



Original Research

Cisatracurium inhibits the proliferation, migration and invasion of breast cancer cells by regulating the expression of miR-3174

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Abstract: Breast cancer is a type of cancer that begins in the breast tissue. Being a woman is the most important factor in the risk of breast cancer. Although men also get the cancer, women are much more likely to get it. This experiment was founded to investigate the effect and mechanism of Cisatracurium on breast cancer cell proliferation, migration and invasion. Breast cancer cells MDA-MB-231 were cultured in vitro. MDA-MB-231 cells were treated with cisatracurium of different concentrations for 48 h. CCK-8 method detected cell proliferation, Transwell detected cell migration and invasion, Western Blot method detected the expression levels of CyclinD1, p21, MMP-2 and MMP-9 protein in cells, RT-qPCR detected the expression level of miR-3174 in cells. After miR-3174 inhibitor was transfected into MDA-MB-231 in order to down-regulate the expression of miR-3174, the same methods as above were used to observe the effect of the down-regulating miR-3174 expression on MDA-MB-231 cell proliferation, migration and invasion as well as the expression levels of CyclinD1, p21, MMP-2 and MMP-9 protein. After different concentrations of Cisatracurium acted on MDA-MB-231 cells, the cell inhibition rate and p21 protein expression were significantly increased ($p < 0.05$), the number of cell migration and invasion and the expression levels of CyclinD1, MMP-2 and MMP-9 were significantly reduced ($p < 0.05$), and the expression of miR-3174 in cells was significantly reduced ($p < 0.05$). After down-regulating the expression of miR-3174, the cell inhibition rate and p21 protein expression were significantly increased ($p < 0.05$), the number of cell migration and invasion and the expression levels of CyclinD1, MMP-2 and MMP-9 were significantly reduced ($p < 0.05$). Up-regulating miR-3174 expression could reverse the effect of Cisatracurium on the proliferation, migration and invasion of MDA-MB-231 cells. Cisatracurium can inhibit the proliferation, migration and invasion of breast cancer MDA-MB-231 cells, and its mechanism is related to the down-regulation of miR-3174 expression in cells.

Key words: Cisatracurium; Breast cancer; miR-3174; Cell proliferation; Migration; Invasion.

Introduction

Breast cancer is a common malignant tumor threatening women's life and health, whose incidence is increasing with patients getting younger (1). Breast cancer occurs in the cells of the breast. After skin cancer, breast cancer is one of the most common cancers diagnosed in women. Breast cancer is found in both women and men, but is much more common in women. Breast cancer occurs when breast cells grow abnormally. These cells divide and accumulate faster than healthy cells and form a mass. The cells may spread to the lymph nodes or other parts of the body. This type of cancer often begins in the milk ducts and may start in the glandular tissue called the labia or other cells inside the breast (1-2). Researchers believe that hormonal factors, lifestyle and environmental factors are involved in the disease, but they still do not know why some people who do not have the underlying causes of breast cancer develop the disease (2).

Radical mastectomy is a model of surgery performed on the breast. During radical mastectomy, most of the muscles in the chest (including the large and small breasts) and the axillary lymph nodes are removed to treat breast cancer. The surgery may also involve pain around the surgical site, which is a procedure that is more supportive and is delayed during the visit after

surgery. The scar tissue that remains at the site of the incision closure after surgery by stitches or staplers will be about 6 to 8 cm long. Radical mastectomy, as currently the most effective treatment for breast cancer, can significantly improve treatment effect and patient prognosis (2).

Surgical excision of breast tumor requires anaesthetics to sufficiently relax the muscles. In recent years, studies have shown that the use of anesthetics can affect patient prognosis by affecting the malignant biological behavior of tumor cells (3,4). Cisatracurium (Cis) is a nonpolarized muscle relaxant often used as an anaesthetic adjuvant. It has been reported that cisatracurium can inhibit the migration and invasion of colorectal cancer cells (5), but its effect on the malignant biological behavior of breast cancer cells still remains unknown.

A microRNA (miRNA) is a small non-coding RNA molecule found in plants, animals, and some viruses. These RNAs are found in eukaryotes and are about 17-23 nucleotides long. Their main function is to regulate gene expression after transcription; By interacting with the mRNA and shutting it down, they suppress and sometimes activate the production of the desired gene protein. 60% of the genome of mammals, and the human genome can encode about 1,000 of them. There are different types of miRNA in fungi, as well as plants and animals. Also, miRNA is a class of small molecule

non-coding single-stranded RNA that participates in the regulation of cell proliferation, apoptosis, differentiation and other biological processes, and plays an important role in the occurrence and development of various human diseases, especially tumors (6,7). Studies have shown that miR-3174 is up-regulated in colorectal cancer, thus promoting proliferation and cell cycle progression of colorectal cancer cells and inhibiting apoptosis via targeted inhibition of PCBD2 expression, which may be a potential molecular target for colorectal cancer therapy (8). At present, the effect of miR-3174 on the malignant biological behavior of breast cancer cells still remains unknown. In this study with breast cancer MDA-MB-231 cells as the research object, the effects of cisatracurium on the proliferation, migration and invasion of MDA-MB-231 cells and whether it is achieved by miR-3174 expression regulation are discussed, with a view to providing a certain experimental basis for the use of cisatracurium in clinical radical mastectomy.

