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WJGO mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal oncology and covering a wide range of topics including liver cell adenoma, gastric neoplasms, appendiceal neoplasms, biliary tract neoplasms, hepatocellular carcinoma, pancreatic carcinoma, cecal neoplasms, colonic neoplasms, colorectal neoplasms, duodenal neoplasms, esophageal neoplasms, gallbladder neoplasms, *etc*.

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ORIGINAL ARTICLE

Basic Study

Potent bromodomain and extraterminal domain inhibitor JAB-8263 suppresses MYC expression and exerts anti-tumor activity in colorectal cancer models

Xin-Mo Liu, Shao-You Xia, Wei Long, Hai-Jun Li, Gui-Qun Yang, Wen Sun, Song-Yan Li, Xiao-Hui Du

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Abstract

BACKGROUND

The overexpression of the *MYC* gene plays an important role in the occurrence, development and evolution of colorectal cancer (CRC). Bromodomain and extraterminal domain (BET) inhibitors can decrease the function BET by recognizing acetylated lysine residues, thereby downregulating the expression of MYC.

AIM

To investigate the inhibitory effect and mechanism of a BET inhibitor on CRC cells.

METHODS

The effect of the BET inhibitor JAB-8263 on the proliferation of various CRC cell lines was studied by CellTiter-Glo method and colony formation assay. The effect of JAB-8263 on the cell cycle and apoptosis of CRC cells was studied by propidium iodide staining and Annexin V/propidium iodide flow assay, respectively. The effect of JAB-8263 on the expression of c-MYC, p21 and p16 in

CRC cells was detected by western blotting assay. The anti-tumor effect of JAB-8263 on CRC cells *in vivo* and evaluation of the safety of the compound was predicted by constructing a CRC cell animal tumor model.

RESULTS

JAB-8263 dose-dependently suppressed CRC cell proliferation and colony formation *in vitro*. The MYC signaling pathway was dose-dependently inhibited by JAB-8263 in human CRC cell lines. JAB-8263 dose-dependently induced cell cycle arrest and apoptosis in the MC38 cell line. SW837 xenograft model was treated with JAB-8263 (0.3 mg/kg for 29 d), and the average tumor volume was significantly decreased compared to the vehicle control group ($P < 0.001$). The MC38 syngeneic murine model was treated with JAB-8263 (0.2 mg/kg for 29 d), and the average tumor volume was significantly decreased compared to the vehicle control group ($P = 0.003$).

CONCLUSION

BET could be a potential effective drug target for suppressing CRC growth, and the BET inhibitor JAB-8263 can effectively suppress c-MYC expression and exert anti-tumor activity in CRC models.

Key Words: Bromodomain; Bromodomain and extraterminal domain inhibitor; Colorectal cancer; JAB-8263; MYC; p21

Core Tip: After treating colorectal cancer (CRC) cells with the bromodomain and extraterminal domain (BET) inhibitor JAB-8263, we found that MC38 cells undergo cell cycle arrest and apoptosis. In multiple human CRC cell lines, we found that JAB-8263 downregulated c-MYC expression and upregulated p21 and p16 expression, which is associated with the highly potent antiproliferative effects of JAB-8263. JAB-8263 effectively inhibited CRC growth with acceptable tolerance in tumor mouse models. Our studies suggested that BET can be a potential effective drug target for suppressing CRC growth, and JAB-8263 can effectively suppress c-MYC expression and exert anti-tumor activity in CRC models.

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignant tumors, and its morbidity and mortality ranks third among all tumor patients[1], which seriously threatens human health. Traditional treatment methods include surgery, chemotherapy and radiotherapy. However, these treatments are invasive and are often accompanied by side effects[2]. In recent years, targeted therapy and immunotherapy have also developed rapidly as new treatment methods. With the deepening of tumor research, it has been found that the occurrence and development of colorectal tumors are related to the dysregulation of the epigenome[3], and one of the major areas of interest in epigenetic targets is the bromodomain and extraterminal domain (BET).

BET proteins belong to the acetyl-lysine-binding bromodomain (BRD) protein family and have four members, BRD2, BRD3, BRD4 and BRDT[4,5]. BET proteins have two N-terminal bromodomains (BD1 and BD2) that interact with acetylated lysine residues in histones. Then, it binds to transcription factor P-TEFb and RNA polymerase II and induces transcription[6]. BET protein acts as an epigenetic regulator and transcriptional cofactor, and it is closely associated with gene transcription, cell cycle and apoptosis, invasion and metastasis. BET proteins promote aberrant expression of many oncogenes such as *MYC*, *CCND1* and *BCL2L1*[7,8].

MYC is a proto-oncogene that is activated by amplification and chromosomal translocation rearrangement. The overexpression of MYC plays an important role in the occurrence, development and evolution of CRC[9,10]. Overexpression of MYC and dysregulation of MYC target genes can be found in most CRC cells[11]. BET inhibitors bind to the BET protein, occupying the space where it binds to acetylated lysines, thus inhibiting the transcription of its downstream *MYC* oncogenes and MYC-dependent genes[12,13]. A study showed the small molecule BET inhibitor JQ1 occupies the bromodomain pocket of BRD4, resulting in downregulation of *MYC* mRNA and MYC protein[14]. This

provides a rationale for the idea that BET inhibitors may exert anti-tumor activity in CRC cells.

