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Original Article

BET inhibitor OTX015 suppresses gastric cancer through pharmacology and promotion of the immune response

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Abstract: Objective: We aimed to explore the effect of BET inhibitors on the pharmacological and immune checkpoint PD-L1 expressions of gastric cancer cells. Methods: We treated gastric cancer cells with the BET inhibitor OTX015 and determined the apoptosis and proliferation of the gastric cancer cells using flow cytometry and CCK8 experiments, respectively. Then, we determined the expression changes of the proliferation and apoptosis-related genes before and after the OTX015 treatment using qPCR and Western blot. Next, we examined the mRNA and protein expression changes before and after the OTX015 treatment using flow cytometry and qPCR. To demonstrate the interaction between the BRD2/4 protein and the PD-L1 promoter, we performed ChIP experiments. After that, we tested the killing effect of the immune cells co-incubated with gastric cancer cells treated with or without OTX015 by LDH, and we detected the secretion of cytokines using ELISA. Finally, we collected patient samples and tested the expressions and correlations of BRD2/4 and PD-L1 in the patient samples using qPCR. Results: OTX015 can inhibit gastric cancer cell proliferation and promote gastric cancer cell apoptosis through pharmacological effects. In addition, OTX015 can also promote the killing effect of immune cells by inhibiting PD-L1. At the same time, in patient samples, BRD2/4 is consistent with the expression of PD-L1 and related to the clinical characteristics of patients. Conclusion: OTX015 is a drug with a potential dual anticancer effect.

Keywords: Gastric cancer, OTX015, PD-L1, BRD2, BRD4

Introduction

Recently, the incidence of cancer has been rising due to various factors in China [1]. In China, gastric cancer has become the second most lethal and fourth most common type of cancer [2, 3]. However, due to the easy metastasis and recurrence of gastric cancer, we currently lack a method to completely cure gastric cancer [4]. With the continuous progress of basic research, researchers have found that epigenetic genetic modification is also an important determinant in tumors [5-8]. In epigenetics, epigenetic changes such as histone methylation, acetylation, and DNA methylation all are involved in the transcriptional regulation of oncogenes. Therefore, in response to these epigenetic changes, researchers have developed a series of small molecular drugs [9-13]. The bromodomain and the extra-terminal domain (BET) family are important components of epigenetic inheritance, which can recruit transcription factors such as cyclin T1 to the position of acetylated histones as a "reader" protein, thereby promoting target gene expression [14]. Among them, the researchers found that BRD2 and BRD4 can interact with sequences upstream of the promoter region of the cancer-related genes to promote the expressions of related genes [15]. As a result, researchers have developed a series of small molecule inhibitors that target BRD2 and BRD4, including JQ1, OTX015, and PFI. In previous studies, researchers found that, on the one hand, BET protein inhibitors can inhibit the proliferation of cancer cells, and, on the other hand, they can also increase the apoptosis of cancer cells [16, 17].

Recently, some studies found that some small molecule inhibitors related to epigenetic genetic modification can improve the killing effect of immune cells on cancer cells by improving the microenvironment of the tumor cells [18-20]. In autoimmune function, immune checkpoints play an important role [21]. Researchers

found that there are different cell proteins on the cell surface that activate or inhibit the activity of immune cells, namely immune checkpoints, including CD40L, CD137/4-1BB, CTLA4, and CD279/PD1. The immune checkpoint protein on the cell surface and the ligand protein on the tumor surface work together as an immune switch to regulate immune function [21-25].

In the tumor cells of many cancer patients, the cells will overexpress PD-L1, which interacts with the PD-1 expressed by immune cells, causing the immune cells to fail to recognize the tumor cells [26]. Therefore, disrupting the combination of PD1 and PD-L1 is a proven immunotherapy option for cancer [27, 28]. Interestingly, in 2017, researchers found that JQ1, a BET protein inhibitor, significantly reduces the mRNA level of PD-L1 in lymphoma cells [29]. This suggests that the BET protein could be involved in controlling the expressions of immune checkpoint proteins.

We explored the effect of the BET inhibitor OTX015 on gastric cancer cells. On the one hand, we further confirmed that the BET inhibitor OTX015 can promote the apoptosis of gastric cancer cells and inhibit the proliferation of gastric cancer cells, which are time- and dose-dependent. On the other hand, we also found that BRD2 and BRD4 can interact with the promoter region of PD-L1, thereby regulating the expression of PD-L1. After treatment with OTX015, we discovered that the mRNA and protein levels of PD-L1 had decreased. When the expression of PD-L1 decreased, we found that the killing effect of PBMCs on gastric cancer cells increased significantly after the co-incubation of PBMCs and gastric cancer cells, which indicated that OTX015 can also hinder the immune escape of tumor cells. Finally, we measured the mRNA levels of PD-L1, BRD2, and BRD4 in the gastric cancer patient samples. We found that the mRNA level of PD-L1 was consistent with the mRNA levels of BRD2 and BRD4 in the gastric cancer samples. This suggests that OTX015, a BET inhibitor, has dual effects on gastric cancer cells.

Materials and methods

Antibodies and reagents

The antibodies and reagents that we used include: anti-GAPDH (abcam, ab181602, 1:5000), anti-Caspase-3 (abcam, ab32351, 1:2000), anti-BRD2 (abcam, ab245436, 1:1000), anti-Bcl2 (abcam, ab42124, 1:2000), anti-BRD4 (abcam, ab243862, 1:1000), goat anti-rabbit IgG HRP (abcam, ab205718, 1:5000) were purchased from Abcam. PMD18-T (6011) was purchased from Takara (Beijing, China). FastFire qPCR PreMix (SYBR Green) (FP208), a cell genome extraction kit (DP304), and a plasmid extraction kit (DP103, DP108, DP117) were purchased from Tiangen (Beijing, China). A Gel Extraction Kit (CW2302) was purchased from CWBIO (Nanjing, China).

