Comprehensive analysis of BTN3A1 in cancers: mining of omics data and validation in patient samples and cellular models

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Butyrophilin 3A1 (BTN3A1), a major histocompatibility complex-associated gene that encodes a membrane protein with two extracellular immunoglobulin domains and an intracellular B30.2 domain, is critical in T-cell activation and adaptive immune response. Here, the expression of BTN3A1 in cancers was analyzed in eight databases comprising 86,733 patients of 33 cancers, and the findings were validated in patient samples and cell models. We showed that BTN3A1 was expressed in most cancers, and its expression level was strongly correlated with clinical outcome of 13 cancers. Mutations of BTN3A1 were detected, and the mutations were distributed throughout the entire gene. Gene set enrichment analysis showed that BTN3A1 co-expression genes and interacting proteins were enriched in immune regulation-related pathways. BTN3A1 was associated with tumor-infiltrating immune cells and was co-expressed with multiple immune checkpoints in patients with breast cancer (BRCA) and non-small cell lung cancer (NSCLC). We reported that BTN3A1 was downregulated in 46 of 65 (70.8%) NSCLCs, and its expression level was inversely associated with clinical outcome of the patients. BTN3A1 in tumor samples was lower than in counterpart normal tissues in 31 of 38 (81.6%) BRCA s. Bioinformatics analyses showed that BTN3A1 could be a target gene of transcription factor Spi-1 proto-oncogene (SPI1), and our ‘wet’ experiments showed that ectopic expression of SPI1 upregulated, whereas silencing of SPI1 downregulated, BTN3A1 expression in cells. These results suggest that BTN3A1 may function as a tumor suppressor and may serve as a potential prognostic biomarker in NSCLCs and BRCA s.

Abbreviations
BLCA, bladder urothelial carcinoma; BRCA, breast cancer; BTN3A1, butyrophilin 3A1; CHOL, cholangiocarcinoma; COSMIC, Catalogue of Somatic Mutations in Cancer; CTLA-4, cytotoxic T-lymphocyte antigen-4; ESCA, esophageal carcinoma; FDR, false discovery rate; GO, Gene Ontology; GSEA, gene set enrichment analysis; HNSC, head and neck squamous cell carcinoma; Ig, immunoglobulin; IHC, immunohistochemistry; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; MDSCs, myeloid-derived suppressor cells; MESO, mesothelioma; NSCLC, non-small cell lung cancer; PD-L1, programmed cell death 1 ligand; PPI, protein-protein interaction; PRAD, prostate adenocarcinoma; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; SPI1, Spi-1 proto-oncogene; STAD, stomach adenocarcinoma; TGCA, The Cancer Genome Atlas; TGCT, testicular germ cell tumors; TIMER, Tumor Immune Estimation Resource; TSS, transcription start site; UCEC, uterine corpus endometrial carcinoma.
Members of the B7:CD28 family play key roles in regulating T-cell activation and in cancer development and progression [1,2]. Blocking the immune checkpoints including programmed cell death 1 (PD-1)/programmed cell death 1 ligand (PD-L1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4) significantly prolongs the overall survival of 20–30% patients of most subtypes of cancers [3,4]. However, immune checkpoint inhibitors do not provide a long-term benefit to the majority of cancer patients, and there is still an urgent need to explore new targets for the development of cancer immunotherapy [5].

