LncRNA KCNQ1OT1 is a Key Factor in the Reversal Effect of Curcumin on Cisplatin Resistance in the Colorectal Cancer Cells

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Research

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Abstract

Context: The development of cisplatin resistance is a common cause of cancer recurrence in colorectal cancer (CRC). Long non-coding RNA KCNQ1OT1 has been proven to exert its oncogenic function in multiple cancers. Curcumin is a natural phenolic that could effectively suppress cisplatin resistance in CRC.

Objective: To expound the role of KCNQ1OT1 in cisplatin resistance in CRC cells and whether KCNQ1OT1 participates in the reversal effect of curcumin on cisplatin resistance in CRC.

Methods: Cell proliferation and apoptosis were evaluated by CCK-8 assay and flow cytometry assay, respectively. The interplay between KCNQ1OT1 and miR-497 was determined using RNA pull-down assay and dual-luciferase reporter gene assay. The combination of B-cell lymphoma 2 (Bcl-2) and miR-497 was confirmed using dual-luciferase reporter gene assay.

Results: Compared with CRC cell line HCT8, the cisplatin-resistant CRC cell line HCT8/DDP exhibited a higher KCNQ1OT1 expression. Functionally, the silence of KCNQ1OT1 suppressed proliferation and promoted apoptosis in HCT8/DDP cells. KCNQ1OT1 could act as a sponge of miR-497 and removed the suppressive effect of miR-497 on Bcl-2 expression. Curcumin treatment suppressed proliferation and promote apoptosis in HCT8/DDP cells. While KCNQ1OT1 overexpression removed the effect of curcumin on HCT8/DDP cells via miR-497/ Bcl-2 axis. Finally, the *in vivo* experiments confirmed that the ectopic expression of KCNQ1OT1 reversed the suppressive effect of curcumin on the growth of cisplatin-resistant CRC cells.

Conclusion: KCNQ1OT1 promoted cisplatin resistance in CRC cells via the miR-497/Bcl-2 axis. Administration of curcumin could effectively down-regulate KCNQ1OT1 expression, thus reversing cisplatin resistance in CRC cells.

Introduction

Colorectal cancer (CRC) is the most common gastrointestinal malignancy and the third leading cause of cancer-related deaths worldwide [1]. Despite surgical resection combined with chemotherapeutic agents such as cisplatin achieve good treatment outcomes at the early stages. The development of cisplatin resistance after prolonged treatment is a common cause of cancer recurrence [2]. Therefore, clarifying the underlying mechanisms of cisplatin resistance has great significance for the clinical treatment of CRC.

B-cell lymphoma 2 (Bcl-2) is a key member of the proto-oncogene Bcl-2 protein family and responsible for the regulation of cell apoptosis [3]. Increasing evidence showed that Bcl-2 contributed to the development of cisplatin resistance in various cancers. For example, in non-small cell lung cancer, the up-regulation of Bcl-2 was associated with the cytoplasmic repressor/activator protein-1-mediated cisplatin resistance [4].

The Bcl-2 overexpression induced by paxillin conferred 5-fluorouracil resistance in CRC [5]. Notably, the Bcl-2 inhibitor YC137 synergized cisplatin sensitivity in nasopharyngeal carcinoma cells via suppressing Bcl-2 protein expression [6], indicating that Bcl-2 is a potential target to reverse the resistance to cisplatin in cancers.

In the last decade, several studies hypothesized that the occurrence of cancer is associated with the dysregulation of microRNAs (miRNAs), small non-coding RNAs that negatively regulate gene expression at the post-transcriptional level [7, 8]. Through CRC miRNAs profiling datasets analysis, Falzone et al. [9] screened out 19 miRNAs that were differentially expressed in CRC tissues. Among them, miR-497 was significantly down-regulated and its overexpression suppressed the proliferation, migration, and invasion of CRC cells *in vitro* [10]. Besides, Zhu et al. [11] reported that miR-497 decreased cisplatin resistance in human lung cancer cell lines via targeting Bcl-2. Hence, it is reasonable to hypothesize that in CRC, miR-497 sensitizes cells to cisplatin-induced apoptosis through modulating Bcl-2.