Cell culture

The SGC7901 cells were cultured in DMEM medium that contained 10% FBS (Gibco), and 1% penicillin/streptomycin (Gibco). As usual, the SGC7901 cells were maintained at 37°C in a 5% CO_2 atmosphere and passaged every 2 days.

Western blot

A total of 1×10^6 SGC7901 cells treated with or without the indicated concentrations OTX015 were collected and lysed with a RIPA buffer. After that, we detected the protein concentration with the BCA assay, and the same amount of protein was subjected to SDS PAGE gel, 80 V electrophoresis for 90 min, then transferred on N.C membrane with 220 A for 2 h. The NC membrane of the corresponding size band was cut off and blocked with 5% BSA for 1 h, then incubated with a corresponding primary antibody at 4°C overnight. The next day, the bands were incubated with the corresponding secondary antibodies for 2 h at room temperature. The bands were incubated with an ECL luminescent solution in the dark for 3 minutes and then exposed using an exposure system to save the pictures.

Vector construction

Individual sgRNA constructs targeting BRD2 and BRD4 were cloned into lentiCRISPR v 2.0 (addgene 52961). The primer sequence was as follows: sg-BRD2-F: 5'-CGA CCA CTC TCT CTA CGC AT-3'; sg-BRD2-R: 5'-ATG CGT AGA GAG AGT GGT CG-3'; sg-BRD4-F: 5'-ACT AGC ATG TCT GCG CTC AG-3'; sg-BRD4-R: 5'-CTC AGG GCA GAC ATG CTA GT-3'.

ELISA quantification of the antigen IL-2 and TNF-α levels

In a 6-well plate, we added a total of 1 × 10^5 SGC7901 cells treated with or without the indicated concentrations OTX015 and 1 × 10^6 PBMCs. After 48 h, we collected the cell supernatant and then measured the IL-2 and TNF-α using the IL-2 and TNF-α ELISA kit IL-2 (R&D Systems, Inc., Minnesota, USA).

ChIP experiments

We performed chromatin immunoprecipitation (ChIP) experiments (Millipore). In short, SGC7901 cells were cross-linked with 1% formaldehyde for 20 min at room temperature and then quenched with 0.125 M glycine for 10 min. The cells were collected and then lysed with an SDS lysis buffer. The cell genome was disrupted to a size of 200-1000 bp using a sonicator (Bioruptor) for 12 min (8 s on and 8 s off) at 4°C. The broken gene component is divided into three parts, which are the input group, the IgG mock group, and the anti-BRD2/4 group. Then, the IgG mock group and the anti-BRD2/4 group were incubated with the antibody-IgG, the antibody-BRD2, or the antibody-BRD4 at 4°C overnight. We added Protein G/A-labeled Dynabeads to each group at 4°C for 2 h. The elution buffer was used to elute the magnetic bead-linked immunoprecipitate, and then 5 M NaCl was used to uncrosslink. The proteinase and RNA were digested with proteinase K and RNase, respectively, and the DNA was retained. Finally, the immunoprecipitated DNA was subsequently analyzed using qPCR. The primer sequence was as follows: BRD2-F: 5'-GGA AGAT GAG GAG GAC GAG G-3'; BRD2-R: 5'-TGG GCT TGG ATA TTG GAC CC-3'; BRD4-F: 5'-ATA CCT GCT CAG AGT GGT GC-3'; BRD4-R: 5'-TGT TCC CAT ATC CAT AGG CGT-3'.

Real-time quantitative PCR

SGC7901 cells treated with or without the indicated concentrations of OTX015 in the 6-well plate were digested with 1 ml Trizol for 30 min, and then the total RNA of the cells was extracted according to the Trizol method. The relative expression levels were calculated using the formula of 1/2ΔCt. The primer sequence was as follows: BRD2-F: 5'-GGA AGA TGA GGA GGA CGA GG-3'; BRD2-R: 5'-TGG GCT TGG ATA TTG GAC CC-3'; BRD4-F: 5'-ATA CCT GCT CAG AGT GGT GC-3'; BRD4-R: 5'-TGT TCC CAT ATC CAT AGG CGT-3'; C-myc-F: 5'-CGA CGA GAA GCG TAG CTT TT-3'; C-myc-R: 5'-CTC GCC GTT TCC TCA GTA AG-3'; Cyclin D1-F: 5'-TGC CAC AGA ACA GAA GTT CAT T-3'; Cyclin D1-R: 5'-AGA AGG GCT TCA ATC TGT TCC-3'; PD-L1-F: 5'-CCA TCC CGC TCT TCA TTC AGA-3'; PD-L1-R: 5'-AGG TGG CAG TGA AGC TCG A-3'; β-actin-F: 5'-CAC CTA GGA CCC GCC CCT CTC TAG-3; β-actin-R: 5'-TAA TCT CCT GAT TGC GTT CAC TCA-3'.

Cell counting Kit-8

2 × 10^4 SGC7901 cells were added to each well of the 96-well plates. To measure the OD value, we added 10 μL CCK8 solution to the fresh medium and incubated it at 37°C for 6 hours. Then we determined the OD 450 nanometer value.

Cell apoptosis detection

We measured the number of apoptotic cells using an Apoptosis Detection Kit (Yeasen, China). 1 × 10^6 SGC7901 cells treated with or without the indicated concentrations of OTX015 were collected. We subsequently added 5 μL of PI and 5 μL of Annexin V-FITC and incubated on the solution on ice in the dark for 30 min, then we washed it with PBS twice. Then the number of apoptotic cells was quantified using flow cytometry (Caliber, BD), and we analyzed the data using FlowJo Software.

Primary PBMC cells

The PBMCs were obtained by isolating blood from patients at the Changhai Hospital. The PBMC cells were maintained in a serum-free medium that contained 5 ng/ml IL-2 (R&D) and 10 ng/ml IL-7 (R&D) and 1% penicillin-streptomycin at 37°C in a 5% CO_2 atmosphere.