The BET inhibitor JAB-8263 used in this study is a new type of BET inhibitor, which has a strong affinity with BET proteins and can significantly inhibit BET downstream signals c-MYC and N-MYC at a concentration of less than 1 nmol/L. It can significantly inhibit the proliferation of various tumor cells and induce the expression of cleaved PARP and the activation of caspase3/7, thereby inhibiting the proliferation of tumor cells and inducing apoptosis. Previous *in vivo* studies have shown that JAB-8263 has strong anti-tumor effects in various tumor models such as hematological tumors and small cell lung cancer through the MYC pathway. The pharmacology tests on safety show that JAB-8263 has no adverse effects on the cardiovascular system, respiratory system and central nervous system.

We predicted that JAB-8263 can suppress CRC cells *in vitro* and *in vivo*, and the purpose of this study was to explore the mechanism of its inhibitory effect on CRC cells.

MATERIALS AND METHODS

Cell proliferation

All CRC cell lines (HT29, DLD1, Colo205, H716, SW837, H508 and MC38) used in this study were purchased from ATCC and kept in our laboratory. The CellTiter-Glo method was used in this experiment. CRC cells were plated in cell culture plates and cultured in a cell culture incubator at 37 °C, 5% CO_2 or 100% air and 95% humidity. Compounds were added the next day and incubated for 5 d, and cell viability was detected with the CellTiter-Glo kit. The data were analyzed using GraphPad Prism software, and a four-parameter equation was used to fit a concentration-response curve, from which the IC_{50} of the compound concentration corresponding to 50% cell viability on the curve was calculated. Cell viability (%) = ($Lumi_{test\ compound}$-$Lumi_{blank\ control}$)/($Lumi_{solvent\ control}$-$Lumi_{blank\ control}$) × 100%. Compound information: BET inhibitor JAB-8263 (Jacobio Pharmaceuticals, Beijing, China), purity: 99.10%, storage condition: 4 °C.

Colony formation assay

The cell suspension was serially diluted, and 1000 cells were inoculated in each group of cells per dish, cultured in a cell incubator at 37 °C, 5% CO_2 or 100% air and 95% humidity and stained with crystal violet solution after 5 d. Cells exposed to the drug were compared to controls (treated with DMSO) assayed in triplicate.

Cell cycle analysis

Six-well plates were seeded with MC38 cells in logarithmic growth phase, 5 × 10^5 cells per well. Diluted JAB-8263 compound was added to each well, and 0.1% DMSO was added to the control group; the incubation time was 3 d and 5 d, respectively. Cells were then trypsinized, washed with PBS and stained with propidium iodide (PI) solution for 30 min in a dark room. Cell DNA content was analyzed by flow cytometry in triplicate.

Apoptosis assay

Six-well plates were seeded with MC38 cells in logarithmic growth phase, 5 × 10^5 cells per well. Diluted JAB-8263 compound was added to each well, and 0.1% DMSO was added to the control group; the incubation time was 3 d and 5 d, respectively. Cells were then trypsinized and washed with PBS. Cells were stained (Thermo Annexin V Apoptosis Detection Kit, APC) and incubated for 30 min at room temperature in a dark room. Analysis was performed in triplicate using a drain cytometer in triplicate.

Western blotting

Cells were harvested, and cellular protein collection was performed after addition of lysate. The protein concentration was detected according to the BCA instructions. The samples added to loading buffer were electrophoresed by discontinuous SDS-PAGE denaturing gel. The protein was transferred to PVDF membrane and detected by ECL exposure. Antibodies information: Anti-c-MYC antibody (ab32072, Abcam, United Kingdom); p21 Waf1/Cip1 (12D1) Rabbit mAb (#2947, GST, United States); p16 INK4A (E6N8P) Rabbit mAb (#18769, GST, United States); GAPDH (D16H11) XP® Rabbit mAb (#18769, GST, United States).

In vivo studies

All animal care and use-related experimental protocols and changes to the experimental protocols of animals in this experiment were reviewed, approved and guided by the Jacobio Animal Care and Use Management Committee.

SW837 xenograft mouse model: 12 female NOD-SCID mice were subcutaneously inoculated with 1 × 10^7 SW837 cells on the right back. When the tumor grew to an average of 121 mm^3, the mice were randomly divided into two groups according to tumor size and body weight. The experiment was

divided into a vehicle control group and a JAB-8263 0.3 mg/kg treatment group. The JAB-8263 0.3 mg/kg treatment group and vehicle control group were administered by gavage once every 2 d. The anti-tumor activity was evaluated according to the relative tumor growth inhibition (TGI) rate. TGI (%) = $(1-T_{RTV})/C_{RTV} \times 100\%$ (T_{RTV}: mean RTV of the treatment group; C_{RTV}: mean RTV of the vehicle control group; RTV = V_t-V_0, V_0 is the volume of the subcutaneous transplanted tumor of the mouse at the time of grouping, and V_t is the volume of the subcutaneous tumor of the mouse after treatment). The safety was evaluated according to the changes in animal body weight, drug withdrawal and death.

MC38 syngeneic murine model: 16 female C57BL/6 mice were subcutaneously inoculated with 1×10^6 MC38 cells on the right back. When the tumors grew to an average of 103 mm^3, they were randomly divided into two groups according to the tumor size and the weight of the mice. The experiment was divided into a vehicle control group and a JAB-8263 0.2 mg /kg treatment group. JAB-8263 0.2 mg/kg treatment group and vehicle control group were administered by gavage once every 2 d. The anti-tumor activity was evaluated according to the relative TGI rate, and the safety was evaluated according to the changes in animal body weight, drug withdrawal and death.