Long non-coding RNA (LncRNA) KCNQ1 opposite strand/antisense transcript 1 (KCNQ1OT1) is located at the KCNQ1 cluster on human chromosome 11p15.5 and has been found to be strongly correlated with the development of cancer [12]. Nakano [13] et al. observed the high expression of KCNQ1OT1 transcription in CRC tissues and cell lines. What's more, the research of Qi et al. [14] demonstrates that the KCNQ1OT1 knockdown promoted the chemosensitivity to cisplatin in osteosarcoma cells, hinting that KCNQ1OT1 may play roles in the cisplatin resistance in cancer. Moreover, increasing evidence confirmed that LncRNAs could function as competing endogenous RNAs (ceRNAs), binding to miRNA and removing the suppressive effect of them on mRNA expression [15]. Using bioinformatics software, we found there were potential binding sites between KCNQ1OT1 and miR-497. Therefore, we wondered whether KCNQ1OT1 took part in the cisplatin resistance in CRC via removing the inhibitory effect of miR-497 on Bcl-2.

Curcumin is a natural phenolic compound that is extracted from *Curcuma longa* and exhibits a strong anti-tumor effect [16]. Over the years, several researchers revealed the potential effect of curcumin to fight against cisplatin-resistant cancer cells while the specific mechanism remains unclear [17]. A recent study showed that curcumin suppressed cisplatin resistance in ovarian cancer via restoring LncRNA MEG3 expression [18], suggesting that curcumin may modulate cisplatin resistance in cancer cells via regulating LncRNAs.

Based on the previous studies, the present study set out to explore the role of KCNQ1OT1 in cisplatin-resistant CRC cells and whether KCNQ1OT1 participated in the reversal effect of curcumin on cisplatin resistance in CRC.

Materials And Methods

Cell culture and transfection

Human CRC cell line HCT8 and cisplatin-resistant CRC cell line HCT8/DDP were purchased from Oulu Biotechnology (China) and maintained in RPMI-1640 medium (Gibco, USA) containing 10% fetal calf

serum (GIBCO, USA) supplemented with penicillin (100 U/ml; Sigma, USA) and streptomycin (100 mg/ml; Sigma, USA). The cells were cultured in a 37°C incubator with 5% CO₂.

RNAi vectors (si-KCNQ1OT1 and miR-497 inhibitor), overexpression vectors (KCNQ1OT1 vector and miR-497 mimic), and relative negative controls (si-NC, inhibitor NC, vector, and mimic NC) were produced by RIBOBIO (China). Cells were transfected with 5 µg plasmid using Lipofectamine 3000 (Invitrogen, USA) according to the manufacturer's protocol. Six hours later, the original medium was replaced with fresh RPMI-1640 complete medium. Forty-two hours later, the transfection efficiency was analyzed by qRT-PCR.

Quantitative Real-time Pcr (qrt-pcr)

Total RNA was extracted from cells or tumor tissues of mice using the Trizol reagent (Invitrogen, USA), and the concentration and purity of RNA were measured by the ultraviolet spectrophotometer. Reverse transcription was conducted for the synthesis of cDNA using the SuperScript IV First-Strand Synthesis System (Invitrogen, Germany) or High Capacity RNA-to-cDNA Kit (Thermo Fischer Scientific, USA). The qRT-PCR assay was conducted using the Platinum Quantitative RT-PCR ThermoScript One-Step Kit (Invitrogen) or TaqMan® Non-coding RNA Assays (Thermo Fischer Scientific) in ABI 7500 Real-Time PCR System (Applied Biosystems, USA). β-actin or U6 was used as an endogenous control.