Cytotoxicity assay

1 × 10^5 PBMCs were co-cultured with or without 1 × 10^4 SGC7901 cells treated with different concentrations of OTX015 in DMEM with 10% FBS, 5 ng/ml IL-2 (R&D), 1% penicillin-streptomycin, and 10 ng/ml IL-7 (R&D) at 37°C in a 5% CO_2 atmosphere for 24 h. then, we used a CytoTox 96 Nonradioactive Cytotoxicity Assay Kit to measure the released lactate dehydrogenase (LDH) in the supernatants.

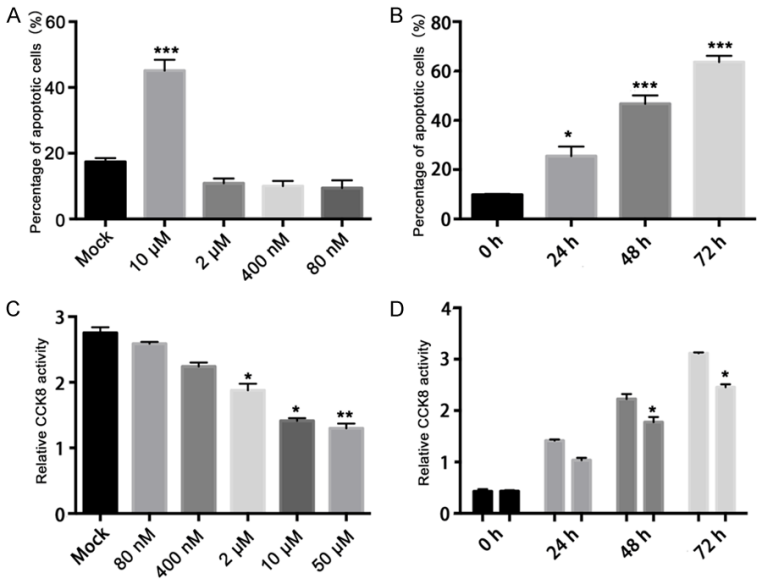

Figure 1. OTX015 promotes apoptosis and inhibits the proliferation of SGC7901 cell culture models. A. SGC7901 cells were treated with 10 μM, 2 μM, 400 nM, and 80 nm of OTX015 for 48 h and measured using flow cytometry and presented as fluorescence histograms. B. SGC7901 cells were treated with 10 μM OTX015 for 0 h, 24 h, 48 h and 72 h, then measured using flow cytometry and presented as fluorescence histograms. C. SGC7901 cells were treated with 10 μM, 2 μM, 400 nM, 80 nM and 16 nM of OTX015 for 48 h and then the cell viability was measured using the CCK-8 method. Changes in OD450 between the different drug concentrations indicated the percentage of cell viability. D. SGC7901 cells were treated with 10 μM OTX015 for 0 h, 24 h, 48 h or 72 h and then the cell viability was measured using the CCK-8 method. Changes in OD450 between the different times indicated the percentage of cell viability. Each data point represents the mean ± SD of three independent experiments and was analyzed with a T-test. *P<0.05, **P<0.01, ***P<0.001.

Statistical analysis

Each experiment was performed in triplicate independently and error bars are standard errors (SD). In order to calculate whether the results are statistically different, we performed t-tests using SPSS version 17.0 (SPSS Inc., Chicago), and statistical significance was indicated at *P<0.05, **P<0.01, or ***P<0.001.

Results

OTX015 can promote the apoptosis of SGC7901

OTX015, a BET protein inhibitor, has entered into clinical trials of various cancers. In 2015, researchers found that OTX015 promoted apoptosis in acute leukemia cells [30]. However, in gastric cancer cells, there is no relevant published study. Therefore, we first explored the effect of OTX015 on the gastric cancer cell line SGC7901. First, we treated SGC7901 cells for 48 h with different concentrations of OTX015. The results showed that OTX015 can promote the apoptosis of SGC7901 at a high concentration (10 um) (**Figure 1A**). Then, in order to further explore the time-dose relationship between OTX015 and SGC7901 apoptosis, we conducted a time-dependent experiment. We found that the amount of apoptosis in the SGC7901 cells significantly increased over time after treatment with OTX015 (**Figure 1B**). This suggests that OTX015 may be a good antineoplastic drug when used at high concentrations and given enough time to act.

OTX015 inhibits the proliferation of SGC7901 cells in a dose- and time-dependent manner

In addition to directly promoting the apoptosis of cancer cells, anti-cancer drugs can also hinder the progress of cancer by inhibiting its proliferation. Therefore, we further explored the action of OTX015 on the proliferation of SGC7901 cells. We measured the proliferation of SGC7901 cells treated with or without the indicated concentrations OTX015 using CCK8. We found that the proliferation of SGC7901 decreased significantly when treated with increased concentrations of OTX015, and the effect became more significant the higher the concentration (**Figure 1C**). In addition, we also examined the relationship between OTX015 inhibition on SGC7901 cell proliferation over time. Then we found that OTX015 inhibited the proliferation of the SGC7901 cells, and the effect increased with time (**Figure 1D**). These results suggest that OTX015, as an antineoplastic drug, can also alleviate the progression of tumors by inhibiting the proliferation of SGC7901 cells.

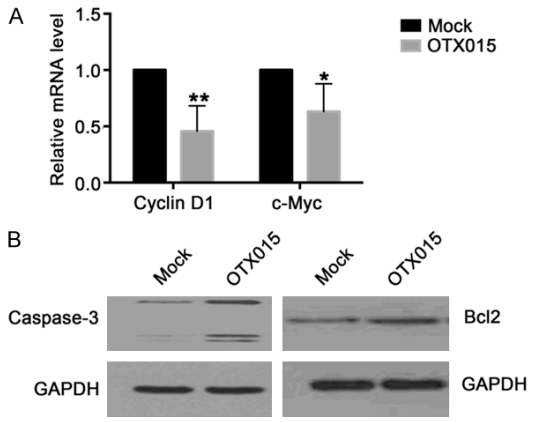

Figure 2. Inhibitory mechanisms of OTX015 on SGC7901. A. SGC7901 cells were treated with or without 10 μM OTX015 for 48 h. Then total RNA was extracted from the cells, and cyclin D1 and C-Myc were quantified using qPCR after reverse transcription of the cDNA. Each data point represents the mean ± SD of three independent experiments which were analyzed with a T-test. *P<0.05, **P<0.01, ***P<0.001. B. The effect of OTX015 on the expression of the apoptotic pathway proteins. The expression of the Caspase-3 and Bcl2 levels in the total protein lysates were measured using Western blot in the SGC7901 cells treated with or without 10 μM OTX015 for 48 h.

