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**Original Article**

# The Effect of SLC2A3 Expression on Cisplatin Resistance of Colorectal Cancer Cells

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**Abstract**

**Background:** To study the molecular mechanism of cisplatin chemotherapy resistance in colorectal cancer cells and to explore the effect of miRNA in regulating the expression of glucose transporter 3 (SLC2A3) and the proliferation and migration of colon cancer cells.

**Methods:** All samples were obtained from the People's Hospital of Wuhai, Wuhai, China between June 2019 and June 2020. Real-time quantitative PCR (qRT-PCR) was carried out to check the expression of miR-103a in these cell lines. Western blotting and Luciferase reporter gene detection confirmed the regulation of the miR-103a/SLC2A3 axis. Western blotting detected the activation of SLC2A3, caspased-9 and -3.

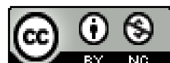
**Results:** The expression of SLC2A3 protein in colon cancer cell lines was significantly higher than that of normal colon cancer cells, while the expression of SLC2A3 miRNA showed no significant difference ($P$<0.05). Then, through clone formation analysis, SLC2A3 was closely related to the proliferation of human colon cancer cells. Functional recovery experiments showed that increasing the expression of miR-103a could reverse the abnormal proliferation caused by overexpression of SLC2A3.

**Conclusion:** Overall, miR-103a can inhibit the proliferation of human colon cancer cells by targeting SLC2A3, and this result will provide a potential target for the treatment of colon cancer.

**Keywords:** Colon cancer; MiRNA; Cisplatin resistance

## Introduction

Colon cancer is a common malignant tumor of the digestive tract in the colon, and its incidence ranks third among gastrointestinal tumors (1). The junction of the rectum and the sigmoid colon is its predisposition site (2). The incidence rate is the highest in the 40-50 age group, where the ratio of male to female is about 3:1(3). As we all know, tumor cell invasion and metastasis are the main causes of death of colon cancer patients. The factors that cause tumor cell metastasis are complex, involving cancer genes, tumor suppressor genes, and a series of regulatory factors, which are multi-factor and multi-step regulation (4, 5). MiRNAs (microRNA) play a role in



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tumor metastasis, providing new ideas and methods for inhibiting tumor cell metastasis (6). Platinum chemotherapy is one of the most important strategies for the treatment of colon cancer. The platinum-based chemotherapeutic drug cisplatin is widely used and shows a wide range of anti-cancer activities. The uptake of cisplatin can cause cell DNA damage, thereby inducing cancer cell apoptosis. Although cisplatin is widely used in the treatment of colon cancer, the drug resistance of colon cancer cells is currently the biggest problem affecting the efficacy (7, 8). How to overcome the drug resistance of tumors, improve the sensitivity of cancer cells to chemotherapeutic drugs, reduce the amount of clinical chemotherapeutic drugs, thereby reducing chemotherapy-related side effects, and successfully completing the chemotherapy process has important clinical significance for improving the therapeutic effect.

In recent years, the research of miRNAs and colon cancer has made great progress (9-11). The transfection of miR-let-7a in colorectal cancer cells can significantly inhibit the growth of tumor cells and the expression of RAS protein (12); overexpression of miR-143 in colorectal cancer LOVO cells can significantly inhibit the proliferation of tumor cells (13); research showed Transfection of HT-29 and LOVO cells with miR-195 can down-regulate the targeted anti-apoptotic protein Bcl-2, thereby inducing tumor cell apoptosis (14).

Based on the recent research progress of SLC2A3 in tumor treatment and the potential role of miRNAs in colon cancer (15-17), this study used bioinformatics methods to find slc2a3 targeted miRNAs and study the role of slc2a3 targeted miRNAs in the occurrence and development of colon cancer, to provide new strategies for the treatment of colon cancer.