Western Blot

The determination of protein levels of Bcl-2 and cleaved caspase 3 were done by western blot. Total protein samples were purified from cell lysate and tumor tissues by RIPA lysis buffer (Beyotime, China). Equal amounts of protein samples were separated on SDS-polyacrylamide gels and transferred to the membrane (Millipore, USA). After blocked with 5% skimmed milk for 1 hour at room temperature, the membrane was incubated with primary antibodies to Bcl-2 (1:1000; Abcam, UK) and cleaved caspase 3 (1 µg/ml; Abcam) for 12 h at 4 °C. After washed three times, the membranes were incubated with the Goat Anti-Rabbit IgG H&L (HRP) (1:2000; Abcam) for 1 hour at room temperature. The blots were observed using the gel imagine system (Bio-Rad, CA, USA). GAPDH (1:10000; Abcam) was used as an internal control.

Cell proliferation assay

Cell proliferation assay was determined by Cell Counting Kit-8 (CCK-8; CoWin Biosciences, China). After indicated transfection, HCT8/DDP cells were seeded in 96-well plates with a concentration of 2×10^3 cells/well (n = 5 for each time point) and cultured in 100 µL RPMI-1640 medium with or without curcumin (10 mM). After incubation for 0, 24, 48, 96 h, 10 µl CCK-8 was added into each well and cultured in a 37°C incubator with 5% CO₂ for 2 hours. The optical density (OD) value of each well was detected at 450 nm by a microplate reader (Bio-Rad, USA).

Cell Apoptosis Analysis

Cell apoptosis was evaluated by flow cytometry using Annexin V-FITC Apoptosis Detection Kit (Univ-bio, China). Briefly, HCT8/DDP cells were resuspended in binding buffer at a concentration of 1×10^6 cells/mL. Then, 50 μ l cell suspension was incubated with 5 μ l Annexin V-FITC for 15 min at room temperature in the dark. Next, 5 μ l propidium iodide staining solution (PI) was added to the cells in the dark. Fifteen minutes later, cell apoptosis was measured by the CytoFLEX flow cytometer (Beckman Coulter) immediately.

Dual-luciferase Reporter Gene Assay

The binding sites between KCNQ10T1 and miR-497 were predicted by LncBase v.2 and the binding sites between miR-497 and Bcl-2 were predicted by Miranda. To confirm the combination of KCNQ10T1 and miR-497, the HCT8/DDP cells were cotransfected with KCNQ10T1 wild type (WT) luciferase reporter/KCNQ10T1 mutant type (MUT) luciferase reporter and miR-497 mimic/ mimic NC. Forty-eight hours later after transfection, cells were harvested and the luciferase activity was measured by dual-luciferase reporter assay system (Promega, USA). The combination of miR-497 and Bcl-2 was confirmed using the same way.

Rna Pull-down Assay

RNA-pull down assay was performed as previously described [19]. The biotinylated KCNQ10T1 probe (Bio-KCNQ10T1 probe) and its negative control (Bio-probe NC) were constructed with the Biotin RNA Labeling Mix (Roche, Basel, Switzerland) and T7 RNA polymerase (Roche, Basel, Switzerland). Then, the Bio-KCNQ10T1 probe and Bio-probe NC were, respectively, incubated with whole-cell lysates from 1.5×10^7 HCT8/DDP cells, followed by the incubation of streptavidin agarose magnetic beads. One hour later, miR-497 in the Bio-KCNQ10T1 probe pull-down complex was detected by qRT-PCR with the Bio-probe NC pull-down complex as a negative control.

