



Effect of miRNA-203 on cervical cancer cells and its underlying mechanism

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ABSTRACT. miRNA-203 is involved in the development and progression of various types of cancer. However, its role in cervical cancer remains unclear. The aim of this study was to investigate the effect of miRNA-203 on the proliferation and migration of HeLa cervical cancer cells, as well as survivin expression in these cells. A miRNA-203 primer probe was designed according to a sequence obtained from NCBI. The expression of miRNA-203 in cervical epithelial cells and cervical cancer cells was detected by quantitative reverse transcriptase-polymerase chain reaction. The miRNA-203 expression pattern was compared between these two cell lines. The cervical cancer cells were transfected with miRNA-203 mimic or inhibitor to determine their effects on proliferation and migration. The expression of the miRNA-203 target protein (survivin) was analyzed by western blot. Cervical cancer cells showed reduced miRNA-203 expression compared to cervical epithelial cells. Transfection of miRNA-203

mimic upregulated the expression of miRNA-203, suppressed cell proliferation and migration, and downregulated survivin expression ($P < 0.05$). However, downregulation of miRNA-203 expression did not affect proliferation, migration, and survivin expression in cervical cancer cells ($P > 0.05$). In conclusion, upregulation of miRNA-203 in cervical cancer cells inhibits the proliferative and migratory capacities of these cells by downregulating the expression of survivin.

Key words: Cervical cancer; Survivin; miRNA-203; Proliferation; Migration

INTRODUCTION

Cervical cancer is one of the most common types of cancer affecting women worldwide (Schmid and Oehler, 2015; Yang et al., 2015). It is among the top three malignant cancers, and has the fifth highest mortality rate (7.3%) in both men and women. Approximately 20% of all cancer-related deaths can be attributed to cervical cancer. A larger number of youths have been afflicted with this type of cancer over the past few years. It also accounts for 75-80% of all cancers in developing countries. Locally, there has been an increase in the incidence of cervical cancer, especially in the youth, which has a serious impact on human health (Zeng et al., 2015).

A number of studies are currently attempting to elucidate the pathogenesis of cervical cancer; however, its underlying molecular mechanism remains unclear. Therefore, further research into the pathogenesis of cervical cancer is of paramount importance (Chen et al., 2013). A majority of the research into the pathogenesis of cervical cancer is focused on human papilloma virus (HPV) infection-induced cervical cancer. Previous studies have suggested that HPV disrupts the cell cycle by inserting itself into the host cell genome and inducing genetic mutations, resulting in the development of cervical cancer (Chintharlapalli et al., 2011). Previous studies have also demonstrated the important role of cell apoptosis in the initiation and development of cervical cancer. The imbalance between oncogenes and anti-oncogenes induces an over-expression in oncogenes, which in turn transforms normal cells to cancerous cells, ultimately resulting in cervical cancer. Cell adhesive factors play an important role in cell cycle, and the changes in expression of these factors are mainly responsible for the pathogenesis of cancer (Debeb et al., 2010). Taken together, these studies indicate that abnormalities or imbalances in the expression of genes related to the pathogenesis of cervical cancer could induce the development and progression of this disease. Therefore, specific gene expression and its modulatory behavior must be elucidated in order to provide a theoretical basis for the pathogenesis of cervical cancer.

Recent studies have focused on exploring the role of microRNA (miRNA) in cancer. miRNA are small RNA molecules that modulate gene expression post-transcription. Studies have shown that miRNA are abnormally expressed in cancer. miRNA participate in the tumor cell differentiation, proliferation, and apoptosis processes (Gaca et al., 2012; Ji et al., 2012; Jin et al., 2013; Huang et al., 2014; Liu et al., 2015). A previous report suggested that miRNA-203 plays an important role in the pathogenesis of many types of cancer (Kapinas et al., 2015). In this study, we investigated the role of miRNA-203 in cervical cancer, based on previous related studies, in order to explore the modulatory mechanism of miRNA in cervical cancer, and provide a theoretical basis for the pathogenesis and targeted therapy of cervical cancer.

MATERIAL AND METHODS

Reagents

Cervical epithelial cells and cervical cancer cells were purchased from ATCC (Manassas, VA, USA). The qRT-PCR pre-mix and RNA isolation kit were purchased from Promega (Madison, WI, USA) and the cell transfection reagents were purchased from Yingjun (Shanghai, China). Cell culture media RPMI1640 and PS were purchased from Gibco (Waltham, MA, USA) and Biyuntian (Beijing, China), respectively. The antibody against survivin and the secondary antibody were purchased from Santa Cruz (Dallas, TX, USA). The remaining reagents were also purchased from Biyuntian. The primers were synthesized and the genes were detected by Yinjun.