OTX015 plays an anti-tumor role by changing the expression of proliferative and apoptotic genes

We wanted to explore the reason why OTX015 inhibits the proliferation of SGC7901 cells, so we examined the expressions of the genes related to proliferation [31, 32]. The mRNAs of c-Myc and cyclin D1 were positively correlated with cell proliferation. Therefore, we first determined the effect of OTX015 on the mRNA of c-Myc and cyclin D1 using qPCR. We found that the mRNA levels of c-Myc and cyclin D1 in the SGC7901 cells treated with OTX015 decreased significantly, suggesting that OTX-015 can hinder the proliferation of SGC7901 cells by inhibiting c-Myc and cyclin D1 (**Figure 2A**). However, we also wanted to know why OTX015 promotes the apoptosis of gastric cancer cells. Caspase-3 and Bcl-2 proteins are the core proteins in the apoptotic process [33, 34]. Therefore, we decided to explore the impact of OTX015 on Caspase 3 and Bcl-2 using Western blot. We found that there were more Caspase-3 and Bcl2 proteins after the OTX015 treatment, suggesting that OTX015 promotes apoptosis by initiating the classic apoptotic pathways of cells (**Figure 2B**).

OTX015 inhibits the PD-L1 in SGC7901 cells

Some researchers have found that JQ1 can inhibit the mRNA and protein levels of PD-L1 in lung cancer cells [29]. Therefore, we decided to explore whether OTX015 has a similar effect on gastric cancer cells. So, we first activated PD-L1 in SGC7901 cells using IFN-α, and then we treated the activated cells with different concentrations of OTX015 to explore the action of OTX015 on PD-L1. We found that as the OTX015 concentration increased, the level of PD-L1 decreased significantly suggesting that OTX015 not only inhibits the transcription of PD-L1, but we also discovered that it has a dose-dependent effect after the analysis using qPCR and flow cytometry (**Figure 3A**, **3C**). In addition, we further examined the time dependence of OTX015 on PD-L1. The results from qPCR and flow cytometry showed that the mRNA and protein levels of PD-L1 were reduced with the prolongation of the OTX015 treatment (**Figure 3B**, **3D**). In conclusion, OTX015 can inhibit PD-L1 in gastric cancer cells just as JQ1 does in lung cancer cells, suggesting that OTX015 may also be a potential immunosuppressant.

BRD2 and BRD4 promote the transcription of PD-L1 by interaction with the promoter of PD-L1

Since we already knew that OTX015 is an inhibitor of BET proteins, we speculated that BRD2 and BRD4 of the BET protein family may interact with the promoter of PD-L1. Therefore, we first determined whether BRD2 and BRD4 can interact with the PD-L1 promoter region using a chromatin immunoprecipitation assay. The results showed that both BRD2 and BRD4 can bind to the promoter region of PD-L1 (**Figure 4A**, **4B**). When the SGC7901 cells were treated with OTX015, we found that the interaction of BRD2 and BRD4 with the region of PD-L1 was significantly reduced using qPCR (**Figure 4C**, **4D**). These results further support our hypothesis. Finally, in order to exclude the possibility that the unknown pharmacological effects of OTX015 may decrease the PD-L1, we detected it by knocking out the BRD2 and BRD4 proteins in the SGC7901 cells. The results showed that the induced expression levels of PD-L1 in the BRD2 or BRD4 knockout cells treated with IFN-α were lower than they were in the non-knockout cells treated with