Materials and Methods

Cells and experiment reagents

Breast cancer cell line MDA-MB-231 (Chinese Academy of Sciences, Shanghai Cell Bank), cisatracurium (batch number 20181215, Jiangsu Hengrui Medicine Co., Ltd.), fetal bovine serum (FBS) (Zhejiang Tianhang Biotechnology Co., Ltd.), RPMI 1640 medium (Beijing Solarbio Science & Technology Co., Ltd), cell counting kit-8 (CCK 8) and bicinchoninic acid (BCA) protein detection kit (Shanghai Beyotime Biotechnology Co., Ltd.), trypsin (Sigma, USA), reverse transcription kit and PCR kit (Shenzhen Jingmei Biological Engineering Co., Ltd.), Trizol reagent and LipofectamineTM 2000 kit (Invitrogen, USA), PCR primer (Sangon Biotech (Shanghai) Co., Ltd.), miR-3174 inhibitor (anti-miR-3174), inhibitor control (anti-miR-NC), miR-3174 mimics (miR-3174), mimics negative control (miR-NC group) (Shanghai GenePharma Co., Ltd.), rabbit anti-human matrix metalloproteinase 2 (MMP-2), matrix metalloproteinase 9 (MMP-9) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) polyclonal antibody (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.), rabbit anti-human cell cycle protein (CyclinD1) and P21 monoclonal antibody (Santa Cruz, USA).

Experimental methods

Cell culture and transfection

MDA-MB-231 cells were cultured in RPMI 1640 medium containing 10% FBS and placed in an incubator at 37°C with 5% CO₂ volume fraction and 97% humidity. The medium was changed once every 2 days. When the cell growth density reached 80%~90%, 0.25% trypsin solution was digested and subcultured at a ratio of 1:3. MDA-MB-231 cells in the logarithmic phase were seeded in 6-well plates with 1×10⁵ cells per well. When the cell growth density reached 60%, it was replaced by RPMI 1640 medium without FBS. Anti-miR-3174, anti-miR-NC, miR-3174 mimics, miR-NC groups were transfected into MDA-MB-231 cells according to the specification of LipofectamineTM 2000

kit. 6 h after transfection, it was replaced by RPMI 1640 medium containing 10% FBS. After 48h of further culture, the cells were collected for subsequent experiments.

Cell grouping

The untransfected MDA-MB-231 cells were divided into the control group (Con group), low-dose cisatracurium group (Cis-L group), medium-dose cisatracurium group (Cis-M group) and high-dose cisatracurium group (Cis-H group). Con group cells were cultured in the conventional medium for 48 h. Cells of Cis-L group, Cis-M group and Cis-H group were cultured with medium containing 10, 20 and 40 μmol/L^[5] cisatracurium respectively for 48 h. The cells transfected with anti-miR-3174 and anti-miR-NC were cultured in the conventional medium for 48 h, which were recorded as an anti-miR-3174 group and anti-miR-NC group, respectively. The cells transfected with miR-3174 mimics and miR-NC were cultured with medium containing 40 μmol/L cisatracurium for 48 h, which were recorded as Cis+ miR-3174 group and Cis+ miR-NC group, respectively.

Cell proliferation detection by cell counting kit -8 (CCK-8)

The cells were seeded in 96-well plates with 0.5×10⁴ cells per well and grouped according to 1.2.2, with 3 parallel holes in each group. After the culture, 100 μL CCK-8 reagent was added to each well and incubated in the incubator for 2 h. Absorbance (A) was determined at 450 nm by the microplate reader. Cell inhibition rate (%) = (1 - A_{experimental group} / A_{control group}) × 100%

Transwell detection of cell migration and invasion

Cell concentration was adjusted to 5×10⁴ cell /mL using RPMI 1640 medium without FBS. Cell migration experiment: Transwell chamber was placed in a 24-well plate. 200 μL cell suspension was added to the upper chamber, and 600 μL RPMI 1640 medium containing 10% FBS was added to the lower chamber. According to the grouping in 1.2.2, 3 parallel holes were set for each group. After culture, the Transwell chamber was removed. Cells on the upper side of the filter membrane were removed by a cotton swab. At room temperature, the membrane was fixed with 4% paraformaldehyde for 30 min and stained with 0.4% crystal violet for 15 min. By inverted microscope observation, 5 fields were randomly selected to count the migration cells. Cell invasion experiment: after melted at 4°C, Matrigel was diluted with RPMI 1640 medium at a ratio of 1:8, and then placed in the upper Transwell chamber. After natural drying, 100 μL cell suspension was added. The subsequent operation was consistent with that of the cell migration experiment.