Butyrophilin (BTN) family belongs to the immunoglobulin (Ig) superfamily, whose family members have cytoplasmic and extracellular regions. The cytoplasmic region contains B30.2 domain, which is also known as PRY/SPRY domain and has an important role in protein–protein interaction (PPI) [6,7], mediating its interaction with proteins including major histocompatibility complex family proteins and proteins associated with Interleukin-1β secretion [8–10]. The extracellular domain of BTN is similar to that of the B7 family proteins, suggesting that BTN family members may also have immunomodulatory properties. Recent studies show that BTN family members regulate the immune responses of T cells, especially the γδ T cells [11], and targeting these molecules is emerging as an attractive strategy for cancer immunotherapy [12]. Butyrophilin 3A1 (BTN3A1), also known as CD277, is a member of the BTN3A subfamily. It can be found in stressed cells, malignant cells, and immune cells such as T cells, natural killer cells, and monocytes [12]. BTN3A1 harbors a B30.2 domain in the cytoplasmic region and B7-like domain in the extracellular region. The most well-known function of BTN3A1 is mediating the activation of γδT cells [12], using its B30.2 domain to bind the phosphorylated antigens to stimulate the cells [13–15]. BTN3A1 can inhibit tumor responsive αβ T-cell receptor activation by preventing N-glycosylated CD45 from dissociation from the immune synapse [16]. In addition, activation of BTN3A1 can significantly enhance the TCR-induced T-cell proliferation and cytokine secretion [17]. It is reported that BTN3A1 plays a critical role in cytosolic DNA- or RNA-mediated type I interferon (IFN) responses through promoting interferon regulatory factor 3 phosphorylation and IFN-β secretion [18].

Although the significance of the BTN3A1 in the activation of γδ T cells has been documented in previous studies, its role in carcinogenesis remains unclear. In this study, 8 databases comprising 33 cancer types and 86 733 cases were accessed to analyze the expression, mutation, enrichment of related signal pathways of BTN3A1, and its relationship with immune cell infiltration and the prognosis of cancer patients. The findings of bioinformatics analyses were validated by experiments in patient samples and cell models. Our results demonstrated that BTN3A1 was perturbed in cancers and this gene might have an important role in cancer development and progression.

**Materials and methods**

**The expression of BTN3A1 in human cancers**

The expression of BTN3A1 in tumor and counterpart normal tissues of various types of cancers was analyzed using the datasets of Oncomine database (https://www.oncomine.org/resource/login.html) [19], The Cancer Genome Atlas (TCGA; http://cBioportal.org), and Tumor Immune Estimation Resource (TIMER; https://cistrome.shinyapps.io/timer/) [20].

**Pan-cancer survival analysis**

The potential association between the expression level of BTN3A1 and the clinical outcome of cancers was analyzed by using the ONLINE SURVIVAL ANALYSIS Software (the Kaplan–Meier Plotter; http://kmplot.com/analysis/) [21] and the Gene Expression Profiling Interactive Analysis (gepia.cancer-pku.cn) [22].

**The mutation analysis of BTN3A1 in human cancers**

cBio Cancer Genomics Portal (http://cBioportal.org) [23] and Catalogue of Somatic Mutations in Cancer (COSMIC) database (V92) (https://cancer.sanger.ac.uk/cosmic/) [24] were used to analyze mutation and copy number variation of BTN3A1 in Pan-cancer.

**Gene set enrichment analysis**

The biological process categories of Gene Ontology (GO) for the gene set enrichment analysis (GSEA) in Linkedomics database (http://www.linkedomics.org/login.php) were analyzed to determine the enriched pathways that are in correlation with BTN3A1 [25]. The rank criterion was false discovery rate (FDR) ≤ 0.05, and 10 000 simulations were performed.

**BTN3A1 and the immune cell infiltration**

TIMER and TIMER2.0 were utilized to analyze the correlation between BTN3A1 expression level and the abundance of immune infiltrates and tumor purity [26]. GEPIA was
employed to analyze the correlation between the expression of BTN3A1 and immune checkpoints.

**Potential regulatory mechanism of BTN3A1**

The transcription factors that may regulate BTN3A1 expression level were predicted by gcbi online software (https://www.gcbi.com.cn), Cistrome Data Browser (http://cistrome.org/db/) [27] and GeneCards (https://www.genecards.org) [28]. The PPI network of BTN3A1 was validated by String Database (V11.0; https://string-db.org/) [29].