Xenograft Animal Experiments

Male six-week-old nude mice (n = 24) were purchased from SLAC Laboratory Animal Co., Ltd (China). The lentivirus-KCNQ10T1 (lenti-KCNQ10T1) and negative control (lenti-NC) were produced by RIBOBIO (China). Mice were divided into 4 groups: vehicle (dimethyl sulfoxide; n = 6), curcumin (n = 6), curcumin + lenti-NC (n = 6), curcumin + lenti-KCNQ10T1 (n = 6). In the vehicle or curcumin group, 1×10^6 HCT8/DDP cells were subcutaneously injected into the back of mice. Once the tumor size reached 100 mm^3 , the mice were randomly intraperitoneally injected with curcumin (1 g/kg/week) or equal vehicle. In curcumin + lenti-NC or curcumin + lenti-KCNQ10T1 groups, 1×10^6 HCT8/DDP cells transfected with lenti-KCNQ10T1 or lenti-NC were suspended in Matrigel matrix (BD Biosciences, USA) and subcutaneously injected into

the back of mice. Once the tumor size reached 100 mm³, the mice intraperitoneally injected with vehicle (dimethyl sulfoxide) or curcumin (1 g/kg/week). The tumor volume of each mouse was measured at 7-day intervals from day 7 to day 42 after tumor cell implantation. Then, the mice were sacrificed and the tumor tissues were collected. All experiments were approved by the Ethics Committee of The First Affiliated Hospital of Wenzhou Medical University.

Statistical analysis

Data were shown as mean $\pm$ SD. Statistical analysis was performed using GraphPad Prism 6.0. Data were analyzed by student's t-test (for two experimental groups) or one-way ANOVA (for multiple experimental groups). Results were considered statistically significant when $P < 0.05$.

Results

KCNQ1OT1 was up-regulated in HCT8/DDP cells

The cisplatin-resistant CRC cell line HCT8/DDP and its wild-type counterpart HCT8 were selected to conduct ours *in vitro* experiments. As depicted in Fig. 1A and B, compared with HCT8 cells, the HCT8/DDP cells displayed higher KCNQ1OT1 expression and lower miR-497 expression. Also, apparent increases in mRNA and protein levels of Bcl-2 expression were observed in HCT8/DDP cells (Fig. 1C and D). Meanwhile, the cleaved caspase 3, a pro-apoptosis protein [20], was down-regulated in HCT8/DDP cells (Fig. 1E).

KCNQ1OT1 functioned as a regulator of HCT8/DDP cells' proliferation and apoptosis

To investigate the functions of KCNQ1OT1 on the proliferation and apoptosis of cisplatin-resistant CRC cells, si-KCNQ1OT1#1, #2, #3 and si-NC were transfected into HCT8/DDP cells. As shown in Fig. 2A, qRT-PCR analyses confirmed that KCNQ1OT1 expression was efficiently repressed in HCT8/DDP cells which were transfected with si-KCNQ1OT1#2. The viability of si-KCNQ1OT1#2-transfected HCT8/DDP cells was markedly decreased compared with those cells with si-NC transfection (Fig. 2B). Flow cytometry assay demonstrated that si-KCNQ1OT1#2 transfection resulted in a significant elevation of the apoptotic HCT8/DDP cells (Fig. 2C and D).

Kcnq1ot1 Released Bcl-2 Expression Via Sponging Mir-497

Using bioinformatics software (LncBase v.2), we found there were several potential binding sites between KCNQ1OT1 and miR-497 (Fig. 3B). In order to confirm the endogenous combination between KCNQ1OT1 and miR-497 in HCT8/DDP cells, RNA pull-down assay and dual-luciferase reporter assay were performed. As depicted in Fig. 3A, compared with Bio-probe NC, a great quantity of miR-497 was detected in the complex pulled down by the Bio-KCNQ1OT1 probe. Meanwhile, miR-497 mimic, rather than mimic NC, markedly decreased the luciferase activity of KCNQ1OT1 WT (Fig. 3C). What's more, the miR-497

