Department of Gastroenterology[1], The First Affiliated Hospital of Bengbu Medical College; Graduate School of Bengbu Medical College[2], Clinical Medical Colleges of Bengbu Medical College[3], Bengbu, P.R. China.

Aloperine can reverse the cisplatin resistance of colorectal cancer cells *via* suppressing the HIF-1α/ERK signaling pathway

Hailun Zheng[1], Ajie Zhang[1], Dapeng Li[1], Xiquan Ke[1], Qizhi Wang[1], Shanjun Yan[1], Yongju Xue[1], Xiaojing Deng[1,2,3*]

Received July 29, 2020, accepted August 25, 2020

**Corresponding author: Xiaojing Deng, Department of Gastroenterology, The First Affiliated Hospital of Bengbu Medical College, Bengbu, Anhui 233004, P.R. China.*
13955238107@163.com

Pharmazie 75: 581-585 (2020) *doi: 10.1691/ph.2020.0672*

Background: Aloperine can exert antitumor effects in colorectal cancer; however, it remains obscure whether aloperine can reverse the cisplatin resistance in colorectal cancer (CRC). *Objective*: To explore the roles of aloperine in the chemosensitivity of the DDP-resistant colorectal cancer cell line HT-29 (HT-29/DDP) and the related mechanism. *Results*: Aloperine can inhibit the proliferation of both HT-29 and HT-29/DDP cells in a dose-dependent manner; moreover, aloperine can significantly increase the sensitivity of HT-29/DDP cells to DDP; finally, HIF-1α and p-ERK was upregulated in HT-29/DDP cells and transient over-expression of HIF-1α has blocked aloperine+DDP induced anti-proliferative and pro-apoptotic effects on HT-29/DDP cells. *Conclusion:* We are reporting for the first time that aloperine can increase the sensitivity of HT-29/DDP cells to DDP and reverse cisplatin resistance *via* downregulating the HIF-1α/ERK signaling pathway.

1. Introduction

Colorectal cancer (CRC) is one of the mostly diagnosed cancers worldwide. Based on previous data, it was estimated that in the year of 2012, more than 1.4 million people were newly diagnosed with CRC and about 700,000 patients died (Enkhbat et al. 2018; Peng et al. 2018). Current anti-CRC methods included surgery, radiotherapies and chemotherapy, however, because of the rapid progression, metastasis and recurrence of the disease, none of the traditional methods have achieved desired therapeutic effects, leading to poor prognosis of the disease (Nakurte et al. 2018; Suehiro et al. 2018). Therefore, to further investigate the pathogenesis and develop novel methods for the treatment of CRC with improved efficacy is in a great demand.

Cisplatin is one of the first-line medications used for the treatment of CRC. It can lead to the arrest of cell cycle and increase the apoptosis of the cancer cells (Chen et al. 2017; Zhang et al. 2018). However, in recent years, the therapeutic efficacy of current cisplatin-based therapies is going worse, and the most important reason is chemoresistance that develops due to various factors (He et al. 2017; Ping et al. 2018; Xie et al. 2016). Thus, to further explore the underlying mechanisms of CRC cisplatin resistance would improve the clinical application of cisplatin.

Aloperine is an alkaloid that isolated from *Sophora alopecuroides* L., which is said to have anti-inflammatory and anti-allergic activities (Gao and Lagerstrom 2015; Wang et al. 2018; Yang et al. 2015; Zhao et al. 2018). Studies have shown that aloperine can exert antitumor effects in different types of tumors, including myeloma, lung cancer and osteosarcoma and CRC (Chen et al. 2018; Ling et al. 2018; Tian et al. 2018; Xu et al. 2017). However, it remains obscure whether aloperine cancer reverse the cisplatin resistance in CRC.

In the present study, we focuses on the roles of aloperine in the chemosensitivity of the DDP-resistant colorectal cancer cell line HT-29 (HT-29/DDP) and the related mechanism. Our study may provide a theoretical basis for the clinical application of aloperine to reverse the chemoresistance of current cisplatin based anti-CRC therapies.

