



MiR-218 Affects the Invasion and Metastasis of Cervical Cancer Cells by Inhibiting the Expression of SFMBT1 and DCUNIDI

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ARTICLE INFO

Original paper

Article history:

Received: September 25, 2021

Accepted: December 18, 2021

Published: February 28, 2022

Keywords:

cervical cancer, micro RNA-218, cell invasion, cell metastasis

ABSTRACT

As the second most common gynecological tumor in the world, cervical cancer has always been a female killer. Some studies have shown that miR-218 can inhibit the invasion and migration of cervical cancer cells through two target genes, SFMBT1 and DCUNIDI. On this basis, this paper presents the study that miR-218 affects the invasion and migration of cervical cancer cells by inhibiting the expression of target genes, SFMBT1 and DCUNIDI. In this paper, we used the control experiment to study. In the fluorescence experiment, we confirmed that SFMBT1 and DCUNIDI are indeed the downstream target genes of miR-218. After that, we down-regulated or up-regulated the expression of SFMBT1 and DCUNIDI in cervical cancer cells through specific small interfering RNA and over-expression plasmids, so we carried out cell migration and cell invasion experiments. The results of the cell invasion experiment showed that the average value of cell invasion ability of the miR-218 group was 286 and 218, that of SFMBT1-siRNA1 was 264, that of DCUNIDI-siRNA1 was 179, that of SFMBT1-siRNA 2 was 245, that of DCUNIDI-siRNA2 was 196, and that of SFMBT1 + miR-218 was 401. The average cell invasiveness of DCUNIDI + miR-218 was 672.

DOI: <http://dx.doi.org/10.14715/cmb/2022.68.2.12>

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Introduction

The abnormal expression of miRNA is closely related to the formation of a variety of tumors, and we all know that miR-218 is a tumor suppressor gene, so its reduced expression may inhibit the development of tumors. On the other hand, SFMBT1 and DCUNIDI are the target genes of miR-218, which may be related to the invasion and metastasis of cervical cancer cells. Therefore, it is of great significance for the study of cervical cancer to study the change of invasion and metastasis ability of miR-218 by inhibiting the expression of SFMBT1 and DCUNIDI.

Pandurangan reported the whole exon sequence analysis of 115 normal matched cervical cancer specimens (1-2), the transcriptome sequence analysis of 79 cervical cancer specimens and the whole genome sequence analysis of 14 normal matched cervical cancer specimens (3-4). In addition, in this study, pandurangan also observed the somatic Elf3 (13%) and CBF3 (8%) mutations in 24 cases of adenocarcinoma (5-6). The results showed that compared with adenocarcinoma, the frequency of nucleotide substitution of cytosine producing cells in

squamous cell carcinoma before thymine (TP * C site) was higher, and the gene expression level of HPV integration site in HPV integrated tumor was significantly higher than that of the same gene in the tumor without integration site (7-8). These results show some recurrent genomic changes in cervical cancer and suggest a new strategy to combat this disease (9-10). However, the results of this study are accidental and need to be improved.

The research on miRNA has always been the focus of scholars. MiRNA regulates the stability or translation efficiency of target mRNA. New evidence suggests that miRNAs may be involved in the pathogenesis of cancer in a variety of human diseases. Malignant glioma is the most common and fatal primary brain tumor in adults. More and more evidences show that microRNAs (miRNAs) have an effect on the regulation of apoptosis signal. For example, a song study found that miRNA 218 (miR-218) was down-regulated in human glioma (11-14). He studied the effect of miR-218 on the proliferation and tumorigenicity of glioma cells by cloning in vitro and xenotransplantation in vivo (15). In this study, he

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Cellular and Molecular Biology, 2022, 68(2): 81-86

used flow cytometry to detect apoptosis and terminal Deoxynucleotidyltransferase deoxyuridine triphosphate nick-end labeling to detect apoptosis (16). On the other hand, Song determined the downstream target of miR-218 by bioinformatics analysis, and further verified the analysis by Western blot and Luciferase Report (17-18). This study showed that overexpression of miR-218 induced apoptosis of glioma cells and inhibited the activity, proliferation and tumorigenicity of glioma cells (19-20). This research has certain reference value but lacks data explanation and persuasion.

