Original Research

Brexpiprazole Prevents the Malignant Progression of Human Colorectal Cancer Cells and Increases Its Sensitivity to EGFR Inhibition

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1. Background

Colorectal cancer (CRC) is a major cause of mortality and morbidity. A study proved that brexpiprazole, as a novel dopamine receptor partial agonist, can also prevent CRC cell proliferation. Therefore, clarifying the molecular mechanism of brexpiprazole is vital to developing a novel therapeutic strategy for CRC. **Methods:** The effect of brexpiprazole on human colorectal cancer cell proliferation was measured with Cell Counting Kit-8 (CCK-8) kits. Cell migration capability was measured using wound healing and transwell. Cell apoptosis was evaluated with a flow cytometer. Western blots and immunohistochemical staining were used to evaluate protein expression. The effects observed in vitro were also confirmed in xenograft models. **Results:** Brexpiprazole remarkably inhibited the proliferation, suppressed the migration ability, and induced apoptosis of colorectal cancer cells. Mechanism study showed that brexpiprazole exerted these effects by inhibiting the EGFR pathway. Brexpiprazole enhanced HCT116 cells’ sensitivity to cetuximab, and a combination of brexpiprazole and cetuximab inhibited xenograft tumor growth in vivo. **Conclusions:** Our finding suggested that brexpiprazole inhibits proliferation, promotes apoptosis, and enhances CRC cells’ sensitivity to cetuximab by regulating the EGFR pathway and it might be an efficacious treatment strategy for CRC.

**Keywords:** colorectal cancer; proliferation; apoptosis; drug resistance; brexpiprazole

1. Background

Colorectal cancer (CRC) is a major cause of mortality and morbidity [1,2]. CRC is mainly caused by gastrointestinal dysfunction, slow intestinal peristalsis, and increased garbage in the intestine [3]. Several risk factors for CRC have been identified, which include age, gender, ethnic background, family history, diet, lifestyle, etc. [1]. According to the latest statistics, 20% of the patients were found to have distant metastases, and there is a trend of younger people among these patients [4]. Therefore, clarifying CRC’s molecular mechanism is of great importance for developing a novel therapeutic strategy.

Chemotherapy is commonly used before/after surgery in cancer therapy [5]. However, one major obstacle to chemotherapy is inevitably the development of drug resistance. It has been reported that most CRC patients showed drug resistance-related treatment failure [6]. Therefore, how to overcome drug resistance has attracted many researchers’ attention and is of great importance in cancer treatment.

Brexpiprazole is a novel atypical antipsychotic with unique pharmacologic targeting, primarily used as an adjuvantive treatment for schizophrenia and depressive disorders [7]. Brexpiprazole can produce a high affinity for multiple monoamine receptors [8]. Brexpiprazole is a partial agonist at 5-hydroxytryptamine (5-HT) 1A (5-H1A) 5-H1A and dopamine D2 receptors and an antagonist at 5-H2A receptors [9]. Recent research confirmed that brexpiprazole could suppress the cell viability of lung and pancreatic cancer [10]. Besides, brexpiprazole could inhibit proliferation and de novo lipogenesis in CRC via the AMPK/SREBP1 pathway [11]. Brexpiprazole also could reduce the resistance of glioma stem cells to epidermal growth factor receptor (EGFR)-TKI osimertinib by reducing Survivin [12]. Generally, brexpiprazole has an inhibitory effect on cancer progression, including CRC, and is associated with EGFR.

Overexpression of EGFR and EGFR-like peptides is involved in CRC development [13]. Targeting EGFR has shown promising results in treating CRC [14]. For example, two anti-EGFR monoclonal antibodies (moAbs), cetuximab and panitumumab, are effective in patients with RAS wild-type (RAS-WT) mCRC [15]. The key limitation of cetuximab is resistance [16]. It has been shown that resistance to EGFR blockade is parallel to RAS mutations or KRAS amplification, etc. [17,18]. However, the exact mechanisms of Cetuximab resistance and how to sensitize CRC cells to Cetuximab remain unclear and require extensive investigation.

Our study aims to investigate the effect of brexpiprazole on the biological functions of CRC cells and to elaborate on underlying mechanisms, that will benefit CRC treatment.
2. Materials and Methods

2.1 Drugs

Brexpiprazole, a novel Dopamine and Serotonin modulator in antipsychotic medication was purchased from Bidepharmtech Ltd. (Shanghai, China). Cetuximab was acquired from Merck Serono China (Beijing, China). Oxaliplatin was kindly provided by Heng Rui Pharmaceuticals (Jiangsu, China). For in vitro experiments, Brexpiprazole was dissolved in dimethylsulfoxide to 40 mM. For in vivo applications, Brexpiprazole was solubilized in 5% Arabic gum in sterile Phosphate Buffered Saline (PBS).