2. Investigations and results

2.1. Cytotoxicity of aloperine on HT-29 and cisplatin-resistant HT-29/DDP cells

First, HT-29 and HT-29/DDP cells were cultured with 0, 50, 150 or 250 μM aloperine, and the cytotoxicity of the cells was evaluated by MTT assay. As shown in Fig. 1A, aloperine has exhibited anti-proliferative effect on HT-29 cells in a dose-dependent manner ($p<0.05$); moreover, aloperine also significantly decreased the viability of HT-29/DDP cells dose-dependently (Fig. 1B, $p<0.05$). Based on these results, 150 nM aloperine has been applied in the further analysis.

2.2. Aloperine can increase the sensitivity of HT-29/DDP cells to DDP

Furthermore, we evaluated whether aloperine can increase the sensitivity of HT-29/DDP cells to DDP *in vitro* by MTT and flow cytometry methods. It was observed that DDP had no significant effect on cell viability and apoptosis on HT-29/DDP cells, while either aloperine or the combination of aloperine and DDP markedly decreased the viability (Fig. 2, $p<0.01$) and increased apoptosis (Fig. 3, $p<0.01$) of HT-29/DDP cells compared with the DDP group; moreover, the combination of aloperine and DDP has shown better anti-proliferative and pro-apoptotic effects than aloperine alone on HT-29/DDP cells ($p<0.05$).

2.3. Aloperine can decrease the expressions of HIF-1α and p-ERK in HT-29/DDP cells

Next, to investigate the association between HIF-1α and ERK and the chemoresistance of HT-29 cells, the expressions of HIF-1α and p-ERK in HT-29 cells and HT-29/DDP cells were compared. The expressions of both HIF-1α and p-ERK were significantly increased in HT-29/DDP cells compared with HT-29 cells on protein level (Fig. 4, $p<0.01$). Moreover, treatment of DDP had no significant effect on the expressions of HIF-1α and ERK, while

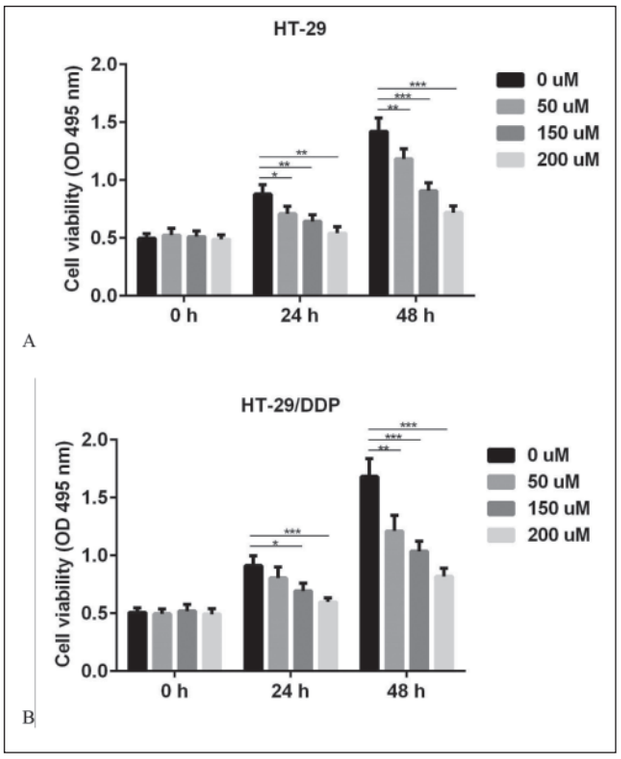

Fig. 1: Cytotoxicity of aloperine on HT-29 and cisplatin-resistant HT-29/DDP cells. (A) Viability of HT-29 treated with different concentration of aloperine. (B) Viability of HT-29/DDP cells treated with different concentration of aloperine. *p<0.05, **p<0.01, ***p<0.001.