expression level was suppressed by KCNQ10T1 overexpression (Fig. 3F) and promoted by KCNQ10T1 knockdown (Fig. 3H). These data verified the interaction between KCNQ10T1 and miR-497 in HCT8/DDP cells. Zhu et al. [21] revealed the combination of miR-497 and Bcl-2 in human glioma cells. To determine whether miR-497 could also target Bcl-2 in HCT8/DDP cells, the following experiments were conducted. As shown in Fig. 3D and E, the luciferase activity of Bcl-2 3'UTR WT was significantly suppressed in the presence of miR-497 mimic. Besides, the miR-497 overexpression suppressed the Bcl-2 protein level (Fig. 3G) and miR-497 knockdown elevated the Bcl-2 protein level (Fig. 3I). What's more, the negative regulation of miR-497 on the Bcl-2 protein level was eliminated by KCNQ10T1 (Fig. 3G and I), demonstrating that KCNQ10T1 functioned as a sponge of miR-497 and removed its regulatory effect on Bcl-2.

KCNQ10T1 mediated the regulatory effect of curcumin on proliferation and apoptosis of HCT8/DDP cells

We next sought to determine whether KCNQ10T1 was involved in the suppressive effect of curcumin on cisplatin resistance in CRC cells. Firstly, as shown in Fig. 4A and B, curcumin supplementation appreciably inhibited cell proliferation and KCNQ10T1 expression. Considering curcumin exhibited the best inhibition effect at a concentration of 10 μ M, we selected this concentration in the next experiments. Secondly, KCNQ10T1 was overexpressed in HCT8/DDP cells by transfecting the KCNQ10T1 vector (Fig. 4C). The curcumin + KCNQ10T1 vector-treated HCT8/DDP cells had a much higher Bcl-2 protein level and a lower cleaved caspase 3 protein level than that of the curcumin + vector-treated cells (Fig. 4D). What's more, the curcumin treatment effectively reduced cell proliferation and elevated the apoptotic cell numbers, while the overexpression of KCNQ10T1 reversed the effect of curcumin (Figure E, F, and G).

KCNQ10T1 mediated the regulatory effect of curcumin on proliferation and apoptosis of HCT8/DDP cells through miR-497

In order to further explore whether KCNQ10T1 took part in the suppressive effect of curcumin on cisplatin resistance in CRC cells through functioning as a sponge of miR-497, we overexpressed miR-497 in curcumin-treated HCT8/DDP cells which had been overexpressed KCNQ10T1 (Fig. 5A). Importantly, the miR-497 overexpression abrogated the KCNQ10T1 overexpression-induced up-regulation of Bcl-2 expression (Fig. 5B) and cell viability (Fig. 5C). Also, in response to the transfection of miR-497 mimic, the apoptosis rate retarded by KCNQ10T1 overexpression was elevated again (Fig. 5D and E).

KCNQ10T1 reversed the suppressive effect of curcumin on the growth of cisplatin-resistant CRC cells *in vivo*

To confirm whether KCNQ10T1 could mediate the regulatory effect of curcumin on cisplatin-resistant CRC cells *in vivo*, a mouse xenograft model was established and KCNQ10T1 was ectopically overexpressed using lenti-KCNQ10T1 in curcumin-treated xenograft model mice (Fig. 6C). As shown in Fig. 6A and B, compared with vehicle, the curcumin decreased the levels of tumor volume (Fig. 6A) and weight (Fig. 6B) in xenograft model mice. What's more, be similar to the results of *in vitro* experiments, the curcumin treatment decreased KCNQ10T1 expression (Fig. 6C) and Bcl-2 protein level (Fig. 6E), while

elevated miR-497 expression (Fig. 6D). On the contrary, the KCNQ10T1 overexpression removed the promoting effect of curcumin on miR-194 expression (Fig. 6D) and the suppressive effect of curcumin on Bcl-2 protein (Fig. 6E), ultimately elevated the levels of tumor volume (Fig. 6A) and weight (Fig. 6B) in xenograft model mice.