## Materials and Methods

### *Clinical samples*

All samples were obtained from the People's Hospital of Wuhai, Cina between June 2019 and June 2020. All participants had signed informed consent forms. Our experiments were approved by the Ethic Community of The People's Hospital of Wuhai.

### *Cell Culture*

Human normal colon epithelial cell line NCM460 and HT29, COLO25, SW620, SW480, NCM460 cells were supplied by American Type Culture Collection (Rockville, MD, USA) and cultured at 37 °C in a humidified incubator containing 5% CO2 according to the manufacturer's recommendations.

### *RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)*

Total RNA from tissues and cells was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. RNA (1 µg) was reverse transcribed to cDNA using the Prime Script RT Master Mix kit (Takara Bio, Inc.). After RT, qPCR analysis was conducted using SYBR Premix Ex Taq™ II (Takara Bio, Inc.) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The $2^{-\Delta\Delta Cq}$ method was used to calculate relative mRNA expression. NADPH was used as a control RNA to determine the relative expression of miR-103a. Table 1 for all the Qrt-PCR primer sequences used in this research.

**Table 1:** The Qrt-PCR primer sequence

| *Gene* | *Primers* |
| --- | --- |
| SLC2A3 | F:5'-TCCCCTCCGCTGCTCACTATTT-3' |
| | R:5'-ATCTCCATGA CGCCGTCCTTTC-3' |
| miR-103a | F:5'-TCAATGCCTTCATAGCCCTGT-3' |
| | R:5'-TTACAGTGCTGCCTTGTTGC-3' |
| GAPDH | F:5'-GTCGGAGTCAACGGATT-3' |
| | R:5'-AAGCTTCCCGTT CTCAG-3'. |

### Oligonucleotides, Plasmid Construct, and Cell Transduction

Solute Carrier Family 2 Member 3 (SLC2A3) small interfering RNA (siRNA) was purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Human mature miR-103a (5'-CTGAGCAGCATTGTACAGGGC-3'), 2'-O-methyl-modified anti-miR-103a (5'-UCAUAGCCCUGUACAAUGCUGCU-3'), and negative control oligonucleotides (NCOs; 5'-CAGUACUUUUGUGUAGUACAA-3') were purchased from GenePharma Co., Ltd. (Shanghai, China). Recombinant SLC2A3 plasmid overexpression was performed by inserting the open reading frame region of human SLC2A3 gene into the pcDNA3.1 eukaryotic expression vector (Invitrogen). For transfection, cells were seeded into six-well plates overnight. Subsequently, the cells were transfected using Lipofectamine 2000 (Invitrogen) with 50 pmol/ml oligonucleotides and 2 μg/ml plasmid in OptiMEM (Gibco, Waltham, MA, USA) according to the manufacturer's protocol.

### Flow cytometry analysis

The apoptosis rates were assessed by flow cytometry via an Annexin V/PI kit (BD Biosciences, USA). Briefly, stably transfected cells were seeded in 6-well plates ($10^5$ cells per well). After washed by PBS several times, cells were resuspended in binding buffer, incubated with 5 μL FITC-Annexin V and 5 μL PI for 15 min in the dark at room temperature. Cells were then analyzed by the FACScan flow cytometry system (Becton Dickinson, San Diego, CA, USA).

### Luciferase Reporter Assay

All luciferase reporter plasmids (pmiR-SLC2A3-3'-UTR-WT, pmiR-SLC2A3-3'-UTR-WT and their corresponding MUT) were constructed by Synthgene Biotech (Nanjing, China). AEC IIs were seeded in 24 well plates with 60% confluence. AEC IIs were co-transfected with luciferase reporter plasmids and miR-20b mimics using Hi-Trans™ LipoPlus Reagent (Synthgene Biotech, Nanjing, China) according to the manufacturer's protocol. Dual-luciferase activity was detected by Dual-Luciferase Reporter Assay (Promega, Shanghai, China).