**Patient samples**

The study was approved by the research ethics committee of Chinese Academy of Medical Sciences Cancer Institute and Hospital. All samples were collected with written informed consent, and the study methodologies conformed to the guidelines set by the Declaration of Helsinki. The diagnosis of non-small cell lung cancer (NSCLC) and breast cancer (BRCA) was confirmed by at least two pathologists. NSCLC and BRCA tissue samples were obtained at the time of surgery and quickly frozen in liquid nitrogen. A tissue microarray containing tumor tissues and adjacent nontumor tissues isolated from 30 BRCA patients was purchased from Shanghai Outdo Biotech (Shanghai, China). The baseline demographic characteristics of patients with NSCLC and BRCA are listed in Tables 1 and 2, respectively.

**Immunohistochemistry assay**

Immunohistochemistry (IHC) assay was performed to test BTN3A1 expression in NSCLC and BRCA patient samples. Tissue specimens were fixed in formalin and embedded in paraffin, subjected to a heat-induced epitope retrieval step in citrate buffer solution after deparaffinized through xylene and graded alcohol. The sections were then blocked in paraffin, subjected to a heat-induced epitope retrieval step in citrate buffer solution after deparaffinized through xylene and graded alcohol. The sections were then blocked step in citrate buffer solution after deparaffinized through xylene and graded alcohol. The sections were then blocked. Immunoreactions were detected using 3,3'-diaminobenzidine (DAB, Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) and hematoxylin. The immunoreactivity score was calculated as IRS (0–12) = RP (0–4) × SI (0–3), where RP is the percentage of staining-positive cells and SI is staining intensity.

**Luciferase assay**

Human breast cancer cell line MCF-7 and NSCLC line H520 were cultured in Dulbecco’s modified Eagle medium supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA). The cells were transfected with plasmids containing BTN3A1 promoter-driven luciferase, constructs containing Spi-1 proto-oncogene (SPI1) coding sequence, or small interfering RNAs (siRNAs) (Table 3). Total RNA of the cells was extracted with TRIZOL reagent (Invitrogen, Frederick, MD, USA), and the expression of interested genes was tested by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) using primers listed in Table 3. Luciferase activity was measured using the Dual-luciferase reporter assay system (Promega, Madison, WI, USA).

**Chromatin immunoprecipitation**

A total of $2 \times 10^7$ cells were fixed with 1% formaldehyde for 10 min at room temperature and then stopped by 0.125 M Glycine. The protein-bound chromatin was fragmented by sonication and divided equally into two parts for ChIP assay or control. 95% of the sonication-treated chromatin was immunoprecipitated at 4 °C overnight with an anti-SPI1 antibody (#2258S; Cell Signaling Technology, Boston, MA, USA, 1 : 100) for ChIP or normal rabbit IgG (#2729; Cell Signaling Technology, 1 : 100) as a control while the remaining 5% was termed ‘Input’. Protein A/G PLUS-agarose (#sc-2003; Santa Cruz Biotechnology, Dallas, TX, USA, 1 : 50) was added and incubation at 4 °C for 6 h to bind and precipitate the antibodies which combined with chromatin DNA. Eventually, the immunoprecipitated DNA and Input DNA was de-crosslinked by incubation at 65 °C and purified, and then, the two DNA samples were used to perform PCR and qRT-PCR to test whether the antibodies can bind the promoter region of BTN3A1. The primers were listed in Table 3.

