

# circBIRC6 regulates cisplatin resistance of choroidal melanoma via positively modulating ERK by sponging miR-503-3p

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## Research article

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# Abstract

## *Background/Aims*

circRNA plays a key regulatory role in various human tumors. This study was carried out to investigate the molecular mechanism of circBIRC6 in the regulation of choroidal melanoma cisplatin (DDP) resistance.

## *Methods*

The expression of circBIRC6 in choroidal melanoma tissues and cells (MUM-2B, MUM-2B / DDP) was detected by RT-qPCR. The function of circBIRC6 in DDP resistance of choroidal melanoma was analyzed by functional loss and overexpression experiments. DDP inhibition rate was detected by MTT method. Western blot was used to analysis the protein levels of P-gp, MRP1 and ERK. The relationship between circBIRC6, miR-503-3p and ERK was analyzed by CircRIP and luciferase reporter vector assay.

## *Result*

circBIRC6 was up-regulated in choroidal melanoma, and it was significantly raised in choroidal melanoma tissues and cells that are resistant to DDP. CircBIRC6 regulated DDP resistance of choroidal melanoma cells by binding to miR-503-3p. ERK was directly targeted by miR-503-3p, and ERK inhibited miR-503-3p-induced DDP resistance. Finally, the expression of circBIRC6 was positively correlated with ERK, and it was verified that circBIRC6 regulated ERK by targeting miR-503-3p.

## *Conclusion*

CircBIRC6 regulated the expression of ERK choroidal melanoma cells and DDP resistance via miR-503-3p. These results suggested that the knockout of circBIRC6 may provide an effective treatment strategy for choroidal melanoma.

# Background

Choroidal melanoma is the most common type of uveal malignant tumor, and it is also a common intraocular malignant tumor in adults. It is mainly derived from melanocytes, with high malignancy and mortality[1]. Its incidence is second only to that of retinoblastoma in children, and it is considered as the "number one killer" in ophthalmic diseases[2]. Studies have shown that its incidence has increased in recent years[3]. At present, according to the demands of patients, their own vision and general conditions, the treatment mainly includes transpupillary thermotherapy and local resection. Most patients with choroidal melanoma are already at the advanced stage at the time of treatment, and platinum-based chemotherapy is the majority of patients with advanced choroidal melanoma[4]. Cisplatin (DDP) is the most common platinum-based first-line chemotherapy drug[5]. Its main mechanism of action is to inhibit DNA replication, affect cell transcription and translation, and promote tumor cell apoptosis[6]. Patients resistant to cisplatin is an important factor affecting the survival prognosis of patients with treatment

failure[7]. In-depth study of the molecular mechanism of drug-resistant tumor cells may help us to understand the proliferation and metastasis of tumor cells, and obtain the ability to avoid programmed cell death.

Circular RNA (circRNA) is involved in regulating the expression level of genes on multiple levels such as epigenetics, transcription level, post-transcription level, and the occurrence and development of tumors[8]. It has been found that circRNA is widely involved in genome regulation, including X-chromosome silencing, transcriptional interference or activation, genome imprinting, chromatin modification, intranuclear transport and other important life processes, and is closely related to many diseases such as malignant tumors[9, 10]. With the discovery of new circRNAs and the gradual recognition of their functions, the role of circRNAs in tumor pathogenesis and drug resistance has become a hot topic in the field of tumor research[11]. Studies have shown that circBIRC6 is expressed in a variety of malignant tumors, and can regulate the biological processes of related tumors, such as liver cancer[12]. However, its effect on cisplatin resistance in tumor cells has not been reported. Therefore, it is very important to explore the mechanism of cisplatin tolerance of choroidal melanoma cells by circBIRC6.

CircRNA regulate tumor resistance by a variety of mechanisms, which can affect mRNA expression or competitively bind miRNA to affect the expression of downstream target genes[13]. MiRNA are involved in important life processes such as cell proliferation and apoptosis, gene expression regulation, and individual development[14]. miRNA is closely related to tumorigenesis, development, and resistance to chemotherapy[15]. MiR-503-3p is a newly discovered mi RNA that is involved in the proliferation of certain tumors[16]. For example, research has found that miR-503-3p promotes epithelial-mesenchymal transition in breast cancer [17]. Studies found that the expression level of miRNAs is up-regulated or down-regulated, which directly causes the abnormal expression of target gene protein, and ultimately changes the drug sensitivity of tumor cells through cell signaling pathways[18]. MAPK signaling, including extracellular signal-regulated protein kinases (ERK) and c-Jun NH-terminal kinase (JNK) and stress-activated p38 MAP kinase/p38/SAPK, is very important for regulating the key functions (apoptosis, differentiation) of tumor cells[19]. Targeting these signaling pathways can induce apoptosis. Several studies have reported the treatment of cisplatin leads to the activation of ERK in several cancers[20]. The aim of this study was to elucidate the mechanism of cisplatin resistance regulated by circBIRC6 through miR-503-3p/ERK axis in choroidal melanoma, so as to provide new theories and new means to guide the individualized treatment strategy of choroidal melanoma in the era of precision medicine.

## Methods

### Tissue sample

The cancerous tissue and adjacent non-cancerous tissues of 40 patients who underwent primary choroidal melanoma surgery were collected from the the Second Clinical Medical College of Jinan University, Shenzhen People's Hospital. All patients received DDP chemotherapy in The Second Clinical Medical College of Jinan University, Shenzhen People's Hospital. The specific chemotherapy regimen was

as follows. Patients were treated with intravenous infusion of 75mg /m<sup>2</sup> DDP, repeated every 21 days for a maximum of six courses. According to the literature, classification of patients' DDP sensitivity or resistance was performed. Patients resistant to DDP did not undergo surgery and all patients signed informed consent.

### **Cell culture and transfection**

Normal human choroid melanocytes (CM), choroid melanoma cell line (MUM-2B) and MUM-2B / DDP cells were purchased from Shanghai Fudan (IBS) Cell Resource Center. MUM-2B was cultured in RPMI1640 medium containing 10% fetal bovine serum. MUM-2B/DDP was stably cultured in RPMI1640 medium with cisplatin concentration of 0.1 mg/L and 10% FBS. Both MUM-2B and MUM-2B/DDP were cultured at 37 °C with 5% CO<sub>2</sub> saturation humidity. The cells in logarithmic growth stage were transfected and the fusion rate reached 60%. The transfection operation was strictly performed in accordance with Lip2000 and gene fragment instructions. The experiments were divided into blank groups, NC group, and transfection group. CircBIRC6, miR-503-3p mimics and miRNA-590-5p inhibitors were designed and synthesized by RiboBio (Guangzhou, China). To construct circBIRC6, ERK and NF-kb overexpression plasmids, their sequences were cloned into the pCDS-At cloning vector (BioVector, Beijing, China). Lipofectamine 2000 (Invitrogen, Carlsbad, CA) was used to transfect oligonucleotides to MUM-2B and MUM-2B / DDP in the cell.

