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MiR-203 inhibits cell proliferation, invasion, and migration of ovarian cancer through regulating RGS17

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Abstract: It has been reported that G-protein signaling 17 (RGS17) oncogene is involved in the occurrence and development of ovarian cancer (OC), but the regulatory mechanism of OC is still unclear. MicroRNA (miRNA) negatively regulates gene expression, and its maladjustment is related to tumorigenesis. We understand the role of miRNA in OC modulators induced by RGS17.

To characterize the regulatory effect of miR-203 on RGS17, we used ovarian cancer cell lines SKOV3 and OVCAR3, and constructed miR-203 and RGS17 overexpression vectors. CCK8 kit was used to detect cell proliferation, while Transwell analysis was used to measure cell invasion and migration. RT-qPCR and Western blotting were used to analyze the expression of miR-203 and RGS17 in ovarian cancer. The interaction between miR-203 and RGS17 was detected by fluorescent enzyme reporter gene method. The expression of miR-203 was significantly decreased in tumor cells, while the expression of RGS17 mRNA was increased. Luciferase reporter gene assay showed that miR-203 down regulated RGS17 expression by directly integrating into RGS17 mRNA. Our results showed that miR-203 was down regulated during tumorigenesis and inhibited the proliferation and invasion of OC cells. MiR-203 inhibits the proliferation, invasion and migration of ovarian cancer cell lines SKOV3 and OVCAR3 by targeting RGS17. After RGS17 overexpression, the regulatory effect of miR-203 was inhibited.

Keywords: Invasion and migration, ovarian cancer, miR-203, proliferation, RGS17

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1.Introduction

Ovarian cancer (OC) is the fifth leading cause of cancer-related deaths in women worldwide, resulting in 14240 deaths per year1. Although the treatment of ovarian cancer has made great progress in the past few decades, the prognosis of OC patients is still unsatisfactory no matter in surgery, chemotherapy or radiotherapy2. Therefore, it is an urgent need to understand the molecular mechanism of the disease related to the occurrence and development of OC. Long noncoding RNA (lncRNA) has been found to be a key regulator in various biological processes. MicroRNA (miRNA) is a small non coding RNA, which can play a role by regulating the expression of genes in a variety of biological processes3. More and more evidences suggest that miRNA plays an important role in controlling the invasion and metastasis of cancer cells4-6 . For example, the tumor suppressor gene miR-203 in ovarian cancer the expression of tumor growth factor Snai27. MiR-203 inhibits ovarian tumor metastasis by targeting BIRC5 / surviving and weakening TGFβ pathway8. The key regulatory point of G protein activity is the inactivation of G protein by GTP hydrolysis, through which GTPase activating protein (GAP) is significantly enhanced in cells. The G-protein modulator signal transduction (RGS) protein is a highly diverse heterotrimeric G-protein used as gap to accelerate the inactivation of protein family, thus terminating the signal transduction initiated by GPCR9,10. We have recently shown that RGS protein inhibits LPA stimulated growth signals in ovarian cancer cells 9,11, and more than ten RGS transcripts were identified in ovarian cancer cells, many of which were differential expression of cell lines in normal ovarian cells and ovarian cancer12.

However, bioinformatics and functional analysis showed that miR-203 could target RGS17 gene. In this study, we investigated the interaction of miR-203-RGS17 inhibited the progression of ovarian cancer, and it was found that the expression of miR-203 significantly increased to inhibite the migration, invasion and diffusion of ovarian cancer cells induced by RGS17. In addition, we identified miR-203 as a regulator cell line to inhibit RGS17 expression in ovarian cancer.

2.Materials and methods

2.1Cell culture

Ovarian cancer cell lines SKOV3 and OVCAR3 were obtained from ATCC and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS (Hyclone, USA), 100 U / ml penicillin and 100 μg / ml streptomycin (Invitrogen, USA). HEK293 cells were cultured in DMEM medium containing 10% FBS, 100 U / ml penicillin, 100 μg / ml streptomycin, 1% glutamine.

2.2 Cell transfection

First, HEK293 cells were seeded into 96 well plates at

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a density of about 1 × 106 / well. Add DMEM medium to each well and incubate for another 24 hours. After the cell adheres to the wall, the cells are transfected. MiR-203 mimic or overexpression of RGS17 vector was transfected into cells according to the instructions of lipofectaminetm2000 (Invitrogen, USA). The related indexes were detected 48 hours later. Three groups are established: NC group (negative control), miR-203 mimic (SKOV3 and OVCAR cells transfected with miR-203 mimic) and miR-203 mimic + RGS17 (SKOV3 and OVCAR cells transfected with miR-203 mimic and RGS17).

2.3MTT determination

According to the manufacturer's instructions, use the MTT assay kit to check cell proliferation. Ovarian cancer cells were cultured (8000 cells / well) in 96 well plate. At different time points, 10 μl MTT reagent (10 mg / ml) was added to each pore and incubated for about 4 h. The reaction was terminated by the addition of 100 μ l cracking reagent and incubated in darkness at 22 ℃ for 2 hours. The absorbance was measured at 570nm on Bio-Rad.

