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Long-stranded non-coding RNA HCG11 regulates glioma cell proliferation, apoptosis and drug resistance via the sponge MicroRNA-144COX-2 axis

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ABSTRACT

In the current study, we analyzed long non-coding RNA HCG11 regulates the proliferation, apoptosis and drug resistance of glioma cells by spongy microRNA-144COX-2 axis. For this purpose, glioma cells at the logarithmic growth stage were divided into blank, up-regulated and down-regulated groups. The blank group did not undergo any treatment. Bacteria were inserted into the complete culture medium of the up-regulated group and down-regulated group for co-culture for 24 h. The down-regulated group was transfected with Mir-HCG11 inhibitor. The expressions of Mir-HCG11, Mir-144-3p and COX-2 in each group were observed, and the proliferation and apoptosis of glioma cells were analyzed, and their drug resistance was analyzed. Results showed that compared with the blank group, the expression of Mir-HCG11 and Mir-144 was increased and the expression of COX-2 was decreased in the up-regulated group ($P < 0.05$). Compared with the up-regulated group, the down-regulated group increased the expression of Mir-HCG11 and Mir-144 and decreased the expression of COX-2 ($P < 0.05$). Compared with the blank group, the proliferation rate of glioma cells in the up-regulated group (24h, 48h, 72h) was increased ($P < 0.05$); Compared with the up-regulated group, the proliferation rate of glioma cells in the down-regulated group (24h, 48h, 72h) was decreased ($P < 0.05$); Compared with the blank group, apoptosis rate of glioma cells in the up-regulated group (24h, 48h, 72h) was decreased ($P < 0.05$); Compared with the up-regulated group, the apoptosis rate of glioma cells in the down-regulated group (24h, 48h, 72h) was increased ($P < 0.05$); The IC50 values of Imatinib, VP-16 and TMZ in blank group and up-regulated group was compared ($P > 0.05$). Compared with the blank group and up-regulated group, the IC50 values of Imatinib, VP-16 and TMZ in the down-regulated group were decreased ($P < 0.05$). In general, down-regulation of long non-coding RNA-HCG11 can regulate the microRNA-144COX-2 axis in glioma, thus reducing the proliferation rate of glioma cells and improving the apoptosis rate of glioma cells. In addition, down-regulation of long non-coding RNA-HCG11 is also involved in the drug resistance mechanism of Imatinib, VP-16 and TMZ chemotherapy drugs.

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Introduction

The main clinical types of glioma include astrocytoma, retinoblastoma and oligodendroglioma (1). The treatment and prognosis of glioma vary according to the type of glioma (2, 3). Gliomas are common clinical malignancies of the central nervous system (4). While low-grade gliomas have a better prognosis, high-grade gliomas are known as glioblastomas, which are the most aggressive malignant primary brain tumors (5, 6). The current clinical treatment for glioma is surgery, chemotherapy and radiotherapy, but the median survival for all treatment modalities is only about 15 months, so many scholars have turned their research to working with cells and genes (7). Long-stranded non-coding RNAs have been a hotspot for oncology research in recent years (8). At present, the clinical pathogenesis of glioma has not yet been elucidated; requiring in-depth research to find precise therapeutic targets and develop targeted drugs.

MicroRNAs, a class of non-coding single-stranded RNA molecules of about 21-23 nucleotides in size encoded by endogenous genes, are involved in cell differentiation, proliferation, apoptosis, invasion and metabolism, and play an important role in the development of tumours.The role of MicroRNA-144 in glioma has been noted, but its resistance to glioma

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has not been studied (6). In this paper, we investigated the MicroRNA-144COX-2 axis in order to find precise therapeutic targets and targeted drug development for glioma, and analyzed the long-stranded non-coding RNA HCG11 to regulate glioma cell proliferation, apoptosis and drug resistance through the sponge MicroRNA-144COX-2 axis, providing some clinical data.

Materials and methods

Materials

Subject: Brain stromal tumor cell line U251 was purchased from Shanghai Kanglang Biotechnology Co.

Drugs and reagents: Optical microscope was purchased from Shanghai Puch Biotechnology Co., Ltd; ELISA was purchased from Wuhan Chengming Instrument Co. DMEM medium was purchased from Wuhan Yipu Biotechnology Co., Ltd; TUNEL kit was purchased from Shanghai Ulva Biotechnology Co. Total RNA extraction kit was purchased from Shanghai Valan Biotechnology Co. Cox-2 protein was purchased from Epitmics, USA.