Discussion

Cisplatin is widely used for chemotherapy of cancers, including CRC [22]. Nevertheless, chronic cisplatin exposure of CRC cells give rise to the cisplatin resistance, ultimately resulted in the chemotherapy failure [23]. Herein in this study, we demonstrate that KCNQ10T1 promoted proliferation and inhibited apoptosis of cisplatin-resistant CRC cells via the miR-497/Bcl-2 axis. Meanwhile, the reversal effect of curcumin on cisplatin resistance in CRC cells was dependent on its negative regulation of KCNQ10T1 expression.

KCNQ10T1 has been reported to be a promoter of cancer progression via regulating cancer cell proliferation, invasion, and apoptosis. For instance, Wang et al [24]. reported that KCNQ10T1 aggravated cell proliferation and migration in bladder cancer via sponging miR-145-5p. Kang et al. [25] found that KCNQ10T1 contributed to the progression of non-small cell lung cancer by releasing autophagy-related gene 3 expressions. All of these findings manifested the importance of KCNQ10T1 in the development of cancer. Recently, the role of KCNQ10T1 in cisplatin resistance of cancer cells aroused wide interest among researchers. Zhang et al. [26] revealed that KCNQ10T1 could enhance cisplatin resistance in tongue cancer via acting as a ceRNA of miR-211-5p. In our present study, we found that compared with CRC cell line HCT8, the cisplatin-resistant CRC cell line HCT8/DDP exhibited a higher expression level of KCNQ10T1 (Fig. 1). In addition, KCNQ10T1 overexpression suppressed apoptosis of cisplatin-resistant CRC cells *in vitro* (Fig. 2) and accelerate tumorigenesis *in vivo* (Fig. 6). Interestingly, the research of Li et al. [27] demonstrated that KCNQ10T1 enhanced oxaliplatin resistance in CRC via inducing protective autophagy. Combined our experimental results with previous studies, KCNQ10T1 could function as a positive regulator in chemoresistance of various cancers, including CRC.

Increasing evidence supported that apoptosis failure is a crucial reason for the development of chemotherapy resistance [28]. As a key anti-apoptotic protein, Bcl-2 has been found to be involved in the multidrug resistance of CRC cells [29]. In line with the previous study, an apparent elevation of the Bcl-2 protein level was observed in HCT8/DDP cells (Fig. 1). Besides, the subsequent study showed that Bcl-2 was the target of miR-497 while KCNQ10T1 released Bcl-2 expression via sponging miR-497 (Fig. 3). miR-497 is a well-known anti-oncogene and the positive role of the miR-497/Bcl-2 axis in enhancing chemotherapy sensitivity has been reported in myeloma [30]. In the present study, miR-497 overexpression abrogated the promoting effect of KCNQ10T1 on cell viability and the inhibitory effect of KCNQ10T1 on cell apoptosis in HCT8/DDP cells via inhibiting Bcl-2 (Fig. 5), proving that miR-497/ Bcl-2 axis could also suppress cisplatin resistance in CRC cells.

Curcumin is a natural phenolic compound and has the advantages of small adverse reactions, good safety, and low price [31]. Numerous studies clarified that curcumin had the property of reversing

chemotherapy drug resistance of tumors [32]. For example, in CRC, curcumin reversed oxaliplatin resistance via regulating the process of epithelial-mesenchymal transition [33]. What's more, Liu et al. [34] reported that the regulatory effect of curcumin on LncRNAs in CRC. Therefore, we speculated that curcumin may reserve chemotherapy drug resistance in CRC via modulating LncRNAs. As we speculated, in the present study, curcumin dose-dependently decreased the viability of HCT8/DDP cells and the expression of KCNQ10T1 (Fig. 4). KCNQ10T1 overexpression abrogated the reversal effect of curcumin on cisplatin-resistant *in vitro* (Fig. 4) and removed the inhibitory effect of curcumin on tumorigenesis *in vivo* (Figs. 6), proving that curcumin suppressed cisplatin resistance via down-regulating KCNQ10T1.

