International Journal of Biochemistry and Cell Biology 108 (2019) 84–91

Contents lists available at ScienceDirect

International Journal of Biochemistry and Cell Biology

journal homepage: www.elsevier.com/locate/biocel

B7-H3 is regulated by BRD4 and promotes TLR4 expression in pancreatic ductal adenocarcinoma

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ARTICLE INFO

Keywords:
Pancreatic ductal adenocarcinoma (PDAC)
BET inhibitors
B7-H3
BRD4
TLR4

ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies worldwide. PDAC is resistant to chemotherapy and radiotherapy which leads to the poor prognosis of PDAC patients and a 5-year survival rate of less than 5%. Exploring the mechanism of the pancreatic cancer tumorigenesis is the key to finding a novel therapeutic strategy for cancer treatment. B7-H3 belongs to the B7 family of immunoregulatory proteins, and the overexpression of B7-H3 is found in various types of cancer. The regulation of B7-H3 expression in pancreatic cancer is still unclear. Here, we showed that B7-H3 acted as a negative prognostic biomarker in PDAC and promoted cell proliferation, invasion and metastasis in pancreatic cancer. Next, we applied the drug screening method to identify bromodomain and extra-terminal motif (BET) inhibitors that decreased the protein and mRNA levels of B7-H3 in pancreatic cancer cells. Moreover, we verified that BRD4 was responsible for regulating the expression of B7-H3 at the transcriptional level. Finally, our data indicated that the BRD4/B7-H3 axis modulated the expression of TLR4 in pancreatic cancer cells. Taken together, our results elucidated the regulation of B7-H3 expression in pancreatic cancer and uncovered the importance of BRD4/B7-H3/TLR4 pathway. The targeting of B7-H3 by the BET inhibitors may be a novel therapeutic strategy to overcome the immunotherapy and chemotherapy resistance in pancreatic cancer.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC), which arises in the exocrine components of the pancreas is one of the most lethal malignancies worldwide (Bray et al., 2018). Since two thirds of patients are diagnosed with pancreatic cancer in the late stage, they lose the opportunity for curative surgical resection therapy (Chari et al., 2015). Moreover, PDAC is resistant to chemotherapy and radiotherapy which leads to the poor prognosis of PDAC patients with a 5-year survival rate of less than 5% (Silvestris et al., 2014). Therefore, exploring the mechanism of pancreatic cancer tumorigenesis is the key to finding a novel therapeutic strategy for cancer treatment.

In recent years, immune checkpoint blockade therapy has made great progress in the treatment of many types of malignancies (Dong et al., 2002, 1999; Freeman et al., 2000; Ishida et al., 1992). B7-H3 (CD276), a novel member of the B7 family of immunoregulatory proteins, consists of two isoforms: 2IgB7H3 and 4IgB7H3. B7-H3 is a member of the immune modulators family and participates in modulating the antitumor immunity (Flies and Chen, 2007; Loos et al., 2010; Wang et al., 2014). It has been reported that B7-H3 is up-regulated in pancreatic cancer and is associated with poor prognosis (Inamura et al., 2018; Zhao et al., 2013). Moreover, B7-H3 functions as an anti-apoptotic molecule and is closely associated with gemcitabine resistance in pancreatic cancer (Li et al., 2017a, 2017b). Recently, an antibody designed to target B7-H3 effectively inhibited PDAC progression in combination with chemotherapy (Kasten et al., 2018). Thus, B7-H3 might be an ideal target for cancer therapy.

The regulation of B7-H3 expression in pancreatic cancer is still unclear. In this study, we examined the clinic relevance of B7-H3 in PDAC and found that B7-H3 was upregulated and associated with poor prognosis in pancreatic cancer patients. Then, we investigated the role of B7-H3 in the cell proliferation, invasion and metastasis of PDAC and showed that B7-H3 promoted cancer progression in pancreatic cancer. Moreover, drug screening indicated that bromodomain and extra-