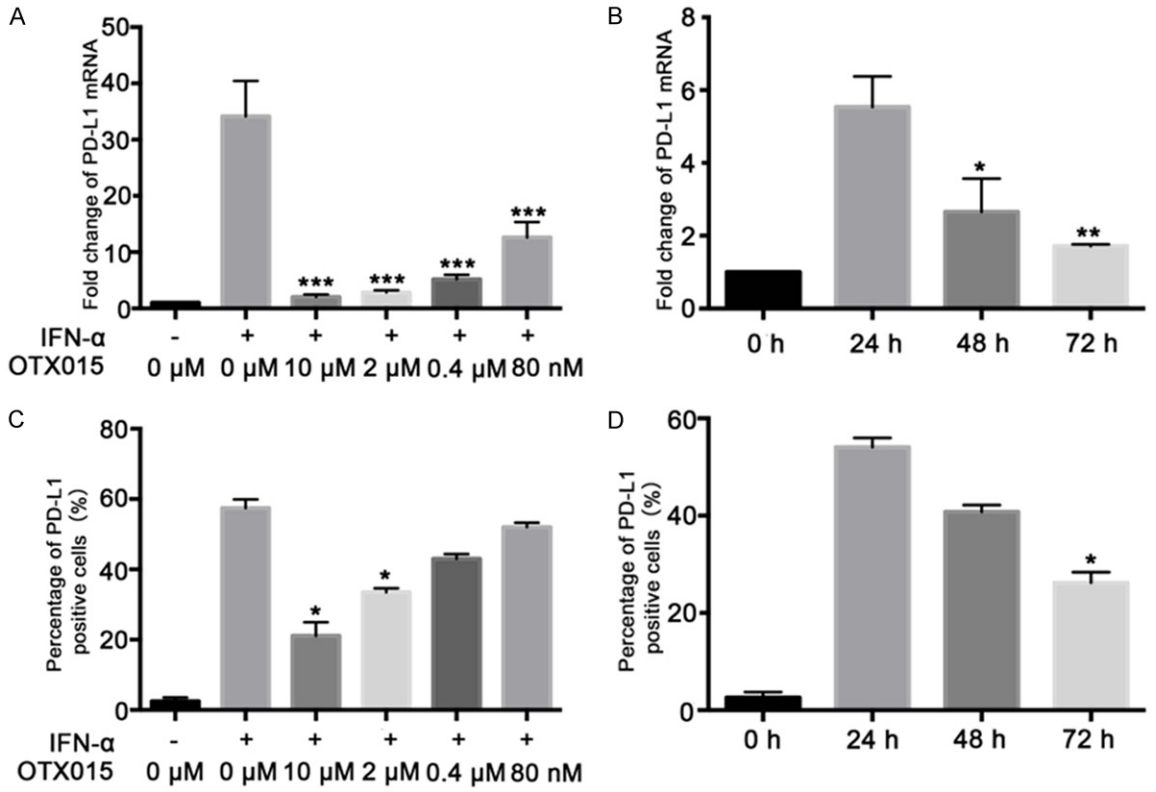

Figure 3. OTX015 inhibits the expression of PD-L1 in the SGC7901 cells. (A, B) SGC7901 cells were treated with IFN-α alone or with 20 μM, 2 μM, 200 nM, or 20 nm of OTX015 for 48 h. Then total RNA was extracted from the cells, and PD-L1 was measured using qPCR after the reverse transcription of cDNA (A) and measured by flow cytometry using anti-CD279-FITC and presented as fluorescence histograms (B). (C, D) SGC7901 cells were treated with 20 μM OTX015 for 0 h, 24 h, 48 h, and 72 h, then the total RNA was extracted from the cells, and PD-L1 was determined using qPCR after the reverse transcription of cDNA (C) and measured using flow cytometry using anti-CD279-FITC (D). Each data point represents the mean ± SD of three independent experiments which were analyzed with a T-test. *P<0.05, **P<0.01, ***P<0.001.

IFN-α (**Figure 4E**). Based on this evidence, it is reasonable to conclude that OTX015 inhibits the transcription of PD-L1 by limiting the interaction of BRD2 and BRD4 with the promoter region of PD-L1.

OTX015 promotes the killing of SGC7901 cells using immune cells

PD-L1 can help cancer cells escape destruction via immune cells [21, 24]. We know that OTX015 can inhibit PD-L1. Therefore, we want to further understand whether OTX015 can attenuate immune escape by inhibiting PD-L1. To this end, we first isolated PBMC from human peripheral blood. We incubated the PBMCs and SGC7901 cells treated with or without the indicated concentrations of OTX015 for 24 h, then we detected the killing effect of PBMCs on the SGC7901 cells using an LDH detection kit. The results showed that the PBMCs enacted a more obvious killing effect on the SGC7901 cells treated with OTX015 than it did with the untreated SGC7901 cells (**Figure 5A**). In addition, we want to confirm the killing effect of the PBMCs on the SGC7901 cells by cytokine secretion, so we used ELISA to determine the secretion levels of the cytokines related to this killing effect in the cells' supernatant after co-incubation. After treatment with OTX015, the secretions of TNF-α and IL-2 in the supernatant increased, and as the OTX015 concentration increased, the secretions of TNF-α and IL-2 were observed to gradually increase as well (**Figure 5B**, **5C**).

Analysis of the BRD2, BRD4, and PD-L1 expressions in the patient samples

Regarding the SGC7901 cells, we concluded that BRD2 and BRD4 promote the transcription of PD-L1 by interacting with the promoter