Collectively, we reported that KCNQ10T1 was a contributor to cisplatin resistance in CRC cells and the administration of curcumin could reserve the cisplatin resistance in CRC cells through down-regulating KCNQ10T1 expression. We hope that our findings could provide new intervention targets for the prevention and reversal of cisplatin resistance during CRC therapy.

Abbreviations

CRC	colorectal cancer
Bcl-2	B-cell lymphoma 2
CCK-8	cell counting kit-8
miRNAs	microRNAs
LncRNA	long non-coding RNA
KCNQ10T1	KCNQ1 opposite strand/antisense transcript 1
ceRNAs	competing endogenous RNAs
WT	wild type
MUT	mutant type
lenti	lentivirus

Declarations

Ethics approval and consent to participate

All experiments were approved by the Ethics Committee of The First Affiliated Hospital of Wenzhou Medical University.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

None

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Authors' contributions

Zhi-hai Zheng: Conceptualization, Writing- Original draft preparation.

He-yi You: Project administration.

Yu-jie Feng: Data analysis.

Zhong-tao Zhang: Complete examination of manuscript.

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Figures

Figure 1

KCNQ10T1 was up-regulated in HCT8/DDP cells. The expression levels of (A) KCNQ10T1 and (B) miR-497 were measured by qRT-PCR in HCT8 cells and cisplatin-resistant HCT8 cells (HCT8/DDP). The (C) mRNA and (D) protein levels of B-cell lymphoma 2 (Bcl-2) were respectively measured by qRT-PCR and western blot in HCT8 cells and HCT8/DPP cells. GAPDH was used as an internal control. (E) The protein level of cleaved caspase 3 was measured by western blot in HCT8 cells and HCT8/DPP cells. GAPDH was used as an internal control. *** $P < 0.001$ vs HCT8.

Figure 2

KCNQ10T1 functioned as a regulator of HCT8/DDP cells' proliferation and apoptosis. (A) The inhibitory efficiencies of si-KCNQ10T1#1, #2, and #3 transfection on KCNQ10T1 expression were evaluated by qRT-PCR in HCT8/DDP cells. (B-D) HCT8/DDP cells were transfected with si-KCNQ10T1#2 or its negative control (si-NC) for 48 h. (B) Cell viability was measured by CCK8 assay at the indicated time points. (D) Flow cytometry assay was performed to detect cell apoptosis. (C) The percentage of apoptotic cells was calculated in (D). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs control.

Figure 3

KCNQ10T1 released Bcl-2 expression via sponging miR-497. (A) Detection of miR-497 using qRT-PCR in the samples pulled down by the biotinylated KCNQ10T1 probe (Bio-KCNQ10T1 probe) and negative control (Bio-probe NC). *** $P < 0.001$ vs Bio-probe NC. (B) The potential binding sites between KCNQ10T1 and miR-497 were forecasted by LncBase v.2. (C) Relative luciferase activities of KCNQ10T1 wild type (WT) and KCNQ10T1 mutant type (MUT) in 293T cells transfected with miR-497 mimic or negative control (mimic-NC) were measured using the dual-luciferase reporter assay system. *** $P < 0.001$ vs mimic NC. (D) Putative binding sites between miR-497 and Bcl-2 were forecasted by Miranda. (E) Relative luciferase activities of Bcl-2 3'UTR wild type (WT) and Bcl-2 3'UTR mutant type (MUT) in 293T cells transfected with miR-497 mimic or mimic-NC were measured using the dual-luciferase reporter assay system. ** $P < 0.01$ vs mimic NC. HCT8/DPP cells were transfected with mimic NC/ miR-497 mimic/ miR-497 mimic+ control vector (vector)/ miR-497 mimic+ KCNQ10T vector (KCNQ10T), 48 h later, (F) miR-497 expression level and (G) Bcl-2 protein level were measured. *** $P < 0.001$ vs mimic NC; ### $P < 0.001$ vs miR-