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https://doi.org/10.1016/j.biocel.2019.01.011
Received 11 October 2018; Received in revised form 14 January 2019; Accepted 17 January 2019
Available online 18 January 2019
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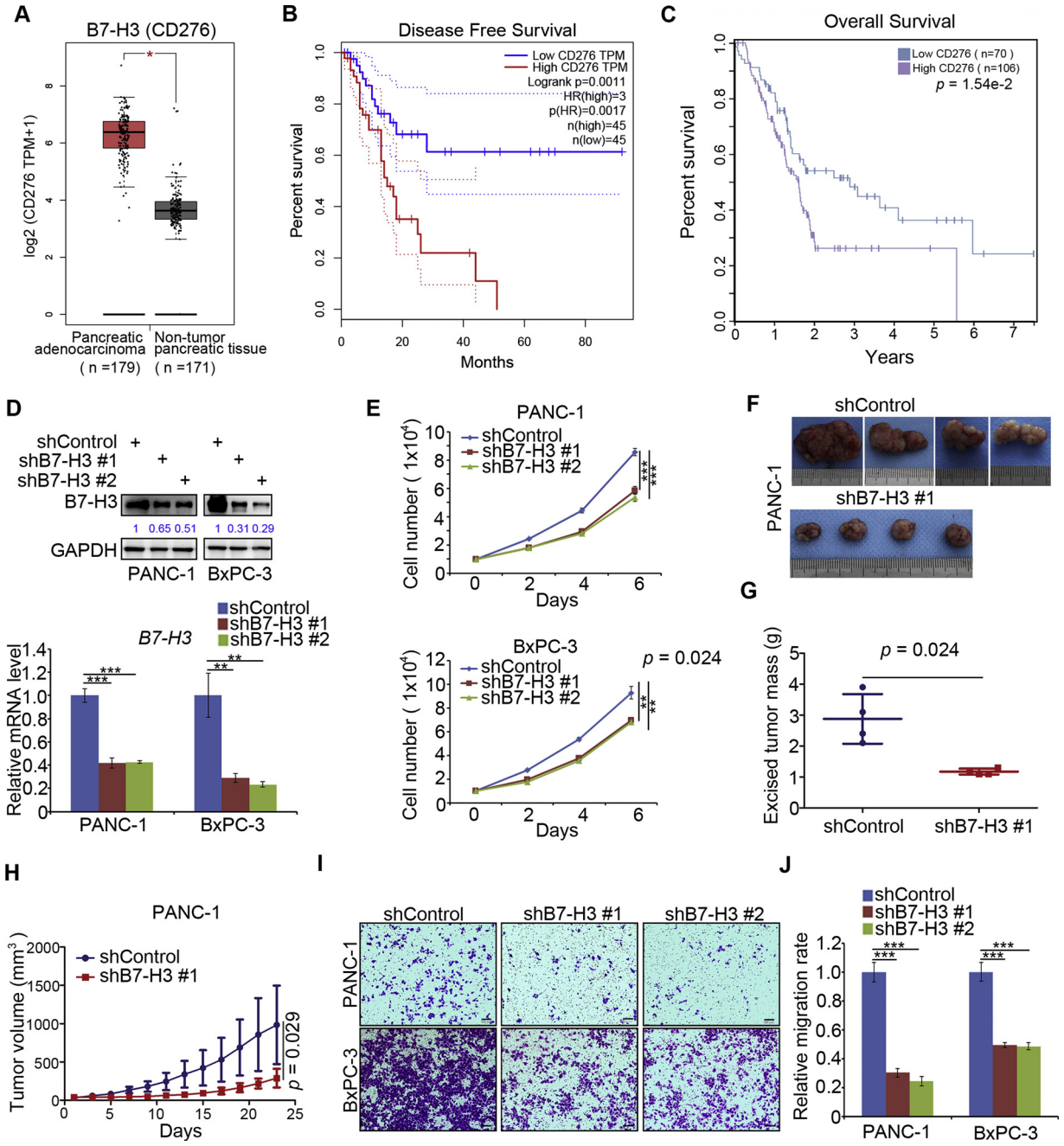

Fig. 1. Aberrant expression of B7-H3 promotes cancer progression in PDAC. (A) The GEPIA database revealed that *B7-H3* expression was significantly upregulated in the HCC tissues. The boxplot analysis used log2 (TPM + 1) for log-scale. (B) The disease-free survival of the patients with PDAC was computed with the GEPIA web tool. (C) The overall survival of the patients with PDAC was computed with the Human Protein Atlas. (D) PANC-1 and BxPC-3 cells were infected with lentivirus vectors expressing control or B7-H3-specific shRNAs. Then, 48 h post-infection, cells were harvested for Western blot analysis and RT-qPCR analysis (D), a cell proliferation assay (E). The data shown are the mean values ± SD from three replicates. **, $p < 0.01$; ***, $p <$ 0.001. The B7-H3 protein was quantified and normalized to the quantified value of GAPDH using ImageJ software. (F, G and H) PANC-1 cells were infected with control or B7-H3-specific shRNAs. Then, 72 h post-infection, the cells were injected subcutaneously into the right dorsal flank of nude mice. After 24 days, the tumours were harvested, photographed and measured. The data are presented as the means ± SD (n = 4), the *P* value is indicated in the figure G and H. (I and J) PANC-1 and BxPC-3 cells were infected with lentivirus vectors expressing control or B7-H3-specific shRNAs. Then, 48 h post-infection, cells were used for Matrigel invasion assays. Representative images of invasion assay are shown in I, and the quantification results are shown in J. The data are mean ± S.D. from experiments with three replicates. ***, $p <$ 0.001.

terminal motif (BET) inhibitors decreased B7-H3 expression and that B7-H3 was regulated by BRD4 in pancreatic cancer cells. Furthermore, we demonstrated that the BRD4/B7-H3 axis regulated the expression of TLR4 in pancreatic cancer cells. Together, our data contribute to a better understanding of the role of B7-H3 in PDAC.

2. Materials and methods

2.1. Cell culture and cell transfection

All pancreatic cancer cell lines including PANC-1 and BxPC-3 were purchased from the Chinese Academy of Science Cell Bank (Shanghai, China). These cell lines were cultured in the Dulbecco's modified Eagle