### **Quantitative reverse transcription PCR (qRT-PCR)**

Total RNA in tissues and cells was extracted using TRIzol reagent (Biosntech, Beijing, China). The quality of RNA was analyzed using NanoDrop 1000 (NanoDrop Technologies; Thermo Fisher Scientific, Inc.). SYBR-Green (Takara Biotechnology, Co., Lt. (Dalian, China) was used in by qRT-PCR. Amplification was performed by ABI 7,500 real-time PCR system. A qScript microRNA cDNA synthesis kit (Quantabio, Beverly, California, USA) was used for cDNA synthesis. The expression levels of circBIRC6 and miR-503-3p were calculated using the 2<sup>-ΔΔCT</sup> method. The expression levels of miRNA were standardized by U6[21]. The primer sequences were as follows:

circBIRC6: forward 5'- CTAATTCACCTGGCGGGAG-3';

circBIRC6: reverse 5'- CGCTTGGATTCCAAGGG-3'

miR-503-3p: forward: 5'- CTGAATGTGAAGGGAATGT-3',

miR-503-3p: reverse: 5'-GTTCTTCCACATCGGGGCCG-3'

GAPDH: forward: 5'-CGAGAGAATCCGCGGACAT-3'

GAPDH: reverse: 5'-TTGTGCAATACAGCGTGGAC-3'

U6: forward: 5'-GACAGATTCGGTCTGTGGCAC-3'

U6: reverse: 5'-GATTACCCGTCGGCCATCGATC-3'

### **DDP sensitivity assay**

SGC7901 cells and SGC7901/DDP cells of blank group, NC group, transfection group were inoculated into 96 well plates with 5000 cells/well respectively, and cultured at 37 °C, 5% CO<sub>2</sub> for 24 hours in saturated humidity environment. After 24 hours of culture, the original culture solution was aspirated, and the cisplatin-containing culture solution with a final concentration of 0, 2, 4, 8, 16, 32 μmol/L was added[22]. The cells were not inoculated, but only the holes with medium were used as the zeroing holes, and the holes without medicine were used as the control group. Each concentration was set with 5 multiple holes, after 48 h of culture, the absorbance at 490 nm was detected.

### **Western blot**

MUM-2B and MUM-2B / DDP cells in the blank group, NC group, and transfection group were collected after transfection. The supernatant was discarded after centrifugation, lysate was added. After 30 min, it was freezed at -20°C overnight. The supernatant was centrifuged at 4°C. After protein quantification, PAGE-SDS gel was used for electrophoresis separation of protein samples for 1.5 h. After transferred to PVDF blotting membrane for 1.5 h, it was sealed in TPST containing 5% skimmed milk for 4 h. Then, it was incubated with P-gp (abcam, 1: 1000, UK), MRP1 (1: 1000, CST, US), SMAD3 (1: 1000, CST, US), ERK (1: 1000, CST, US), and anti-GAPDH antibody (1: 1000, Abcam, Cambridge, UK) overnight at 4 ° C. After that, anti-rabbit secondary antibody (1: 1000, Cell Signaling Technology, Boston, MA, USA) was added for 1 h of incubation [23].

### **CircRIP**

The biotin-labeled circBIRC6 probe was designed and synthesized by Gene Pharma (Shanghai, China). The cells were seeded in a petri dish for 4 hours. Next, the cells were transfected with a specific biotin-labeled probe or control probe (200 nM) for 24 hours. It was incubated with formaldehyde for 10 minutes and terminated with a glycine solution. The supernatant was centrifuged. After mixed with streptavidin-labeled magnetic beads (M-280, Invitrogen, CA (USA, USA), it was incubated overnight. Then, the mixture was washed and reverse-crosslinked by lysis buffer containing proteinase K. Finally, miRNeasy Mini Kit (Qiagen, Dusseldorf, Germany) was used for subsequent quantitative detection of total RNA.

### **Luciferase reporter assay**

Wild-type or mutant circBIRC6 was subcloned it into the pm-irGLO vector. PmirGLO, pmirGLO-circBIRC6 or pmirGLO-circBIRC6-mut was co-transfected into cells with miR-503-3p mimic by Lipofectamine 2000 (Invitrogen). After 48 h, luciferase activity was measured using a dual luciferase assay kit (Promega). After centrifugation, luciferase activity was measured by a Modulus TD20 / 20 luminometer (Turner Biosystems, CA).

### **Statistical methods**

The monitoring data were analyzed by SPSS19.0 statistical software. The results of data analysis were represented as mean  $\pm$  standard deviation (mean  $\pm$ SD). Multigroup data analysis was founded on one-way ANOVA. LSD test was used for subsequent analysis.  $P < 0.05$  indicated the difference was significant.

## Results

### **circBIRC6 was upregulated in choroidal melanoma and related to DDP resistance**

First, the role of circBIRC6 in DDP resistance of choroidal melanoma was analyzed. The patients with choroidal melanoma were classified based on DDP sensitivity, and the expression of circBIRC6 in different types of choroidal melanoma patients and normal tissues was measured. The expression level of circBIRC6 in choroidal melanoma tissues was significantly up-regulated contrasted with that in the Normal group ( $P < 0.05$ , Fig.1A and 1B). Contrasted with DDP-sensitive tissues, circBIRC6 expression was significantly increased in DDP-resistant tissues ( $P < 0.05$ ). In addition, the expression level of circBIRC6 in the MUM-2B group was significantly up-regulated compared with that in the normal cell line (CM) ( $P < 0.05$ , Fig.1C). In MUM-2B /DDP cells, the expression level of circBIRC6 was significantly raised with the extension of DDP treatment time ( $P < 0.05$ , Fig.1D).