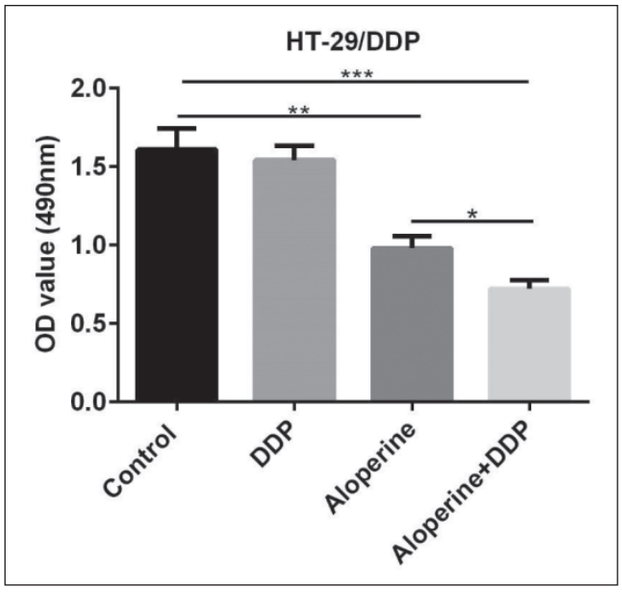

Fig. 2: Aloperine can inhibit the proliferation of HT-29/DDP cells treated with DDP. *p<0.05, **p<0.01, ***p<0.001.

the combination of aloperine and DDP markedly decreased the expressions of both HIF-1α and p-ERK in HT-29/DDP cells (Fig. 5, p<0.01).

2.4. Aloperine can reverse the DDP resistance of HT-29/DDP via suppressing the expression of HIF-1α and p-ERK

Finally, to assess whether aloperine increased the sensitivity of HT-29/DDP cells to DDP by inhibiting HIF-1α/ERK signaling

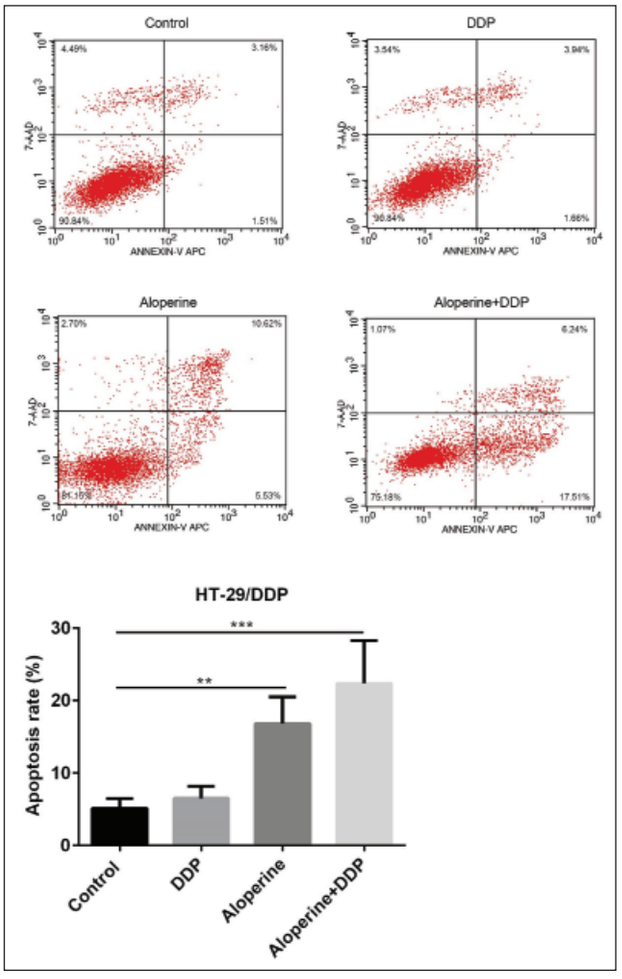

Fig. 3: Aloperine can promote the apoptosis of HT-29/DDP cells treated with DDP. **p<0.0, ***p<0.001.