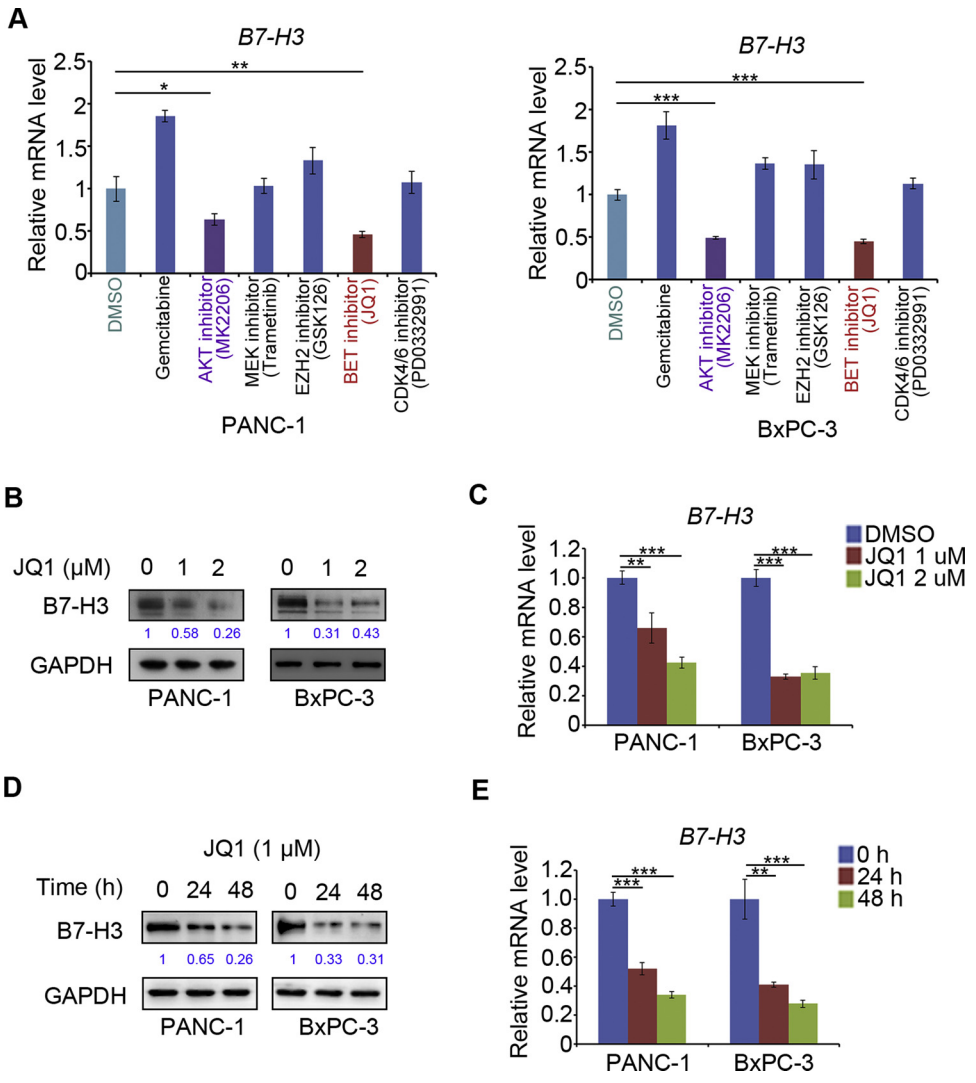

Fig. 2. A BET inhibitor decreases B7-H3 expression in pancreatic cancer cells. (A) PANC-1 and BxPC-3 cells were treated with indicated small molecular inhibitors (DMSO as control; Gemtabine 10 u M; MK2206 2 μM; Trametinib 10 μM; GSK126 10 μM; JQ1 2 μM; PD0332991 2 μM). After 24 h, cells were harvested for RT-qPCR analysis. The data shown are the mean values ± SD from three replicates. **, $p < 0.01$; ***, $p < 0.001$. (B and C) PANC-1 and BxPC-3 were treated with DMSO, 5 μM or 10μM of JQ1. 24 h after treatment, cells were harvested for western blotting analysis (B) and RT-qPCR analysis (C). For panel B, B7-H3 proteins were quantified by ImageJ software and normalized to the quantified value of GAPDH. For panel C, the data are mean ± S.D. from experiments with three replicates. ***, $p < 0.001$. (D and E) PANC-1 and BxPC-3 cells were treated with JQ1 (1 μM), and at different time points, cells were harvested for Western blots (D) and RT-qPCR analysis (E). For panel D, B7-H3 proteins were quantified by ImageJ software and normalized to the quantified value of GAPDH. For panel E, the data are mean ± S.D. from experiments with three replicates. **, $p <$ 0.01; ***, $p <$ 0.001.

medium (DMEM) medium (Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS) (HyClone, USA). All cell lines were routinely maintained at 37 °C in a 5% CO_2 incubator. Transfections were conducted using the lipid-based method (Lipofectamine 2000, Thermo Fisher Scientific, USA) following the manufacturer's instructions.

2.2. Antibodies and chemicals

B7-H3 antibody (ab227670) was purchased from Abcam (working dilution 1:1000); GAPDH antibody (ab8245) was purchased from Abcam (working dilution 1:5000); TLR4 antibody (ab13556) was purchased from Abcam (working dilution 1:500), and BRD4 (13440) was purchased from Cell signalling Technology (working dilution 1:1000). JQ1 and puromycin were purchased from Sigma-Aldrich (Shanghai, China), and MK 2206, Trametinib, GSK126 and Palbociclib (PD0332991) were purchased from Selleckchem (Houston, USA). Gemcitabine was obtained from Eli Lilly and Company (Indianapolis, USA).

