kidney cancer and leukemia. *BTK* expression is absent in infants and young children[48] but has not been demonstrated in adults. No studies have reported the expression of *BTK* in lung cancer, kidney cancer and sarcoma, which may indicate an innovative result of our work.

Our study demonstrated that *BTK* expression may be connected to MSI in 12 tumor types and TMB in 13 tumor types, further suggesting a potential prognostic relationship between *BTK* and tumors. We studied the expression of *BTK* in pan-cancer and adjacent tissues or normal tissues in data from 33 cancer types from TCGA database. We observed elevated expression of *BTK* in BRCA[49], CHOL, GBM[50], HNSC[51], KICH, KIRC[52] and KIRP, while lower expression was observed in BLCA, COAD, LUAD, LUSC, PAAD and

READ. We also found that *BTK* expression was associated with the clinical stage of 10 types of cancers, including ACC, BLCA, ESCA, KICH, KIRP, LUAD, SKCM, STAD, TGCT and THCA. To further analyze the relation of *BTK* and prognosis, we conducted survival analysis with survival data from TCGA database and Kaplan–Meier analyses. High expression of *BTK* in CSEC, HNSC, LUAD and SKCM was associated with a better prognosis of patients, while a high expression of *BTK* in DLBC, LGG and ESCA was associated with a worse prognosis of patients. Combined with the results of Cox analysis, the prognostic value of *BTK* for HNSC, LGG, LUAD and SKCM was more reliable.

We then used the ESTIMATE method to evaluate the stromal cell score and immune cell score of the pan-cancer samples, and the correlation between *BTK* expression and TCM score of the two tumors was analyzed. We focused on HNSC, LGG, LUAD and SKCM and found that the expression of *BTK* was positively correlated with TCM score of HNSC and SKCM. The infiltration of 22 kinds of immune cells in pan-carcinoma tissues was evaluated by the CIBERSORT method, and the relationship between *BTK* and the degree of infiltration of immune cells was analyzed. We focused on HNSC, LGG, LUAD and SKCM in this analysis. *BTK* expression was correlated with memory B cells, active dendritic cells, naive B cells, resting dendritic cells, M0 macrophages and active dendritic cells, while M1 macrophages, monocytes, M1 macrophages, resting mast cells, NK cells, plasma cells, activated CD4 memory T cells, and resting CD4 memory T cells, regulatory T cells, and CD8 T cells are correlated. We found that *BTK* was co-expressed with 46 immune-associated genes in HNSC, LGG, LUAD and SKCM, and our findings indicate that *BTK* may be involved in the positive regulation of some immune functions and pathways.

A previous study in NSCLC showed that *BTK* inhibits proliferation, invasion and migration of tumor cells induced by cinnamaldehyde, which is extracted from cinnamon[53]. Moreover, another report showed that *BTK*-tyrosine kinase inhibitors may re-sensitize drug-resistant NSCLC to standard-of-care chemotherapy (cisplatin, gemcitabine and pemetrexed)[54]. In LUAD, *BTK* expression was negatively correlated with clinical stage and distant metastasis and positively correlated with patient survival[27]. Yue et al.[55] reported that *BTK* can act as an independent prognostic factor for LUAD, which is consistent with our study results. In HNSC, *BTK* expression was positively related with nasopharyngeal carcinoma B cells, memory B cells and TME score, which may indicate some prognostic value. A recent study reported that ibrutinib inhibited the cancer stem cell–like phenotype of OSCC cells by abnormal regulation of *BTK/CD133* signaling. In SKCM, *BTK* was reported to be connected with the progression/metastasis of melanoma[56]. No study has examined the relation of *BTK* with the prognosis of LGG. We believe that the results of this study can provide certain prognostic value for the four tumor diseases.

Conclusions

Our results demonstrated that *BTK* is associated with immune infiltration and influences the prognosis of pan-cancer. The prognostic value of *BTK* may be more profound for HNSC, LGG, LUAD and SKCM and may be related to the different infiltration patterns of different immune cells in TME. In addition, *BTK* can work as a key biomarker for the prognosis of pan-cancer. These discoveries may furnish insights into immune-related anti-cancer strategies, including modulating the invasion pattern of the TME.

Materials And Methods

BTK expression in human cancers

We first assessed the expression of BTK in cancer tissues and corresponding normal tissue using ONCOMINE databases (<https://www.oncomine.org>). We downloaded RNA expression data, somatic mutation data (SNPs and small indels), clinical staging and survival data for 33 kinds of cancers from the University of California, Santa Cruz Xena (UCSC Xena) database (<https://xenabrowser.net>). We extracted BTK expression data from TCGA database and used Wilcox test to analyze the differences of BTK expression in normal tissue and cancer tissues, setting FDR <0.05 as the cut-off value. R package “ggpubr” was used to draw boxplots. In addition, the changes of BTK gene expression in different cancer types were calculated using the Cbioportal database (<http://www.cbioportal.org/>), which extracted from 32 TCGA research outcome involving a total of 10953 tumor patients.