Detection of mir-3174 expression by real-time fluorescent quantitative PCR (RT-qPCR)

Total RNA was extracted from the cells using Trizol reagent and reversely transcribed to cDNA for PCR amplification according to the instructions of the reverse transcription kit. Amplification procedures: 95°C 10 min, 95°C 15 s, 60°C 30 s, 72°C 30 s, a total of 35 cycles. Primer sequence: miR-3174 forward primer 5'-GTC-

CGAAGTCGATGCCCGT-3', reverse primer 5'-GCAT-TAAGCTC GCTAGTTTGTCA-3'; U6 forward primer 5'-CTCGCTTCGGCAGCACA-3', reverse primer 5'-AACGCTTCACGAATTTGCGT-3'. With U6 as an internal reference, the relative expression level of miR-3174 in cells was calculated by the $2^{-\Delta\Delta Ct}$.

Detection of CyclinD1, p21, MMP-2 and MMP-9 protein expressions in cells by Western Blot

Total protein was extracted from RIPA cell lysate, and protein concentration was detected by the BCA protein kit. An appropriate amount of protein solution was weighed, added with 1×loading buffer, mixed evenly, and boiled at 100°C for 5 min. After protein denaturation, SDS polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 20 µg proteins per well. After electrophoresis, it was transferred to polyvinylidene fluoride (PVDF) membrane, sealed in 5% skim milk powder for 2 h. Then, CyclinD1 (1:1000), p21 (1:1000), MMP-2 (1:8000), MMP-9 (1:8000) and GAPDH (1:1000) primary antibodies were added and incubated overnight at 4°C. After washing the membrane with TBST, horseradish peroxidase-labeled sheep anti-rabbit IgG (1:500) was added and incubated at room temperature for 1 h. After washing the membrane with TBST, ELC chemiluminescence reagent was added. After development in darkness, the gel imaging system was exposed and photographed, and the gray value of the protein strip was analyzed using Image J software.

Statistical analysis

SPSS.22.0 software was used to analyze the experimental data. The measurement data conforming to normal distribution were expressed as mean ± standard deviation ($\bar{x} \pm s$). An independent sample t-test was used for comparison between two groups. One-way analysis

of variance was used for comparison among multiple groups, and the *SNK-q* test was used for further pairwise comparison. $P < 0.05$ indicates statistically significant differences.

Results

Effect of cisatracurium on the proliferation of breast MDA-MB-231 cells

Compared with the control group, after different concentrations of cisatracurium act on MDA-MB-231 cells, cell inhibition rate is significantly increased ($p < 0.05$), CyclinD1 protein expression in the cells is significantly decreased ($p < 0.05$), p21 protein expression is significantly increased ($p < 0.05$), indicating that cisatracurium can inhibit the proliferation of breast cancer MDA-MB-231 cells, as shown in Figure 1 and Table 1.

Effect of cisatracurium on the migration and invasion of breast cancer MDA-MB-231 cells

Compared with the control group, after different concentrations of cisatracurium act on MDA-MB-231 cells, the number of migrating and invasive cells is significantly reduced ($p < 0.05$), MMP-2 and MMP-9 protein expression in the cells is significantly reduced ($p < 0.05$), suggesting that cisatracurium can inhibit the migration and invasion of breast cancer MDA-MB-231 cells, as shown in Figure 2 and Table 2.

Effect of cisatracurium on miR-3174 expression in breast cancer MDA-MB-231 cells

Compared with the control group, after different concentrations of cisatracurium act on MDA-MB-231 cells, miR-3174 expression in the cells is significantly reduced ($p < 0.05$), indicating that cisatracurium can inhibit miR-3174 expression in breast cancer MDA-

Table 1. Effect of cisatracurium on the proliferation of breast cancer MDA-MB-231 cells ($\bar{x} \pm s$, n=9).

Group	Inhibition rate (%)	CyclinD1 protein	p21 protein
Con	0.00±0.00	0.68±0.05	0.32±0.03
Cis-L	27.02±2.60*	0.56±0.05*	0.49±0.05*
Cis-M	43.08±3.93*#	0.41±0.04*#	0.63±0.05*#
Cis-H	69.13±5.35*#&	0.25±0.03*#&	0.77±0.05*#&
F	687.421	166.560	158.964
P	0.000	0.000	0.000

Note: Compared with Con group, * $p < 0.05$; compared with Cis-L group, # $p < 0.05$; compared with Cis-M group, & $p < 0.05$.