2.3. Western blot analysis

Cells were harvested and lysed with lysis buffer containing 1% protease and phosphatase inhibitors for 15 min on ice as described previously (Jin et al., 2017a, b). Then, the cell lysates were centrifuged at 12,000 × g for 20 min at 4 °C to remove undissolved impurities, and the supernatants were collected. The protein concentration was determined with a protein assay kit (Pierce Biotechnology, USA). For each sample, equal amounts of protein for each sample were separated using SDS-PAGE gels and transferred onto PVDF membranes (Pierce Biotechnology, USA). The membranes were blocked in 5% nonfat milk for 1 h at room temperature and then incubated with a primary antibody overnight at 4 °C. The membranes were then washed with 1x TBST and incubated with a secondary antibody for 1 h. Finally, the membranes were treated with ECL detection reagents and exposed to X-ray film.

2.4. Real-time RT-PCR

Total RNA was extracted from the cells using TRIzol reagent (Thermo Fisher Scientific, USA). First-strand cDNA was synthesized from 2 μg of RNA using a cDNA Reverse Transcription kit (PrimeScript™ RT reagent kit, code no. RR037 A), and real-time PCR analysis was carried out with a PCR kit (TB Green™ Fast qPCR Mix, code no. RR430 A) according to the manufacturer's protocols. The two kits were purchased from Takara Bio Inc. (Shiga, Japan). All the values were normalized against actin, and the 2-ΔCt method was used to quantify fold change. Primer sequences for RT-qPCR were as follows: *B7-H3*, forward 5′-GGGCTGTCTGTCTGTCTCAT -3′ and reverse 5′ - GAGTGTT TCAGAGGCTGCAG-3′; *TLR4*, forward 5′-GTGCCTCCATTTCAGCT CTG-3′ and reverse 5′-CAAAGATACACCAGCGGCTC -3′; *GAPDH*, forward 5′-ATGACAATGAATACGGCTACAGCA-3′and reverse 5′-GCAGCG AACTTTATTGATGGTATT-3′.