497 mimic+ vector. HCT8/DDP cells were transfected with miR-497 inhibitor/ the negative control of miR-497 inhibitor (inhibitor NC)/ miR-497 inhibitor+ si-NC/ miR-497 inhibitor+ si-KCNQ10T, 48 h later, (H) miR-497 expression level and (I) Bcl-2 protein level were measured. ***P<0.001 vs inhibitor NC; ###P<0.001 vs miR-497 inhibitor+si-NC.

Figure 4

KCNQ10T1 mediated the regulatory effect of curcumin on the proliferation and apoptosis of HCT8/DDP cells. (A-B) HCT8/DDP cells were incubated with different concentrations of curcumin (0, 1, 3, 10 μM). Forty-eight hours later, (A) Cell viability was measured by CCK8 assay. (B) The KCNQ10T1 expression level was determined by qRT-PCR. (C-G) HCT8/DDP cells were divided into 4 groups: control, curcumin (10

μM), curcumin+vector, curcumin+ KCNQ10T1. (C) The overexpression efficiency of the KCNQ10T1 vector on KCNQ10T1 expression was evaluated by qRT-PCR. (D) The protein levels of Bcl-2 and cleaved caspase 3 were measured by western blot. GAPDH was used as an internal control. (E) Cell viability was measured by CCK8 assay at the indicated time points. (G) Flow cytometry assay was performed to detect cell apoptosis. (F) The percentage of apoptotic cells was calculated in (G). *P<0.05, **P<0.01, ***P<0.001 vs curcumin (0 μM) or control; #P<0.05, ###P<0.001 vs curcumin+vector.

Figure 5

KCNQ10T1 mediated the regulatory effect of curcumin on the proliferation and apoptosis of HCT8/DDP cells through miR-497. HCT8/DDP cells were divided into 5 groups: control, curcumin (10 μM), curcumin+ KCNQ10T1, curcumin+ KCNQ10T1+mimic NC, curcumin+ KCNQ10T1+miR-497 mimic. (A) The miR-497 expression level was determined by qRT-PCR. (B) The protein levels of Bcl-2 and cleaved caspase 3 were measured by western blot. GAPDH was used as an internal control. (C) Cell viability was measured by CCK8 assay at the indicated time points. (E) Flow cytometry assay was performed to detect cell apoptosis. (D) The percentage of apoptotic cells was calculated in (E). *P<0.05, **P<0.01, ***P<0.001 vs control; #P<0.05, ###P<0.001 vs curcumin; &P<0.05, &&P<0.01, &&&P<0.001 vs curcumin+ KCNQ10T1+mimic NC.

Figure 6

KCNQ10T1 reversed the suppressive effect of curcumin on the growth of cisplatin-resistant CRC cells in vivo. The xenograft model mice were divided into four groups, vehicle (dimethyl sulfoxide, n=6), curcumin (1 g/kg/week, n=6), curcumin+lentivirus-KCNQ10T1 (lenti-KCNQ10T1, n=6), curcumin+negative control of lenti-KCNQ10T1 (lenti-NC, n=6). (A) The tumor volumes in the 4 groups of mice were measured at 7-day intervals from day 7 to day 42. Forty-two days after tumor cell implantation, the mice were sacrificed and the tumor tissues were collected. (B) The tumor weights in the 4 groups of mice were measured. The expression levels of (C) KCNQ10T1 and (D) miR-497 in tumor tissues were determined by qRT-PCR. (E) The Bcl-2 protein level in tumor tissues was measured using western blot. GAPDH was used as an internal control. **P<0.01, ***P<0.001 vs vehicle; #P<0.05, ##P<0.01, ###P<0.001 vs curcumin+lenti-NC.