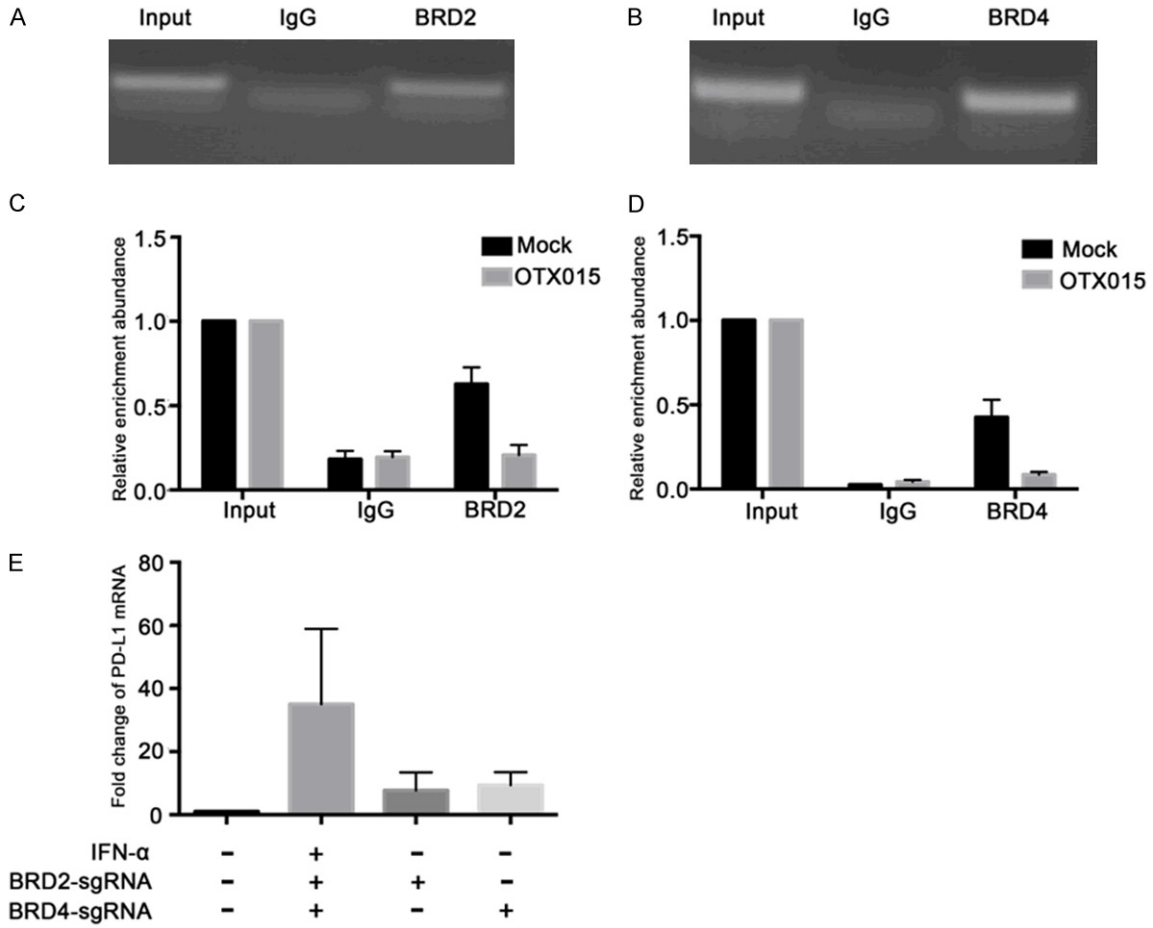

Figure 4. OTX015 reduces BRD2 and BRD4 recruitment to the PD-L1 promoter and inhibited PD-L1 transcription. A, B. ChIP assays were performed using antibodies against BRD2, BRD4, and normal mouse IgG. PCR primers specific for the PD-L1 promoter were used to amplify the DNA isolated from the immunoprecipitated chromatin. C, D. SGC7901 cells were mock-treated or stimulated with OTX015 (2 μM) for 6 h. ChIP assays were performed using antibodies against BRD2, BRD4, and normal mouse IgG. qPCR primers specific for the PD-L1 promoter were used to amplify the DNA isolated from the immunoprecipitated chromatin. E. SGC7901 cells were first transfected with PX459 plasmids targeting BRD2 and BRD4, and then selected with Puro for 3 days. Then the SGC7901 cells were treated with IFN-α alone or with 20 μM OTX015 for 48 h. Then total RNA was extracted from the cells, and PD-L1 was determined using qPCR after reverse transcription of the cDNA. Each data point represents the mean ± SD of three independent experiments, which were analyzed with a T-test. *P<0.05, **P<0.01, ***P<0.001.

region of PD-L1. However, there are distinct individual differences between patients, so we decided to further analyze the mRNA levels of BRD2, BRD4, and PD-L1 in the patient samples. We first collected paraffin samples of 30 patients with gastric cancer who were treated in Shanghai Hospital from 2012 to 2018. After RNA was extracted from the samples, we measured the PD-L1 BRD2, and BRD4 levels using qPCR. We found that PD-L1 increased significantly in 20 out of 30 patients, and the BRD2 and BRD4 also increased significantly in 16 of 20 patients. This indicated that PD-L1, BRD2, and BRD4 were positively correlated in patients (**Figure 6**). To further explore the relationship of these proteins in cancer patients, we analyzed the relative mRNA levels of PD-L1, BRD2, BRD4, and the clinical data of gastric cancer. We found that PD-L1, BRD2, and BRD4 were significantly correlated with TMN and tumor size. However, there was no significant correlation with the clinical features such as age, sex, or metastasis (**Table 1**). This indicates that BRD2, BRD4, and PD-L1 are correlated with tumor progression in vivo. Therefore, OTX015 may be a good target drug.

Discussion

Due to dietary habits and other reasons, gastric cancer is a cancer type with a high incidence in China, and it is a complex disease with

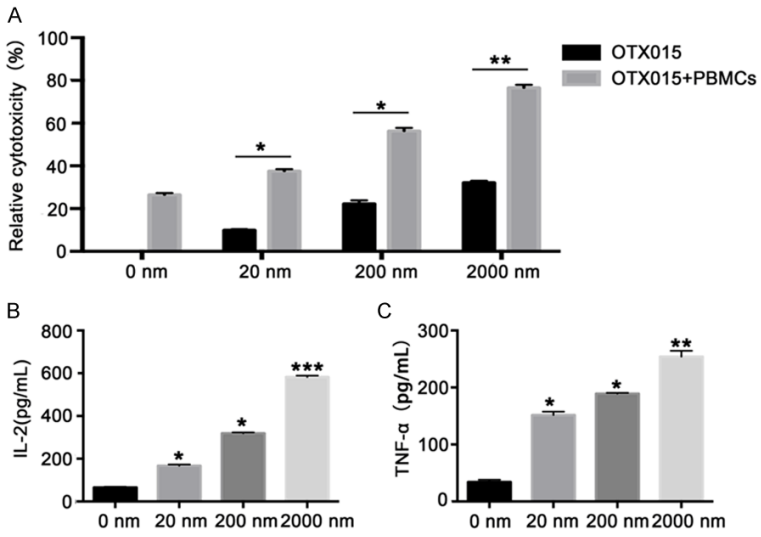

Figure 5. OTX015 can promote the killing effect of PBMC on SGC7901. (A) SGC7901 cells were mock-treated with OTX015 (2000 nM, 200 nM, and 20 nm) for 48 h. These cells were then incubated with PBMCs at a ratio of 1:10 for 24 hours. The killing effect of PBMC on SGC7901 was tested using a LDH assay. (B, C) The supernatants of the co-incubated cells were collected and IL-2 (B) and TNF-α (C) secreted by PBMCs were determined using an ELISA kit. Each data point represents the mean ± SD of three independent experiments and was analyzed with a T-test. *P<0.05, **P<0.01, ***P<0.001.

a high mortality rate [1, 2]. Currently, the main treatment for gastric cancer is surgical resection, but after the surgery, the patients also need to take medicine to control the metastasis and spread of the gastric cancer cells. In the past, controlling the spread and recurrence of gastric cancer was mainly achieved through chemotherapeutic drugs. However, in recent years, more and more attention has been paid to targeted therapy, and the BET protein has become one of the most popular targets. Some studies have found that that BET protein inhibitors can control cancer cell proliferation and promote cancer cell apoptosis by inhibiting the expression of oncogenes. BET protein inhibitors have now entered the clinical research stage due to their good therapeutic effects in vitro.