Cell culture and grouping

Glioma cells preserved in liquid nitrogen were removed and lysed in a 37°C water bath. The cells were incubated at 37°C in a 5% CO_2 incubator using DMEM medium containing 10% FBS, penicillin (100μl/mL) and streptomycin (100μl/mL). The culture medium was changed every 2-3 d. When the cells reached 80%-90% fused growth, the old culture medium was discarded. The cells were repeatedly washed with PBS buffer and digested with trypsin. When the cells changed from prismatic to round, trypsin was discarded and the digestion was terminated by adding medium. When glioma cells grew to log phase, they were inoculated at 8×104 into 9-well culture plates and cultured at constant temperature for 24h. The glioma cells were divided into control, down-regulated and up-regulated groups. The up-regulation group was given miR-HCG11 mimics to perform up-regulation transfection, and the down-regulation group was given miR-HCG11 inhibitor to perform down-regulation transfection.

Cell proliferation rate determination

The proliferation rate of glioma cells cultured for 24h, 48h and 72h in different grouping treatments was determined using the MTT method. The glioma cells to be examined were passage into 96-well plates with 3000 cells and 100μL of cell culture solution per plate. 5 replicate wells were set up for each group and incubated for 48h. The wells were washed with PBS buffer and 150μL of DMSO solution was added to each well. The plates were slowly shaken for 10min at room temperature using a constant temperature shaker. Cell proliferation rate = OD experimental wells/OD control wells x 100%.

Determination of apoptotic cell death rate

Glioma cell apoptosis rates were determined by flow cytometry in different subgroups cultured for 24h, 48h and 72h. The cells to be examined were passage into 96-well cell culture plates. The cells were washed in PBS buffer and digested by trypsin so that the cells changed from prismatic to round when the medium was added to terminate the digestion. The cells were blown into a cell suspension and processed by centrifugation. The supernatant was discarded and washed with PBS buffer. After collecting (1~5)×105 cells, 5μL each of Annexin V-FITC and PI staining solution at a concentration of 250μL/mL was added, mixed thoroughly, incubated for 5~15min at room temperature and protected from light. The DNA content of each group of glioma cells was determined using flow cytometry. Apoptosis rates were calculated using MultiCycle software for analysis.

Western blot method to determine the relative expression of Cox-2

The proteins were extracted 48 hours after transfection and the protein concentration was determined by the BCA method. The extracted proteins were mixed with buffer and boiled at 100°C for 5 min before electrophoresis. The protein was transferred to the NC membrane at 90V, 4°C for 90min. The blocking condition was the addition of 5% fetal bovine serum albumin and incubation for 60min at room temperature. The NC membranes were incubated at 4°C in a 1:900 dilution of primary antibody and incubated in secondary antibody for 1h at room temperature before colour development. The relative expression of Cox-2 in cells was measured (Fig. 1).

RT-PCR for miR-HCG11, miR-144 expression

The TRIzol reagent was added within the specimen to be tested and subsequently left to stand for 10min at 37°C. After dissolution, 600 μL of trichloromethane was added and stirred until the solution was milky white and then left to stand for 10 min at 4°C. The supernatant was then extracted by centrifugation. The supernatant was placed in a centrifuge tube and centrifuged for 15min after 1:1 addition of isopropanol. 1mL of 75% ethanol was added to extract the total RNA. The purity and content of the extracted total RNA were tested, followed by reverse transcription processing to obtain cDNA. Primers were designed using Primer 5.0 software and calculated using the $2^{-\Delta\Delta Ct}$ method with internal reference U6. The reverse transcription reaction conditions were set to 25°C for 10 min, 40°C for 60 min and 85°C for 5 min. The amplification conditions were set at 94°C for the 20s, 72°C for 30s, 60°C for 30s, with 35 cycles. The expression of miR-HCG11 and miR-144 to be detected was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical processing

The data were analysed using SPSS 25.0. The measures were tested for chi-square by the Levene method and normally distributed by the Shapiro-Wilk test. Repeated measures analysis of variance was performed with mean ± standard deviation ($\bar{x} \pm s$). Comparisons between two groups at the same time point were made using the independent samples t-test. Count data were expressed as a rate (%) using the X2-test, and F-test was performed between multiple groups. Differences were considered statistically significant at P<0.05.