Cell culture and transfection

HeLa cells (stored in liquid nitrogen) were thawed, incubated at 37°C for 5 min, and transferred to sterile 1.5-mL EP tubes in a biosafety unit. The cells were centrifuged at 500 g for 3 min, the supernatant was removed, and the cell pellet was suspended in fresh complete 1640 medium with 10% fetal bovine serum. The cells were counted using 10 µL cell suspension. The cells were then cultured on dishes at a concentration of 5 x 10⁶ cells/dish at 37°C and under 5% CO₂ for 24 h. Cells at a confluence rate of 85% were transfected with the miRNA-203 mimic (GUGAAAUGUUUAGGACCACUAG), miRNA-203 inhibitor (GUGAAAUGUUUAGGACCACUAG), or the control (UCACAACCUCCUAGAAAGAGUAGA) synthesized by SwitchGear Genomics (Carlsbad, CA, USA) using Lipo2000 (Biyuntian) according to the manufacturer instructions. Accordingly, the cells were separated into the blank control, mimic, and inhibitor groups. Each group contained three replicates.

Effect of miRNA-203 on cervical cancer cell proliferation

The HeLa cells were transfected with miRNA-203 for 24, 48, and 72 h. The proliferative capacity of transfected HeLa cells was analyzed using the MTT method (Biyuntian) according to manufacturer protocol, using cells cultured in normal medium as the negative control. The absorbance of the cells was detected at an optical density of 490 nm. The cell proliferation rate was calculated using the following equation:

$$\text{Proliferation rate} = \frac{(\text{OD of the control group} - \text{OD of the experimental group})}{(\text{OD of the control group})} \times 100\%$$

Effects of miRNA-203 on cervical cancer cell migration

The effect of miRNA-203 on the migratory ability of cervical cancer cells was determined by a wound healing assay. HeLa cells were cultured on six-well plates with fresh complete medium at a concentration of 1 x 10⁵ cells/well for 24 h. The cells were “wounded” (area without cell growth) with a sterile pipette tip. The cell debris was washed with PBS and the cells were transfected with the miRNA-203 mimic or inhibitor using Lipofectamine2000. The cells were cultured, and the wound distance between cells was detected at 24, 48, and 72 h. Each cell group was analyzed in triplicate and the average value was used.

Effect of miRNA-203 on survivin expression in cervical cancer cells

The survivin RNA and protein was isolated from cervical cancer cells incubated for 24 h, using standard RNA and protein isolation kits. Survivin RNA and protein expression in miRNA-203-transfected cervical cancer cells was measured by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) and western blot. The reaction mixture was composed of 10 μ L qRT-PCR Mix, 0.8 μ L forward primer, 0.8 μ L reverse primer, 1.0 μ g cDNA, and 20 μ L ddH₂O. The reaction conditions were set as follows: initial denaturation at 95°C for 3 min; 40 cycles of denaturation at 95°C for 10 s and amplification at 60°C for 30 s; and a final amplification at 55°C for 10 s. Western blot analysis was performed using primary and secondary antibodies diluted to 1:500 and 1:1000, respectively.

RESULTS

Effect of miRNA-203 on the proliferative ability of cervical cancer cells

Cervical cancer cells transfected with the miRNA-203 mimic showed significantly downregulated proliferation, compared to cells in the control and inhibition groups ($P < 0.05$). However, the cells in the control and inhibition groups showed no significant differences ($P > 0.05$). miRNA-203 over-expression could inhibit the proliferation of HeLa cervical cancer cells (Figure 1).

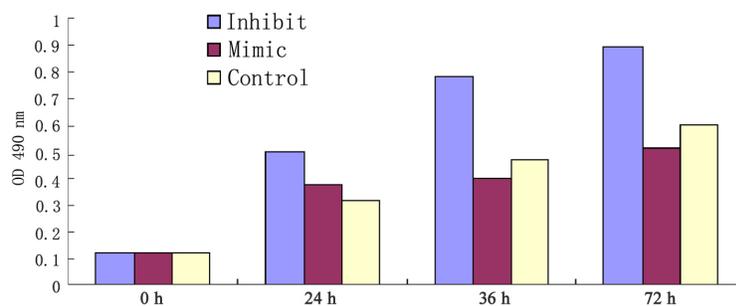


Figure 1. Proliferation ability of HeLa cells.

Effect of miRNA-203 on cervical cancer cell migration

The effect of miRNA-203 expression on the migration ability of cervical cancer cells was determined by a wound healing assay. The wound distance was measured at 24, 48, and 72 h and three replicates were made for each group. The results of this experiment, summarized in Table 1, indicate that the upregulation of miRNA-203 significantly inhibits cervical cancer cell migration, while its downregulation had no effect on the cell migration.

Effect of miRNA-203 on survivin expression in cervical cancer cells

The expression of miRNA-203 in each group is summarized in Table 2. Transfection of miRNA-203 mimic or inhibitor resulted in a significant increase or decrease in miRNA-203

expression in the cervical cancer cells. Moreover, we observed that survivin expression corresponded with mi-RNA-203 expression; that is, variations in miRNA expression induced a change in survivin expression (Figure 2).