### **circBIRC6 increased the DDP resistance of choroidal melanoma cells**

Next, the effect of circBIRC6 on DDP resistance in choroidal melanoma cells was investigated. As shown in Fig.2A, contrasted with the si-NC group, the expression level of circBIRC6 of MUM-2B/DDP cells was significantly reduced in si-circBIRC6-1, si-circBIRC6-2 and si-circBIRC6-3 group ( $P < 0.05$ ), while the expression level of circBIRC6 in MUM-2B / DDP cells was significantly increased in the pCDS-circBIRC6 group ( $P < 0.05$ , Fig.2B). The inhibitory effect of MUM-2B cells in MUM-2B / DDP cells was significantly reduced contrasted with that in the MUM-2B group ( $P < 0.05$ ), si-circBIRC6 significantly enhanced the inhibitory effect of DDP on MUM-2B cells ( $P < 0.05$ ), and pCDS-circBIRC6 significantly inhibited the inhibitory effect of DDP on MUM-2B cells ( $P < 0.05$ , Fig.2C). Similarly, compared with MUM-2B cells, pCDS-circBIRC6 effectively enhanced the increase of IC50 in DDP-resistant cells and the protein expression levels of P-gp and MRP1 ( $P < 0.05$ ), while si-circBIRC6 effectively decreased the increase of IC50 in DDP-resistant cells and inhibited P-gp and MRP1 Protein expression levels (( $P < 0.05$ , Fig.2D, 2E). These results indicated that circBIRC6 played an key role in the regulation of DDP sensitivity in choroidal melanoma cells.

### **miR-503-3p was targeted by circBIRC6**

We predicted with online prediction tool Starbase v2.0 and miR-503-3p was identified as a potential target for circBIRC6 (Fig.3A). In addition, it was found that miR-503-3p expression level was significantly reduced in the DDP-sensitive group and DDP-resistant group contrasted with that in the Normal group ( $P < 0.05$ ), and miR-503-3p expression level was decreased more significantly in the DDP-resistant group (Figure 3B). Contrasted with the CM group, the miR-503-3p expression level in the MUM-2B group and

MUM-2B/DDP group were significantly decreased ( $P < 0.05$ ), and the miR-503-3p expression level in the MUM-2B/DDP group was decreased more significantly (Fig.3C). The expression level of circBIRC6 was negatively correlated with miR-503-3p in choroidal melanoma tissues (Fig.3D). And the luciferase activity was significantly reduced in miR-503-3p mimic and circBIRC6-WT co-transfected cells ( $P < 0.05$ ), but the luciferase activity of circBIRC6-MUT did not change significantly (Fig.3E). Contrasted with the MUM-2B cells treated with NC probe, the expression level of circBIRC6 in the cells treated with miR-503-3p probe was significantly increased ( $P < 0.05$ , Fig.3F). Contrasted with the Ctrl group, the expression level of miR-503-3p was significantly increased in the si-circBIRC6 group ( $P < 0.05$ ), and the expression level of miR-503-3p in the pCDS-circBIRC6 group was significantly reduced ( $P < 0.05$ , Fig. 3G).

### **miR-503-3p targeted ERK and declined the DDP resistance of choroidal melanoma cells**

We predicted with TargetScan version 7.2 and ERK was identified as a potential target for miR-503-3p (Fig.4A). The luciferase activity of co-transfected cells with miR-503-3p mimic and ERK-WT was significantly reduced ( $P < 0.05$ ), but the luciferase activity of ERK-MUT did not change significantly (Fig.4B). In addition, it was found that the expression level of miR-503-3p was significantly raised in the DDP-sensitive group and DDP-resistant group contrasted with that in the Normal group ( $P < 0.05$ ), and the expression level of miR-503-3p was raised more significantly in the DDP-resistant group ( $P < 0.05$ , Fig.4C). The expression level of miR-503-3p was negatively correlated with Erk in choroidal melanoma (Fig.4D). Contrasted with the CM group, the mRNA and protein expression levels of Erk in the MUM-2B group and the MUM-2B/DDP group were significantly increased ( $P < 0.05$ ), and the mRNA and protein expression levels of Erk was increased more significantly in the MUM-2B / DDP group ( $P < 0.05$ , Fig.4E, 4F).

### **miR-503-3p reduced the DDP resistance of choroidal melanoma cells by ERK**

As shown in Fig.5A, the expression level of ERK protein in the miR-503-3p mimic group was significantly reduced contrasted with that in the Ctrl group ( $P < 0.05$ ), while co-transfection of miR-503-3p mimic with pCDS-ERK reversed the effect of miR-503-3p mimic on the expression level of ERK protein ( $P < 0.05$ ). In addition, miR-503-3p mimic significantly inhibited the viability of MUM-2B / DDP cells ( $P < 0.05$ ), while co-transfection of miR-503-3p mimic with pCDS-ERK reversed the effect of miR-503-3p mimic on cell viability ( $P < 0.05$ , Fig.5B, 5C). Similarly, miR-503-3p mimic effectively reduced IC50 value in DDP-resistant cells and decreased the protein expression levels of P-gp and MRP1 ( $P < 0.05$ ), while co-transfection of miR-503-3p mimic with pCDS-ERK reversed the effect of miR-503-3p mimic on the protein expression levels of P-gp and MRP1 ( $P < 0.05$ , Fig.5D). These results indicated that miR-503-3p reduced DDP resistance of choroidal melanoma cells through ERK.

### **circBIRC6 regulated ERK expression and DDP resistance in choroidal melanoma via sponging to miR-503-3p**

As shown in Fig.6A, the expression level of ERK in MUM-2B/DDP cells transfected with si-circBIRC6 was significantly reduced contrasted with that in the Ctrl group, and co-transfection of si-circBIRC6 with miR-503-3p inhibitor or pCDS-ERK reversed the effect of si-circBIRC6 on ERK expression level. In addition, si-

circBIRC6 significantly inhibited MUM-2B / DDP cell viability ( $P < 0.05$ ), while co-transfection of si-circBIRC6 with miR-503-3p inhibitor or pCDS-ERK reversed the effect of si-circBIRC6 on cell viability ( $P < 0.05$ , Fig.6B and 6C). Similarly, si-circBIRC6 significantly reduced the protein expression levels of P-gp and MRP1 ( $P < 0.05$ ), and co-transfection of si-circBIRC6 with miR-503-3p inhibitor or pCDS-ERK reversed the effect of si-circBIRC6 on P-gp and MRP1 protein expression levels ( $P < 0.05$ , Fig.6D). These results indicated that the circBIRC6 / miR-503-3p / ERK axis was essential for regulating DDP resistance in choroidal melanoma cells.