### Western Blot Analysis

Total proteins in tissues and cells were lysed using RIPA lysate buffer with protease inhibitor cocktail (Roche Applied Science). SDS-PAGE was conducted to isolate 20 μg/lane of protein and transfer it to PVDF membranes (EMD Millipore). After blocking with 5% skimmed milk solution, membranes were incubated overnight with primary antibodies at 4˚C and then incubated with HRP-conjugated secondary antibody (1:5,000; Sigma-Aldrich; Merck KGaA). Western blotting analysis were preformed according to previously description7. The used specific antibodies as follows: SLC2A3, caspased-9, caspased-3, GAPDH. All of the antibodies were purchased from Abcam, Shanghai, China.

### Colony Formation Assay

SW480 and HCT116 cells (5×102) in different groups were seeded into 6-well plates and cultured for 10 days. Subsequently, the cells were washed with PBS at least three times, fixed with methyl alcohol for 15 min and stained with 0.1% crystal violet for 15 min. After washing with PBS, the colonies in each group were imaged and counted.

### Statistical Analysis

All statistical data were showed as the mean ± standard deviation (SD) at least three independent experiments. The Student's *t*-test was used to analyze significant differences. Asterisks indicated significant differences (*$P<0.05$; ** $P<0.01$; ***$P<0.001$) compared with the control.

## Results

### SLC2A3 protein is Upregulated in colon cancer

To investigate the role of SLC2A3 on colon cancer, 14 pairs of colon cancer tissues and adjacent

tissues were used to measure the expression levels of SLC2A3. As shown in Fig. 1A and B, when compared with the normal tissues, the protein expression levels of SLC2A3 in colon cancer tissues were significantly up regulated. We also used these samples to measure the mRNA levels of SLC2A3. Intriguingly, there were no strikingly differences between cancer and adjacent tissues in SLC2A3 mRNA levels (Fig. 1C). These results suggested that SLC2A3 may play a role in colon cancer pathogenesis, and SLC2A3 was regulated post-transcriptionally in colon cancer.