Multifaceted survival and clinical stage correlativity test based on the levels of BTK gene expression in human cancer

Survival data for all tumor samples were obtained from TCGA database, and the correlations between pan-cancer survival and the expression of BTK were further analyzed. Survival analysis was conducted for OS, DFI, DSS and PFI. OS reflected the time from the date of first diagnosis to the date of death. DFI indicated the period of time between the date of diagnosis and the date of the initial new cancer progression after the patient was disease-free. DSS referred to the time from the initial diagnosis to death caused by the diagnosed cancer. These new tumor progression events usually include local recurrence, distant metastasis, occurrence of new primary tumor or death caused by the same malignant tumor. PFI indicated the time from the date of diagnosis to the occurrence of the first new tumor event, which includes tumor progression, locoregional recurrence, broad metastasis, novel primary cancer or death due to cancer. Kaplan–Meier method was used to estimate the survival rate, and log-rank test was used to compare the survival differences. $P < 0.05$ was considered to indicate statistical significance.

Patients were split into high-risk and low-risk groups according to the median expression level of BTK in various types of cancer. R-package “survivor” and “survival” were used to display the survival curve according to the high and low risk value. We also performed Cox analysis to investigate the association between BTK expression and the prognosis of different cancers. The results of Cox analysis were displayed using R package “forestplot” to plot forest plots. The correlation between clinical stage and BTK expression level was analyzed using R package “limma” and “ggpubr.”

Association between BTK expression and TMB and MSI in Pan-cancer

TMB was calculated based on the somatic mutation data in TCGA database. We used Perl script to calculate the TMB of 33 kinds of cancers and corrected it to the number of mutated bases per 1 million bases (divided by the total length of exons). The MSI score data were also from TCGA database. Spearman’s method was used to analyze the correlation between BTK gene expression and TMB or MSI, and the R-package “fmsb” was used to visualize the correlation between BTK and TMB and MSI.

Correlation of BTK expression with the immunologic microenvironment and immune cell infiltration in endemic carcinoma

To explore the relationship between BTK and the tumor immune microenvironment and immune cell infiltration, we first estimated the immune and stromal cell score using R package “estimate” and “limma” to analyze the relationship between BTK expression and infiltrating immune cells and stromal cells in pan-cancer [57, 58]. We further used CIBERSORT to estimate the infiltration levels of 22 types of immune cells in 33 cancers [59]. R package “ggplot2,” “ggpubr” and “ggextra” were used to analyze the correlation between BTK expression and tumor immune microenvironment and immune cell infiltration ($P < 0.001$ as a cut-off value).

Co-expression analysis and BTK pathway analysis of immunoreacted genes in Pan-cancer

We extracted the expression data of 47 immune-related genes (including immune checkpoint- and immunotherapy-related genes) in pan-cancer [60, 61] and analyzed the co-expression of BTK and the 47 tumor immune-associated genes with R package “limma.” Correlation heat map was performed with R package “reformae2” and “rcolor brewer.” In addition, Gene Set Enrichment Analysis (GSEA) was conducted found on the BTK expression. We downloaded Gene Ontology (GO) and Kyoto Encyclopedia of genes and genomes (KEGG) data from GSEA database. R packages “limma,” “org.hs.eg.db,” “clusterprofiler” and “enrichplot” were used to annotate the go function of BTK and enrich KEGG pathway [62].

Abbreviations

Table 2. Abbreviation list

Abbreviations	Full name
ACC	Adrenocortical carcinoma
BLCA	Bladder urothelial carcinoma
BRCA	Breast invasive carcinoma
BTK	Bruton's tyrosine kinase
CHOL	Cholangiocarcinoma
COAD	Colon adenocarcinoma
CSEC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
DFI	Disease-free interval
DLBC	Lymphoid neoplasm diffuse large B cell lymphoma
DSS	Disease-specific survival
ESCA	Esophageal carcinoma
ESTIMATE	Estimation of STromal and Immune cells in MAlignant Tumour tissues using Expression data
GBM	Glioblastoma multiforme
GSEA	Gene Set Enrichment Analysis
HNSC	Head and neck squamous cell carcinoma
ICI	Immune checkpoint inhibitor
KICH	Kidney chromophobe
KIRC	Kidney chromophobe
KIRP	Renal papillary cell carcinoma
LGG	Brain lower grade glioma
LIHC	Liver hepatocellular carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
MSI	Microsatellite instability
NSCLC	Non-small cell lung cancer
OS	Overall survival
PAAD	Pancreatic adenocarcinoma
PDAC	Pancreas ductal adenocarcinoma

PFI	Progression-free interval
READ	Rectum adenocarcinoma
SKCM	Skin cutaneous melanoma
STAD	Stomach adenocarcinoma
TCGA	The Cancer Genome Atlas
TGCT	Testicular germ cell tumors
TMB	Tumor mutation burden
TME	Tumor microenvironment
UCEC	Uterine corpus endometrial carcinoma

Declarations

Ethics approval and consent to participate

Not necessary.