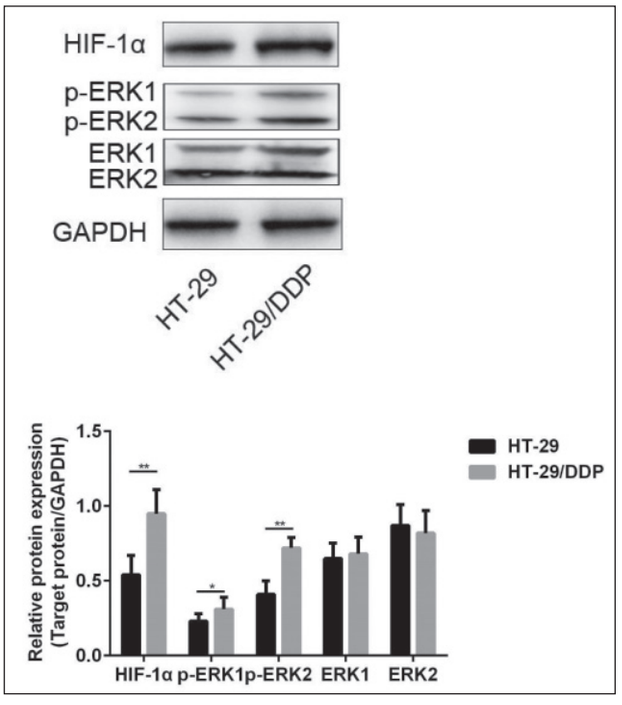

Fig. 4: Comparison of the expressions HIF-1α and ERK in HT-29/DDP cells and HT-29 cells. *p<0.05, **p<0.01.

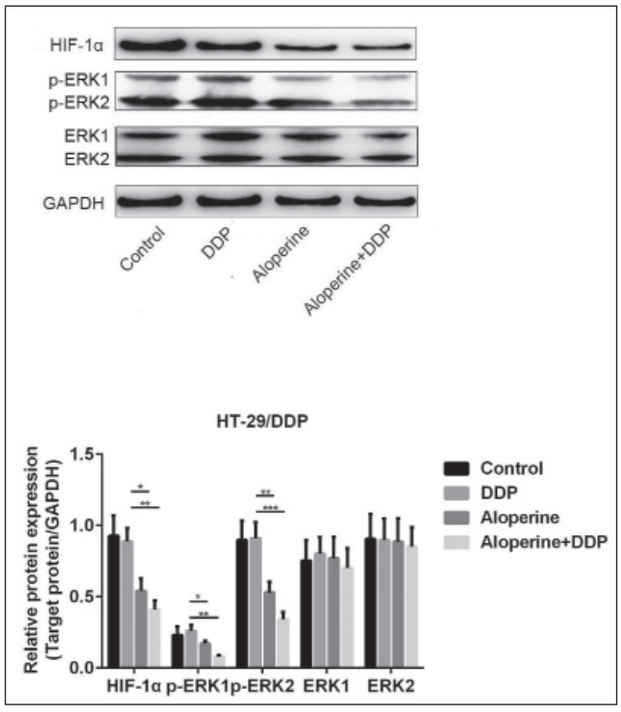

Fig. 5: Protein expression of HIF-1α and p-ERK in HT-29/DDP cells of different treatment by Western blot assay. *p<0.05, **p<0.01, ***p<0.001.