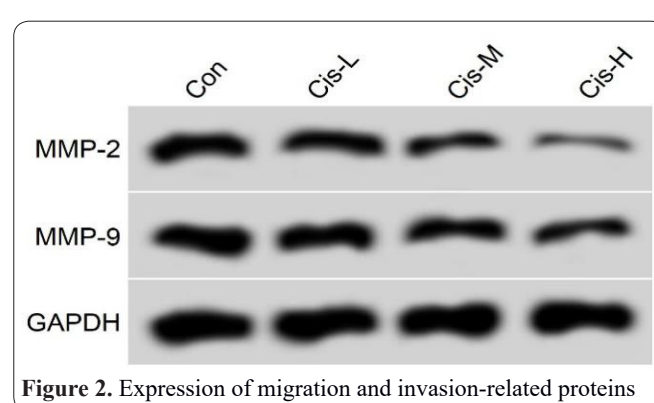
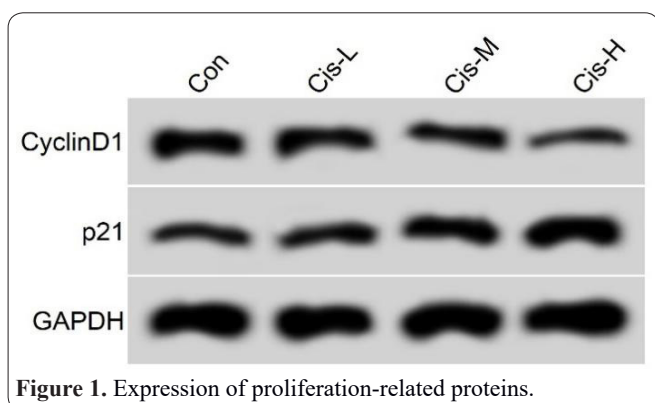


Table 2. Effect of cisatracurium on the migration and invasion of breast cancer MDA-MB-231 cells ($\bar{x} \pm s$, n=9).

Group	Number of migrating cells	Number of invasive cells	MMP-2 protein	MMP-9 protein
Con	115.76±9.77	95.75±7.69	0.61±0.04	0.83±0.06
Cis-L	87.48±6.79*	78.32±6.77*	0.46±0.04*	0.70±0.05*
Cis-M	70.41±5.02*#	62.20±5.91**	0.34±0.03*#	0.55±0.05*#
Cis-H	54.69±4.21*#&	44.67±3.82**&	0.20±0.02*#&	0.37±0.03*#&
<i>F</i>	133.340	111.426	243.400	148.642
<i>p</i>	0.000	0.000	0.000	0.000

Note: Compared with Con group, * $p < 0.05$; compared with Cis-L group, # $p < 0.05$; compared with Cis-M group, & $p < 0.05$.

Table 3. Effect of cisatracurium on miR-3174 expression in breast cancer MDA-MB-231 cells ($\bar{x} \pm s$, n=9).

Group	miR-3174
Con	1.00±0.05
Cis-L	0.78±0.06*
Cis-M	0.63±0.04*#
Cis-H	0.45±0.04*#&
<i>F</i>	210.194
<i>p</i>	0.000

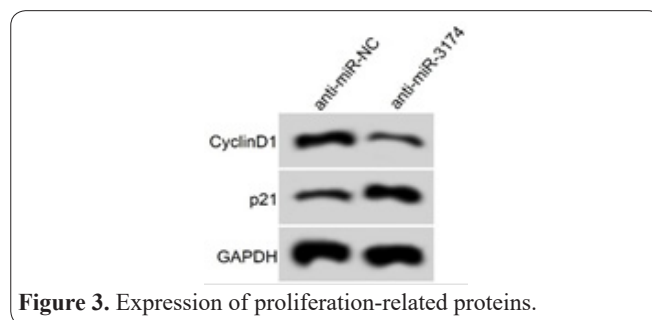
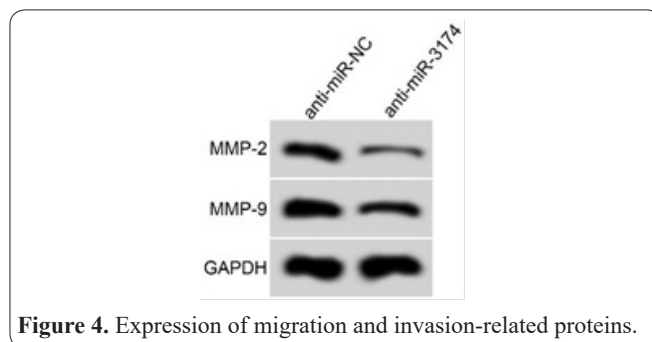
Note: Compared with Con group, * $p < 0.05$; compared with Cis-L group, # $p < 0.05$; compared with Cis-M group, & $p < 0.05$.