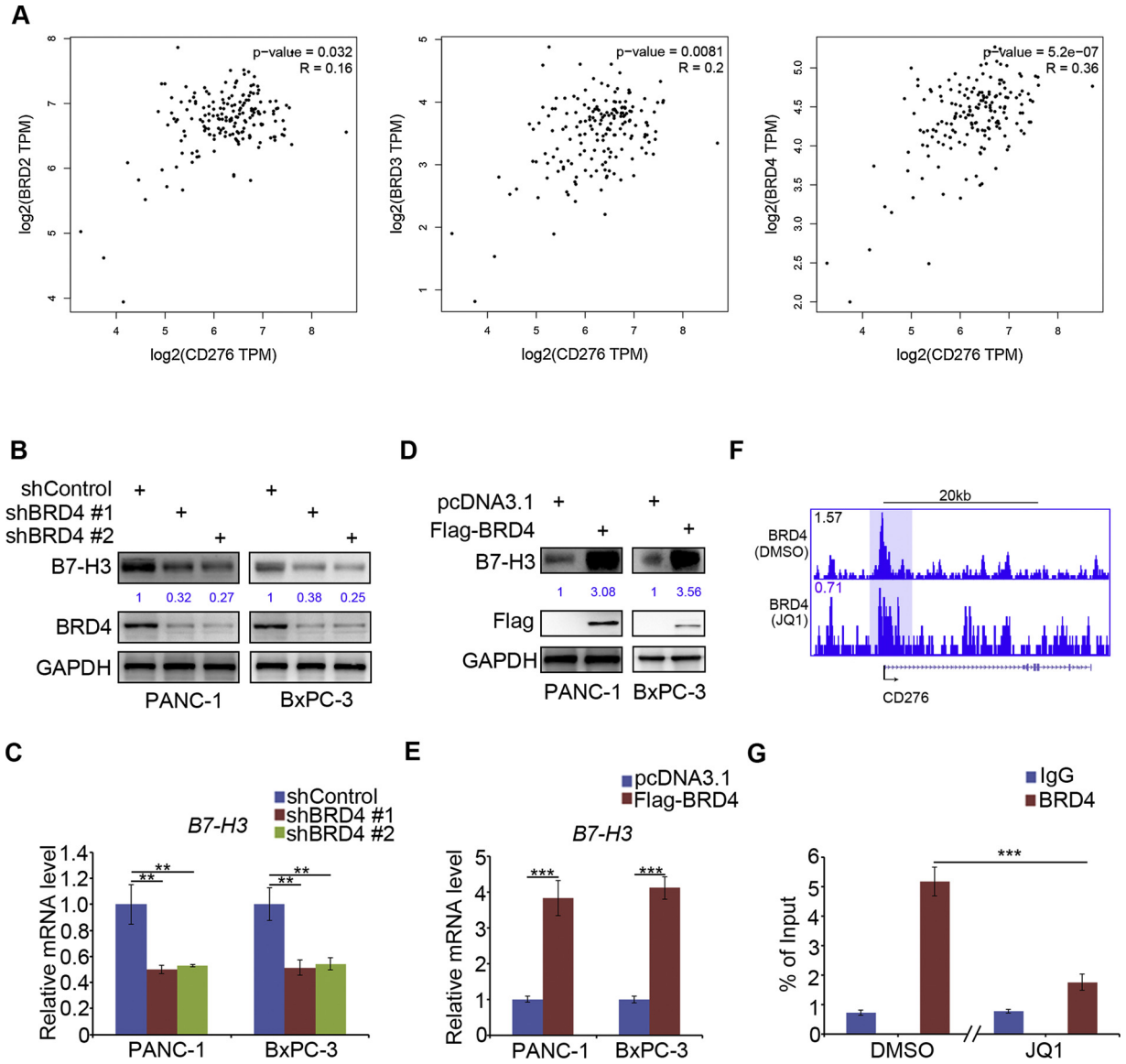

Fig. 3. B7-H3 is transcriptionally regulated by BRD4 in pancreatic cancer cells. (A) The GEPIA web tool was used to determine the correlation between the mRNA expression levels of *BRD2*, *BRD3* or *BRD4* and *B7-H3 (CD276)* in human pancreatic cancer samples. (B and C) PANC-1 and BxPC-3 cells were stably infected with control or two independent BRD4-specific shRNAs for 3 days and harvested for Western blots (B) and RT-qPCR analysis (C). The data are mean ± S.D. from experiments with three replicates. **, $p < 0.01$. (D and E) PANC-1 and BxPC-3 cells were transfected with indicated constructs. 24 h post transfection, cells were harvested for Western blots (D) and RT-qPCR analysis (E). (F) UCSC Genome Browser screenshots showing BRD4 ChIP–seq signal profiles in the *B7-H3 (CD276)* gene locus (Zhang et al., 2017). (G) BRD4 ChIP-qPCR of *B7-H3* in PANC-1 cells after treatment with or without 5 μM of JQ1 for 24 h. All data are shown as the mean values ± SD from three replicates. ***, $p < 0.001$.

2.5. RNA interference

Lentivirus-based control and gene-specific shRNAs were purchased from Sigma-Aldrich. Lipofectamine 2000 was used to transfect 293 T cells with shRNA plasmids and viral packaging plasmids (pVSV-G and pEXQV). At 24 h post-transfection, the medium was replaced with DMEM containing 10% FBS. The virus culture medium was collected at 48 h posttransfection and added to cells. The cells were cultured in medium for 24 h, then puromycin (0.75 μg/ml; Sigma-Aldrich) was added to the medium. At 72 h after puromycin selection, the cells were harvested for further analysis. (Jin et al., 2017a; Wang et al., 2018). The following shRNA sequences were used: shB7-H3-1, CCGGGCTTGTTTG ATGTGCACAGCACTCGAGTGCTGTGCACATCAAACAAGCTTTT TTG;shB7-H3-2,CCGGCTCTGAAACACTCTGACAGCACTCGAGTGCTGT CAGAGTGTTTCAGAGTTTTTTG;shBRD4-1,GTACCGGTGAACCTCCCTG ATTACTATACTCGAGTATAGTAATCAGGGAGGTTCATTTT TTG;shBRD4-2,CCGGCCTGGAGATGACATAGTCTTACTCGAGTAAGAC TATGTCATCTCCAGGTTTTTG;shTLR4,AACCCGGAGGCCATTATGCTA.

2.6. Survival analysis and correlation analysis using GEPIA web tool

The online database Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancerpku.cn/index.html) (Tang et al., 2017) was used to analyze RNA sequencing expression data related to our project based on The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) projects. GEPIA performs survival analyses based on gene expression levels and uses a log-rank test for hypothesis evaluation. GEPIA also performs a pairwise gene correlation analysis for any given sets of TCGA and/or GTEx expression data using Pearson correlation statistics (Fan et al., 2018b).