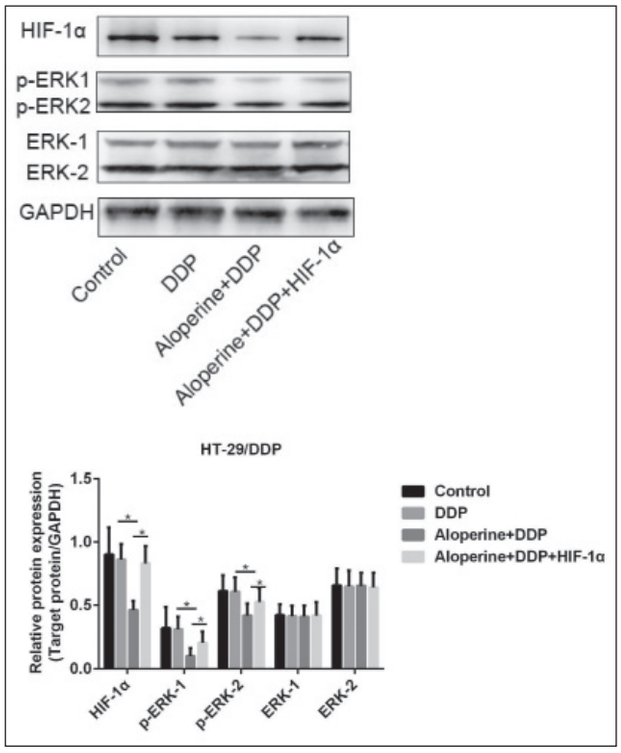

Fig. 6: HIF-1α plasmid can increase the expression of HIF-1α and ERK in aloperine+DDP treated HT-29/DDP cell. *p<0.05, **p<0.01.

pathway, HT-29/DDP cells were transfected with either HIF-1α over-expressions plasmids, and the effects of aloperine on the proliferation and apoptosis of the cells were examined. As shown in Fig. 5, transfection of HIF-1α plasmid increased the expression of HIF-1α and ERK in aloperine+DDP treated HT-29/DDP cell (Fig. 6, p<0.01); meanwhile, transient over-expression of HIF-1α has blocked aloperine+DDP induced anti-proliferative and pro-apoptotic effects on HT-29/DDP cells (Fig. 7, p<0.01).