2.4Cell migration assay

The chamber system was used to measure the cross whole migration. These cells were inserted into 24 well plates and 30 μ l of 3 × 104 cells in serum-free DMEM were added to the upper chamber. DMEM (supplemented with 10% FBS) was added to the inferior chamber of each well and the cells were incubated for 24 hours. The cells in the lower side of the membrane were fixed with methanol and stained with 0.1% crystal violet. Count the migrating cells in at least three different areas.

2.5Real time quantitative PCR analysis

Trizol reagent (Invitrogen, USA) was used to extract total RNA from tissues and cells. The extracted RNA is reverse transcribed into complementary DNA (cDNA). RT-qPCR was performed with fluorescent RT-qPCR instrument. The specific reaction conditions are as follows: pre-denaturation at 95℃ for 10 minutes, denaturation at 95℃ for 30 minutes. After renaturation for 30 s at 55℃, it was extended for 2 min at 72 ℃ for a total of 35 cycles. The relative expression of miR-203 and RGS17 were quantified by the 2-ΔΔ CT method. The primer sequences used in this study are as follows: RGS17, foward: 5'-CCA GGA ACC CCT CCT TAC TC-3', reverse: 5'-TGT GTC CGA AGG ACT AGG GA-3'; miR-203, forward: 5'-AGG GGT GTA ACA TCC TCG ACT G-3', reverse: 5'-AGT GTC GTG GAG TTT GCG TGT CAT-3'; U6: forward: 5'-GCA ACG GCA GCA CAT ATA CTA AAA T-3', reverse: 5'-CGC TTC AGA ATT TGC GTG TCA T-3',GAPDH, forward: 5'-CGC TCT CTG CTC CTC CTG TTC-3', reverse: 5'-ATC CGT TGA CTC CGA CCT TCA C-3'.

2.6Western blotting (WB) analysis

The protein was extracted from cells using protein lysates. The concentration of the extracted protein was quantified by the analysis of BCA. The extracted protein was electrophoresis and transferred to PVDF membrane. The membrane was then incubated overnight at 4 ℃ with Rabbit anti human RGS17 primary antibody and 3-phosphoglyceraldehyde dehydrogenase (GAPDH). After washing with tris buffer salt water and Tween-20 (TBST), the membrane was incubated with the second antibody of Goat anti rabbit immunoglobulin G (IgG) labeled with horseradish peroxidase for 1 hour at 37℃. After washing, use enhanced chemiluminescence (ECL) method to develop color (Thermo Fisher, USA).

2.7Statistical analysis

Use Students' t-test or F-test for statistical analysis. All P values were positive and negative, $P < 0.05$ was considered statistically significant. For all statistical analysis.

3.Results

3.1Expression of miR-203 and RGS17 in ovarian cancer

To study the role and mechanism of miR-203 and RGS17 in ovarian cancer. Here, we used RT- qPCR to analyze the expression of miR-203 and RGS17 in SKOV3 and OVCAR cell lines. As expected, miR-203 was negatively correlated with RGS17 and significantly decreased in OC cell lines compared with normal IOSE144 cells (Fig. 1A), whereas RGS17 was up-regulated in OC cell lines (Fig. 1B). There is a specific regulatory relationship between miR-203 and RGS17 mRNA.

3.2MiR-203 inhibited tumorigenicity and metastasis in vitro

In order to identify the effect of miR-203 on ovarian cancer cell proliferation, migration, and invasion, both SKOV3 and OVCAR3 cells were transfected with the miR-203 overexpression mimic vector. After 48 h of transfection, expression level of miR-203 was detected by RT-qPCR. Result showed that the expression of miR-203 was significantly increased in both SKOV3 and OVCAR3 cells compared with the control group (Fig. 2A). Cell proliferation was then determined using a cell-counting kit, with 2 ×103 cells used as the initial concentration. After culturing for different times (0, 1, 2, and 3 days), cell proliferation was detected by measuring the absorbance at 450 nm. Expression of miR-

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203 significantly suppressed cell proliferation at 2 days in both SKOV3 and OVCAR3 cells. However, there was no significant difference after transfection with the NC vector compared with the control group (Fig. 2B, C). To determine the effect of miR-203 on metastasis, Transwell detected migration and invasion assays were carried out using SKOV3 and OVCAR3 cells transfected with the miR-203 mimic, NC, or their respective controls. The miR-203-transfected ovarian cancer cells showed significantly lower migration and invasiveness than the control or NC group (Fig. 2D-G). Together, these results showed that miR-203 inhibited ovarian cancer cell proliferation, migration, and invasion in vitro.