A single-dose MC38 model: In addition, we used the above method to establish a single-dose MC38 model. Nine female C57BL/6 mice were randomly divided into two groups according to the tumor size and the weight of the mice. The experiment was divided into a vehicle control group, a JAB-8263 0.1 mg/kg treatment group and a JAB-8263 0.2 mg/kg treatment group. One hour after the treatments were administered, the experiment was terminated, all mice were euthanized, and tumor tissues were collected.

Statistical analysis

All experimental results were expressed as mean ± SD. The *t*-test method was used to compare the data of the treatment group and the control group for statistical differences. All data were analyzed with SPSS 22.0, and $P < 0.05$ was considered statistically significant.

RESULTS

JAB-8263 dose-dependently suppressed CRC cell proliferation and colony formation in vitro

We found seven CRC cell lines that were sensitive to JAB-8263 in cell proliferation assays, including human CRC cell lines (HT29, DLD1, Colo205, H716, SW837 and H508, Figure 1A) and murine CRC cell line (MC38, Figure 1B). The IC_{50} values of six human CRC cell lines including HT-29, DLD-1, Colo205, H716, SW837 and H508 were 0.09-1.24 nmol/L, and the IC_{50} of the murine CRC cell line MC38 was 1.25 nmol/L.

In the colony formation assay, five groups of CRC cell lines were sensitive to the JAB-8263 compound. Compared with the control group (DMSO), the colony formation of the cell lines in each group was significantly reduced with increasing drug concentration (Figure 1C). Taken together, these data suggest that JAB-8263 dose-dependently suppressed CRC cell proliferation and colony formation *in vitro*.

JAB-8263 suppressed CRC cell MYC expression and promoted p21 and p16 expression

Western blot assays on MYC, p21 and p16 levels were performed in human CRC cell lines with JAB-8263 treatment. Compared with the control group (DMSO), the expression of MYC was downregulated in all cell lines with the treatment of different concentrations of JAB-8263 (1 nmol/L, 10 nmol/L and 100 nmol/L). The p21 expression of MC38, DLD-1, H508, HT29, SW837 and Colo205 was upregulated, and the expression of p16 in H716, HT29 and colo205 was upregulated (Figure 2A-C). This data suggest that JAB-8263 dose-dependently downregulated the expression of c-MYC in CRC cells and upregulated the expression of p21 and p16 in some of the CRC cell lines.

JAB-8263 dose-dependently induced cell cycle arrest and apoptosis in the MC38 cell line

We conducted further cell cycle and apoptosis assays on the murine CRC cell line MC38 to explore the mechanism of JAB-8263 suppressed CRC cell proliferation. In the cell cycle assay, the MC38 cell cycle was arrested in the subG0 phase compared with the control group after 3 d and 5 d of treatment with JAB-8263 in different concentrations. JAB-8263 dose-dependently decreased the G2/M phase ratio and increased the subG0 prophase ratio in MC38 cells, indicating that JAB-8263 induced cell cycle arrest in the G0 phase. (Figure 3A and B). In the apoptosis assay, the apoptotic ratio of MC38 was increased compared with the control group after 3 d and 5 d of treatment with JAB-8263. Furthermore, the apoptotic ratio increased with the compound concentration (Figure 3C and D). This data indicates that JAB-2485 suppressed tumor cell activity in two ways by inducing MC38 cell cycle arrest and apoptosis.

JAB-8263 suppressed in vivo CRC growth

After 29 d of treatment in the SW837 xenograft model, the average tumor volume in the vehicle control