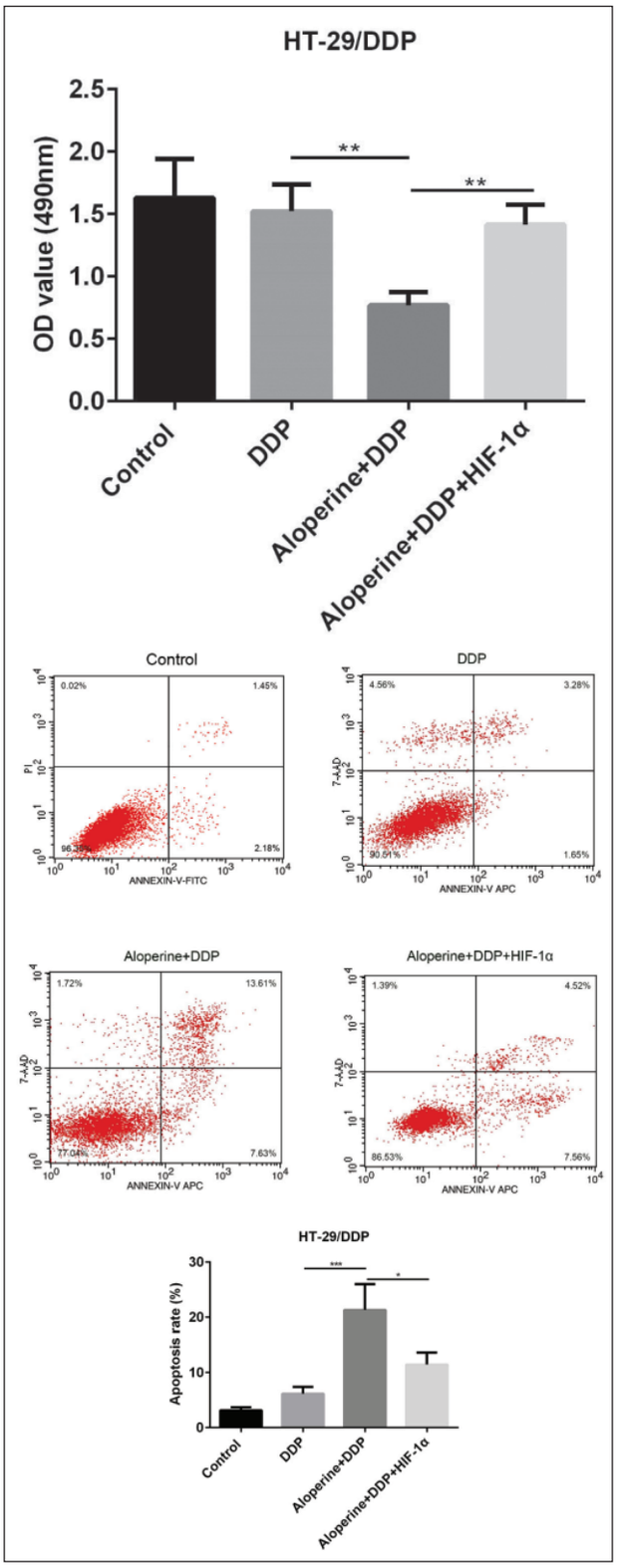

Fig. 7: HIF-1α over-expression plasmid can block aloperine induced anti-proliferative and pro-apoptotic effects on DDP treated HT-29/DDP cells. *p<0.05, **p<0.01, ***p<0.001.

3. Discussion

In the present study, the roles of aloperine in the chemoresistance of CRC cell line HT-29/DDP cells were examined. We observed that aloperine can reverse the DDP resistance of HT-29/DDP *via* suppressing the HIF-1α/ERK signaling pathway.

The tumor-suppressive roles of aloperine in different type of cancers have been discussed previously. Tian et al. (2018) reported that aloperine can inhibit the proliferation, migration and invasion and induce the apoptosis of breast cancer cells by blocking Ras signaling pathway. Ling et al. (2018) performed both *in vitro* and *in vivo* analysis and suggested that aloperine can execute antitumor effects in prostate cancer (Ling et al. 2018). Lee et al. (2018) reported that aloperine can promote the apoptosis of human thyroid cancer cells *via* caspase-dependent signaling pathway. In the case of CRC, Zhang et al. (2014) suggested that aloperine can induce the G2/M phase cell cycle arrest and increase the apoptosis of HCT116 cells, and here, we observed that aloperine can inhibit the proliferation of HT-29 cells, which is consistent with Zhang et al.'s finding. Taken together, these results suggested that aloperine may also exert anti-tumor effects in CRC.

Natural compounds can increase the sensitivity of DDP resistant cells to DDP and consequentially reverse chemoresistance. For example, it has been reported that *Glycyrrhiza glabra* extract and quercetin can reverse cisplatin resistance in triple-negative breast cancer cells *via* inhibiting the cytochrome P450 1B1 enzyme (Sharma et al. 2017); moreover, gambogic acid has been observed to reverse the hypoxia-induced cisplatin resistance in osteosarcoma cells (Zhao et al. 2016). Furthermore, curcumin has been reported to suppress cisplatin resistance in ovarian cancer (Zhang et al. 2017). However, it remains unclear whether aloperine could increase chemosensitivity of CRC cells. In the present study, we observed that aloperine can also inhibit the proliferation of HT-29/DDP cells; moreover, while DDP had no significant effect on the proliferation and apoptosis of HT-29/DDP cells, the combination of aloperine and DDP markedly decreased the viability and increased the apoptosis of HT-29/DDP cells, suggesting that aloperine can increase the sensitivity and reverse the chemoresistance of HT-29/DDP cell to DDP.