MB-231 cells, as shown in Table 3.

Effect of down-regulation of miR-3174 expression on proliferation of breast cancer MDA-MB-231 cells

Compared with the anti-miR-NC group, anti-miR-3174 group cells have significantly reduced miR-3174 expression level ($p < 0.05$), suggesting successful transfection of anti-miR-3174 and down-regulated miR-3174 expression in the cells. Compared with the anti-miR-NC group, the anti-miR-3174 group has significantly increased cell inhibition rate ($P < 0.05$), significantly decreased CyclinD1 protein expression in the cells ($p < 0.05$), significantly increased p21 protein expression ($p < 0.05$), suggesting that down-regulation of miR-3174 can inhibit the proliferation of breast cancer MDA-MB-231 cells, as shown in Figure 3 and Table 4.

Effect of down-regulation of miR-3174 expression on the migration and invasion of breast cancer MDA-MB-231 cells

**Figure 3.** Expression of proliferation-related proteins.**Figure 4.** Expression of migration and invasion-related proteins.

Compared with the anti-miR-NC group, the anti-miR-3174 group has significantly reduced the number of migrating and invasive cells ($p < 0.05$), significantly reduced MMP-2 and MMP-9 protein expression in the cells ($p < 0.05$), indicating that down-regulation of miR-3174 expression can inhibit the migration and invasion of breast cancer MDA-MB-231 cells, as shown in Figure 4 and Table 5.

Upregulation of miR-3174 reverses the effect of cisatracurium on proliferation, migration and invasion of breast cancer MDA-MB-231 cells

Table 4. Effect of down-regulation of miR-3174 expression on proliferation of breast cancer MDA-MB-231 cells ($\bar{x} \pm s$, n=9).

Group	miR-3174	Inhibition rate(%)	CyclinD1 protein	p21 protein
anti-miR-NC	1.00±0.06	6.37±0.63	0.69±0.05	0.31±0.03
anti-miR-3174	2.84±0.25*	48.19±4.33*	0.28±0.03*	0.71±0.05*
<i>t</i>	21.470	28.673	21.094	20.580
<i>p</i>	0.000	0.000	0.000	0.000

Note: Compared with the anti-miR-NC group, * $p < 0.05$.

Table 5. Effect of down-regulation of miR-3174 expression on the migration and invasion of breast cancer MDA-MB-231 cells ($\bar{x} \pm s$, n=9).

Group	Number of migrating cells	Number of invasive cells	MMP-2 protein	MMP-9 protein
anti-miR-NC	119.83±9.54	98.08±8.70	0.62±0.05	0.86±0.05
anti-miR-3174	65.37±5.35*	57.74±4.61*	0.26±0.02*	0.45±0.05*
<i>t</i>	14.937	12.291	20.055	17.395
<i>p</i>	0.000	0.000	0.000	0.000

Note: Compared with the anti-miR-NC group, * $p < 0.05$.

Table 6. Upregulation of miR-3174 reverses the effect of cisatracurium on proliferation, migration and invasion of breast cancer MDA-MB-231 cells ($\bar{x} \pm s$, n=9).

Group	miR-3174	Inhibition rate (%)	Number of migrating cells	Number of invasive cells
Cis+miR-NC	1.00±0.04	67.92±5.30	52.40±5.37	43.17±3.92
Cis+miR-3174	2.36±0.22*	23.69±2.38*	92.45±8.92*	81.44±5.91*
<i>t</i>	18.246	22.839	11.540	16.189
<i>p</i>	0.000	0.000	0.000	0.000
Group	CyclinD1 protein	p21 protein	MMP-2 protein	MMP-9 protein
Cis+miR-NC	0.24±0.02	0.78±0.06	0.19±0.02	0.36±0.03
Cis+miR-3174	0.57±0.05*	0.41±0.03*	0.50±0.04*	0.72±0.05*
<i>t</i>	18.384	16.547	20.795	18.522
<i>p</i>	0.000	0.000	0.000	0.000

Note: Compared with the Cis+miR-NC group, * $p < 0.05$.

Compared with the Cis+ miR-NC group, Cis+ miR-3174 group has significantly increased miR-3174 expression ($p < 0.05$), significantly decreased cell inhibition rate and p21 protein expression ($p < 0.05$), significantly increased the number of migrating and invasive cells as well as CyclinD1, MMP-2 and MMP-9 protein expression in the cells ($p < 0.05$), as shown in Figure 5 and Table 6.