## Discussion

Choroidal melanoma is a severe primary intraocular malignant melanoma that often occurs in adults. Statistical analysis shows that the incidence of intraocular malignant melanoma has continued to increase in recent years, and the incidence rate of developing countries has gradually approached western developed countries[24]. The death rate of malignant melanoma is as high as 50%[25]. Clinical studies have shown that the median survival time of intraocular malignant melanoma such as choroidal melanoma is only 4 to 5 months[26]. Since cisplatin has been used as a chemotherapy drug, it has become the most important drug for cancer treatment[27]. However, about 25% of patients with choroidal melanoma will develop drug resistance within 6 months of platinum chemotherapy[28]. Platinum resistance is an important factor in the recurrence of choroidal melanoma[29]. Therefore, it is particularly important to study the mechanism of platinum resistance to choroidal melanoma and find an effective treatment for choroidal melanoma.

CircRNA is a type of endogenous RNA that is widely present in cells and has diverse biological functions. With the development of bioinformatics and whole gene sequencing technology, circRNA is gradually discovered by scholars, and its function in cells is gradually being studied and understood[30]. CircRNA has rich biological functions, which can not only regulate the cell cycle but also promote cell self-proliferation [31]. It can not only play the role of oncogenes, but also play the role of tumor suppressor genes. It has a great correlation with the invasion, distant metastasis and prognosis of tumor patients[32]. Studies have shown that certain circRNAs are related to chemotherapy resistance in osteosarcoma, pancreatic cancer, and breast cancer. For example, studies have found that expression of circ\_000401 is raised in NSCLC, and circ\_0004015 is involved in the progression of NSCLC through miR-1183/PDPK1 signaling, including TKI resistance[33]. However, few researches have been reported on the role of circRNA in choroidal melanoma resistance, and the relationship between circRNA and choroidal melanoma resistance is currently unknown. CircBIRC6 is a recently discovered CircRNA, research has found that it is abnormally expressed in a variety of malignant tumors[34]. For example, it has been found that circBIRC6 is raised in lung cancer tissues and increases cell viability[35]. The occurrence of cancer resistance is related to the abnormality of multiple genes and their products, including multidrug resistance gene 1 (MDR1), lung resistance-related protein (LRP), etc[36]. P-gp is an MDR1 gene expression product and is an ATP-dependent transporter that can transport intracellular chemotherapeutic drugs out of the cell, thereby reducing the efficacy of the drug and leading to the emergence of drug resistance[37]. This study found that pCDS-circBIRC6 inhibited P-gp and MRP1 protein

expression, and si-circBIRC6 increased the resistance to adverse reactions of DDP. The above results indicated that the expression of circBIRC6 was closely related to the regulation of DDP resistance in choroidal melanoma.

circRNA can be used as a sponge for miRNA, targeting mRNA to promote cell autophagy, which shows that circRNA may also be involved in regulating cell apoptosis[38]. In the process of tumorigenesis and development, miRNA can not only promote tumorigenesis by regulating the expression of oncogenes, but also inhibit tumorigenesis by inhibiting the expression of oncogenes[39]. Many studies have shown that miRNAs play a pivotal role in the occurrence and regulation of cisplatin resistance[40]. For example, miR-218 can up-regulate expression and further increase the sensitivity of esophageal cancer cells to cisplatin[41]. MiR-503-3p is a tumor found in recent years. It is found that miR-503-3p overexpression can induce breast cancer apoptosis [42]. In this study, miR-503-3p was screened as a target gene of circBIRC6. miR-503-3p was reduced in choroidal melanoma tissues and cell lines, and its expression was closely related to the regulation of DDP resistance in choroidal melanoma. It was verified that circBIRC6 regulated DDP resistance in choroidal melanoma cells through miR-503-3p.

MAPK / ERK signaling pathway play a key role in the process of signal transfer from cell surface to nucleus, and regulates cell proliferation and differentiation by activating transcription factors in the nucleus[43]. ERK is a very important member of its family, and it is related to cell growth, transformation, drug sensitivity and other biological behaviors[44]. Studies in a variety of tumors suggest that the activity of ERK protein increases in a variety of malignant tumors, cells form a state of high proliferation and low apoptosis, and the defense ability of various biochemical or other stimulations to promote apoptosis is greatly improved[45]. Therefore, it was speculated that this mechanism may lead to tumor cells bypassing cisplatin induced apoptosis and producing cisplatin resistance. In this study, miR-503-3p was selected as Target gene of ERK through the database. Co-transfection of si-circBIRC6 with miR-503-3pinhibitor or pCDS-ERK reversed the effect of si-circBIRC6 on cell viability and protein expression levels of P-gp and MRP1. It demonstrated that circBIRC6 targeted and inhibited miR-503-3p/ERK expression, thereby increasing the DDP sensitivity of choroidal melanoma cells.

## Conclusion

In this study, it was focused on circBIRC6 and confirmed the upregulation of circBIRC6 in choroidal melanoma. In addition, circBIRC6 released ERK by acting as a miR-503-3p sponge, and increased DDP resistance in choroidal melanoma cells. This may provide new horizons for the treatment of choroidal melanoma.

## Abbreviations

Not applicable

## Declarations

## Ethics approval and consent to participate

This study was approved by the Ethics Committee of The Second Clinical Medical College of Jinan University, Shenzhen People's Hospital. Written informed consent was obtained from each individual participant.

## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

WL and AN performed the molecular studies. LJ performed the animal experiments. YC provided experimental technical support and performed the statistical analysis. NX designed the study and helped to draft the manuscript. All authors read and approved the final manuscript.