Cervical cancer, as a female killer, has always been a frequent disease of women, which has puzzled many women's physical and mental health. Therefore, this disease once became a research hotspot. Therefore, this paper has carried out experiments. In this paper, we set up a control experiment to study the cell migration and cell invasion through specific small interfering RNA and overexpression plasmid to down-regulate or up-regulate the expression of SFMBT1 and DCUN1D1 in cervical cancer cells. The results of the cell invasion experiment and cell migration experiment showed that except miR-NC group and vector + miR-218 group, other interference factor groups had a significant difference, namely $P < 0.01$.

Materials and methods

Cervical Carcinoma

The prevention, diagnosis and treatment of cervical cancer are receiving more and more attention. What is the clinical manifestation of cervical cancer? Generally speaking, vaginal bleeding, irregular menstruation and leucorrhea like rice soup may be the clinical symptoms of cervical cancer (21-22). On the other hand, there are no visible lesions in the cervical microinvasive carcinoma, and the cervical smooth or corrosive changes can be seen in the gynecological examination. With the development of the disease, different symptoms may appear, and vegetable like vegetations may be observed. When the cancerous tissue is necrotic and falling off, the formation of ulcers or cavities may be accompanied by a stench (23).

Let's look at the treatment of cervical cancer. Generally speaking, cervical cancer can be treated by surgery, radiotherapy or chemotherapy as adjuvant treatment. The treatment of early cervical cancer is

mainly surgery. According to the needs of the disease, cervical conization, total hysterectomy and radical hysterectomy can be selected. In this way, we all know that surgery is a method and link in the treatment of cervical cancer, so after surgery, we should timely and appropriate consolidate and strengthen the effect of surgical treatment, and supplement the shortcomings of surgical treatment. On the other hand, after radical hysterectomy, according to the postoperative pathological lymph node metastasis, positive margin, parauterine metastasis and so on are the indications of adjuvant radiotherapy or concurrent radiotherapy and chemotherapy.

MiRNA

MicroRNA (miRNA) is a kind of small RNA, which does not have extensive eukaryotic coding. MiRNA has obvious structural characteristics. Mature miRNA is usually a single strand structure composed of 25 nucleotides. The 5' end is phosphoryl and the 3' end is hydroxylamine. MiRNA is first transcribed from RNA polymerase II to original miRNA (pro miRNA), the latter is about 70m, which is an incomplete pairing of stem structure and can bind to dgcr8 of dsRNA, which is the binding protein of dsRNA. MiRNA binds to the 3' untranslated genes (UTRs) of the target mRNA gene. By inducing translation inhibition, the target mRNA breaks into other regulatory mechanisms, thus inhibiting the expression of the target gene.

MiR-218

miR-218.1 encoded in the Slit2 intron has low expression in a variety of human malignant tumors and plays an important role in tumor inhibition (24). MiR-218 can be down-regulated to adapt to a variety of human malignant tumors, such as malignant glioma, malignant astrocytoma, neuroblastoma, breast cancer, prostate cancer, pancreatic cancer, thyroid cancer, gastric cancer, colon cancer, gastrointestinal stromal tumor, kidney cancer, head and neck squamous cell carcinoma, NPC, oral cancer, esophageal cancer, liver cancer, osteosarcoma, etc It has an inhibitory effect on all these tumors (25).

Research Object

The source of these cell lines is 100 cervical cancer patients from December 2017 to December 2019 who

underwent surgical resection in a hospital. The pathological diagnosis of these patients is clear, and they did not receive chemotherapy or radiotherapy before the collection of tissue samples.