In addition to targeted therapy, immune checkpoints such as PD-1 and CTAL4 are also important targets in immunotherapy research. In previous studies, researchers have found that the PD-1 receptor molecule is present on the surfaces of immune cells, and tumor cells can achieve immune escape by promoting the interaction of PD1 and PD-L1. Interestingly, in 2017 researchers found that JQ1, one of the BET inhibitors, can down-regulate the PD-L1 in NCSCL, which may be a new treatment idea for BET inhibitors.

However, is JQ1 only a BET inhibitor, or does it have another mechanism of action that impacts PD-L1? Is it a specific treatment for lung cancer alone, or does it have the same effect on multiple types of cancer? This information has not been elucidated. Therefore, we intended to preliminarily explore the answers to the above questions using OTX015, another BET inhibitor, in gastric cancer cells.

We first found that OTX015 could promote the apoptosis of SGC7901 cells and inhibit the proliferation of SGC7901 cells at high concentrations. Furthermore, we discovered that the apoptotic and inhibition effects of the drug are achieved by inhibiting the expressions of the proliferating genes and promoting the expression of the apoptotic genes. In addition, we found that OTX015 can also inhibit PD-L1 in SGC7901 cells and that this inhibition was achieved on the RNA level. We further noted that BRD2 and BRD4 of the BET protein family can interact directly with the promoter region of PD-L1. After knocking out these two proteins, the transcription of PD-L1 is relatively reduced, which means that BRD2 and BRD4 can promote the expression of PD-L1. We then found that when the SGC7901 cells were treated with OTX015, the PD-L1 was decreased, and the killing effect of PBMCs on SGC7901 cells was increased, which indicated that OTX015 has a dual anti-tumor effect on gastric cancer.

Although we have preliminarily demonstrated the dual anti-tumor effect of OTX015, there are still some shortcomings to this study. First, the effect of OTX015 on gastric cancer cells is mainly focused on the SGC7901 cell model, and we did not analyze any other models in this study. Second, the conclusions we have drawn were all obtained by using in vitro experiments, and the results may differ if performed in vivo. However, despite these shortcomings, our

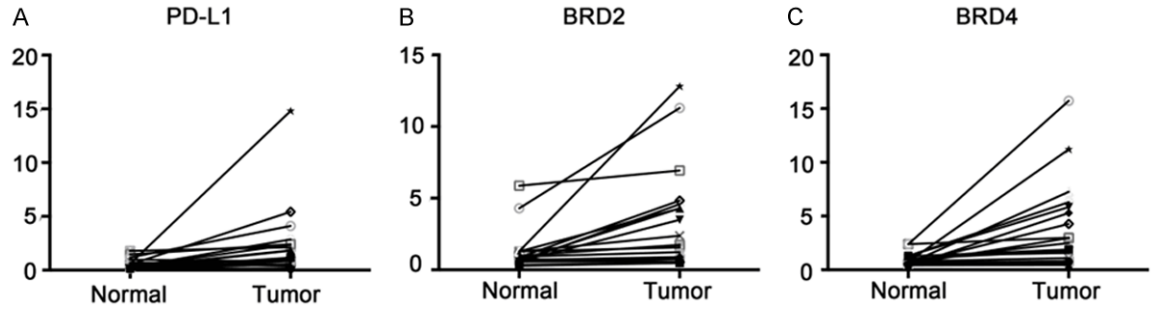

Figure 6. The expressions of PD-L1, BRD2, and BRD4 in the patient samples. (A-C) The expressions of PD-L1 (A), BRD2 (B), and BRD4 (C) were detected using qPCR after the retroviral transcription of the RNA from the cancer tissues and the adjacent tissues.

Table 1. Analysis of the correlation between the expression of PD-L1 and the clinical characteristics of the patients

Parameter	n	PD-L1	*P* value
Sex			0.678
Male	14	3.271	
Female	6	4.021	
Age			0.825
≤60 years	8	3.427	
>60 years	12	3.854	
TNM			0.019
I-II	4	1.857	
III-IV	14	6.225	
Tumor diameter			0.043
≤5 cm	12	2.142	
>5 cm	8	5.924	
Lymph node metastasis			0.804
No	6	2.802	
Yes	14	2.571	
Degree of differentiation			0.987
Poorly differentiated	14	2.88	
Medium to high differentiation	6	2.967	

research lays the groundwork for further studies and provides many useful references for subsequent researchers.

In conclusion, we confirmed for the first time that OTX015 has dual antineoplastic effects on gastric cancer cells, suggesting that OTX-015 is not only an epigenetic targeting drug, but it is also a potential promoter of cellular immunotherapy.

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Disclosure of conflict of interest

None.

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References

[1] Bode AM, Dong Z and Wang H. Cancer prevention and control: alarming challenges in China. Natl Sci Rev 2016; 3: 117.

[2] Nie Y, Wu K, Yu J, Liang Q, Cai X, Shang Y, Zhou J, Pan K, Sun L, Fang J, Yuan Y, You W and Fan D. A global burden of gastric cancer: the major impact of China. Expert Rev Gastroenterol Hepatol 2017; 11: 651-661.

[3] Zhang XY and Zhang PY. Gastric cancer: somatic genetics as a guide to therapy. J Med Genet 2016; 54: 305-312.

[4] Roy PS and Saikia BJ. Cancer and cure: a critical analysis. Indian J Cancer 2016; 53: 441-442.

[5] Goldberg AD, Allis CD and Bernstein E. Epigenetics: a landscape takes shape. Cell 2007; 128: 635-638.