Discussion

Radical surgery refers to the surgical procedure that removes the circulatory system, lymph nodes, and sometimes adjacent structures from a damaged organ during surgery. In oncology surgery, radical surgery is a procedure that can be used to help diagnose and treat a disease by removing a tumor or cluster lymph nodes and capturing their contents. (Such as radical mastectomy) (2, 9).

Radical surgery is the dominant treatment for breast cancer, which is inseparable from the use of anesthetic drugs. Studies have shown that both anesthetic drugs and anesthesia methods can affect patient prognosis. Cisatracurium is currently one of the most widely used anesthetic-inducing drugs in clinical practice, which can reduce the migration and invasion of esophageal squamous cell carcinoma by inhibiting epithelial-mesenchymal transition (EMT) and TGF- β /Smad signaling pathway (9). So far, the effect of cisatracurium on proliferation, migration and invasion of breast cancer cells remains unknown. CyclinD1 participates in the regulation of the cell cycle, which can promote cell transition from the G1 phase to the S phase and accelerate cell proliferation (10). p21 is an already known tumor growth suppressor, whose increased expression can inhibit cell proliferation (11). In this study, compared with the control group, after different concentrations of cisatracurium acted on MDA-MB-231 cells for 48 h, cell inhibition rate and p21 protein expression were significantly increased and CyclinD1 protein expression was significantly decreased, suggesting that cisatracurium could effectively inhibit the proliferation of MDA-MB-231 cells. Inhibition of tumor cell migration and invasion as the main causes of tumor recurrence and metastasis can effectively reduce the rate of tumor recurrence and metastasis (12). Matrix metalloproteinases are a type of enzymes that can degrade the extracellular matrix and promote cell migration and invasion by

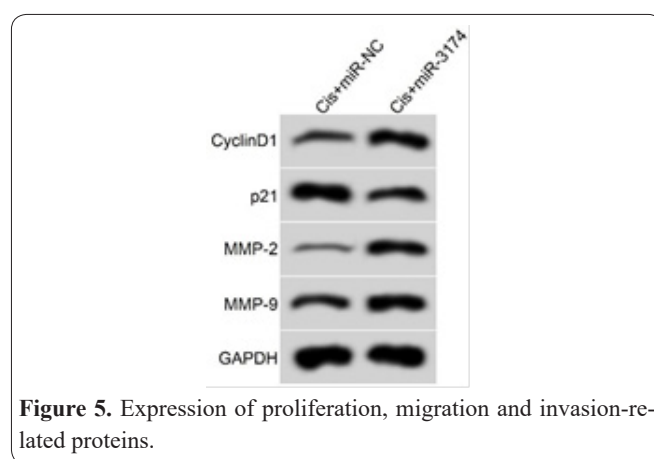


Figure 5. Expression of proliferation, migration and invasion-related proteins.

degrading extracellular matrix (13). In this study, after cisatracurium acts on MDA-MB-231 cells, the number of migrating and invasive cells decreased, with MMP-2 and MMP-9 protein expression in the cells decreased, indicating that cisatracurium could inhibit the migration and invasion of MDA-MB-231 cells.

miRNA widely present in eukaryotes plays an important regulatory role in life processes like cell proliferation, migration, invasion, which is a potential molecular target for tumor therapy (14). miR-3174 is a newly discovered miRNA with up-regulated expression in tumors of liver cancer (15), stomach cancer (16), etc. Down-regulation of miR-3174 expression can inhibit the proliferation of tumor cells, induce apoptosis of tumor cells, and delay tumor development. However, the effect of miR-3174 on the malignant biological behavior of breast cancer cells remains unknown. In this study, after down-regulating miR-3174 expression by transfection of the miR-3174 inhibitor to MDA-MB-231 cells, cell ability of proliferation, migration and invasion was decreased, suggesting that down-regulating miR-3174 expression could inhibit the proliferation, migration and invasion of MDA-MB-231 cells. miR-3174 may also be a potential molecular target for breast cancer therapy. To explore the molecular mechanism of cisatracurium in inhibiting the proliferation, migration and invasion of breast cancer MDA-MB-231 cells, RT-qPCR was taken in this study to detect the influence of cisatracurium on miR-3174 expression in MDA-MB-231 cells. The results showed that cisatracurium could inhibit miR-3174 expression in MDA-MB-231 cells. At the same time, this study also revealed that the upregulation of miR-3174 expression reversed the inhibition of

cisatracurium on the proliferation, migration and invasion of MDA-MB-231 cells, suggesting that cisatracurium inhibited the proliferation, migration and invasion of MA-MB-231 cells by down-regulating miR-3174 expression. The associations of non-coding RNAs with genomic lesions, colorectal lesions, in cancers, and even in the rate of fetal growing emphasize the significance of miRNAs (17-19).