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## Figures

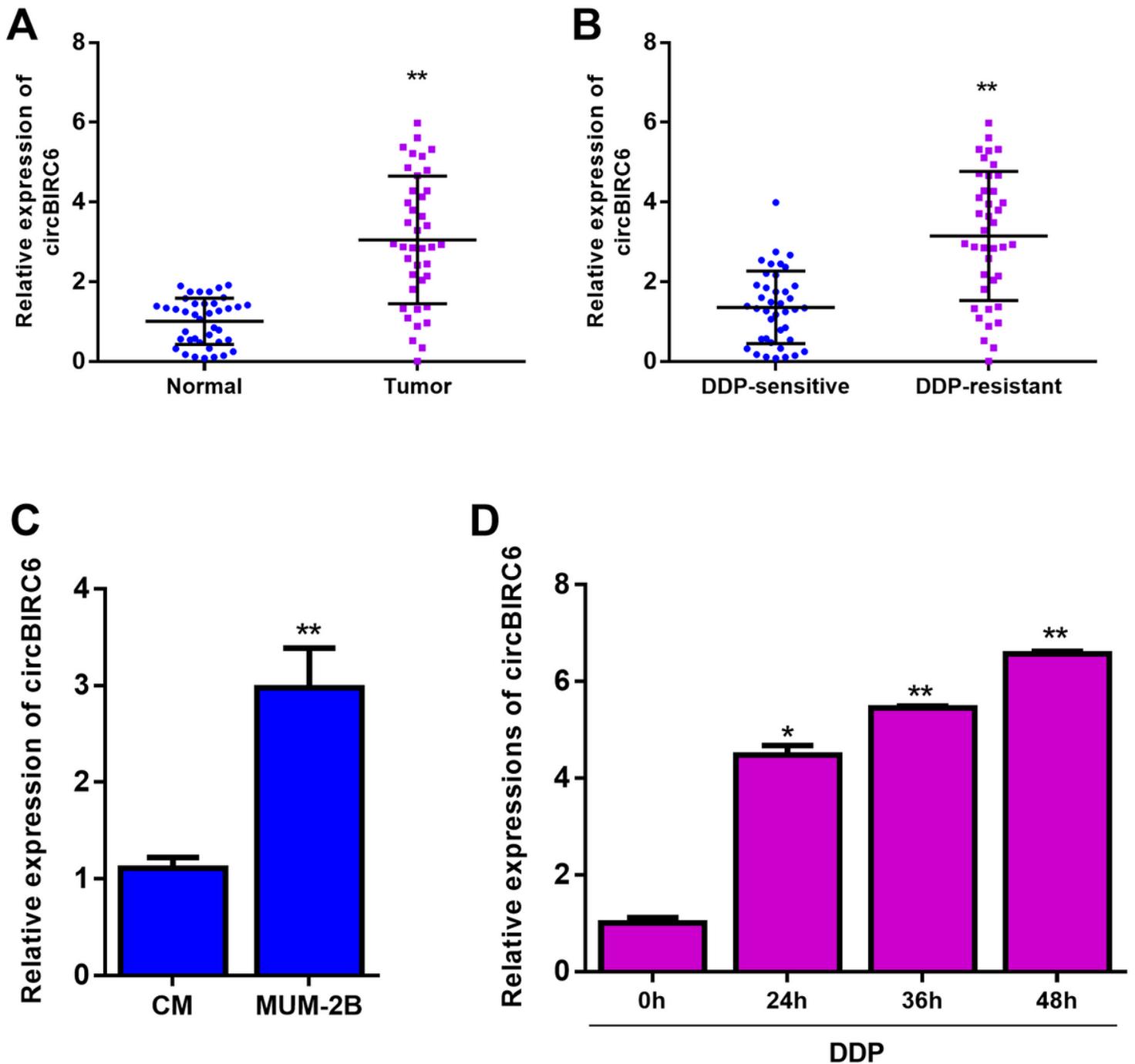
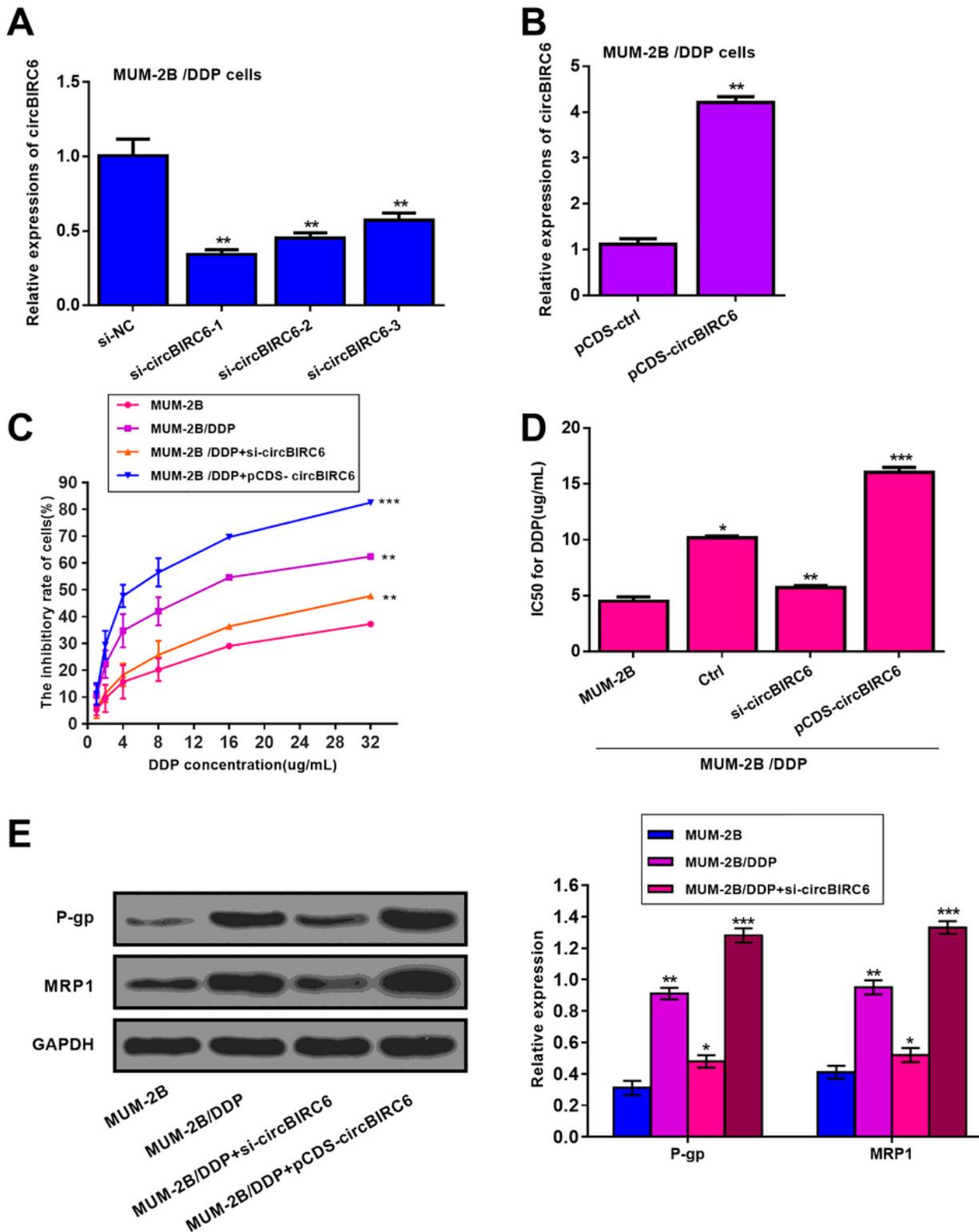