| Table 1. Baseline demographic characteristics of the 65 NSCLC patients. |
|----------------|----------------|----------------|----------------|
| Variable       | Cases, n (%)  | High, n (%)   | Low, n (%)     | P values*     |
| Total          | 65            | 19 (29.2)     | 46 (70.8)      |              |
| Age            |               |               |                |              |
| ≤ 60           | 37 (56.9)     | 12 (32.4)     | 25 (67.6)      | 0.51         |
| > 60           | 28 (43.1)     | 7 (25)        | 21 (75)        |              |
| Gender         |               |               |                |              |
| Male           | 45 (69.2)     | 14 (31.1)     | 31 (68.9)      | 0.62         |
| Female         | 20 (30.8)     | 5 (25)        | 15 (75)        |              |
| Tobacco smoke  |               |               |                |              |
| Smoker         | 35 (53.8)     | 10 (28.6)     | 25 (71.4)      | 0.90         |
| Nonsmoker      | 30 (46.2)     | 9 (30)        | 21 (70)        |              |
| Stage          |               |               |                |              |
| I              | 23 (35.4)     | 8 (34.8)      | 15 (65.2)      | 0.20         |
| II             | 11 (16.9)     | 1 (9.1)       | 10 (90.9)      |              |
| III–IV         | 29 (44.6)     | 10 (34.5)     | 19 (65.5)      |              |
| Not recorded   | 2 (3.1)       | 0 (0)         | 2 (100)        |              |

*P values were calculated using a two-sided Fisher’s exact test.
**Western blot**

Cells and tissues were lysed with RIPA buffer, protein extracts were quantitated, subjected to 10% SDS/PAGE, electrophoresed, and transferred onto a PVDF membrane. The membrane was washed and incubated with the indicated primary and secondary antibodies after blocking with 5% nonfat milk in Tris-buffered saline and then detected by electrochemiluminescence in Luminescent Image Analyzer LSA 4000 (GE, Fairfield, CO, USA). Antibodies used included rabbit anti-β-Actin (#ab8227; Abcam, Cambridge, UK; 1:5000) and rabbit anti-BTN3A1 (#25221-1-AP; Proteintech; 1:500) antibodies.

**Statistical analysis**

The data generated in Oncomine were displayed with P values, fold changes, and ranks. Survival curves were generated by the Kaplan–Meier plots and GEPIA. The results of Kaplan–Meier plots and GEPIA were displayed with hazard ratio and P values from a log-rank test. The correlation of gene expression was evaluated by Pearson’s correlation. The strength of the correlation was determined using the following guide for the absolute value: 0.00–0.29 ‘weak’, 0.30–0.49 ‘moderate’, 0.50–0.79 ‘strong’, 0.80–1.0 ‘very strong’. P values < 0.05 were considered statistically significant.

**Results**

**The mRNA expression levels of BTN3A1 in different types of human cancers**

The expression of BTN3A1 in tumor and adjacent normal tissues was analyzed in TIMER database containing TCGA data, and the results showed that BTN3A1 expression was significantly higher in tumor samples of cholangiocarcinoma (CHOL), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), and stomach adenocarcinoma (STAD), compared to their counterpart normal tissue controls (Fig. 1A). In contrast, BTN3A1 expression was significantly lower in breast cancer (BRCA), kidney chromophobe (KICH), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), and uterine corpus endometrial carcinoma (UCEC), in comparison with that in counterpart normal tissue controls (Fig. 1A). In Oncomine database, the expression of BTN3A1 was upregulated in tumor tissues than in normal tissue controls of patients with cancers of brain, cervical, esophageal, head and neck, kidney, and liver, and was downregulated in tumor samples of patients with cancers of breast, lung, ovarian, and prostate (Fig. 1B).

**Prognostic potential of BTN3A1 in human cancers**

We employed the Kaplan–Meier plotter database to evaluate the relationship between BTN3A1 expression level and patient outcome in multiple cancer types. In datasets derived from the Affymetrix gene chips (Fig. 2A), higher expression of BTN3A1 was associated with better prognosis in breast cancer, ovarian cancer, gastric cancer, and NSCLC. In datasets based on RNA-seq (Fig. 2B), higher expression of BTN3A1
was associated with better prognosis in bladder urothelial carcinoma (BLCA), rectum adenocarcinoma (READ), sarcoma (SARC), and UCEC. However, higher expression of BTN3A1 indicated poor prognosis in patients with testicular germ cell tumors (TGCT) (Fig. 2C). In GEPIA datasets, higher BTN3A1 expression was associated with longer overall survival in patients with BLCA, mesothelioma (MESO), KIRC, and skin cutaneous melanoma (SKCM) (Fig. 2D), whereas higher BTN3A1 expression was associated with worse prognosis in patients with brain lower grade glioma (LGG) (Fig. 2E).