Table 1. Cervical cancer cell migration (wound healing assay).

Groups	0 h	24 h	48 h	72 h
miRNA-203 mimic (μm)	35	35.12	35.2	35.34
miRNA-203 inhibitor (μm)	35	34.24	15.88	10.34
Control (μm)	35	30.02	13.36	5.78
P value	>0.05	<0.05	<0.05	<0.05

Table 2. MicroRNA-203 expression in each group of cervical cancer cells.

Groups	0 h	24 h	48 h	72 h
Mimic	1.51 ± 0.31	4.67 ± 0.58	6.47 ± 0.79	8.45 ± 1.34
Inhibit	1.43 ± 0.24	1.76 ± 0.34	3.04 ± 0.34	3.66 ± 0.24
Control	1.38 ± 0.15	2.25 ± 0.34	3.65 ± 0.34	4.45 ± 0.36
P value	>0.05	<0.05	<0.05	<0.05

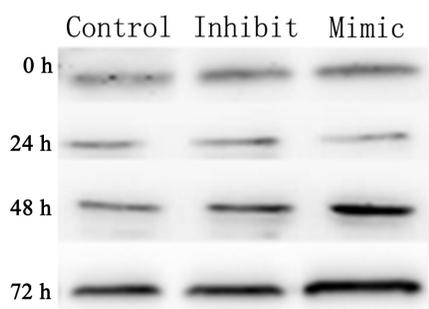


Figure 2. Expression of survivin in each group.

DISCUSSION

miRNA is a non-coding, 22-25 nucleotide-long, single-stranded RNA. miRNA employs two different mechanisms to bind with the target genes because of complementary base pairing. Previous studies have shown that miRNA plays a major role in various cell functions, such as cell metabolism, cell proliferation, cell differentiation, and apoptosis. miRNA has been reported to specifically bind to mRNA and negatively modulate target gene expression. It participates in tumor pathogenesis by modulating the tumor-promoting as well as tumor-inhibitory genes (Saini et al., 2011; Ma et al., 2013). A complete match between miRNA and the mRNA results in degradation of the mRNA. Incomplete binding of miRNA with the 5'-UTR, 3'-UTR, or CDS post-transcription inhibits gene expression, which in turn leads to a decrease in protein expression (Shen et al., 2012; Chen et al., 2013; Singh and Chakrabarty, 2013; Weirauch et al., 2013). Incomplete matching of the miRNA with mRNA is known to modulate the expression of several proteins. In contrast, a protein can also be modulated by various miRNA (Wu et al., 2014; Yu et al., 2014). The technology for miRNA research is currently well established and its modulatory role in human disease has been identified. This

has led to the use of miRNA as clinical biomarkers for early-stage tumor detection and as markers for targeted therapy (Yuan et al., 2015). Therefore, elucidating the modulatory role of miRNA in disease-related genes will provide evidence for disease pathogenesis as well as targets for clinical therapy (Zhang et al., 2012, 2013).

In this study, survivin expression was analyzed as it belongs to the IAP family, which has recently been cloned. Survivin participates in the cell cycle, and is expressed specifically in the G2/M phase. The promoter region of survivin presents a sequence similar to that of the cell cycle-dependent complex. Previous studies have reported that survivin is specifically expressed in tumor tissues. Therefore, it could be an ideal therapeutic target for tumor, which would provide novel avenues for tumor treatment. Previous studies have also reported a close association between survivin gene expression and the pathogenesis of various types of tumors. The changes in survivin expression directly affect tumor radiation therapy. Patients with differing survivin gene expressions present different responses to radiation therapy. Moreover, the change in survivin expression is a major criterion for tumor diagnosis. However, we cannot exclude the involvement of other miRNA-203 target genes, such as *c-jun* and *p63*, in the pathogenesis of cervical cancer; in fact, the major limitation of this study is that the expressions of *c-jun* and *p63* were not measured after transfection with the miRNA-203 mimic and the inhibitor.

In this study, we determined the changes in miRNA-203 expression in cervical cancer cells, and its effects on the proliferation and migration of cervical cancer cells. We discovered that the expression of survivin in cervical cancer cells is modulated by miRNA-203 in combination with the changes in survivin gene expression, which in turn inhibits the proliferation of cervical cancer cells. These results indicated that survivin expression might be correlated with the effects of miRNA-203 on cervical cancer. Therefore, it is essential to investigate its expression and effect on cervical cancer cells. Cervical cancer diagnosis mainly includes techniques such as pathological examination, viral DNA detection, and identification of biomarkers in plasma; the results of this study could further supplement current biomarker detection methods for cervical cancer diagnosis.

Conflicts of interest

The authors declare no conflict of interest.

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