2.2 Cell Culture

The human normal colonic epithelial cells (2 × 10⁶ NCM460 cells) and human CRC cells (1 × 10⁶ HCT116 cells and 1 × 10⁶ SW620 cells) were obtained from ATCC. NCM460 was maintained in RPMI-1640, HCT116 was cultured in McCoy’s 5A medium (Solarbio, Pleasanton, CA, USA), and SW620 was grown in DMEM with 10% FBS and 1% pen/strep (Boster, Wuhan, China). All cells were grown at 37 °C and 5% CO₂. All cells were validated through STR and tested negative for mycoplasma.

2.3 Cell Counting Kit-8 (CCK-8) Assay

Cells were placed in a 96-well plate (6 × 10³ cells/well), and were treated with 5, 10, 20, and 40 µM Brexpiprazole for 24 or 48 h, or treated with 1.5 mg/mL Cetuximab for 72 h, or received both Cetuximab (1.5 mg/mL) and Brexpiprazole (10 µM) for 72 h, respectively. After treatment, 10 µL CCK-8 (Beyotime, Suzhou, China) was provided and kept at 37 °C for 1.5 h, and OD450 was recorded.

2.4 Wound Healing Assay

HCT116 and SW620 cells were cultured in 6-well plates (8 × 10⁵ cells/well) for 24 h. After making a scratch with a 200 µL pipette tip, cells were PBS-washed twice, and treated with 20 µM Brexpiprazole in a serum-free medium for 48 h. Wound closure was represented by the formula (the scratched area before incubation – the scratched area after incubation)/(the scratched area after incubation) × 100%.

2.5 Migration Assay

Migration assay was carried out with transwell inserts of an 8-µm filter. HCT116 and SW620 cells were resuspended in a serum-free medium after starvation. Cell suspension (5 × 10⁴ cells) was inoculated in the upper transwell chambers and incubated in the lower chamber with 600 µL complete medium containing Brexpiprazole (20 µM) for 48 h. Cells on transwell inserts were fixed with methanol and stained with a 1% crystal violet solution. Cells on the lower surface of the chamber membrane were photographed with a Leica DMi3000 microscope at 200× magnification. The average counting number of 5 random high-power fields was recorded.

2.6 Apoptosis Assay

Cells were incubated with 0, 10, 20, and 40 µM Brexpiprazole for 24 h, collected, and washed. After resuspension in 500 µL binding buffer, the cells were stained with Annexin V-FITC and PI (Beyotime) in the dark for 15 min and apoptosis was monitored with a BD Accuri C6 cytometer. The raw data were analyzed with FlowJo v10.3 (Tree Star Inc., Ashland, OR, USA).

2.7 Nude Mice Xenograft Model

Four-week-old male nude mice and weight of 20 ± 2 g from Beijing Laboratory Animal Center were routinely maintained at the Animal Experimental Center of North Sichuan Medical College. All procedures followed the guidelines of Sichuan and were approved by the Experimental Animal Ethics Committee of our university. After one week of adaption, 1 × 10⁷ HCT116 cells were inoculated subcutaneously into each mouse. When the tumor reached 100 mm³, the mice were divided into four groups, 6 nude mice per group: Group 1, 5% gum arabic was gavaged and PBS was intraperitoneally injected. Group 2, Brexpiprazole in 5% gum Arabic was gavaged (5 mg/kg/day). Group 3, cetuximab was injected intraperitoneally with a dose of 1 mg twice a week. In group 4, both Brexpiprazole and cetuximab were administered. Tumor volume was calculated by measuring the length (a) and width (b) with a caliper every 2 days. After 3-week treatment, nude mice were anesthetized with 1% pentobarbital and then killed by cervical dislocation, and tumors were collected for immunochemical staining or Western blotting.