**Variations of BTN3A1 in human cancers**

The cBioportal was used to investigate the mutations of this gene, and the results showed that BTN3A1 was mutated in cancers of uterine, CHOL, melanoma, and others (Fig. 3A). A total of 188 (1.72%) somatic mutations were seen in 10,953 patients with different types of cancers. The mutation frequency reached 32/529 (6.05%) in UCEC (Fig. 3A). Among the 383 BTN3A1 nucleotide substitutions, 175 (58.14%) ones were nonsense or missense mutations (Fig. 3B). The C:G>T:A mutations were the most frequently detected nucleotide substitutions (46.71%) (Fig. 3C). The mutations were distributed throughout the entire gene (Fig. 3D), which is characteristic of mutations in potential tumor suppressors. The potential relationship between BTN3A1 mutation and the well-known driver mutations was analyzed, and the results showed that patients with mutant BTN3A1 had higher mutation frequency in 24 genes including TP53, PTEN, EGFR, STK11, and others, than patients with wild type BTN3A1 (Fig. 3E).

**BTN3A1 correlated signal pathways in BRCA, LUAD, and LUSC**

Gene Ontology biological process for the GSEA of the Linkedomics database was employed to analyze the signal pathways that may be in correlation with BTN3A1 expression during carcinogenesis. The results showed that pathways involving adaptive immune response and immune response-regulating signaling were significantly enriched and were positively correlated with BTN3A1 expression levels in BRCA, LUAD, and LUSC (Fig. 4A–C). Other immune-related pathways, such as response to interferon gamma, regulation of leukocyte activation, and positive regulation of cytokine production, also appeared in the GSEA dataset (Fig. 4A–C).

**BTN3A1 is correlated with immune infiltration and immune checkpoint expression**

The tumor-infiltrating lymphocytes are associated with prognosis and response rate of immunotherapy [30]. We analyzed the correlation between the expression levels of BTN3A1 and the infiltration status of immune cell in
BRCA, LUAD, and LUSC by using the TIMER database. The results showed that BTN3A1 expression was negatively associated with tumor purity and positively correlated with infiltration levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells (Fig. 5A–C). Furthermore, the TIMER2.0 database was employed to determine whether other immunocyte infiltration levels were correlated with BTN3A1 expression. In this database, higher infiltration levels of monocytes, NK cells, M1, and M2 macrophages, and γδ T cells were positively associated with increased expression of BTN3A1 in BRCA, LUAD, and LUSC (Fig. 5D–H). The numbers of myeloid-derived suppressor cells (MDSCs) in BRCA, LUAD, and LUSC were negatively associated with the BTN3A1 expression (Fig. 5I). In addition, the correlation between the expression of BTN3A1 and the typical immune checkpoints was explored, and the results showed that the expression levels of PD-1, TIM3, CTLA-4, LAG3, TIGIT, CD39, STING, PD-L1, PD-L2, HLA-E, SIGLEC10, and IDO1 were positively associated with BTN3A1 expression levels (Fig. 5J). The above data suggested that BTN3A1 may have a role in modulating the immune microenvironment of cancers.