Results and discussion

The miR-HCG11, miR-144, Cox-2 expression

As shown in Table 1, miR-HCG11 and miR-144 expression was increased and Cox-2 expression was decreased in the up-regulated group compared with the blank group, with statistical differences (P<0.05); miR-HCG11 and miR-144 expression was decreased and Cox-2 expression was increased in the down-regulated group compared with the up-regulated group, with statistical differences (P<0.05).

Table 1. Expression of miR-HCG11, miR-144 and Cox-2 in each group ($\bar{x} \pm s$)

Group	miR-HCG11	miR-144	Cox-2
Blank group	7.42 ± 1.06	9.34 ± 2.05	8.25 ± 0.61
Up-regulated group	14.29 ± 2.71a	15.32 ± 3.05a	3.51 ± 0.22a
Down-regulated group	8.61 ± 0.85ab	12.64 ± 1.05ab	6.94 ± 0.94ab

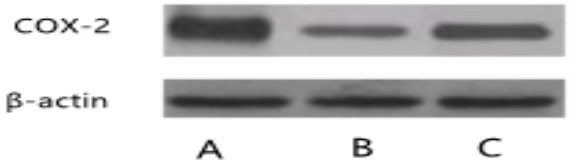

Figure 1. Western blot of Cox-2 protein expression. Compared with the blank group, aP < 0.05; compared with the up-regulated group, bP<0.05.Note: A: Blank group; B: Up-regulated group; C: Down-regulated group.

Comparison of proliferation rates of glioma cells in each group

As shown in Table 2, the proliferation rate of glioma cells (24h, 48h, 72h) increased in the up-regulation group compared with the blank group, with statistical difference (P < 0.05); the proliferation rate of glioma cells (24h, 48h, 72h) decreased in the down-regulation group compared with the up-regulation group, with statistical difference (P < 0.05).

Table 2. Comparison of proliferation rates of glioma cells in each group ($\bar{x} \pm s$)

Group	Proliferation rate		
	24 h	48 h	72 h
Blank group	8.32 ± 0.87	14.95 ± 1.02	12.38 ± 0.96
Up-regulated group	13.15 ± 1.11a	19.24 ± 1.55a	16.12 ± 1.17a
Down-regulated group	6.55 ± 1.26ab	8.17 ± 0.84ab	8.14 ± 0.79ab

Note: aP < 0.05 compared to the blank group; bP < 0.05 compared to the up-regulated group.

Comparison of apoptosis rates of glioma cells in each group

As shown in Table 3, the apoptosis rate of glioma cells (24h, 48h, 72h) was reduced in the up-regulated group compared with the blank group, with a statistical difference (P < 0.05); the apoptosis rate of glioma cells (24h, 48h, 72h) was increased in the down-regulated group compared with the up-regulated group with a statistical difference (P < 0.05) (Fig. 2).

Table 3. Comparison of apoptosis rates of glioma cells in each group ($\bar{x} \pm s$)

Group	Apoptosis rate		
	24 h	48 h	72 h
Blank group	8.11 ± 0.92	9.61 ± 0.94	10.79 ± 1.00
Up-regulated group	5.87 ± 0.77a	6.79 ± 0.74a	8.93 ± 0.76a
Down-regulated group	19.84 ± 1.15ab	26.74 ± 2.34ab	39.15 ± 3.11ab

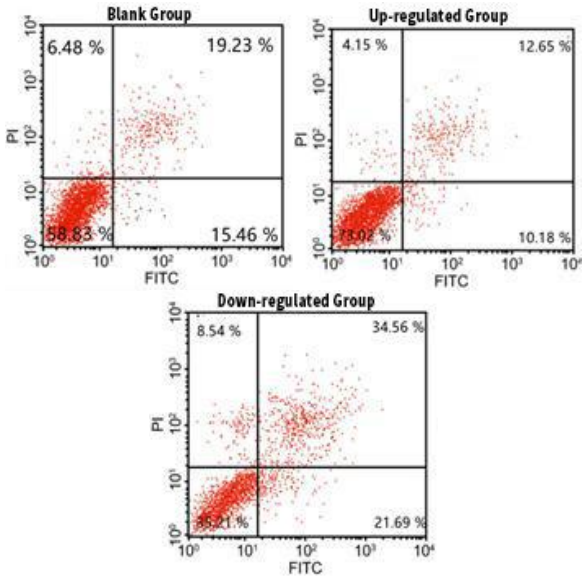

Figure 2. Graph of apoptosis rate of glioma cells in each group; Note: aP< 0.05 compared to the blank group; bP< 0.05 compared to the up-regulated group.