[6] Egger G, Liang G, Aparicio A and Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. Nature 2004; 429: 457-463.

[7] Nebbioso A, Tambaro FP, Dell'Aversana C and Altucci L. Cancer epigenetics: moving forward. PLoS Genetics 2018; 14: e1007362.

[8] You JS and Jones PA. Cancer genetics and epigenetics: two sides of the same coin? Cancer Cell 2012; 22: 9-20.
[9] Bennett RL and Licht JD. Targeting epigenetics in cancer. Annu Rev Pharmacol 2017; 58: 187.
[10] Kristensen LS, Nielsen HM and Hansen LL. Epigenetics and cancer treatment. Eur J Pharmacol 2009; 625: 131-142.
[11] Peedicayil J. The role of DNA methylation in the pathogenesis and treatment of cancer. Curr Clin Pharmacol 2012; 7: 333-340.
[12] West AC and Johnstone RW. New and emerging HDAC inhibitors for cancer treatment. J Clin Invest 2014; 124: 30-39.
[13] Falkenberg KJ and Johnstone RW. Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. Nat Rev Drug Discov 2014; 13: 673-691.
[14] Filippakopoulos P and Knapp S. Targeting bromodomains: epigenetic readers of lysine acetylation. Nat Rev Drug Discov 2014; 13: 337-356.
[15] Lovén J, Hoke HA, Lin CY, Lau A, Orlando DA, Vakoc CR, Bradner JE, Lee TI and Young RA. Selective inhibition of tumor oncogenes by disruption of super-enhancers. Cell 2013; 153: 320-334.
[16] da Motta LL, Ledaki I, Purshouse K, Haider S, De Bastiani MA, Baban D, Morotti M, Steers G, Wigfield S, Bridges E, Li JL, Knapp S, Ebner D, Klamt F, Harris AL and McIntyre A. The BET inhibitor JQ1 selectively impairs tumour response to hypoxia and downregulates CA9 and angiogenesis in triple negative breast cancer. Oncogene 2017; 36: 122-132.
[17] Coudé MM, Braun T, Berrou J, Dupont M, Bertrand S, Masse A, Raffoux E, Itzykson R, Delord M, Riveiro ME, Herait P, Baruchel A, Dombret H and Gardin C. BET inhibitor OTX015 targets BRD2 and BRD4 and decreases c-MYC in acute leukemia cells. Oncotarget 2015; 6: 17698-17712.
[18] Peedicayil J. The role of DNA methylation in the pathogenesis and treatment of cancer. Curr Clin Pharmacol 2012; 7: 333-340.
[19] West AC and Johnstone RW. New and emerging HDAC inhibitors for cancer treatment. J Clin Invest 2014; 124: 30-39.
[20] Falkenberg KJ and Johnstone RW. Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. Nat Rev Drug Discov 2014; 13: 673-691.
[21] Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012; 12: 252-264.
[22] Topalian SL, Drake CG and Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell 2015; 27: 450-461.
[23] Stromnes IM, Hulbert A, Pierce RH, Greenberg PD and Hingorani SR. T-cell localization, activation, and clonal expansion in human pancreatic ductal adenocarcinoma. Cancer Immunol Res 2017; 5: 978-991.
[24] Gomes-Silva D, Mukherjee M, Srinivasan M, Krenciute G, Dakhova O, Zheng Y, Cabral JMS, Rooney CM, Orange JS, Brenner MK and Mamonkin M. Tonic 4-1BB costimulation in chimeric antigen receptors impedes T cell survival and is vector-dependent. Cell Rep 2017; 21: 17-26.
[25] Öckinger J, Hagemann-Jensen M, Kullberg S, Engvall B, Eklund A, Grunewald J, Piehl F, Olsson T and Wahlström J. T-cell activation and HLA-regulated response to smoking in the deep airways of patients with multiple sclerosis. Clin Immunol 2016; 169: 114-120.
[26] Couzin-Frankel J. Breakthrough of the year 2013. Cancer immunotherapy. Science 2013; 342: 1432-1433.
[27] Nicodemus CF. Antibody-based immunotherapy of solid cancers: progress and possibilities. Immunotherapy 2015; 7: 923-39.
[28] Prasad V and Kaestner V. Nivolumab and pembrolizumab monoclonal antibodies against programmed cell death-1 (PD-1) that are interchangeable. Semin Oncol 2017; 44: 132-135.
[29] Hogg SJ, Vervoort SJ, Deswal S, Ott CJ, Li J, Cluse LA, Beavis PA, Darcy PK, Martin BP, Spencer A, Traunbauer AK, Sadovnik I, Bauer K, Valent P, Bradner JE, Zuber J, Shortt J and Johnstone RW. BET-bromodomain inhibitors engage the host immune system and regulate expression of the immune checkpoint ligand PD-L1. Cell Rep 2017; 18: 2162-2174.
[30] Coudé MM, Braun T, Berrou J, Dupont M, Bertrand S, Masse A, Raffoux E, Itzykson R, Delord M, Riveiro ME, Herait P, Baruchel A, Dombret H and Gardin C. BET inhibitor OTX015 targets BRD2 and BRD4 and decreases c-MYC in acute leukemia cells. Oncotarget 2015; 6: 17698-17712.
[31] Kim JK and Diehl JA. Nuclear cyclin D1: an oncogenic driver in human cancer. J Cell Physiol 2010; 220: 292-296.
[32] Xu AG, Li SG, Liu JH and Gan AH. Function of apoptosis and expression of the proteins Bcl-2, p53 and C-myc in the development of gastric cancer. World J Gastroenterol 2001; 7: 403-406.
[33] Porter AG and Jänicke RU. Emerging roles of caspase-3 in apoptosis. Cell Death Differ 1999; 6: 99-104.
[34] Chao DT and Korsmeyer SJ. BCL-2 family: regulators of cell death. Annu Rev Immunol 1998; 16: 395-419.