2.8 Immunoblotting

RIPA buffer, containing 1% PMSF (Dingguo, Guangzhou, China) and 1% phosphatase inhibitor (Solarbio), was used for total protein extraction from tumor tissues or treated cells. The supernatant was taken after the centrifugation. After quantification via BCA assay, proteins (30 µg) were resolved by 10% SDS-PAGE, and electro-blotted into PVDF membranes. After blocking, membranes were probed with first antibodies at 4 °C, followed by second antibodies such as goat anti-rabbit IgG HRP (Huabio, HA1031, Hangzhou, China, 1:5000), goat anti-mouse IgG HRP (Huabio, HA1006, Hangzhou, China, 1:5000). ECL reagent (Bio-Rad, Hercules, CA, USA) was used for development, and images were quantified with ImageJ 1.52a Software (National Institutes of Health, Washington, DC, USA).

Primary antibodies that were used in this study were ERK1/2 (Abcam, ab28481, Cambridge, UK) and Ser473 (Proteintech, 28731-1, Wuhan, China).

2.9 Immunohistochemistry

Tissue was fixed with 4% formalin, paraffin-embedded, and cut to 4 µm sections, followed by deparaffin and rehydration, and antigen retrieval. After
**Fig. 1. Brexpiprazole remarkably inhibited the proliferation of colorectal cancer (CRC) cells.** Cells were treated with Brexpiprazole at various concentrations for different time courses. (A) Viability of HCT116, (B) viability of SW620, (C) morphology of HCT116, (D) morphology of SW620 cells, and (E) NCM460 (scale: 50μm). *p < 0.05, **p < 0.01, ***p < 0.001. (n = 3)

blocking with 3% H\textsubscript{2}O\textsubscript{2}, sections were probed with antibodies against survivin (Abcam, ab76424, Cambridge, UK, 1:500). After PBS-washing, sections were probed with a second antibody goat anti-rabbit IgG HRP (Huabio, HA1031, Hangzhou, China, 1:200). DAB kit (ZSGB-BIO) was applied to stain the slides. After hematoxylin counterstaining and mounting, sections were observed under the Olympus microscope. Five random fields (×400) were selected for intensity analysis. Image-Pro Plus 4.5 software (Media Cybernetics, Silver Spring, MD, USA) was used for quantitation.

### 2.10 Statistical Analysis

Data were presented as mean ± standard deviation (SD). SPSS 24.0 software (SPSS Inc., Chicago, IL, USA) was used for one-way ANOVA (>2 groups) and Student t-test (2 groups) analysis. *p-value < 0.05 was defined as significant.

### 3. Results

#### 3.1 Brexpiprazole Remarkably Inhibited the Proliferation of CRC Cells

The effect of brexpiprazole on human CRC cells’ proliferation was first measured. Brexpiprazole treatment dose- and time-dependently inhibited HCT116 and SW620 cells’ viability (Fig. 1A,B), while HCT116 cells were more sensitive to this treatment. The toxicity of brexpiprazole in human normal colonic epithelial cells (NCM460) was also measured. Only a high concentration of Brexpiprazole (40 μM) significantly reduced the cell viability of NCM460 cells.
Brexpiprazole suppressed CRC cells’ migration. Cells were treated by brexpiprazole (20 µM) for 48 h. (A) Wound healing assay of HCT116 cells, (B) wound healing assay of SW620 cells (scale: 50μm), and (C) transwell migration assay of HCT116 cells (scale: 500μm). **p < 0.01. (n=3)

3.2 Brexpiprazole Inhibited CRC Cells’ Migration in Vitro

Knowing that brexpiprazole inhibits CRC cells’ proliferation triggers our interest in studying how brexpiprazole affects CRC cells’ migration. HCT116 and SW620 cells were treated with brexpiprazole (20 µM) for 48 h, and cell migration was monitored using wound healing and tran-
swell assay. Wound healing experiments showed that brexpiprazole markedly inhibited the migration of HCT116 and SW620 cells, and HCT116 cells were more sensitive to brexpiprazole than SW620 cells (Fig. 2A,B). Meanwhile, results from transwell assays demonstrated that brexpiprazole dramatically suppressed HCT116 cells’ migration (Fig. 2C), indicating that brexpiprazole can suppress the migration of CRC cells.

3.3 Brexpiprazole Induced CRC Cells’ Apoptosis

Annexin FITC/V and PI staining were performed to measure how brexpiprazole affects the apoptosis of CRC cells. As depicted in Fig. 3A, brexpiprazole treatment increased the percentage of apoptotic cells in a dose-dependent manner, indicating that brexpiprazole could induce apoptosis of CRC cells. Next, we checked the effect of brexpiprazole on apoptosis-related proteins in these cells through Western blot. Brexpiprazole dose-dependently enhanced survivin but decreased cleaved caspase-9, indicating the activation of caspase-9 by brexpiprazole (Fig. 3B). These findings demonstrate that brexpiprazole can induce the apoptosis of CRC cells by regulating survivin and caspase-9.