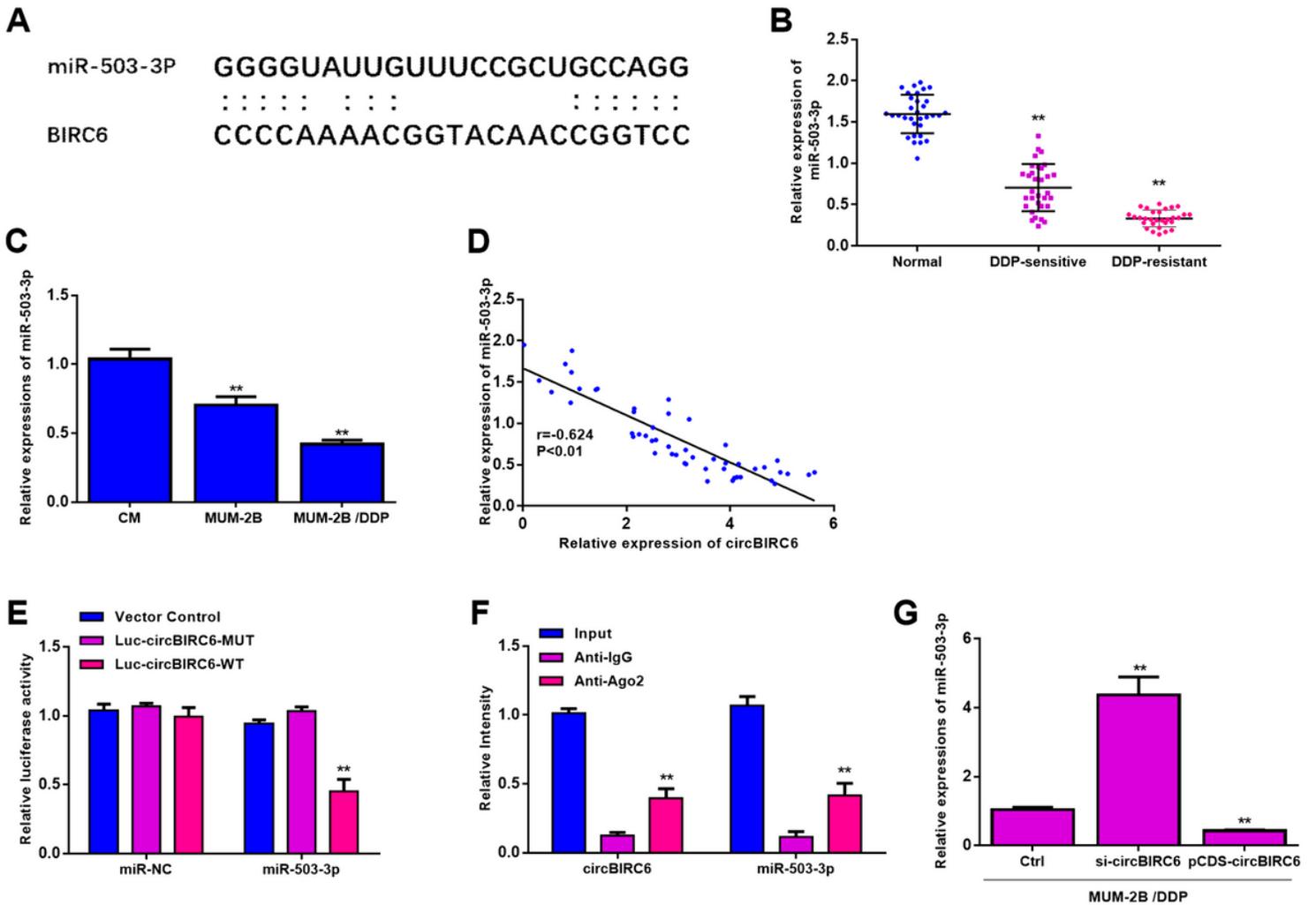
Figure 1

CircBIRC6 was up-regulated in choroidal melanoma and was associated with DDP resistance. (A) The expression levels of circBIRC6 in Normal and tumor tissue. (B) The expression levels of circBIRC6 in DDP sensitive and DDP resistant tissues. (C) The expression level of circBIRC6 in choroidal melanoma cell line. (D) The expression level of circBIRC6 in MUM-2B cells treated with 5  $\mu\text{g} / \text{mL}$  DDP at different times. \*  $P < 0.05$ ; \*\*  $P < 0.01$ ,  $n = 3$ .