HIF-1α is a transcription factor that has been proved to play important roles in the occurrence and development of tumors (De la Garza et al. 2018; Jin et al. 2019; Li et al. 2018; Xu et al. 2018). In CRC, HIF-1α was reported to regulate the growth and metastasis of the cancer cells (Tang et al. 2018; Zhang et al. 2018), and the role of HIF-1α in the chemoresistance has also been discussed (Tang et al. 2018). Some previous studies indicated that HIF-1α may exert its carcinogenic function *via* interacting with different signaling pathways, for example, the ERK signaling pathway activated in many types of cancers (Liu et al. 2016; Zhang et al. 2014; Zhao et al. 2014). In the present study, we observed that the expressions of both HIF-1α and p-ERK were significantly increased in HT-29/DDP cells compared with HT-29 cells, and aloperine significantly decreased the expressions of both HIF-1α and p-ERK in DDT treated HT-29/DDP cells; furthermore, transfection of HIF-1α over-expression plasmids has blocked aloperine+DDP induced anti-proliferative and pro-apoptotic effects on HT-29/DDP cells. Taken together, these results indicated that aloperine can reverse the cisplatin resistance of CRC cells via inhibiting HIF-1α/ERK signaling pathway.

In conclusion, we reported for the first time that aloperine can reverse cisplatin resistance of HT-29/DDP cells, probably *via* downregulating the HIF-1α/ERK signaling pathway. Our results have provided novel evidence for the clinical application of aloperine to reverse the chemoresistance of current cisplatin based anti-CRC therapies.

4. Experimental

4.1. Cells and cell culture

The HT-29 cell line and the HT-29/DDP cell line were purchased from Shanghai Oulu Biotechnology Co., Ltd. (Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco/Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), 1% penicillin and streptomycin solution and incubated in a humidified incubator at 37 °C with 5% CO_2.

4.2. MTT assay

The viability of the cells was determined by MTT assay. Briefly, HT-29 or HT-29/DDP cells were seeded onto 96-well plates and treated with various concentrations of DDP. To determining the viability of the cells, 10 µl MTT solution (5 mg/ml) were added to each well and incubated at 37 °C for 4 h. Then the optical absorbance was measured at 490 nm using a spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA). Each experiment was repeated three times.

4.3. Cell apoptosis analysis

Cells was stained with the Annexin V/ 7-AAD apoptosis detection kit (BD Biosciences, Franklin Lakes, NJ, USA) 48 h after transfection, and the apoptosis rate was analyzed using BD FACSVerse flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

4.4. Cell transfection

To explore the relationship between DDP resistance and HIF-1α, HIF-1α over-expression plasmids (purchased from GenePharma, Shanghai, China) were transfected into HT-29/DDP cells using Lipofectamine 2000 (Invitrogen/Thermo Fisher Scientific) according to the manufacturer's protocols. Cells were harvested 48h after transfection for further analysis.

4.5. Western blot

RIPA buffer (Beyotime, Shanghai, China) was used for cell lysis at 4°C. The total protein samples were collected, and SDS-PAGE was performed to separate the proteins. After that, the separated proteins were transferred onto PVDF membranes and then blocked for 2 h with 5% non-fat dried milk dissolved in 0.1% PBST. The PVDF membranes were then incubated with the primary antibodies (anti-HIF-1α, anti-ERK1/2, anti-p-ERK1/2 and anti-GAPDH, Abcam, Cambridge, USA) overnight at 4 °C. GAPDH (Sigma, USA) was used as the internal control. On day 2, the membranes were incubated with the HRP-conjugated secondary antibodies (Beyotime), and then visualized with ECL Western Blotting Substrate (Beyotime) with a Tanon 5200 imaging system (Tanon, Shanghai, China). All experiments were repeated three times at least.

4.6. Statistical analysis

All experiments were repeated in triplicate. Data were thought as significant by comparing mean values (±standard deviation, SD) by T-test or analysis of variance as appropriate with the program SPSS11.0. $P<0.05$ was considered as statistically significant.

Acknowledgments: This work was supported by the Anhui Provincial Natural Science Foundation. (No. 1808085MH240)

Conflicts of interest: None declared.

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