3.4 Brexpiprazole Inhibited the Signal Transition of the EGFR Pathway in CRC Cells

The activation status of the PI3K pathway in HCT116 and NCM460 was determined through Western blot. As shown in Fig. 4A, phosphor-PI3K subunit P85 in HCT116 was significantly higher than that of NCM460, indicating that the pathway is activated. To determine how brexpiprazole affects downstream pathways of EGFR, proteins related to PI3K and MAPK pathways were measured. Brexpiprazole significantly down-regulated the expression of phosphorylated AKT, P85, P38, ERK1/2, and RAF in HCT116 and SW620 cells without affecting their total expression levels. However, brexpiprazole only down-regulated the expression level of RAS protein in HCT116 cells but had no significant effect on RAS protein in SW620 cells (Fig. 4B,C). Taking the data together, brexpiprazole might regulate CRC cells’ survival and proliferation by affecting the signal transduction of PI3K and MAPK pathways.

3.5 Brexpiprazole Inhibited Tumor Growth in HCT116 Xenograft Models

The anti-colon cancer effect of brexpiprazole was also analyzed in vivo. HCT116 tumor-bearing mice received brexpiprazole (5 mg/kg) daily via intragastrical administration (i.g.) for 17 days. Positive control mice received oxaliplatin (5 mg/kg) twice a week. Tumors developed rapidly in control mice. Both brexpiprazole and oxaliplatin significantly attenuated tumor growth (Fig. 5A). Next, we investigated the change of survivin expression in tumor tissues via immunohistochemical staining. The proportion of survivin-positive cells in brexpiprazole group was less than that of vehicle group, indicating that brexpiprazole increased apoptosis in tumor sections (Fig. 5B). Furthermore, data showed that the expression of phosphorylated AKT, P85, P38, ERK1/2, RAF in tumor tissues was remarkably decreased by brexpiprazole treatment, but the expression of RAS did not change significantly (Fig. 5C). In summary, these data suggest that brexpiprazole inhibits colon cancer xenograft tumor proliferation, possibly through affecting the signal transduction of PI3K and MAPK pathways.

3.6 Brexpiprazole Synergized with Cetuximab to Reduce Cell Viability and Downregulate RAS, Phosphorylated AKT, P85, P38, ERK1/2, and RAF in HCT116 Cells

Previous data showed that HCT116 cells had different sensitivity to brexpiprazole treatment. Therefore, in subsequent studies, we chose HCT116 cells, which are more sensitive to brexpiprazole, as the research object. We first verified the effect of brexpiprazole combined with cetuximab on HCT116 cells’ proliferation. As shown in Fig. 6A, compared with the vehicle group, brexpiprazole group, or cetuximab group, the combination of brexpiprazole and cetuximab significantly reduced the proliferation activity of HCT116 cells. Next, we detected the effect of the combination of the two drugs on the PI3K and MAPK pathway in HCT116. Compared with the vehicle group, brexpiprazole group, or cetuximab group, the combination of brexpiprazole and cetuximab significantly reduced the expression of RAS, phosphorylated AKT, P85, P38, ERK1/2, and RAF proteins (Fig. 6B). These data indicate that brexpiprazole increased the sensitivity of HCT116 cells to cetuximab.

3.7 Brexpiprazole Synergized with Cetuximab to Inhibit Xenograft Tumor Growth in CRC

Next, we validated the effect of brexpiprazole and cetuximab on tumor cell proliferation in the HCT116 xenografts. Mice in the single medication group were given 5 mg/kg of brexpiprazole daily via gavage or 25 mg/kg of cetuximab intraperitoneally twice a week, and the combination medication group received both treatments. Compared with single medication groups, the combined medication observably suppressed HCT116 xenograft growth (Fig. 7A). To verify its underlying mechanism, we also performed immunohistochemical analysis and Western blot. Data indicated that compared to single medication groups, combination medication drastically down-regulated the expression of survivin in tumor tissues (Fig. 7B). Combination medication also remarkably down-regulated the phosphorylation expression levels of PI3K- and MAPK-related proteins in tumor tissues but did not affect the expression of RAS protein (Fig. 7C). Taking the data together, brexpiprazole combined with cetuximab can produce a synergistic anti-tumor effect.