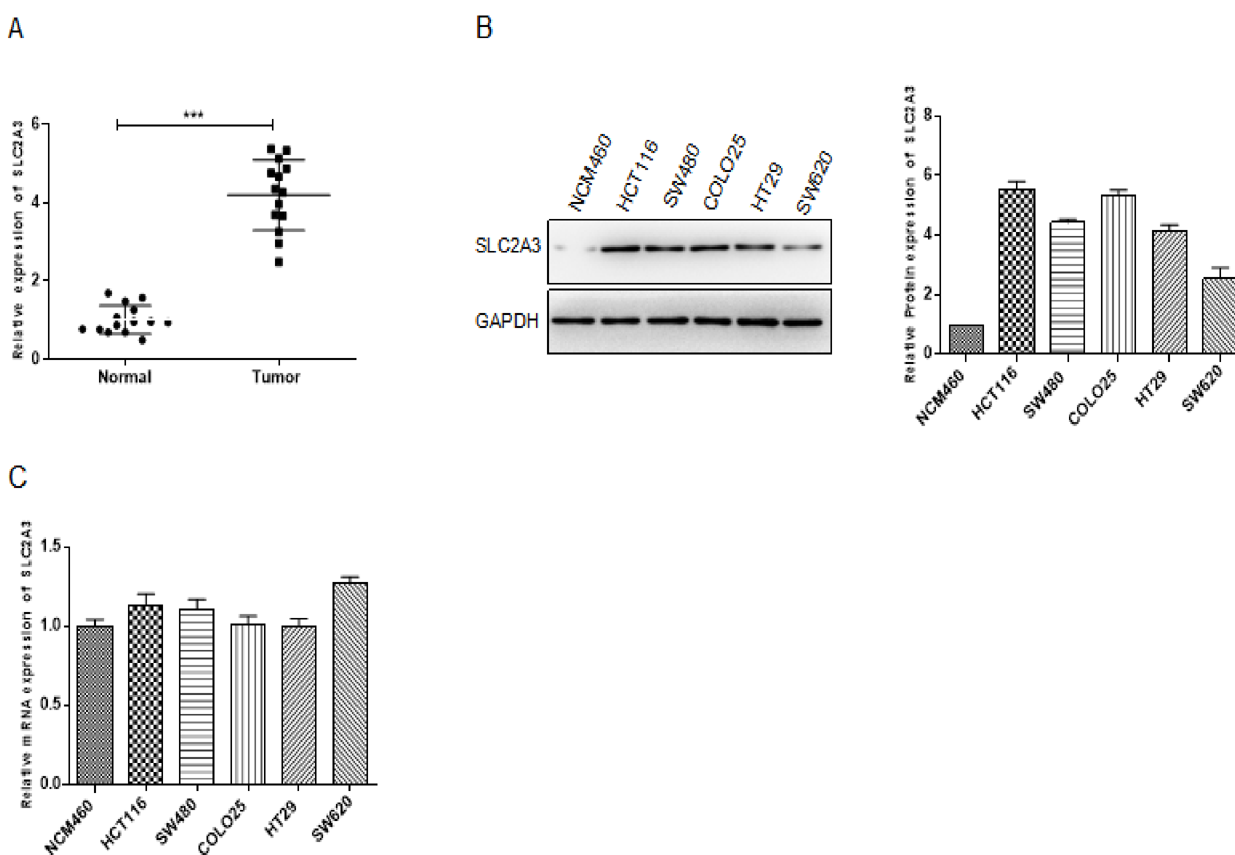


**Fig. 1:** SLC2A3 is upregulated in colon cancer tissues. A, The expression levels of SLC2A3 in tumor tissues and adjust normal tissues. B, The expression level of SLC2A3 protein in colon cancer cell lines. C, Relative expression level of SLC2A3 mRNA in colon cancer cell lines. GAPDH was used as the internal control. * $P<0.05$, ** $P<0.01$, *** $P<0.001$

### *SLC2A3 promotes cells proliferation in vitro*

First, we tested the protein expression level of SLC2A3 in cisplatin-resistant cell lines, and the results showed that compared with normal cell lines, the protein expression level of SLC2A3 in drug-resistant cell lines was significantly increased (Fig. 2A). Then we transfected the two groups of drug-resistant cell lines with control siRNA, control plasma, SLC2A3 siRNA, and SLC2A3 plasma. After successful transfection, the drug-resistant cells were treated with a large dose (10 μg/ml) of cisplatin solution. The results of the clone formation experiment showed Compared with the normal group and the blank control group, the cell density of the SLC2A3 plasma group was significantly higher than that of the other groups, while the cell density of the SLC2A3 siRNA group was significantly reduced, indicating that the cell resistance was reduced (Fig. 2B).