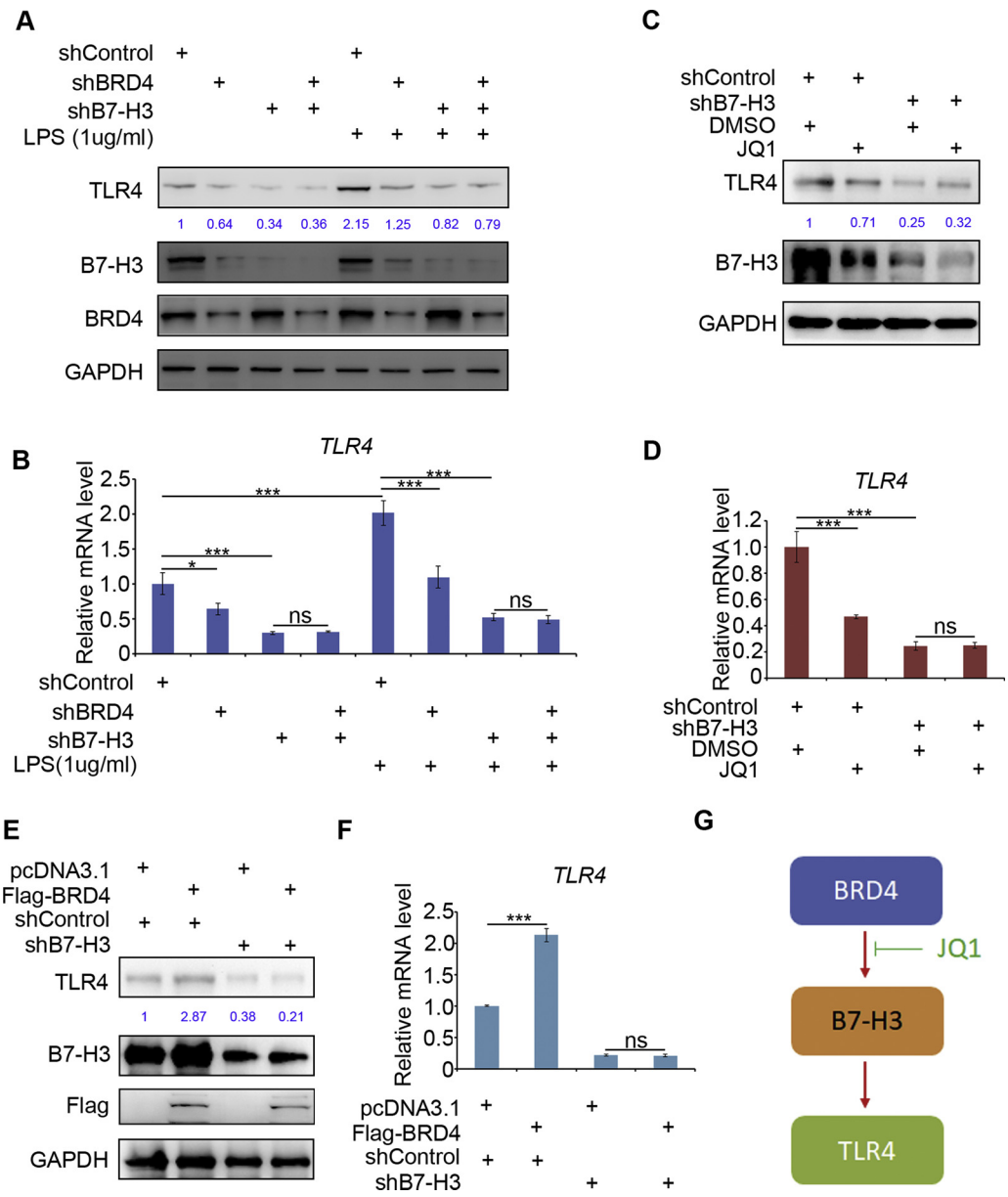

Fig. 4. BRD4/B7-H3 signaling increases TLR4 expression in pancreatic cancer. (A and B) PANC-1 cells were infected with indicated constructs. 72 h post transfection, cells were treat with or without LPS (1 μg/ml) for 30 min before harvested for Western blots (A) and RT-qPCR analysis (B). The data shown are the mean values ± SD from three replicates. ns, not significant;*, $p < 0.05$; ***, $p < 0.001$. For panel A, TLR4 proteins were quantified by ImageJ software and normalized to the quantified value of GAPDH. (C and D) PANC-1 cells were infected with indicated constructs. 72 h post transfection, cells were treated with or without 3 μM of JQ1. After 24 h, cells were harvested for Western blots (C) and RT-qPCR analysis (D). The data shown are the mean values ± SD from three replicates. ns, not significant; ***, $p < 0.001$. For panel C, TLR4 proteins were quantified by ImageJ software and normalized to the quantified value of GAPDH. (E and F) PANC-1 cells were infected with indicated constructs. 72 h post transfection, cells were transfected with pcDNA 3.1 or Flag-BRD4. After 24 h, cells were harvested for Western blots (E) and RT-qPCR analysis (F). The data shown are the mean values ± SD from three replicates. ns, not significant; ***, $p < 0.001$. For panel E, TLR4 proteins were quantified by ImageJ software and normalized to the quantified value of GAPDH. (G) A hypothetical model depicting that BRD4 regulates the expression of B7-H3. The BET inhibitors, JQ1, block the function of BRD4 and inhibit B7-H3 expression. BRD4/B7-H3 signaling promotes the expression of TLR4in pancreatic cancer cells.

2.7. In vitro cell proliferation assay

PANC-1 or BxPC-3 cells were plated in 12-well plates an the initial concentration of 1×10^4 cells/plate. On days 0, 2, 4 and 6, cells were collected for cell counting.

2.8. Generation of PDAC xenografts in mice

BALB/c-nu mice (4–5 weeks of age, 18–20 g) were purchased from Vitalriver (Beijing, China). All animal experiments were performed according to the Guidelines set forth by the Chinese National Institutes of Health and followed the protocols approved by the Ethical Committee on Animal Experiments at the Huazhong University of Science and Technology in Wuhan, China. For the cell proliferation assay, the BALB/c nude mice were randomly divided into two groups (n = 4/group): the shControl group and the shB7-H3-1 group. PANC-1 cells (5×10^6) infected with the indicated lentivirus were subcutaneously inoculated into the right dorsal flank of the mice. On day 24, the animals were euthanized, and the tumors were excised and weighed. Statistical analyses were performed using a two-sided paired Student's *t*-test to compare the difference in the tumor weight between the two groups.

2.9. In vitro invasion assay

The in vitro cell invasion assay was performed using a BioCoat Matrigel invasion chamber (BD Biosciences) according to the manufacturer's protocol. PANC-1 and BxPC-3 cells were cultured in the insert for 24 h. Cells were fixed in methanol for 15 min and then stained with 1 mg/ml crystal violet for 20 min. At least five fields were photographed for each group after staining, and invaded cells were counted (Jin et al., 2017b).