Consent for publication

Not applicable.

Availability of data and materials

The data analyzed in this study were all obtained from public databases. All data can be found here: <https://www.oncomine.org>, <https://xenabrowser.net>, and <http://www.cbioportal.org/>.

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

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Authors' Contributions

TY, LH and JC have contributed equally to this work. ZL, YZ, and TY designed the study. TY, LH, and JC extracted the information from the databases. TY and NS analyzed the data. XZ, KS and YZ supervised the entire study. LH and JC wrote the manuscript. All authors revised the manuscript.

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Figures

Figure 1

Workflow of the study analysis.

Figure 3

Correlation of BTK gene expression with AJCC stage of different cancer types. (A) ACC, (B) BLCA, (C) ESCA, (D) KICH, (E) KIRP, (F) LUAD, (G) SKCM, (H) STAD, (I) TGCT, and (J) THCA.

Figure 4

Correlation between TMB, MSI and BTK gene expression in pan-cancer. (A) The correlations between TMB and BTK expression of various cancer types are shown in the radar chart. The correlation coefficient is represented by the red curve, and the blue numbers serve as the range of correlation coefficient. (B) The correlations between BTK expression and MSI of pan-cancer are shown in the radar chart. The blue curve indicates the correlation coefficient, and the green numbers show the range of the correlation coefficient. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 5

Survival curves of BTK high and low expression patient groups in different cancers. (A) OS of DLBC (n=47), HNSC (n=501), LGG (n=524), LUAD (n=513) and SKCM (n=457). (B) DSS of CESC (n=293), HNSC (n=501), LGG (n=524), LUAD (n=513) and SKCM (n=457). (C) PFI of CESC (n=293), DLBC (n=47), LGG (n=524), LUAD (n=513) and SKCM (n=457). (D) DFI of ESCA (n=161).

Figure 6

Forest maps of Cox analysis of BTK mRNA expression and survival of various cancer types. (A) OS, (B) DSS, (C) PFI and (D) DFI. The red squares represent the HR value, and the black lines represent 95% CI of HR. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 7

Correlation of ESTIMATE score with BTK gene expression in HNSC, LGG, LUAD and SKCM. (A) Scatterplots for the correlation of BTK gene expression with immune score. (B) Scatterplots for the correlation of BTK gene expression with stromal score.

Figure 8

Association between BTK gene expression and infiltrating degrees of various immune cells in pan-cancer. BTK expression showed remarkable association with infiltrating degrees of memory B-cells in LUAD (A), naive B cells in HNSC, LUAD and SKCM (B), activated dendritic cells in HNSC and LUAD (C), activated dendritic cells in LUAD (D), eosinophils in LGG (E), M0 macrophages in HNSC, LUAD and SKCM (F), M1 macrophages in SKCM (G), monocytes in HNSC, LGG, LUAD and SKCM (H), M2 macrophages in HNSC, LUAD and SKCM (I), activated mast cells in HNSC, LGG and SKCM (J), resting mast cells in HNSC and LUAD (K), resting NK cells in HNSC (L), plasma cells in LUAD and SKCM (M), activated memory CD4 T cells in HNSC and SKCM (N), resting memory CD4 T cells in HNSC and LGG (O), regulatory T cells in HNSC (P) and CD8 T cells in HNSC and SKCM (Q).

Figure 9

Co-expression analysis of immune checkpoint genes and BTK gene and GSEA enrichment analysis of BTK in selected cancers. (A) Co-expression analysis between BTK expression and immune checkpoint genes in HNSC, LGG, LUAD and SKCM. The cancer type and immune checkpoint genes are shown on the horizontal and vertical axes of the heatmap, respectively. The asterisk and color in the upper left corner of each small rectangular module represent the P-value, and the color in the lower right corner represents the correlation coefficient. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (B) GSEA-GO functional annotation of the BTK gene in different cancers. (C) GSEA-KEGG pathway analysis of the BTK gene in HNSC, LGG and SKCM. The colored curves represent different functions or pathways regulated by BTK in different cancers, and the peaks of enrichment curves upward and peaks of enrichment curves downward indicating positive regulation and negative regulation respectively.