Cell Culture and Transfection

In the cell culture of this paper, HeLa cells were cultured with high glucose DMEM, in which 10% bovine embryo serum was added and grown at 37 °C, in which the concentration of CO₂ was 5% and the relative humidity was 50%. The HeLa cells were cultured in a cell incubator, digested and dispersed with 0,25% trypsin and - 0,02% EDTA working solution, and then subcultured or inoculated. When the cell sequence reaches 80% to 90%, 0.25% trypsin is used to digest the cells and culture them. At this time, HeLa cells of the logarithmic growth period are selected for the experiment. On the other hand, the cells were inoculated into 6-well plates one day before transfection and then transfected with serum-free medium according to the instructions when the fusion degree of adherent cells reached 40% ~ 60%. After 6 hours of transfection, a fresh and complete culture medium should be replaced, so as to facilitate the continuous expansion of culture, and can be used in subsequent experiments. Among them, a control group was added, and the cells in the control group were added into the complete medium without transfection.

Fluorescence Activity Experiment

In fluorescence experiments, we used Ezol to extract the total RNA of cervical cancer cells and used the spectrophotometer and agarose gel to carry out electrophoresis, so as to detect the concentration and purity of RNA, so that quality control could be detected. On the other hand, we used the TLI RNaseH plus test kit to conduct a real-time quantitative analysis of related mRNA. In the specific experiment process, we strictly follow the instructions of the real-time quantitative PCR detection kit. In this process, we first used the first strand of the synthesized cDNA as the template for real-time PCR amplification, so as to obtain the CT value of each sample, and β - actin as the internal reference to correct the error. After these steps, according to the experimental results, we can determine the relative expression level of related mRNA in cervical cancer cells.

Cell invasion Experiment

In the cell invasion experiment, we took 501aL of BD matrix gum and did not use DMEM medium without serum to dilute 5 times, reaching 2509 L. After that, we put the chamber into a 24-well plate, and add 501aL of diluted matrix glue into each hole, keep the temperature at 37 °C and the concentration at 5% CO₂, and incubate in the incubator for 3 hours. When the cells in the six pore plate grow to 50%, do not use the medium, and add the serum-free DMEM medium. After 12h, add 0, 100, 500 ng/ml rhPGRN. On the other hand, collect the cells and calculate the cell density after 2H. In the follow-up, we took out the chamber, washed it three times with PBS, fixed it with methanol for 20min, washed it once again with PBS, stained it with crystal violet for 30min, wiped off the upper cells with a cotton swab, washed it with PBS, and then immediately observed and photographed under the microscope. To avoid the chance of data, each group of our experiment was repeated three times.

Cell Migration Experiment

In the cell migration experiment, three groups of HeLa cells in a logarithmic growth period were taken, the cell density was $5 \times 10^5 \text{mL}^{-1}$, and then 100 μ L cell suspension was added to the Transwell cell chamber. In addition, put the Transwell cell into a 24 well culture plate, and add 500 μ L 20% serum culture medium. After 24 hours of culture, take out the Transwell cell. At this time, do not use the medium in the hole, and wash twice with PBS, The cells were fixed with formaldehyde for 30 minutes, stained with 1% crystal violet for 20 minutes, and then wiped off the upper layer of non-migrating cells with a cotton swab, washed twice with PBS, and observed three visual fields randomly under 400 times microscope, and observed cell count. To avoid the chance of data, each group of our experiment was repeated three times.

Statistical Methods

In the experiment of this paper, spss21.0 statistical software is used to process and analyze the experimental data. The data obtained is expressed by mean \pm standard deviation. The comparison between the two groups of data is tested by T, and the comparison between multiple groups of data is

analyzed by one-way ANOVA. In this process, $P < 0.05$ is used as the difference, that is to say, at this time, the data has Statistical significance.