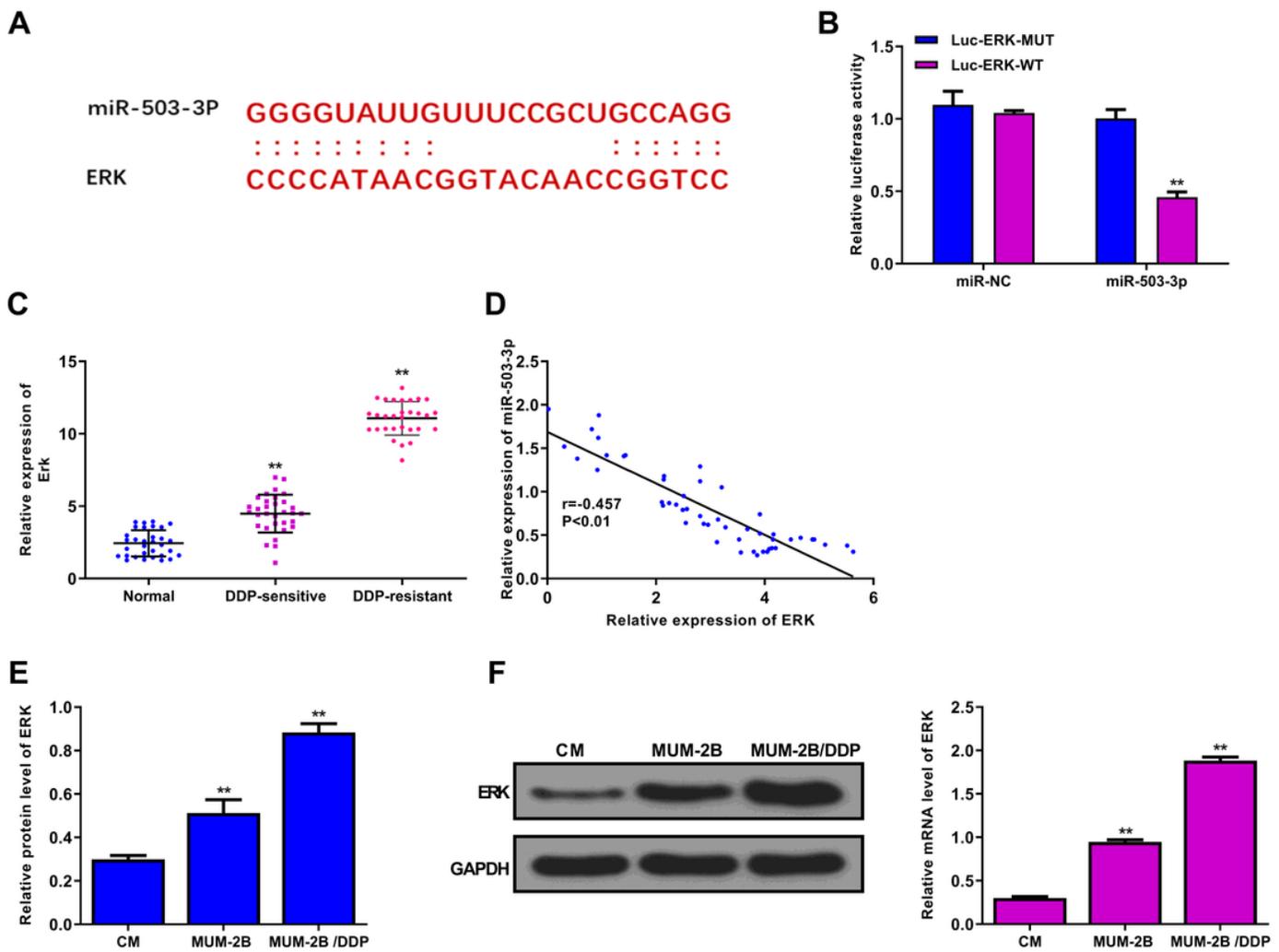
**Figure 2**

CircBIRC6 raised the DDP resistance of choroidal melanoma cells. (A and B) The expression level of circBIRC6 in MUM-2B / DDP cells after transfection was measured by RT-qPCR. (C) The inhibition rate of cells treated with DDP for 48 hours was detected by MTT. (D) The IC 50 of DDP in each group of cells was calculated by GraphPad Prism. (E) Protein expression levels of P-gp and MRP1. \* P <0.05; \*\* P <0.01, n = 3.