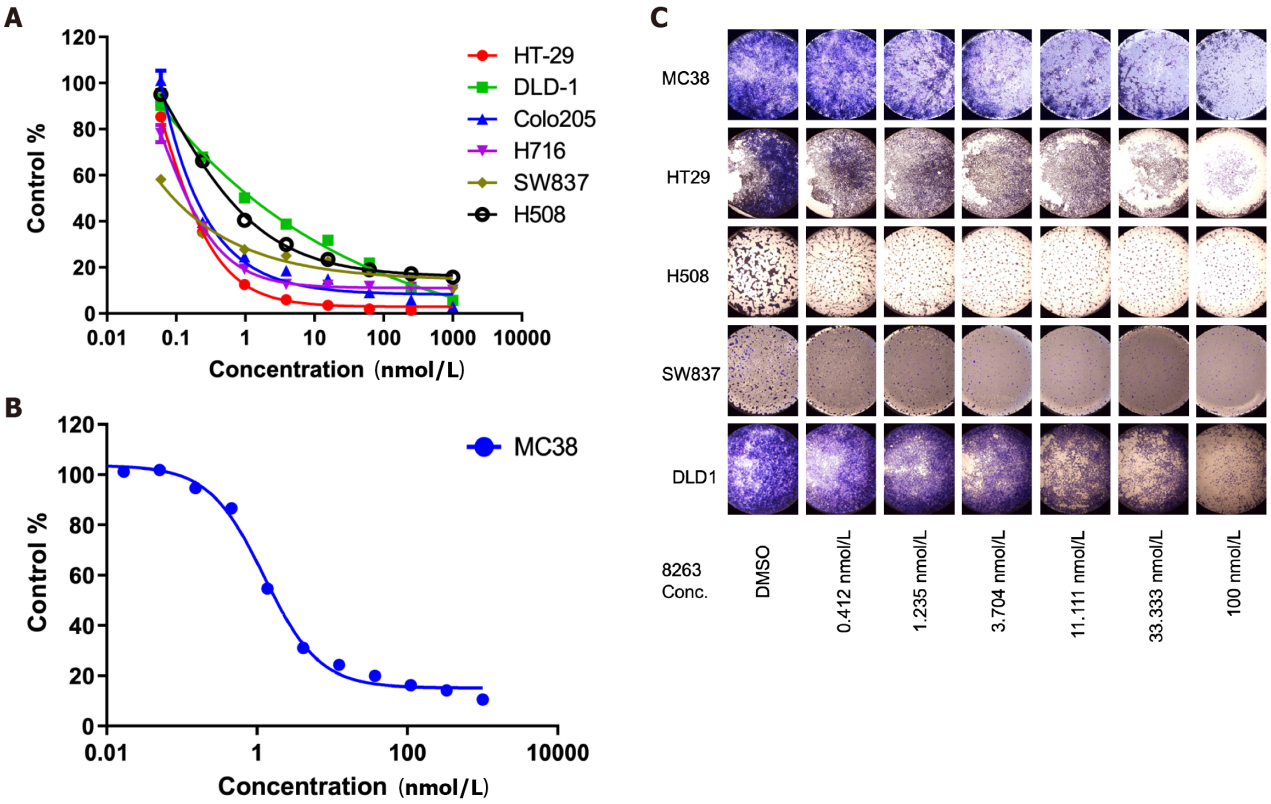

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Figure 1 JAB-8263 dose-dependently suppressed colorectal cancer cell proliferation and colony formation *in vitro*. A: Human colorectal cancer cell lines including HT29, DLD1, Colo205, H716, SW837 and H508 were treated with JAB-8263 for 6 d, and the proliferation was dose-dependently suppressed; B: The MC38 mouse cell line was treated with JAB-8263 for 6 d, and the proliferation was dose-dependently suppressed; C: The IC_{50} values of HT-29, DLD-1, Colo205, H716, SW837, H508 and MC38 were 0.15, 1.24, 0.19, 0.09, 0.57, 0.14 and 1.25 μmol/L. Colony formation assays for six colorectal cancer cell lines including MC38, HT29, H508, SW837 and DLD1 were treated with various concentrations of JAB-8263 for 5 d. Cell proliferation in all cell lines was dose-dependently suppressed. All experiments were performed in triplicate. Conc.: Concentration.

group was 895 mm^3, and the average tumor volume in the JAB-8263 0.3 mg/kg treatment group was 283 mm^3, which was statistically significant compared to the vehicle control group. The relative tumor inhibition rate TGI (%) was 79.0% (Figure 4A). Only one animal in the JAB-8263 treatment group lost 16.6% of body weight at the end of the trial, and animals in the other groups tolerated it well without discontinuation or death (Figure 4B). After 18 d of treatment in the MC38 syngeneic model, the average tumor volume in the vehicle control group was 2580 mm^3, and the average tumor volume in the JAB-8263 0.2 mg/kg treatment group was 686 mm^3. Compared with the vehicle control group, there was a significant statistical difference (P = 0.003) (Figure 4C), and the relative tumor inhibition rate TGI (%) was 76.5%. The body weight change of each treatment group was controlled within 15%, no drug discontinuation or death occurred, and the animals tolerated the treatment well (Figure 4D).

The tumor tissue of the single-dose MC38 model was further subjected to the western blot assay to evaluate the underlying mechanism, and it was found that the expression of c-MYC was significantly decreased by a single dose of JAB-8263 administration (P = 0.013 and P = 0.011) (Figure 4G and H). All data showed that JAB-8263 downregulated the expression of c-MYC in tumor tissue from the single-dose MC38 model.

DISCUSSION

In recent years, BET protein inhibitors have received extensive attention in the application of tumors, and many BET inhibitors have been used in clinical trials, but most are focused on hematological tumors and some solid tumors such as lung cancer and prostate cancer[15-19]. Some previous studies have used JQ1 and other compounds in the study of CRC cells[20,21], but due to the short half-life of most compounds, they are quite challenging for further clinical application. JAB-8263 used in this study has stronger protein affinity, high affinity for BET protein *in vitro*, and the IC_{50} is less than 1 nmol/L.

In the *in vitro* cell proliferation and colony formation experiments, we found that JAB-8263 had an inhibitory effect on CRC cells. To further study its mechanism of action, we performed cell cycle and apoptosis experiments. However, only the mouse CRC cell line MC38 obtained ideal positive results,