Results and discussion

SFMBT1 and DCUNIDI are the Downstream Target Genes of miR-218

In order to determine whether miR-218 directly regulates the expected potential target gene, we conducted a luciferase reporter gene experiment. In this process, we co-transfected mimic and its negative control of miR-218 with the reporter gene vector containing the wild-type 3' non-coding region of the target gene into HeLa cells. After 36 hours, we detected the luciferase activity and calculated the luciferase activity change with the β -gal activity as the internal reference. The results are shown in Figure 1 and Figure 2. From Figure 1 and Figure 2, we know that the luciferase activity of the reporter gene vector of wild-type 3' non-coding region containing SFMBT1 and DCUNIDI is significantly decreased, on the other hand, the luciferase activity of other target genes screened is not significantly decreased, so we can know that SFMBT1 and DCUNIDI is the direct targets of miR-218. That is to say, SFMBT1 and DCUNIDI are the direct downstream target genes of miR-218, and miR-218 can play a role by combining with the 3' non-coding region of SFMBT1 and DCUNIDI.

Analysis of Cell Invasion Experiment Results

The purpose of the cell invasion experiment is to verify that miR-218 can affect the invasive ability of cervical cancer cells by inhibiting the expression of target genes SFMBT1 and DCUNIDI. In this paper, specific small interfering RNA and over-expression plasmids are used to down-regulate or up-regulate the expression of SFMBT1 and DCUNIDI, respectively, so as to carry out a cell invasion experiment. The experimental results are shown in Table 1 and Figure 3.

Table 1. Cell invasion test results

Treated factors	Average cell invasiveness		P
	SFMBT1	DCUNIDI	
MiR-NC	536	569	
MiR-218	286	218	<0.001
SFMBT1/DCUNIDI-siRNA1	264	179	<0.001
SFMBT1/DCUNIDI-siRNA2	245	196	<0.001
Vector+miR-218	246	342	
SFMBT1/DCUNIDI+miR-218	401	672	<0.001