2.10. Chromatin immunoprecipitation (ChIP) and ChIP-qPCR

ChIP was performed following the manufacturer's instructions with the Chromatin Extraction Kit (Abcam, ab117152, USA) and ChIP Kit Magnetic - One Step (Abcam, ab156907, USA) (Fan et al., 2018a). BRD4 (Cell signaling Technology, 13440, dilution 1:50) was used for the ChIP assay. The purified DNA was analyzed by real-time PCR with a PCR kit

(Takara Bio Inc., Japan) according to the manufacturer's protocols (Jin et al., 2018). Primers used for ChIP-qPCR were as follows: B7-H3 ChIP primers, forward 5′ - ACCTAACTCTGGCCCACAAA -3′ and reverse 5′ − CCTTGGGCTCTGTCCTTACA -3′.

2.11. Statistical analysis

Statistical analyses were performed with the two-sided paired Student's *t*-test for single comparisons and one-way ANOVA or a post hoc test for multiple comparisons. p values < 0.05 were considered statistically significant. All values represent the means ± SDs.

3. Results

3.1. Aberrant expression of B7-H3 promotes cancer progression in PDAC

To investigate the specific role of B7-H3 in pancreatic cancer, we first analyzed *B7-H3* (*CD276*) mRNA levels in pancreatic cancer and nontumor pancreatic tissues by using the GEPIA web tool (Tang et al., 2017). The results indicated that the mRNA levels of *B7-H3* in pancreatic cancer were higher than those nontumor pancreatic tissues (Fig. 1A). We then sought to determine the clinical relevance of B7-H3 in pancreatic cancer patients via the GEPIA web-based tool and The Human Protein Atlas, which suggested that overexpression of B7-H3 shortens the disease free survival (Fig. 1B) and overall survival time (Fig. 1C) of PDAC patients. Given that B7-H3 is a negative prognostic biomarker in PDAC, we knocked down of B7-H3 with a specific lentiviral short hairpin RNA to explore the biological function of B7-H3 in PANC-1 and BxPC-3 cells (Fig. 1D). Our results showed that knockdown of B7-H3 not only blocked tumor cell growth in vitro and in vivo (Fig. 1E -H) but also impeded the migration capability of pancreatic cancer cells in vitro (Fig. 1I-J). Taken together, our data indicate that upregulated B7-H3 is associated with poor prognosis in PDAC patients and promotes tumor progression in pancreatic cancer cells.

3.2. A BET inhibitor decreases B7-H3 expression in pancreatic cancer cells

Since B7-H3 functions as an oncogenic protein in pancreatic cancer, it should be an ideal target for cancer therapy. Studying the regulatory mechanism of B7-H3 would shed a light on a novel therapeutic strategy for pancreatic cancer. Here, pancreatic cancer cell lines were treated with different types of small-molecule inhibitors and RT-qPCR assays were applied to evaluate the *B7-H3* mRNA level. It has been reported that B7-H3 is regulated by PI3K/AKT/mTOR signaling in cancer cells (Zhang et al., 2015a, 2015b), and our results also showed that the AKT inhibitor (MK2206) inhibits the expression of B7-H3 in pancreatic cancer cells (Fig. 2A). Interestingly, the most studied BET inhibitors, JQ1, consistently decreased the expression of B7-H3 in both PANC-1 and BxPC-3 cells (Fig. 2A). Moreover, we found that JQ1 decreased the level of B7-H3 protein in PANC-1 and BxPC-3 pancreatic cancer cells at different doses (Fig. 2B). We further showed that different doses of JQ1 downregulated the B7-H3 expression at the transcriptional level in both pancreatic cancer cell lines (Fig. 2C). Furthermore, our results demonstrated that JQ1 undermined the expression of B7-H3 in a time-dependent manner in PANC-1 and BxPC-3 cells (Fig. 2D and 2E). Together, our data suggest that the BET inhibitors represses B7-H3 expression in pancreatic cancer cells

3.3. B7-H3 is transcriptionally regulated by BRD4 in pancreatic cancer cells

Since JQ1 mainly blocks the transcriptional activity of BET proteins, including BRD2, BRD3 and BRD4 (Jin et al., 2018; Yan et al., 2018; Zhang et al., 2017), we first analyzed the correlation of BRD2, BRD3 or BRD4 with B7-H3 in pancreatic cancer specimens by using the GEPIA web-based tool. Consistent with previous results, the *B7-H3* mRNA level was positively correlated with *BRD2*, *BRD3* or *BRD4* in PDAC patients (Fig. 3A). As BRD4 the most studied BET family protein, we sought to verify whether B7-H3 expression is regulated by BRD4 in pancreatic cancer cells. Indeed, the knockdown of BRD4 significantly decreased the protein and mRNA levels of B7-H3 in PANC-1 and BxPC-3 cells (Fig. 3B and C). In contrast, the ectopic overexpression of BRD4 increased the expression of B7-H3 in both PANC-1 and BxPC-3 cells (Fig. 3D and E). Furthermore, we analyzed existing BRD4 ChIP-seq data (Zhang et al., 2017) and noticed that there is a BRD4-binding peak in the promoter of the *B7-H3* (*CD276*) gene and that treatment with JQ1 diminished this binding (Fig. 3F). This result was further confirmed by ChIP-qPCR of PANC-1 cells (Fig. 3G). Therefore, our data suggest that BRD4 transcriptionally regulates B7-H3 expression in pancreatic cancer cells.