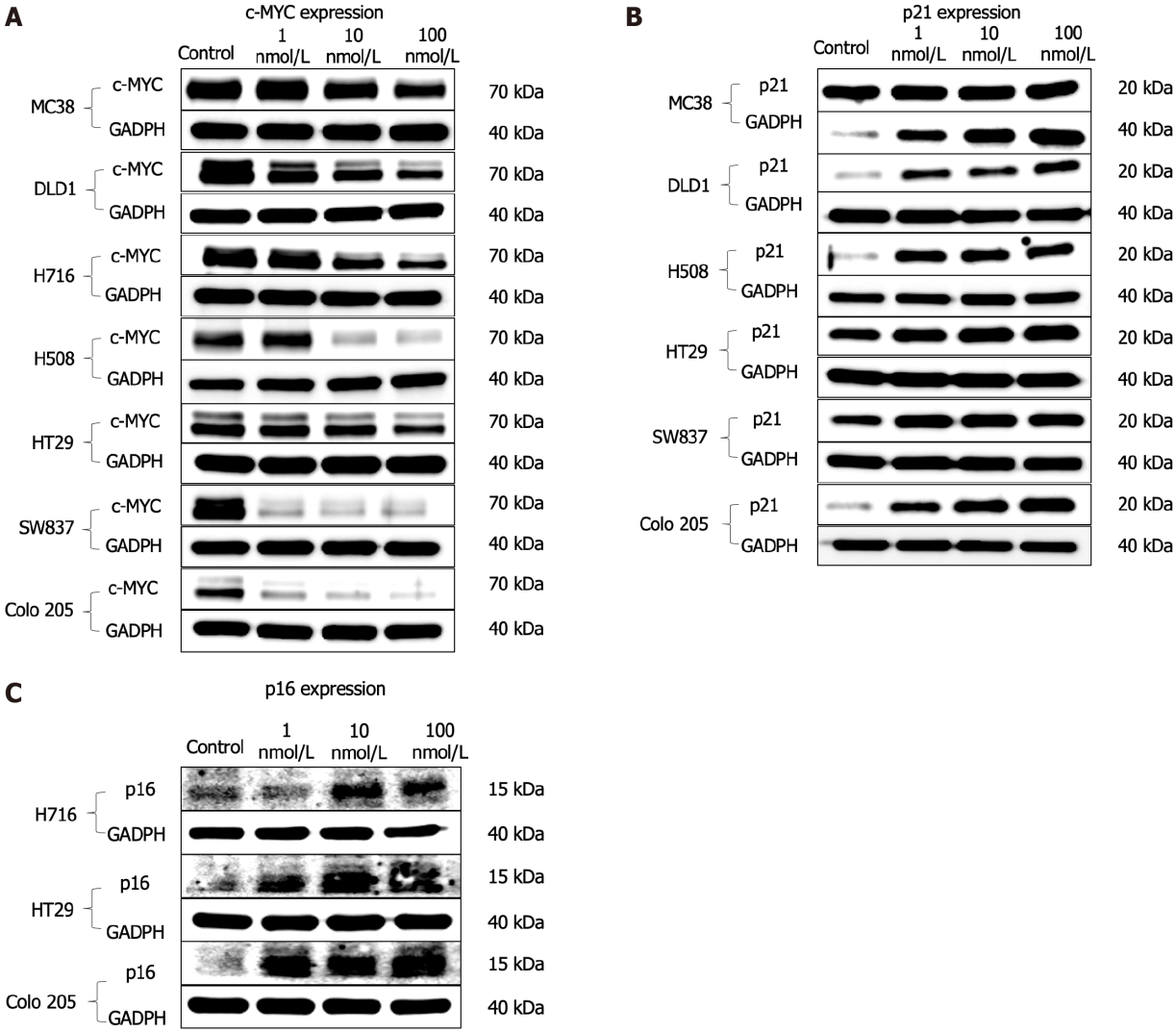

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Figure 2 MYC signaling pathway was dose-dependently inhibited by JAB-8263 in human colorectal cancer cell lines. A-C: Multiple colorectal cancer cell lines were treated with different concentrations of JAB-8263 (1 nmol/L, 10 nmol/L and 100 nmol/L) for 16 h. Western blotting assay was performed to detect levels of MYC, p21 and p16. The expression of MYC were downregulated (A), and the expression of p21 (B) and p16 (C) were upregulated in multiple colorectal cancer cell lines.

and the human CRC cell lines did not have a significant difference from the control group. We conducted the western blot experiments to further explore this result.

MYC plays an important role in the cell cycle, cell death, cellular senescence, and tumorigenesis of CRC cells[9]. Myc-related lnc-RNAs such as MYCLo-2 are overexpressed in CRC cells and have oncogenic functions[14]. Through the *in vitro* and *in vivo* studies of this study, we found that JAB-8263 can effectively suppress the expression of c-MYC and finally suppress CRC cells.

The tumor suppressor genes *p21* and *p16* are regulated by the *MYC* gene[14]. Therefore, we further investigated whether the expression of these two genes was affected by BET inhibitors. p21 (CDKN1A) is involved in the regulation of cell cycle and cellular senescence[22]. In 1993, it was reported that p21 can suppress multiple tumors such as CRC by activating wild-type p53[23]. Moreover, studies have shown that p21 can also suppress tumor growth by inhibiting cyclin kinase complexes and proliferating cell nuclear antigen[24]. JAB-8263 achieves an anti-tumor effect by inducing CRC cell cycle arrest by upregulating p21. However, at the same time, some studies have suggested that p21 has an anti-apoptotic effect, and the apoptosis of hCT116 colon cancer cells can be inhibited by inhibiting p21[25, 26]. This might be one reason why JAB-8263 did not have ideal results in the apoptosis experiments, which also requires further study.

p16 (CDKN2A) can inhibit the function of CDK4, and the combination of CDk4 and cyclin D1 plays a key regulatory role in the G1→S phase of the cell cycle, thereby suppressing the malignant proliferation of cells[27]. The inactivation or decreased expression of the *p16* gene can lead to the malignant proliferation of cells and lead to tumorigenesis[28,29]. JAB-8263 inhibits CDK4 function by upregulation of p16, thereby suppressing CRC cells.