4. Discussion

Metastasis is highly related to the death of CRC patients [2,19]. CRC is likely to migrate to livers, lungs,
Fig. 3. Brexpiprazole induced apoptosis in colon cancer cells. Cells were treated with Brexpiprazole at various concentrations. (A) Flow cytometry analysis of apoptosis, where Q4 is viable cells, Q1 is necrotic cells, Q2 represents late apoptotic cells, and Q3 represents early apoptotic cells. Apoptotic cells include late apoptotic cells and early apoptotic cells. (B) Western blotting analysis of apoptosis-related proteins. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. (n = 3)
Fig. 4. Brexpiprazole inhibited the signal transition of the EGFR pathway in colon cancer cells. Immunoblotting analysis of (A) the activation of the PI3K pathway, (B) the effect of brexpiprazole on PI3K and MAPK pathway-related proteins in (B) HCT116 cells, and (C) SW620 cells. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. (n = 3)

In recent years, the use of old drugs has become a new approach to effective anticancer treatment, such as antipsychotic drugs with anticancer activity [24]. In particular, first-generation antipsychotics, such as thiamphenicol and pimozide, have shown some cancer-inhibitory effects in a variety of cancers in humans [25–28]. Brexpiprazole is a new antipsychotic drug developed in recent years [7,8]. It is a central nervous system 5-HT-dopamine balancer and is mainly used in the treatment of schizophrenia and de-

bones, etc. About 1/5 patients showed synchronous metastases, and up to 2/3 patients showed distant metastases in 5 years [20]. The development of peritoneal metastases is commonly accompanied by malignant ascites, visceral pain, or malnutrition [21]. CRC patients with peritoneal metastases have been shown to have the worst prognosis [22]. About 1/5 of patients showed metastasis at diagnosis [23]. Therefore, it is of great significance to suppress metastasis of CRC cells.
Fig. 5. Brexpiprazole inhibited tumor growth in HCT116 xenograft models. HCT116 tumor-bearing mice received brexpiprazole (5 mg/kg) daily through intragastrical administration (i.g.) for 17 days. (A) Tumor growth curve, (B) immunohistochemical staining of surviving (scale: 50μm), (C) immunoblotting of PI3K and MAPK pathway-related proteins. *\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.001 \), ****\( p < 0.0001 \). NC represents the untreated group, injected intraperitoneally with an equal dose of PBS and gavaged with gum arabic solvent; Brexpiprazole group was represented by intraperitoneal injection of an equal doses of PBS and gavage of brexpiprazole 5 mg/kg prepared with gum arabic; Oxaliplatin group was represented by intraperitoneal injection of an equal dose of oxaliplatin 5 mg/kg prepared with PBS and gavage of gum arabic solvent. (n = 6)
Fig. 6. Brexpiprazole synergized with cetuximab to reduce cell viability and downregulate RAS, phosphorylated AKT, P85, P38, ERK1/2, and RAF in HCT116 cells. HCT116 cells were treated with brexpiprazole, cetuximab, or a combination of brexpiprazole and cetuximab. (A) Cell viability, (B) immunoblotting of the effect of brexpiprazole, cetuximab, or their combination on PI3K and MAPK pathway-related proteins in HCT116 cells. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. There were four groups in total: Group 1, Colorectal cancer cells were added to equal volumes of DMSO and PBS (brexpiprazole – & cetuximab –); Group 2, Equal volumes of 10 μM of brexpiprazole prepared in DMSO and PBS (brexpiprazole + & cetuximab –); Group 3, Equal volumes of DMSO and 0.5 mg/kg of cetuximab prepared in PBS (brexpiprazole – & cetuximab +); Group 4, Equal volumes of 10 μM of brexpiprazole prepared in DMSO and 0.5 mg/kg of cetuximab prepared in PBS. (n = 3).

Brexpiprazole can inhibit a variety of cancer processes including lung and pancreatic cancers [10], and CRC [11]. The latest studies proved that Brexpiprazole could prevent CRC cell proliferation [11]. Our study indicated that administration of Brexpiprazole not only significantly suppressed the migration ability, but also suppressed proliferation and increased apoptosis of CRC cells. The results updated our knowledge of Brexpiprazole in CRC and may provide novel directions for CRC treatment.