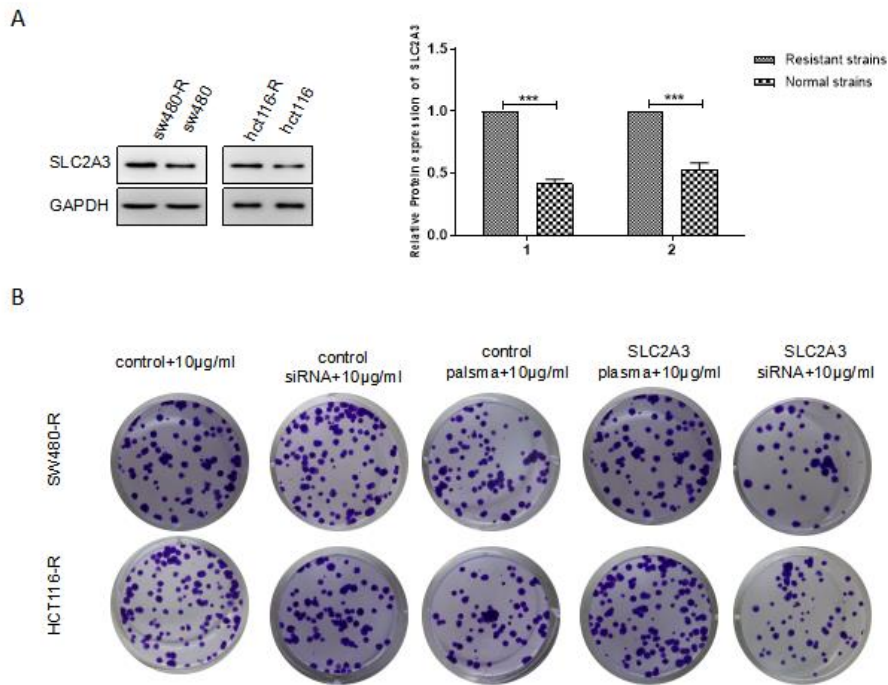


**Fig. 2:** Increasing the expression of SLC2A3 can enhance the drug resistance of colon cancer cells. A, The protein level of SLC2A3 is upregulated in both SW480 and HCT116 resistance cell lines. B, Compared with the blank group and the negative control group, the cells in the SLC2A3 plasma transfected group showed increased resistance to drugs, and the cell proliferation ability did not decrease significantly. * $P<0.05$, ** $P<0.01$, *** $P<0.001$

### *MiR-103a targets SLC2A3 in CRC*

To investigate the relation between SLC2A3 and miR-103a, we performed bioinformatics analysis with TargetScan (www.targetscan.org). SLC2A3 was a potential target of miR-103a (Fig. 3A). To verify whether SLC2A3 is the target gene of miR-103a, the luciferase reporter assay was performed. The luciferase activity significantly decreased following co-transfection with pmiR-SLC2A3-3'-UTR-WT and miR-103a mimic, compared with co-transfection with pmiR-SLC2A3-3'-UTR-Mut and miR-103a mimic, indicating that miR-103a specifically binds to the 3'-UTR of SLC2A3, and regulated the SLC2A3 expression negatively (Fig. 3B).

### *MiR-103a suppresses cell proliferation via regulating SLC2A3 in vitro*

In order to determine whether SLC2A3 KD has beneficial effects on CRC through the miR-103a pathway, SW480 cells were treated with cisplatin (10 μg/ml) followed by SLC2A3 KD or SLC2A3 KD plus miR-103a inhibitor and SLC2A3 KD plus miR-103a mimic for 24 h. Colon formation data showed the inhibitory effects of SLC2A3 KD plus miR-103a mimic on cell viability were stronger than SLC2A3 KD alone (Fig. 4A). Similarly, western blot data found SLC2A3 KD could improve the expression of caspase1, caspase9, which could be covered by miR-103a inhibitor (Fig. 4B), Flow cytometry data also showed similar results (Fig. 4C). In a word, these data suggested that miR-103a can participate in the positive effects of SLC2A3 on cell pyroptosis.