Gene expression and its regulation have always been the topics of concern to researchers in molecular genetics, and research in this area has yielded interesting results. Gene editing is one of the most important achievements in human research and can greatly control gene expression (20-31). In this experiment, miR-3174 was targeted for expression.

To conclude, cisatracurium can effectively inhibit the proliferation, migration and invasion of breast cancer MDA-MB-231 cells, possibly by down-regulating miR-3174 expression in the cells.

References

- Zhou Li. Advances in simplified MRI screening techniques for breast cancer [J]. *J Clin Radiol* 2018; 37(10):1766-1769.
- Liuqin J, Le J, Yanbin L. Comparative study on the effects of rocuronium bromide and cisatracurium besylate on hemodynamics and stress response in patients undergoing radical mastectomy for breast cancer [J]. *Chin Med Herald* 2018; 15(34):108-111.
- Wenwen H, Muhan L, Dongxin W. Impact of conventional anesthesia ways, drug selection and intraoperative management of tumor surgery on the prognosis of elderly patients [J]. *J Clin Anesthesiol* 2018; 34(7):94-98.
- Yuhe ZH, Lili W, Zhitao W. Effect of sevoflurane on invasion and migration of breast cancer cells and its mechanism [J]. *Int J Pathol Clin Med* 2019; 39(2):10-16.
- Yabasin IB, Sanches JG, Ibrahim MM, Huidan J, Williams W, Lu ZL, Wen Q. Cisatracurium retards cell migration and invasion upon upregulation of p53 and inhibits the aggressiveness of colorectal cancer. *Front Physiol* 2018; 9:941.
- Taherdangkoo K, Kazemi NS, Hajjari MR, Tahmasebi BM. miR-485-3p suppresses colorectal cancer via targeting TPX2. *Bratisl Lek Listy* 2020; 121(4):302.
- Sun W, Li W, Chen J, Teng Y, Ding X. MicroRNA-520b targets interleukin-2 to regulate the growth, metastasis and drug sensitivity of liver cancer cells. *Am J Transl Res* 2020; 12(4):1267.
- Zhang DL, Yang N. MiR-3174 functions as an oncogene in rectal cancer by targeting PCBD2. *Eur Rev Med Pharmacol Sci* 2019; 23(6):2417-26.
- Lv W, Wang J, Zhang S. Effects of cisatracurium on epithelial-to-mesenchymal transition in esophageal squamous cell carcinoma. *Oncol Lett* 2019; 18(5):5325-31.
- Chen B, Hu Z, Chen B, Li B. Effects and mechanism of Lanthanum Citrate on the proliferation and apoptosis of hepatocellular carcinoma cell line SMMC-7721. *Turk J Gastroenterol* 2020; 31(3):264.
- Wang W, Li X, Ji D. Molecular mechanism of the effect of angiopoietin-like protein 8 on the proliferation, invasion and migration of placental trophoblasts in preeclampsia. *Exp Ther Med* 2020; 19(6):3461-8.
- Lu M, Huang L, Tang Y, Sun T, Li J, Xiao S, Zheng X, Christopher O, Mao H. ARNTL2 knockdown suppressed the invasion and migration of colon carcinoma: decreased SMOC2-EMT expression through inactivation of PI3K/AKT pathway. *Am J Transl Res* 2020; 12(4):1293.
- Li H, Zhao J, Jia X, Zhang Y, Du Y, Li H, Ma L, Huang JA. miR-21 promotes growth, invasion and migration of lung cancer cells by AKT/P-AKT/cleaved-caspase 3/MMP-2/MMP-9 signaling pathway. *Int J Clin Exp Pathol* 2020; 13(4):692.
- Zhang H, TulaHong A, Wang W, Nuerrula Y, Zhang Y, Wu G, Mahati S, Zhu H. Downregulation of microRNA-519 enhances development of lung cancer by mediating the E2F2/PI3K/AKT axis. *Int J Clin Exp Pathol* 2020; 13(4):711.
- Wang Q, Yang X, Zhou X, Wu B, Zhu D, Jia W, Chu J, Wang J, Wu J, Kong L. MiR-3174 promotes proliferation and inhibits apoptosis by targeting FOXO1 in hepatocellular carcinoma. *Biochem Biophys Res Commun* 2020.
- Li B, Wang L, Li Z, Wang W, Zhi X, Huang X, Zhang Q, Chen Z, Zhang X, He Z, Xu J. miR-3174 contributes to apoptosis and autophagic cell death defects in gastric cancer cells by targeting ARHGAP10. *Mol Ther Nucleic Acids* 2017; 9:294-311.