3.4. BRD4/B7-H3 signaling increases TLR4 expression in pancreatic cancer

TLR4 is the first discovered human Toll homolog, and the overexpression of TLR4 is found in pancreatic cancer. Targeting the expression of TLR4 provides a promising therapeutic strategy for cancer therapy. Since we previously reported that soluble B7-H3 upregulated the expression of TLR4 (Xie et al., 2016), we sought to determine whether BRD4 modulated TLR4 expression via B7-H3 in pancreatic cancer cells. Consistent with other reports, we found that LPS stimulation upregulated the expression of TLR4 in pancreatic cancer cells, and this effect was diminished after knockdown of B7-H3 or BRD4 (Fig. 4A and B). Importantly, co-knockdown of BRD4 and B7-H3 did not further decrease the expression of TLR4 compared with knockdown of B7-H3 alone (Fig. 4A and B), which indicated that BRD4 regulated TLR4 via B7-H3 in pancreatic cancer cells. Similarly, we demonstrated that JQ1 treatment inhibited the protein and mRNA levels of TLR4, and this effect was no longer significant after knockdown of B7-H3 in PANC-1 cells (Fig. 4C and D). Furthermore, our results showed that the ectopic overexpression of BRD4 elevated the expression of TLR4, and this effect also diminished after knockdown of B7-H3 in pancreatic cancer cells (Fig. 4E and F). Thus, our data suggest that BRD4 might regulate the expression of TLR4 via B7-H3 in pancreatic cancer cells (Fig. 4G).

4. Discussion

The abnormal expression of B7-H3 is found in various types of cancer, such as prostate cancer (Comiskey et al., 2018), bladder cancer (Li et al., 2017a, 2017b), gastric cancer (Arigami et al., 2011) or nonsmall cell lung cancer (Zhang et al., 2015a, 2015b). B7-H3 belongs to the B7 family of immunoregulatory proteins and has been implicated in cancer progression and metastasis (Inamura et al., 2018). It has been reported to repress T cell-mediated anti-tumor immunity and is considered a promising target for immune-based anti-tumor therapies (Li et al., 2018). Moreover, beyond its role in immune regulation, B7-H3 is involved in activating the Jak2-STAT3 signaling pathway to modulate the apoptosis of colorectal cancer cells (Zhang et al., 2015a, 2015b). Furthermore, B7-H3 is critical for the drug resistance. Knocking down the expression of B7-H3 increases the sensitivity to dacarbazine (DTIC) chemotherapy and small-molecule inhibitors targeting the MAP kinase (MAPK) and AKT/mTOR pathways in metastatic melanoma cells (Flem-Karlsen et al., 2017). Although B7-H3 is a target for cancer therapy, the regulation of B7-H3 expression in cancer cells is poorly understood. It has been reported that ILT4 drives B7-H3 expression via the PI3K/AKT/mTOR pathway in nonsmall cell lung cancer (Zhang et al., 2015a, 2015b). In our study, we first verified that a specific inhibitor of AKT decreased B7-H3 expression in pancreatic cancer cells (Fig. 2A). Importantly, according to the drug screening results, we uncovered that the BET inhibitor decreases the expression of B7-H3 and confirmed that BET family proteins transcriptionally increase the expression of B7-H3 in pancreatic cancer cells (Figs. 2 and 3).

TLR4 is the first discovered human Toll homolog (El-Omar et al., 2008; Lu et al., 2008). The immune effects and tumorigenesis of TLR4

activation are indeed extensive (Pandey et al., 2018). In addition to its well-known immunological functions, TLR4 is involved in cellular development regulation, chronic inflammation, neurotoxic effects, autoimmune diseases and fibroblast activation (Mishra and Pathak, 2019). It has been reported that chronic inflammation mediated by TLR4 stimulated the development and progression of cancer (Vijay, 2018). The significant upregulation of TLR4 expression was observed in a number of tumors such as prostate cancer, breast cancer, and pancreatic cancer (Grimmig et al., 2016; Khademalhosseini and Arababadi, 2018; Ou et al., 2018). Therefore, TLR4 is a promising target for cancer therapy. In our previous study, we demonstrated that TLR4 was upregulated by TLR4 in pancreatic cancer cells (Xie et al., 2016). This is because of the important functions of TLR4 and its correlation with tumors; however, there is still no specific inhibitor to downregulate TLR4. We detected the levels of TLR4 after interfering with BRD4 or treating with a BET inhibitor, and our results indicated that the BRD4/B7-H3 axis promotes TLR4 expression, which provides a new therapeutic strategy for decreasing the level of TLR4 in pancreatic cancer.

Collectively, our results demonstrate that the aberrant expression of B7-H3 is associated with progression pancreatic cancer. The BET inhibitors block the expression of B7-H3 expression in a dose- and time-dependent manner. B7-H3 is transcriptionally regulated by BRD4 in pancreatic cancer cells. Finally, we showed that the BRD4/B7-H3 axis is critical for regulating the expression TLR4. These findings elucidated the regulation of B7-H3 expression in pancreatic cancer and uncovered the BRD4/B7-H3/TLR4 signaling. Targeting B7-H3 by the BET inhibitor may be a novel therapeutic strategy to overcome the immunotherapy and chemotherapy resistance in pancreatic cancer.

Author contribution

J.Z., Z.M., C.X. and C.Y. performed the experiments, Z.L., S.W., B.W. and P.F. collected data; X.J. and H.W. wrote the paper and analyzed the data.

Conflict of interest

No potential conflicts of interest are disclosed.

Acknowledgement

This work was supported in part by grants from the Chinese National Natural Science Foundation Grant No. 81702374 (X.J.) and No. 81372261 (H.W.).

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