Validation of the expression of BTN3A1 in NSCLC and BRCA patient samples

To validate the results obtained from the databases, we tested the expression of BTN3A1 in NSCLCs and BRCA. Our results showed that BTN3A1 was lower in tumor samples than in counterpart normal controls in 46 (70.8%) of 65 NSCLCs (Table 1), detected by qPCR (Fig. 6A), western blot (Fig. 6B,C), and IHC (Fig. 6D, E). The expression level of BTN3A1 was inversely associated with clinical outcome of NSCLC patients (Fig. 6F). In BRCA, BTN3A1 was lower in tumor samples than in counterpart normal controls in 31 (81.6%) of 38 patients’ samples (Table 2), detected by western blot (Fig. 6G,H) and IHC (Fig. 6I,J) assays.

BTN3A1 is a target of transcription factor Spi-1 proto-oncogene

We investigated the transcription factors that may control BTN3A1 expression by using the GCBI online software. A total of 48 transcription factors were identified (Fig. 7A). In Chip-seq data from Cistrome and GeneCards, we showed that SPI1 (also known as
Fig. 3. Mutations of BTN3A1 in human cancers. (A) The mutation frequencies of BTN3A1 in different cancers in cBioportal database. CNA, copy number alteration. (B) The mutation types of BTN3A1 in cancers. (C) Nucleotide substitutions of BTN3A1 in cancers. (D) The number of mutations and the affected amino acids of BTN3A1 in cancers. (E) The frequency of driver mutations in patients of BTN3A1 altered and unaltered groups (*P < 0.05).

Fig. 4. Significantly enriched GO Biological processes in correlation with BTN3A1 expression in BRCA (A), LUAD (B) and LUSC (C). The x-axis represents the normalized enrichment score, while the y-axis represents the term of GO. FDR, false discovery rate.
PU.1) could bind to the promoter region of BTN3A1 in these three databases (Fig. 7B). By analyzing the sequence of BTN3A1 gene, we found two SPI1 binding sites (PU boxes), 5'-GAGGAA-3', in −1353 to −1348 and −1347 to −1342 bp upstream of the transcription start site (TSS) (Fig. 7C, upper panel). In *in vitro* luciferase reporter assays, we showed that overexpression of SPI1 in MCF-7 and H520 cells enhanced luciferase activity driven by BTN3A1 promoter (Fig. 7C, lower panel). Ectopic expression of SPI1 upregulated
Fig. 6. The expression of BTN3A1 in patients with NSCLC or BRCA. (A) The expression of BTN3A1 in NSCLCs was detected by quantitative RT-PCR. (B, C) The expression of BTN3A1 in NSCLCs was detected by western blot (B), and the western blot bands were assessed by densitometry analysis (C). (D, E) The expression of BTN3A1 in NSCLCs was tested by IHC assay (D), and the immunoreactivity score was calculated (E). (F) Overall survival of the 65 patients with NSCLC. (G, H) The expression of BTN3A1 in BRCA was detected by western blot (G), and the western blot bands were assessed by densitometry analysis (H). (I, J) The expression of BTN3A1 in BRCA was tested by IHC assay (I), and the immunoreactivity score was calculated (J). Scale bar = 100 μm; P values, Student’s t-test, *P < 0.05; **P < 0.01; ***P < 0.001. Error bars, SEM.
BTN3A1 at protein level (Fig. 7D). In the two lines, silencing of SPI1 by siRNAs significantly reduced BTN3A1 promoter-driven luciferase activity (Fig. 7E). Knockdown of SPI1 also led to downregulation of BTN3A1 at protein level (Fig. 7F). ChIP assays were performed in MCF-7 and H520 cells by using an anti-BTN3A1 antibody or a control IgG. The results of RT-PCR (Fig. 7G, upper panel) and qPCR (Fig. 7G, lower panel) confirmed that SPI1 was able to recruit BTN3A1 promoter. The PPI network of BTN3A1 was constructed by mining the STRING database, and the top ten proteins most connected to BTN3A1 were PPL, BTN3A2, IPP, KRP7, IL1F10, BTN3A3, KPNA6, FOXP1, MUC3A, and SMURF1 (Fig. 7H).