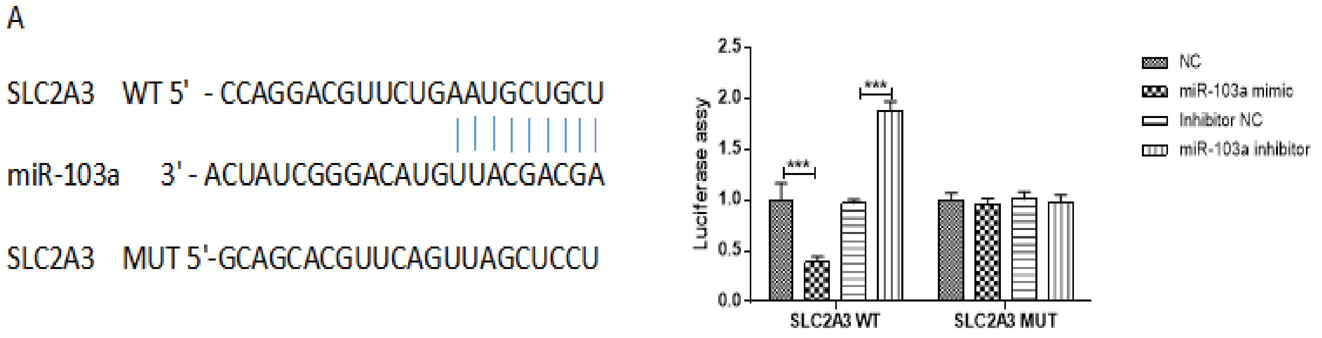


**Fig. 3:** SLC2A3 is a target gene of miR-103a. **A,** 3'-UTR bases pairing diagram of miR-103a and SLC2A3. Replacement of Guanine base with Cytosine (G to C) or replacement of Cytosine bases with Guanine (C to G) can also be used for the construction of mutant reporter. **B,** Prediction results were confirmed with Luciferase assay. GAPDH was used as the internal control. * *P*<0.05, ** *P*<0.01, *** *P*<0.001