3.3RGS-17 overexpression reversed miR-203-induced cell proliferation, migration, and invasion inhibition

Previous studies showed that RGS17 expression played an important role in the maintenance of tumor cell proliferation. To determine whether RGS17 was involved in the suppression of miR-203-mediated tumor cell proliferation, the RGS17 overexpression vector was constructed and successfully transfected into SKOV3 and OVCAR cells. Western blotting showed that the expression of RGS17 was significantly increased in both SKOV3 and OVCAR cells (Fig. 3A, B). Previous studies reported that the expression of miR-203 significantly inhibited proliferation of both SKOV3 and OVCAR cells. However, RGS17 overexpression reversed miR-203-induced proliferation inhibition in both SKOV3 and OVCAR cells (Fig. 3C, D). Transwell detected migration and invasion assays were carried out using SKOV3 and OVCAR cells, showing that RGS17-transfected OC cells significantly reversed the miR-203-induced migration and invasiveness inhibition (Fig. 3E-H). Taken together, the result showed that the antitumor effect of miR-203 on OC cells was decreased after overexpression of RGS17.

3.4RGS-17 was the direct target of miR-203

To determine the possible interaction between miR-203 and RGS17, we first carried out a bioinformatics screen for its possible target genes, using an online 3'-UTR binding site prediction database. A mutated version of the RGS17 3'-UTR was constructed (Fig. 4A). This mutated construct was fused to the luciferase coding region and co-transfected into HEK293T cells along with miR-203 mimic. The relative luciferase activity showed that when the wild-type RGS17 3'-UTR was co-transfected with miR-203 mimic, RGS17 expression was significantly decreased ($P < 0.01$) compared with co-transfection with the control miRNA. However, this effect was not observed after mutant 3'-UTR of RGS17, indicating that miR-203 can specific suppression RGS17 expression by targeting 3'-UTR of RGS17 (Fig. 4B, C). Western blotting and RT-PCR analyses further confirmed that miR-203 expression significantly inhibited RGS17 expression in both protein and mRNA level in vitro (Fig. 4D-F). Our results also showed that inhibiting miR-203 expression with miR-203 inhibitor treatment significantly promoted RGS17 expression in both SKOV3 and OVCAR cells.

4.Discussion

Recently, miR-203 has been reported to be dysfunctional in a variety of cancer types. The down-regulation of miR-203 was found in lung cancer, gastric cancer and glioblastoma13-15. However, it is reported that the expression of miR-203 is up-regulated in ovarian cancer tissues16, indicating that the expression pattern of miR-203 depends on the type of cancer. In addition, some previous and current studies have demonstrated the correlation between the expression of miR-203 and the clinical characteristics and carcinogenic function of miR-203 in ovarian cancer, indicating that miR-203 may be used as a diagnostic marker and treatment target for ovarian cancer. However, the related molecular mechanism of its expression is still unclear. MiR-203 has been shown to regulate the resistance of cancer cells to therapeutic drugs, indicating that miR-203 may be a predictor of chemotherapy response17. The change of miR-203 expression leads to antitumor effect, suggesting that miR-203 plays a crucial role in the regulation of tumorigenesis. Therefore, it may be of great clinical significance to study the potential mechanism of miR-203 in different types of cancer. Our results showed that the expression level of miR-203 in OC cells was lower than that in normal cells. In contrast to miR-203, RGS17 expression induces tumorigenesis. A number of recent studies have shown that the expression of RGS17 was up-regulated and promoted the growth and migration of colorectal cancer. RGS17 is one of the smallest RGS proteins and lacks clear definition functional domain outside RGS domain. RGS17 is a member of RZ (A) subfamily of RGS protein, preferentially inactivating Gi /Go family of G protein18,19. RGS17 also contains a palmitoylation site located in its N-terminal region of the RGS domain. This site may regulate its subcellular location, further affect the selectivity of G protein and receptor. In contrast to miR-203, RGS17 expression is regulated in cancer cells, such as in HCC19 and prostate cancer20. RGS17 increased proliferation, migration and invasion, and even increased resistance to chemotherapy. Therefore, it may be of great clinical significance to study the mechanism of RGS17 mediated tumor metabolism.

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In our study, our results showed that miR-203 expression in ovarian cancer cells was significantly lower than that in normal cells, but RGS17 expression in ovarian cancer cells was significantly increased. We also found that the expression of miR-203 significantly inhibited the proliferation of OC cells and reduced cell migration and invasion. However, over expression of RGS17 reversed miR-203 induced antitumor effect. The double fluorescein test further confirmed that miR-203 was integrated into the 3'-UTR of RGS17 and down regulated the expression of RGS17 after transcription. We also show that miR-203 can inhibit cell proliferation, invasion and migration of OC by down regulation RGS17. However, the exact regulatory mechanism of RGS17. It is still unknown to promote cell proliferation, invasion and migration of OC, which needs further study. In conclusion, this study shows that miR-203 inhibits cell proliferation, invasion and migration in OC by targeting RGS17. Therefore, miR-203 may be a new diagnosis and treatment option for OC patients.

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