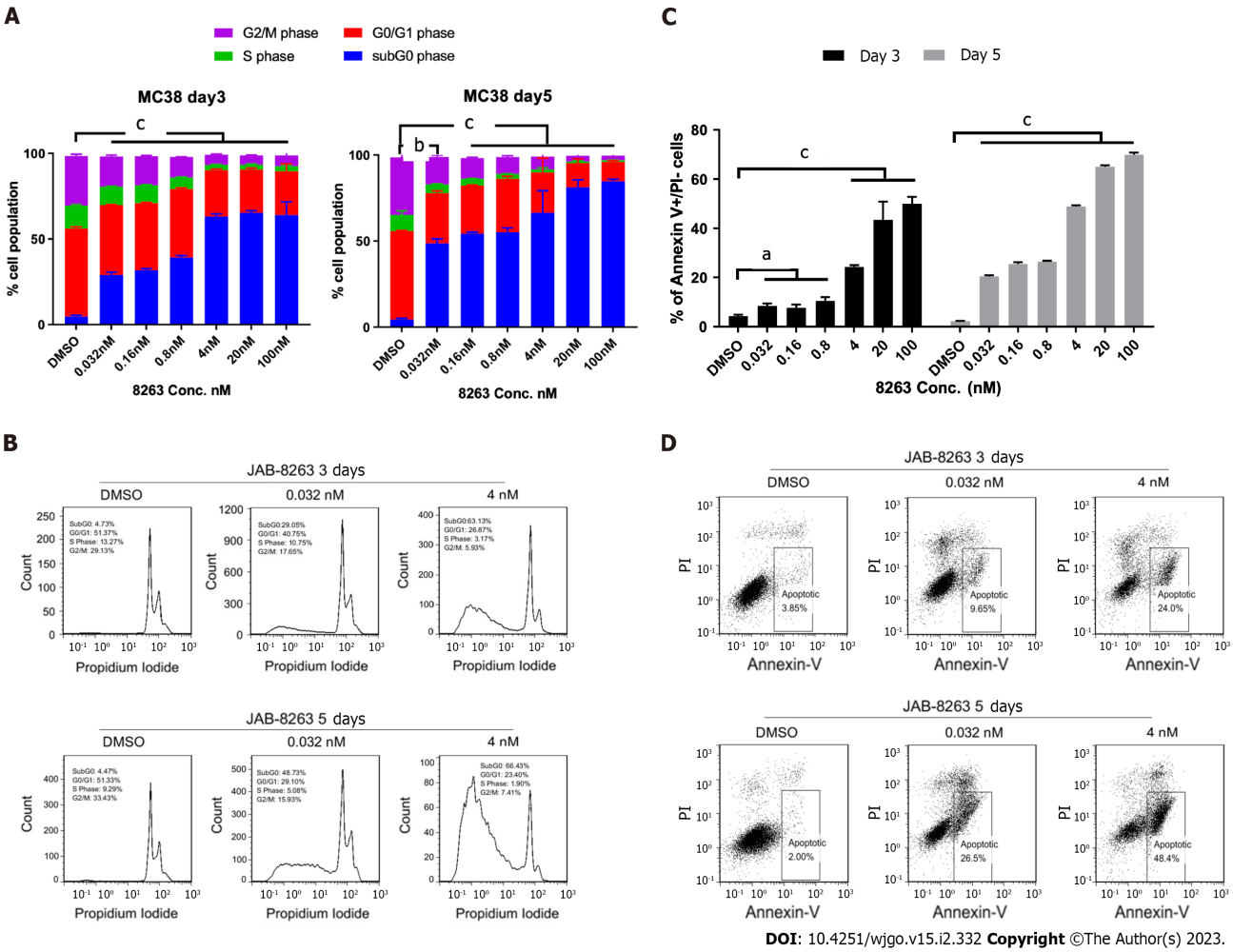

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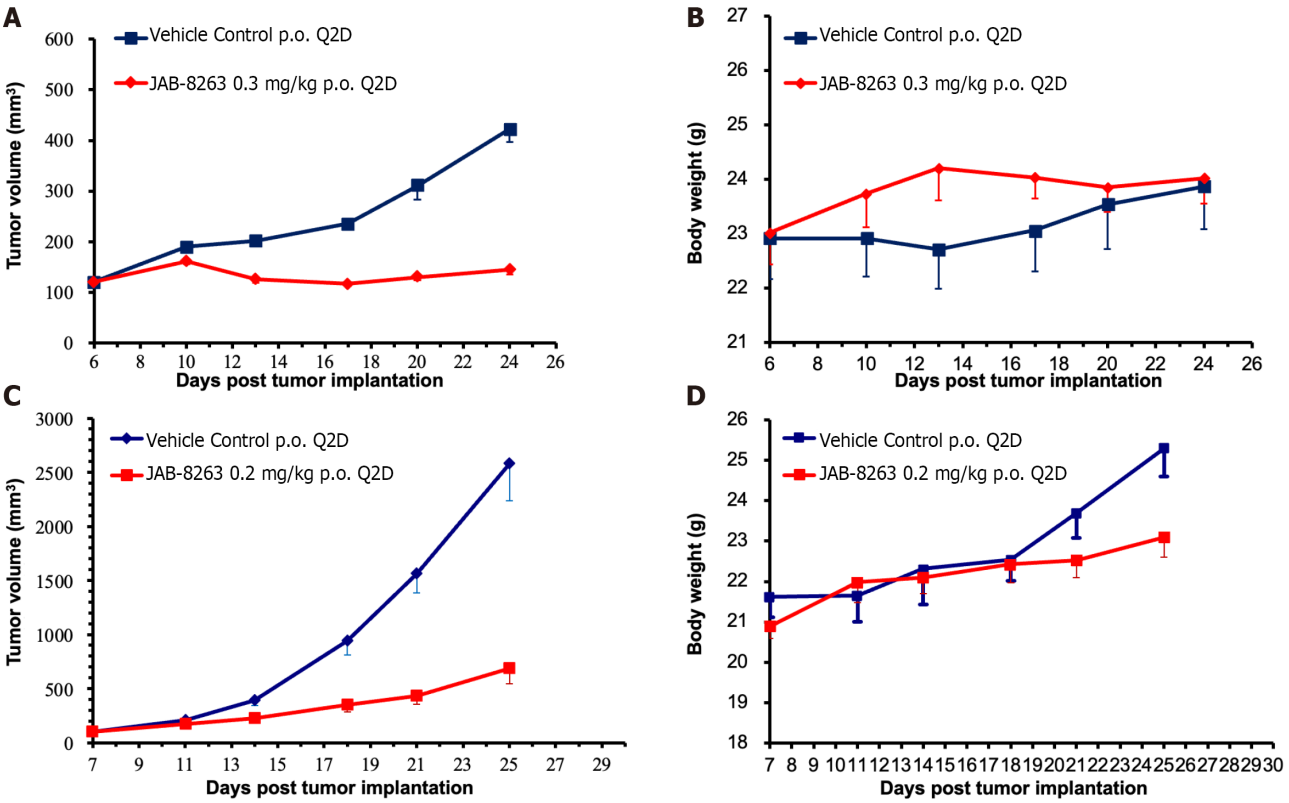
Figure 3 JAB-8263 dose-dependently induced cell cycle arrest and apoptosis in the MC38 cell line. After incubation with different concentrations of JAB-8263 (0, 0.032, 0.16, 0.8, 4, 20 and 100 nmol/L) for 3 d and 5 d, MC38 cells were collected and analyzed for cell cycle and apoptosis assays by flow cytometry. A and B: JAB-8263 dose-dependently decreased the G2/M phase ratio and increased the G0 prophase ratio in MC38 cells, indicating that JAB-8263 induced cells to arrest in the G0 phase; C and D: JAB-8263 dose-dependently induced apoptosis in MC38 after treatment for 3 d and 5 d. All experiments were performed in triplicate. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ *vs* DMSO control. PI: Propidium iodide.