Discussion

Butyrophilin belongs to Ig superfamily and contains both B7 and B30.2 domains. Most BTN family members are reported to play vital roles in regulating immunity. Among them, BTN3A1 is widely expressed in immune cells and tumor cells and can regulate the immune response of T cells, especially the γδ T cells [12,16]. In this study, we performed Pan-cancer analysis to evaluate the role for BTN3A1 to play in tumorigenesis and reported that there was a significant differential expression pattern of BTN3A1 among the 33 cancer types. In patients with breast cancer, ovarian cancer, gastric cancer, NSCLC, BLCA, READ, SARC, UCEC, MESO, KIRC, and SKCM, higher expression of BTN3A1 was associated with better prognosis; but in patients with TGCT and LGG, higher expression of BTN3A1 was associated with worse prognosis. These results suggest that BTN3A1 may play context-dependent roles in different types of cancers; therefore, its activities in different cancer types should be carefully investigated in the future. Breast cancer and lung cancer represent the most commonly diagnosed cancer in female and male, respectively [31]. BTN3A1 expression was downregulated in breast cancer and NSCLC, and was positively associated with clinical outcome of the patients. We showed that BTN3A1 mutations are found in several cancers at the coding regions of each domain. In addition, the frequency of driver gene mutations in patients with mutant BTN3A1 was significantly increased, suggesting that BTN3A1 may play an important role in carcinogenesis.

BTN3A1 can activate gene expression during myeloid and B-lymphoid cell development and has been reported to be expressed in a variety of immune cells and involved in a variety of immune regulatory processes [32]. We showed that BTN3A1 was positively correlated with the infiltration of B cells, CD4+ T cells, CD8+ T cells, macrophages, dendritic cells, γδ T cells, and NK cells, and negatively correlated with the infiltration of MDSCs. BTN3A2, BTN3A3, FOXP1, and IL1F10, which are important for immune response [17,33,34], were included in the PPI network of BTN3A1. The expression of BTN3A1 was significantly decreased in NSCLC and BRCA. In these cancer types, the co-expressed genes of BTN3A1 were mainly distributed in immune regulation-related pathways, such as differentiation and activation of immune cells, cytokine secretion, and immune response. The downregulated BTN3A1 may be associated with the decrease of tumor-infiltrating immune cells such as CD8+ T cells, dendritic cells, γδ T cells, and NK cells, leading to suppression of tumor inhibition effects and worse prognosis of patients. The results suggested that BTN3A1 may have a role in shaping the immunosuppressive tumor microenvironment and may sever as a tumor suppressor in breast cancer and NSCLC by promoting the invasion of innate and adaptive immune cells and inhibition of the invasion of MDSCs.

We screened for transcription factors that can regulate BTN3A1 in Cistrome, GeneCards, and GCBI, and found that SPI1 was the only transcription factor in the three databases that may bind BTN3A1 promoter. The regulatory effects of SPI1 on BTN3A1 were confirmed by our ‘wet’ experiments. The expression of SPI1 in NSCLCs has been reported, and increased number of PU.1+ cells is correlated with favorable prognosis of adenocarcinoma and poor prognosis of
squamous cell carcinoma [35]. PU.1 is also a major transcriptional activator of the tumor suppressor gene LIMD1 [36]. These results suggest that the SPI1-BTN3A1 pathway may have an important role in lung carcinogenesis, but the effects of SPI1-BTN3A1 on immune suppressive microenvironment and related mechanisms remain to be elucidated.

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Conflict of interest

The authors declare no conflict of interest.

Data accessibility

All data generated or analyzed during this study are included in this published article or are available from the corresponding author on reasonable request.

Author contributions

FL and G-BZ conceived and designed the project; FL, CZ, S-HG, F-YY, and G-ZW acquired the data; FL, G-ZW, and HG analyzed and interpreted the data; FL, G-BZ, and G-ZW wrote the paper.

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