EGFR has been proven to regulate cell proliferation and differentiation in cancer [30]. EGFR is increased in
Fig. 7. Brexpiprazole synergized with cetuximab to inhibit xenograft tumor growth in CRC. HCT116 tumor-bearing mice received brexpiprazole, cetuximab, or the combination of brexpiprazole and cetuximab. (A) Tumor growth curve, (B) immunohistochemical staining of surviving (scale: 50μm), and (C) immunoblotting of PI3K and MAPK pathway-related proteins. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. NC represents the untreated group, injected intraperitoneally with an equal dose of PBS and gavaged with gum arabic solvent; Brexpiprazole group was represented by intraperitoneal injection of an equal dose of PBS and gavage of brexpiprazole 5 mg/kg prepared with gum arabic; Cetuximab group was represented by intraperitoneal injection of an equal dose of cetuximab 25 mg/kg prepared with PBS and gavage of gum arabic solvent; Brex+Cetu group was represented by intraperitoneal injection of an equal dose of cetuximab 25 mg/kg prepared with PBS and gavage of brexpiprazole 5 mg/kg prepared with gum arabic. (n = 6)
60–80% of CRC [31]. EGFR has been projected to be a key therapeutic target [32]. EGFR monoclonal antibodies and tyrosine kinase inhibitors have been evaluated in CRC therapy [33]. Several studies proved that Brexpiprazole can sensitize the cells to the targeted drug by down-regulating survivin in EGFR-TKI-resistant tumor cells including NSCLC, pancreatic cancer, and glioblastoma [12]. Survivin, a member of the anti-apoptotic family (IAP), plays a key role in the inhibition of cell apoptosis and the regulation of the cell cycle [34]. Survivin is highly expressed in almost all human malignant tumors [35,36]. Survivin expression is closely associated with the prognosis of CRC patients [37–39]. In addition, survivin is also a vital downstream molecule of the PI3K-AKT pathway [40], which is one of the downstream pathways of EGFR. Accordingly, we hypothesized that Brexpiprazole can inhibit the PI3K-AKT pathway in CRC cells and affect the activation of EGFR to sensitize Brexpiprazole to targeted drugs. However, it is not clear whether Brexpiprazole can alter EGFR-related pathway proteins in CRC cells. The current study indicated that brexpiprazole treatment significantly down-regulated p-AKT, P85, P38, ERK1/2, and RAF in HCT116 cells, suggesting that brexpiprazole suppressed EGFR to inhibit its downstream PI3K and MAPK pathway in CRC cells.

Despite the advancement of cancer treatment, drug resistance remains to be a key challenge [41]. Drug resistance accounts for most cancer relapses [42]. It has been reported that drug resistance is related to over 90% of cancer-related deaths [43]. EGFR is closely related to drug resistance. For example, gefitinib/erlotinib resistance is because of additional mutations in EGFR such as C797S/G, G796S/R, etc. [45]. On the other hand, inhibition of EGFR showed promising results in sensitizing cancer cells to drugs. For example, Jin et al. [46] have reported that inhibition of EGFR can sensitize liver cancer to lenvatinib. Xu et al. [47] have demonstrated that EGFR inhibitors sensitize NSCLC cells to TRAIL-induced apoptosis. Here, we investigated how Brexpiprazole sensitizes CRC cells to cetuximab treatment. Data indicated that Brexpiprazole drastically enhanced the sensitivity of HCT116 cells to cetuximab treatment. Furthermore, results also proved that a combination of brexpiprazole and cetuximab remarkably inhibited xenograft tumor growth in vivo. These novel findings illustrated the great significance of Brexpiprazole or Brexpiprazole/cetuximab in the treatment of CRC. It is worth mentioning that no clinical samples were included in this study. Clinical samples or PDX mouse models will be included in future studies to provide more relevant data. Nevertheless, the current study reveals a novel finding, showing that Brexpiprazole suppresses human CRC cell proliferation and increases its sensitivity to cetuximab treatment.

5. Conclusions

In conclusion, our data indicated that Brexpiprazole can inhibit the proliferation and migration, and enhance apoptosis of CRC cells. In addition, Brexpiprazole can increase the sensitivity of CRC to cetuximab. Mechanistically, Brexpiprazole can activate PI3K/Akt, RAS/Raf/ERK1/2, and p38 pathway in CRC cells. These results highlighted the importance of Brexpiprazole in sensitizing CRC cells, which might shed light on new treatment of CRC.

Availability of Data and Materials

All data can be accessed through the corresponding author, Wei Chen.

Author Contributions

WC and LL designed the experiments. XYL, XJL, LL, and WC performed the experiments. XYL and WJX collected and analyzed the data. WC provided the resource support. XYL, LL, and WC drafted the manuscript. All authors approved the final manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The present study was approved by the Ethics Committee of the North Sichuan Medical College (approval no. 2023004).

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Conflict of Interest

The authors declare no conflicts of interest.

References