Finally, we verified that JAB-8263 has a significant tumor inhibitory effect compared with the control group in the SW837 and MC38 animal models. The animals in the treatment group tolerated the drug well. Since c-MYC expression is disturbed in long-term dosing models, we established a single-dose model. The detection of tumor tissue in single-dose MC38 model also showed that c-MYC was downregulated. This is consistent with the conclusions we obtained in the *in vitro* studies.

According to the conclusion of this study, the BET inhibitor JAB-8263 can inhibit CRC cells mainly by inhibiting the expression of c-MYC. But at the same time, we found that the inhibition of BET inhibitors on CRC has many mechanisms other than the *MYC* gene. Further directions include whether the BET inhibitors still have an anti-tumor effect in cells that do not overexpress MYC, which will provide a theoretical basis for the indications of CRC treatment in future clinical applications.

CONCLUSION

The JAB-8263 compound inhibited the BET target. The expression of BET downstream signaling protein MYC was repressed by JAB-8263, resulting in downregulation of c-MYC and upregulation of p21 and p16. It induced cell cycle arrest, promoted apoptosis of CRC cells and displayed anti-tumor activity. *In vivo*, JAB-8263 was effective in CRC models.


A
Vehicle Control p.o. Q2D
JAB-8263 0.3 mg/kg p.o. Q2D
Tumor volume (mm³)
Days post tumor implantation
B
Vehicle Control p.o. Q2D
JAB-8263 0.3 mg/kg p.o. Q2D
Body weight (g)
Days post tumor implantation
C
Vehicle Control p.o. Q2D
JAB-8263 0.2 mg/kg p.o. Q2D
Tumor volume (mm³)
Days post tumor implantation
D
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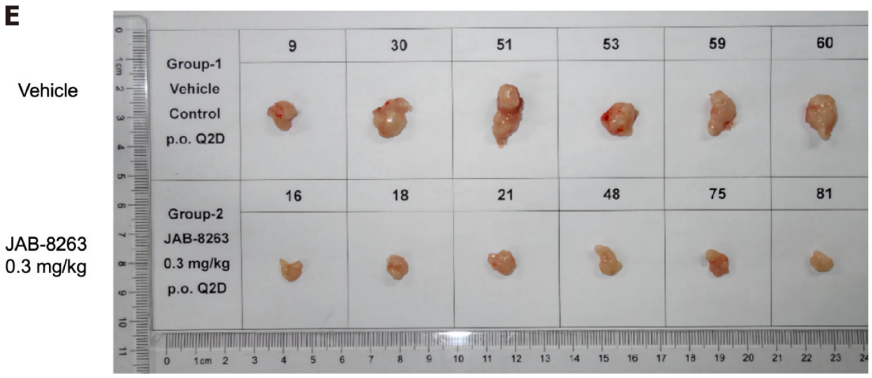
JAB-8263 0.2 mg/kg p.o. Q2D
Body weight (g)
Days post tumor implantation