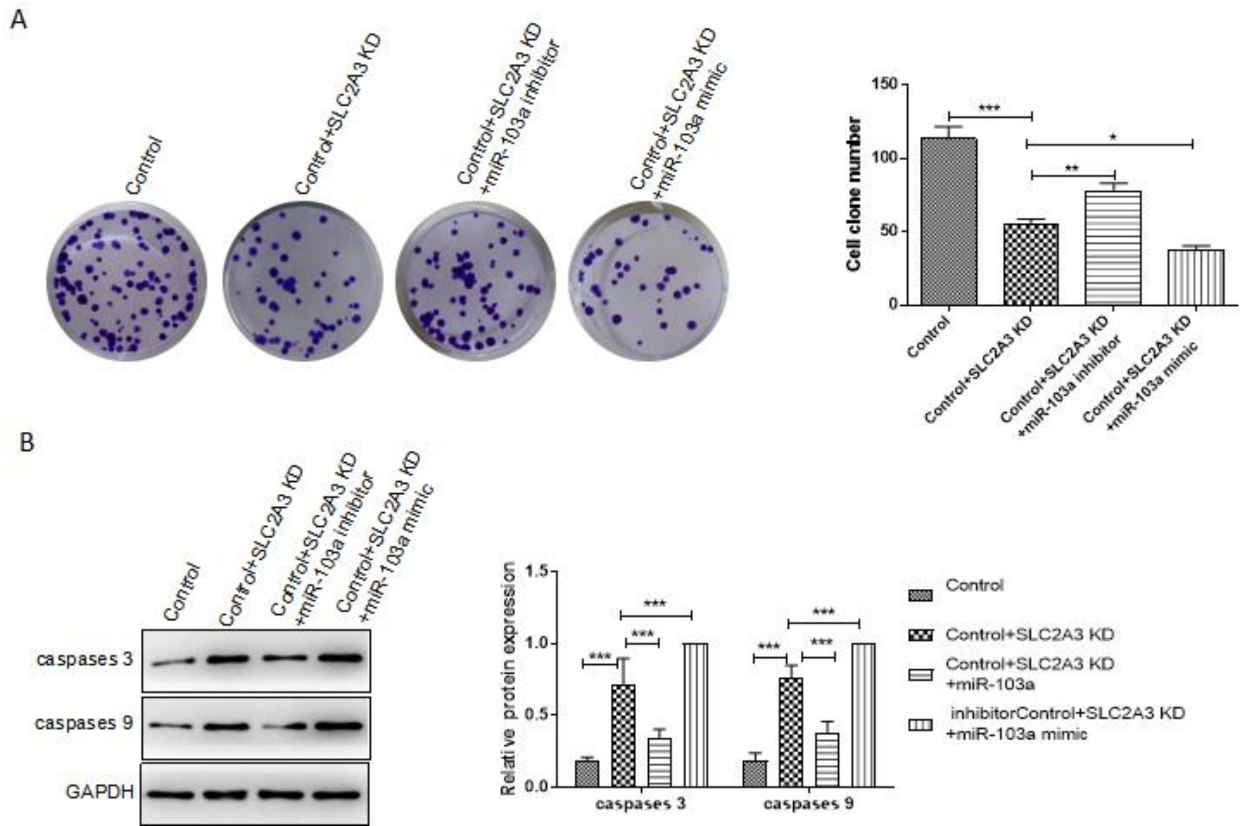


**Fig. 4:** MiR-103a affects the drug resistance of colon cancer cells by affecting the expression of SLC2A3. A, Clone formation assay was used to analyze the effects of the expression level of SLC2A3 and miR-103a on cell proliferation. B, Effects of SLC2A3 and MIR-103 expression on apoptosis-related proteins CAS1 and CAS9, detected by WB. C, Flow cytometry was used to detect the influence on cell apoptosis after transfection. GAPDH was used as the internal control. * *P* <0.05, ** *P* <0.01, *** *P* <0.001

## Discussion

Cisplatin is one of the most effective drugs with broad-spectrum anti-tumor effects and is used in the treatment of colorectal cancer. It relies on the formation of DNA adducts between platinum atoms and the N7 site of DNA purine bases to trigger the inhibition of DNA replication and transcription. In the past 40 yr, more and more evidence (18,19) has shown that the sensitivity of cancer cells to cisplatin is decreasing, and cell resistance is widespread. At the same time, high doses of cisplatin can bring patients including nephrotoxicity, peripheral neurotoxicity, and ototoxicity, with many side effects. How to improve the curative effect of cisplatin chemotherapy and reduce its toxic and side effects is an urgent clinical problem.

This study took human colon cancer drug-resistant cells SW620 and HCT116 as the research objects. By regulating the expression of SLC2A3 on SW620 and HCT116 cells and treated with cisplatin, we observed the changes in the apoptosis and proliferation of colon cancer cells and studied the effects of both whether the combination has a cooperative function. First, we used bioinformatics methods to find miRNAs targeting the SLC2A3 gene, used the luciferase gene reporter system to verify the function of miRNAs targeting the SLC2A3 gene, and then used clone formation experiments to verify the function of miRNAs on colon cancer cells. The proliferation ability of colon cancer cells treated with miRNA decreased significantly, indicating that miRNA targeting SLC2A3 can inhibit the expression of SLC2A3, thereby inhibiting the proliferation and migration of colon cancer cells. Western blotting results showed that the expression of SLC2A3 in the miRNA-treated cell line was significantly decreased.

MiRNA103a is involved in regulating the expression of SLC2A3 protein, and SLC2A3 overexpression can promote the invasion of colon cancer cells and enhance the ability of tumor cells to metastasize.

## Conclusion

MiRNA-103a can inhibit the expression of SLC2A3 in colon cancer cell lines and thereby inhibit the proliferation and migration of tumor cells. This may provide new ideas for cisplatin chemotherapy for colon cancer.

## Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

## Acknowledgements

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## Conflict of interest

The authors declare that there is no conflict of interest.