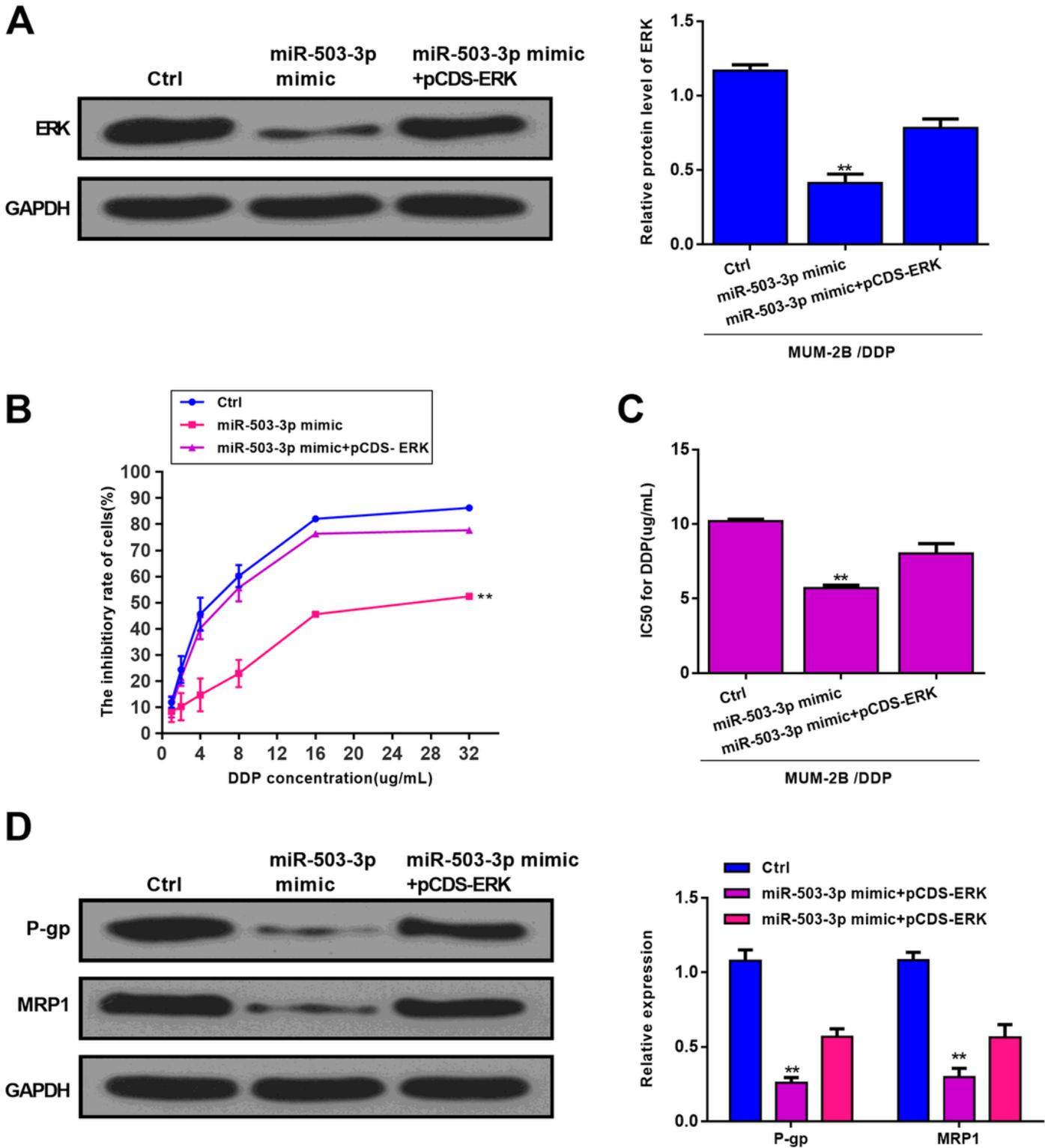
**Figure 3**

MiR-503-3p had a negative regulatory effect on circBIRC6. MiR-503-3p was targeted by circBIRC6. (A) Bioinformatics analysis results showed the combination of circBIRC6 and miR-503-3p. (B and C) Expression levels of miR-503-3p in normal, chemically resistant and chemically sensitive tissues and cells. (D) Correlation between the expression of circBIRC6 and miR-503-3p (E) Detection of luciferase activity by a luciferase reporter assay. (F) CircRIP analysis of the relationship between circBIRC6 and miR-503-3p. (G) Expression level of miR-503-3p. \*  $P < 0.05$ ; \*\*  $P < 0.01$ ,  $n = 3$ .



**Figure 4**

MiR-503-3p targeted ERK and reduced DDP resistance in choroidal melanoma cells. (A) Predicted results of miR-503-3p binding to ERK provided by TargetScan. (B) Luciferase activity was detected by Luciferase Reporter assay. (C) ERK expression levels in normal and tumor tissues. (D) Analysis of correlation between ERK and miR-503-3p expression by Pearson's analysis. (E, F) ERK mRNA and protein expression levels in cell lines. \*  $P < 0.05$ ; \*\*  $P < 0.01$ ,  $n = 3$ .



**Figure 5**

miR-503-3p reduced DDP resistance of choroidal melanoma cells through ERK. (A) ERK protein expression level. (B) Cell inhibition rate was detected by MTT method. (C) the IC<sub>50</sub> of each group of DDP was calculated by GraphPad Prism. (D) P-gp and MRP1 protein expression levels. The experiment was performed in triplicate. \* P < 0.05; \*\* P < 0.01, n = 3.

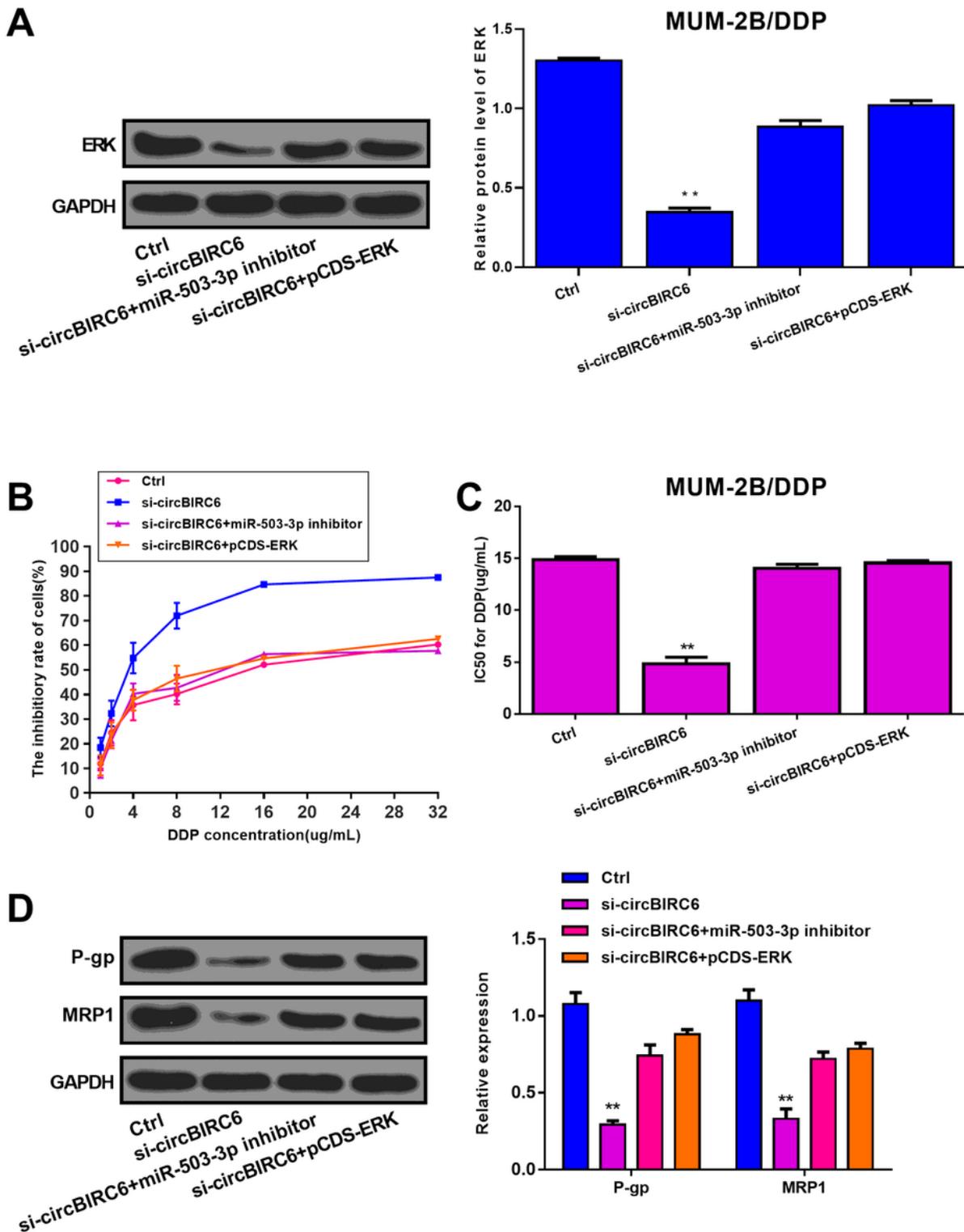


Figure 6

CircBIRC6 / miR-503-3p / ERK / NF-kb axis regulated DDP resistance in choroidal melanoma cells. (A) Erk protein expression level. (B, C) Inhibitory effect of DDP on cell survival and IC50 for DDP. (D) P-gp and MRP1 protein expression levels. \* P < 0.05; \*\* P < 0.01, n = 3.

## Supplementary Files

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