E
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JAB-8263
0.3 mg/kg
Group-1
Vehicle
Control
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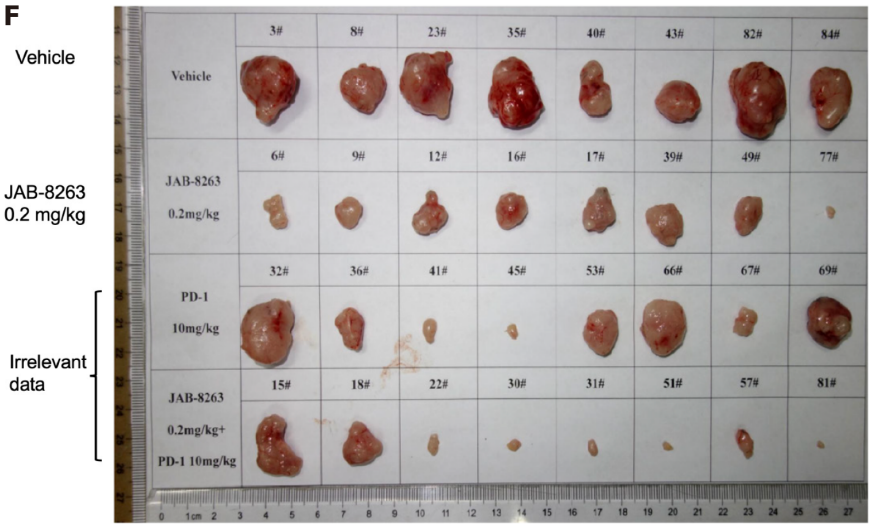
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JAB-8263
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PD-1
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JAB-8263
0.2mg/kg+
PD-1 10mg/kg
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81#

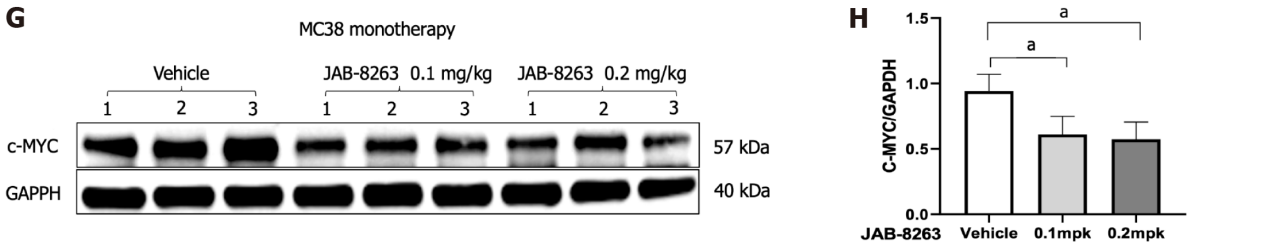

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Figure 4 JAB-8263 suppressed tumor growth in colorectal cancer murine xenograft models. A and E: The SW837 xenograft model (6 mice for each group) was treated with JAB-8263 0.3 mg/kg for 29 d. The average tumor volume was significantly decreased compared to the vehicle control group, $P < 0.001$; B: There was no significant difference in body weight change between groups; C and F: The MC38 syngeneic murine model (8 mice for each group) was treated with JAB-8263 0.2 mg/kg for 29 d. The average tumor volume was significantly decreased compared to the vehicle control group, $P = 0.003$; D: There was no significant difference in body weight change between groups; G and H: The tumor tissue of the MC38 syngeneic murine model (3 mice for each group) after a single dose of JAB-8263 treatment was collected for the Western blotting assay. Compared with the control group, the expression of c-MYC in the treatment group was downregulated, $^{a}P < 0.05$.

ARTICLE HIGHLIGHTS

Research background

The overexpression of the *MYC* gene plays an important role in the occurrence, development and evolution of colorectal cancer (CRC). Bromodomain and extraterminal domain (BET) inhibitors decrease the function of BET, which is the recognition of acetylated lysine residues, thereby downregulating the expression of MYC.

Research motivation

BET proteins are an important target in solid tumors, hematologic tumors and myelofibrosis. The development of BET small-molecule inhibitors has promising therapeutic value.

Research objectives

The study aimed to investigate the inhibitory effect and mechanism of a BET inhibitor on CRC cells.

Research methods

The effect of the BET inhibitor JAB-8263 on the proliferation of various CRC cell lines was studied by the CellTiter-Glo method and colony formation assay. The effect of JAB-8263 on the cell cycle and apoptosis of CRC cells was studied by propidium iodide staining and Annexin V/propidium iodide flow assay, respectively. The effect of JAB-8263 on the expression of c-MYC, p21 and p16 in CRC cells was detected by western blot. To predict the anti-tumor effect of JAB-8263 on CRC cells *in vivo* and to evaluate the safety of the compound, a CRC cell animal tumor model was developed.

Research results

JAB-8263 dose-dependently suppressed CRC cell proliferation and colony formation *in vitro*. The MYC signaling pathway was dose-dependently inhibited by JAB-8263 in human CRC cell lines. JAB-8263 dose-dependently induced cell cycle arrest and apoptosis in the MC38 cell line. The SW837 xenograft model was treated with JAB-8263 0.3 mg/kg for 29 d. The average tumor volume was significantly decreased compared to the vehicle control group, $P < 0.001$. The MC38 syngeneic murine model was treated with JAB-8263 0.2 mg/kg for 29 d. The average tumor volume was significantly decreased compared to the vehicle control group, $P = 0.003$.

Research conclusions

BET can be a potential effective drug target for suppressing CRC growth, and the BET inhibitor JAB-8263 can effectively suppress c-MYC expression and exert anti-tumor activity in CRC models.

Research perspectives

BET proteins are an important target in solid tumors, hematologic tumors and myelofibrosis. The development of BET small-molecule inhibitors has promising therapeutic value. Our study results are encouraging and will motivate further clinical evolution.

FOOTNOTES

Author contributions: Liu XM performed experiments and data analysis and wrote the paper; Xia SY performed experiments; Long W and Li H collected data; Yang GQ and Sun W performed the data analysis; Du XH and Li SY designed and revised the manuscript; All authors contributed to the article and approved the submitted version.

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