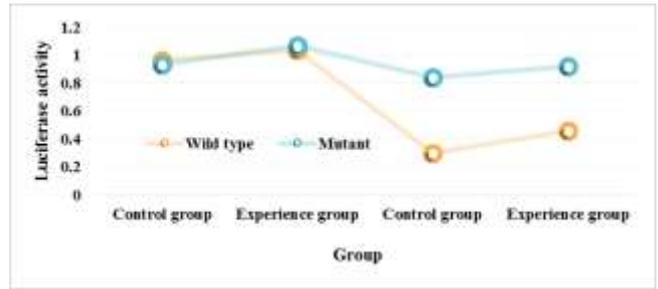


Figure 1. Experimental results of luciferase activity of SFMBT1

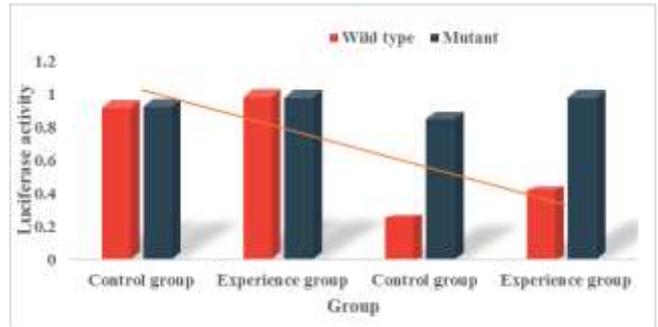


Figure 2. Experimental results of luciferase activity of DCUNIDI

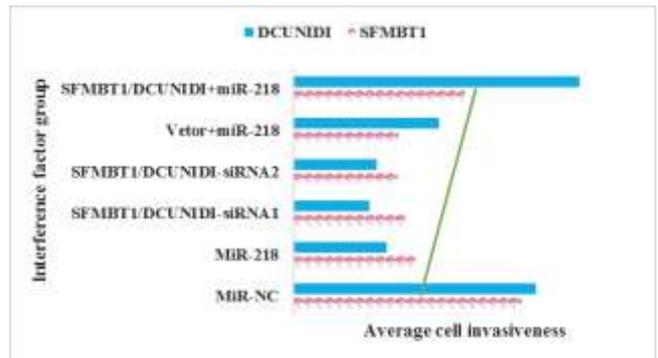


Figure 3. Analysis of cell invasion test results

It can be seen from Table 1 and Figure 3 that except miR-NC group and vector + miR-218 group, other interference factor groups have significant differences. Among them, the average cell invasiveness of the miR-218 group was 286 and 218, that of SFMBT1-siRNA1 was 264, that of DCUNIDI-siRNA1 was 179, that of SFMBT1-siRNA 2 was 245, that of DCUNIDI-siRNA2 was 196, that of SFMBT1 + miR-218 was 401, and that of DCUNIDI + miR-218 was 401, the average value of cell invasion was 672. In other words, the invasion ability of HeLa cells decreased after SFMBT1 or DCUNIDI interfered. On the other hand, the invasion ability of HeLa cells increased after overexpression of SFMBT1 or DCUNIDI. In addition, overexpression of SFMBT1 or

DCUNIDI could reverse the effect of miR-218 on the invasion of HeLa cells.

Analysis of Cell Migration Results

The purpose of the cell migration experiment is to verify that miR-218 can affect the migration ability of cervical cancer cells by inhibiting the expression of target genes SFMBT1 and DCUNIDI. In this paper, specific small interfering RNA and over-expression plasmids are respectively used to down-regulate or up-regulate the expression of SFMBT1 and DCUNIDI in cervical cancer cells to carry out a cell migration experiment. The experimental results are shown in Figure 4.

It can be seen from Figure 4 that the average cell migration ability of the miR-NC group is 756 and 810, that of the vector + miR-218 group is 274 and 356, that of the miR-218 group is 382 and 364, that of SFMBT1-siRNA 1 is 289, that of DCUNIDI-siRNA1 is 242, that of SFMBT1-siRNA 2 is 278, and that of the average cell migration ability of DCUNIDI - siRNA 2 is 213, SFMBT1 + miR-218 and DCUNIDI + miR-218 was 502 and 889 respectively. In other words, HeLa's migration ability decreased after SFMBT1 or DCUNIDI interfered, but on the other hand, HeLa's migration ability increased after overexpression of SFMBT1 or DCUNIDI. In addition, overexpression of SFMBT1 or DCUNIDI could reverse the effect of miR-218 on HeLa cell migration.

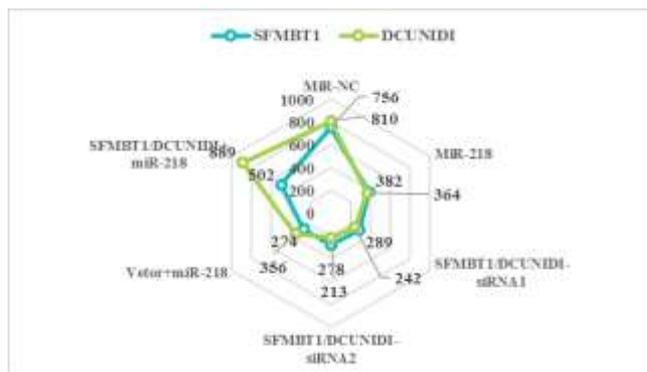


Figure 4. Analysis of cell migration test results

As we all know, cancer has been a serious threat to human health in the world, and cervical cancer is a serious impact on women's physical and mental health. Therefore, many scholars have studied cervical cancer. Some studies have shown that miR-218 is related to the occurrence of the tumor, and its target

genes, SFMBT1 and DCUNIDI, are related to the invasion and migration of cervical cancer cells.

HeLa, a cervical cancer cell line, was selected as the research object in this paper. After we confirmed that SFMBT1 and DCUNIDI were the direct target genes downstream of miR-218, we carried out cell invasion and cell migration experiments. In this study, we used specific small interfering RNA and over-expression plasmids to down-regulate or up-regulate the expression of SFMBT1 and DCUNIDI in cervical cancer cells, so as to carry out cell invasion and cell migration experiments. The results showed that overexpression of SFMBT1 or DCUNIDI could reverse the effect of miR-218 on HeLa cell migration.

Acknowledgments

None.

Conflict interest

The authors declare no conflict of interest.

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