- Taheri Z, Asadzadeh Aghdaei H, Irani S, Modarressi MH, Noor-mohammadi Z. Clinical Correlation of miR-200c/141 Cluster DNA Methylation and miR-141 Expression with the Clinicopathological Features of Colorectal Primary Lesions/Tumors. *Rep Biochem Mol Biol*, 2019; 8(3):208-215.
- Esfandi F, Fallah H, Arsang-Jang S, Taheri M, Ghafouri-Fard S. The Expression of CCAT2, UCA1, PANDA and GHET1 Long Non-coding RNAs in Lung Cancer. *Rep Biochem Mol Biol*, 2019; 8(1):36-41.
- Azari I, Ghafouri-Fard S, Omrani MD, Arsang-Jang S, Kordi Tamandani DM, Saroone Rigi M, Rafiee S, Pouresmaeili F, Taheri M. Expression of Long Non-Coding RNAs in Placentas of Intrauterine Growth Restriction (IUGR) Pregnancies. *Rep Biochem Mol Biol* 2019; 8(1):25-31.
- Akbarabadi A, Ismaili A, Kahrizi D, Firouzabadi FN. Validation of expression stability of reference genes in response to herbicide stress in wild oat (*Avena ludoviciana*). *Cell Mol Biol* 2018; 64(4): 113-118.
- Ghorbani T, Kahrizi D, Saeidi M, Arji I. Effect of sucrose concentrations on *Stevia rebaudiana* Bertoni tissue culture and gene expression. *Cell Mol Biol*, 63(11): 32-36.
- Kahrizi D, Ghari SM, Ghaheri M, Fallah F, Ghorbani T, Beheshti AA, Kazemi E, Ansarypour Z. Effect of KH2PO4 on gene expression, morphological and biochemical characteristics of *stevia rebaudiana* Bertoni under in vitro conditions. *Cell Mol Biol*, 63(7): 107-111.
- Fallah F, Nokhasi F, Ghaheri M, Kahrizi D, Beheshti Ale AA, Ghorbani T, Kazemi E, Ansarypour Z. Effect of salinity on gene expression, morphological and biochemical characteristics of *Stevia rebaudiana* Bertoni under in vitro conditions. *Cell Mol Biol* 2017; 63(7): 102-106.
- Bordbar M, Darvishzadeh R, Pazhouhandeh M, Kahrizi D. An overview of genome editing methods based on endonucleases. *Mod Genet J* 2020; 15(2): 75-92.
- Ghaheri M, Kahrizi D, Bahrami G, Mohammadi-Motlagh HR. Study of gene expression and steviol glycosides accumulation in *Stevia rebaudiana* Bertoni under various mannitol concentrations. *Mol Biol Rep* 2019; 46(1): 7-16.
- Erurygur N, Ucar E, Akpulat HA, Shahsavari K, Safavi SM, Kahrizi D. In vitro antioxidant assessment, screening of enzyme inhibitory activities of methanol and water extracts and gene expression in *Hypericum lydidium*. *Mol Biol Rep* 2019; 46(2): 2121-9.
- Esmaeili F, Ghaheri M, Kahrizi D, Mansouri M, Safavi SM, Ghorbani T, Muhammadi S, Rahmanian E, Vaziri S. Effects of various glutamine concentrations on gene expression and steviol glycosides accumulation in *Stevia rebaudiana* Bertoni. *Cell Mol Biol*, 64(2): 1-5.
- Akbari F, Arminian A, Kahrizi D, Fazeli A, Ghaheri M. Effect of

nitrogen sources on gene expression of *Stevia rebaudiana* (Bertoni) under in vitro conditions. *Cell Mol Biol*, 64(2): 11-16.

29. Ghaheri M, Adibrad E, Safavi SM, Kahrizi D, Soroush A, Mohammadi S, Ghorbani T, Sabzevari A, Ansarypour Z, Rahmanian E. Effects of life cycle and leaves location on gene expression and glycoside biosynthesis pathway in *Stevia rebaudiana* Bertoni. *Cell Mol Biol*, 64(2): 17-22.

30. Kahrizi D, Ghaheri M, Yari Z, Yari K, Bahraminejad S. Investi-

gation of different concentrations of MS media effects on gene expression and steviol glycosides accumulation in *Stevia rebaudiana* Bertoni. *Cell Mol Biol*, 64(2): 23-27.

31. Hashempour S, Ghaheri M, Kahrizi D, Kazemi N, Mohammadi S, Safavi SM, Ghorbani T, Rahmanian E, Heshmatpanaah M. Effects of different concentrations of mannitol on gene expression in *Stevia rebaudiana* Bertoni. *Cell Mol Biol*, 64(2): 28-31.