## References

1. Auclin E, Zaanan A, Vernerey D, et al (2017). Subgroups and prognostication in stage III colon cancer: future perspectives for adjuvant therapy. *Ann Oncol*, 28(5): 958-68.
2. Karim S, Brennan K, Nanji S, et al (2017). Association Between Prognosis and Tumor Laterality in Early-Stage Colon Cancer. *JAMA Oncol*, 3(10): 1386-92.
3. Salem ME, Yin J, Goldberg RM, et al (2020). Evaluation of the change of outcomes over a 10-year period in patients with stage III colon cancer: pooled analysis of 6501 patients treated with fluorouracil, leucovorin, and oxaliplatin in the ACCENT database. *Ann Oncol*, 31(4): 480-6.
4. Shi C, Ding K, Li KZ, et al (2020). Comprehensive analysis of location-specific hub genes re-

lated to the pathogenesis of colon cancer. *Med Oncol*, 237(9): 77.
5. Tarazona N, Gimeno-Valiente F, Gambardella V, et al (2019). Targeted next-generation sequencing of circulating-tumor DNA for tracking minimal residual disease in localized colon cancer. *Ann Oncol*, 30(11): 1804-12.
6. Waissbluth S, Daniel SJ (2013). Cisplatin-induced ototoxicity: transporters playing a role in cisplatin toxicity. *Hear Res*, 299: 37-45.
7. Li Q, Zhang J, Zhou J, et al (2018). lncRNAs are novel biomarkers for differentiating between cisplatin-resistant and cisplatin-sensitive ovarian cancer. *Oncol Lett,* 15(6): 8363-70.
8. Melnikov SV, Soll D, Steitz TA, et al (2016). Insights into RNA binding by the anticancer drug cisplatin from the crystal structure of cisplatin-modified ribosome. *Nucleic Acids Res,* 44(10): 4978-87.
9. Schlormann W, Naumann S, Renner C, et al (2015). Influence of miRNA-106b and miRNA-135a on butyrate-regulated expression of p21 and Cyclin D2 in human colon adenoma cells. *Genes Nutr*, 10(6): 50.
10. Xiao B, Viennois E, Chen Q, et al (2018). Silencing of Intestinal Glycoprotein CD98 by Orally Targeted Nanoparticles Enhances Chemosensitization of Colon Cancer. *ACS Nano*, 12(6): 5253-65.
11. Xu H, Wang X, Wu J, et al (2020). Long Noncoding RNA LINC01094 Promotes the Development of Clear Cell Renal Cell Carcinoma by Upregulating SLC2A3 via MicroRNA-184. *Front Genet*, 11: 562967.
12. Akao Y, Kumazaki M, Shinohara H, et al (2018). Impairment of K-Ras signaling networks and increased efficacy of epidermal growth factor receptor inhibitors by a novel synthetic miR-143. *Cancer Sci,* 109(5): 1455-67.
13. Zhang Y, Wang Z, Chen M, et al (2012). MicroRNA-143 targets MACC1 to inhibit cell invasion and migration in colorectal cancer. *Mol Cancer,* 11: 23.
14. Zampetaki A, Attia R, Mayr U, et al (2014). Role of miR-195 in aortic aneurysmal disease. *Circ Res,* 115(10): 857-66.
15. Zhang Q, Zhang C, Ma JX, et al (2019). Circular RNA PIP5K1A promotes colon cancer development through inhibiting miR-1273a. *World J Gastroenterol,* 25(35): 5300-9.
16. Yao X, He Z, Qin C, et al (2020). SLC2A3 promotes macrophage infiltration by glycolysis reprogramming in gastric cancer. *Cancer Cell Int,* 20: 503.
17. Chen D, Wang H, Chen J, et al (2018). MicroRNA-129-5p Regulates Glycolysis and Cell Proliferation by Targeting the Glucose Transporter SLC2A3 in Gastric Cancer Cells. *Front Pharmacol*, 9: 502.
18. Lee J, You JH, Shin D, Roh JL (2020). Inhibition of Glutaredoxin 5 predisposes Cisplatin-resistant Head and Neck Cancer Cells to Ferroptosis. *Theranostics,* 10(17): 7775-7786.
19. Makovec T (2019). Cisplatin and beyond: molecular mechanisms of action and drug resistance development in cancer chemotherapy. *Radiol Oncol,* 53(2): 148-158.