Drug resistance analysis

As shown in Table 4, the IC50 values of Imatinib, VP-16 and TMZ in the blank and up-regulation groups were not statistically different compared to each other (P > 0.05); the IC50 values of Imatinib, VP-16 and TMZ in the down-regulation group decreased compared to the blank and up-regulation groups and the difference was statistically significant (p < 0.05).

Table 4. Drug resistance analysis ($\bar{x} \pm s$)

Drug	IC50（ug/ml）		
	Blank group	Up-regulated group	Down-regulated group
Imatinib	14.56 ± 3.14	15.53 ± 2.09	9.15 ± 1.02ab
VP-16	168.52 ± 11.26	165.26 ± 10.84	89.56 ± 8.76ab
TMZ	35.62 ± 4.25	33.26 ± 4.81	15.34 ± 3.81ab

Note: aP < 0.05 compared to the blank group; bP < 0.05 compared to the up-regulated group.

As one of the most common types of glioma in the human brain, glioblastoma has a high degree of malignancy and aggressiveness, which seriously affects the physical and mental health of patients and their quality of life (9, 10). Gliomas are treated by a variety of methods, including chemotherapy and surgery, but the recurring nature of gliomas has led to low rates of complete surgical resection and resistance to chemotherapy (11, 12). Therefore, a clinical shift in direction towards glioma genetics is particularly important for the development of treatment options for glioma (13).

Long-stranded non-coding RNAs have diverse transcripts (14). Long-stranded non-coding RNAs are able to regulate genes at the level of chromatin composition and at the level of transcription, thereby affecting cell proliferation, apoptosis or migration (15, 16). In recent years, it has been suggested that long-stranded non-coding RNAs can be used as targets for tumor diagnosis as well as therapy (17, 18). It has been shown that HCG11 regulates apoptosis in hepatocellular carcinoma and is closely related to the development of hepatocellular carcinoma, but it has been less studied in glioma (19). MicroRNAs are non-coding RNAs that are widely found in various organisms that can induce mRNA degradation or inhibit the process of translation. MicroRNA-144 is aberrantly expressed in a variety of cancers, including laryngeal, colorectal and breast cancers. It has been shown that the regulation of long-stranded non-coding RNA-HCG11 is able to regulate the MicroRNA-144COX-2 axis to some extent in gliomas (20). In this study, we found that the expression of MicroRNA-144 and COX-2 was regulated by the long-stranded non-coding RNA-HCG11 in glioma cells. The expression of MicroRNA-144 and COX-2 were negatively correlated, and when MicroRNA-144 was overexpressed, the expression of COX-2 decreased to a certain extent (21).

It has been clinically noted that about 50% of miRNAs are located around tumor-associated fragile loci. This result can prove that miRNAs are closely related to tumor pathogenesis and play an important role in the development of tumors (22, 23). COX-2, an inducible enzyme, has low activity in normal tissue cells and is activated in large quantities when cells are damaged or stimulated (Fig. 1). Long-stranded non-coding RNA-HCG11 acts as a potential target gene for

MicroRNA-144, whose regulation is able to modulate COX-2 (14). Clinical studies have suggested that the possible molecular mechanisms of drug resistance in gliomas include mutations in the targets of genes acting under chemotherapeutic agents and diminished apoptosis of tumor cells. Mutations in key genes along these signaling pathways can lead to drug resistance in tumor cells (17, 24, 25) (Fig. 2). In this study, we found that down-regulation of long-stranded non-coding RNA-HCG11 was able to reduce the proliferation rate and increase the apoptosis rate of glioma cells. Also, down-regulation of long-stranded non-coding RNA-HCG11 reduced the sensitivity of glioma parental cells to Imatinib, VP-16 and TMZ chemotherapeutic drugs, suggesting that long-stranded non-coding RNA-HCG11 was involved in the regulation of chemo-resistance in glioma.

In summary, down-regulation of long-stranded non-coding RNA-HCG11 regulates the MicroRNA-144COX-2 axis in gliomas, thereby decreasing the proliferation rate and increasing the apoptosis rate of glioma cells. Meanwhile, down-regulation of long-stranded non-coding RNA-HCG11 is also involved in the resistance mechanism of Imatinib, VP-16 and TMZ chemotherapeutic agents.

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Interest conflict

None.

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