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MicroRNA-138 inhibits proliferation of cervical cancer cells by targeting c-Met

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Abstract. – OBJECTIVE: MicroRNAs (miRNAs) function as important post-transcriptional regulators involved in a wide range of biological behaviors. MicroRNA-138 (miR-138) has been shown to play a critical role in tumor pathogenesis, the present study aimed to investigate the role of miR-138 in cervical cancer.

MATERIALS AND METHODS: CCK-8 assay was performed to measure the viabilities of cancer cells. Quantitative real-time PCR (qRT-PCR) and western blot were used to detect the mRNA and protein expression, respectively. Moreover, the miRNA target genes were validated with luciferase activity assay.

RESULTS: In the current study, we found that the expression of miR-138 was significantly down-regulated in cervical cancer tissues compared to the adjacent non-cancer tissues. CCK-8 assay showed that over-expression of miR-138 suppressed the proliferation of four cervical cancer cell lines including HeLa, SiHa, C33A and CaSki. By contrast, down-regulation of miR-138 promoted the growth of cervical cancer cells. In addition, increased expression of miR-138 led to a reduction in c-Met expression, whereas inhibition of miR-138 enhanced c-Met levels in cervical cancer cells. The luciferase reporter assay showed that c-Met was a direct target of miR-138 in cervical cancer cells.

CONCLUSIONS: These findings demonstrated that miR-138 inhibited cervical cancer cells proliferation via c-Met, providing a novel target for the molecular treatment of cervical cancer.

Key Words:
Cervical cancer, miR-138, c-Met, Proliferation.

Introduction

Cervical cancer has been remained as one of the most frequent malignancies with an estimated global incidence of 470,000 new cases and over 200,000 deaths annually in the world[1]. Multiple causes including high-risk HPV, genetic, epigenetic, and environmental factors all contribute to the pathogenesis of cervical carcinoma[2]. Surgery and radiotherapy are the primary treatment for cervical cancer, and sometimes chemotherapy is performed on advanced patients with metastasis or recurrence[3]. A wealth of information demonstrates that microRNAs (miRNAs) play diverse roles in regulation of tumor initiation, progression and molecular targeted therapy by regulating oncogenes or tumor suppressor genes[4,5]. Thus, it may lead to novel therapeutic targets to elucidate the biological function of miRNAs in cervical cancer.

MiRNAs are a group of small (< 22 nt), noncoding RNA molecules that exhibit different roles in the biological behaviors, such as cell growth, apoptosis, differentiation, and tumor progression[6]. A couple of recent studies have reported the role of miRNAs in modulating cervical cancer cell invasion and metastasis[7,8]. For instance, Deng et al[9] showed that miRNA-142-3p inhibited the proliferation and invasion of cervical cancer cells by targeting Frizzled 7 receptor (FZD7), suggesting a potential therapeutic approach for cervical cancer[9]. Another study demonstrated that miR-506 served as a tumor suppressor by regulating the hedgehog pathway transcription factor Gli3 in human cervical cancer[10]. MicroRNA-138 (miR-138) has been reported to be down-regulated in different cancers including aggressive papillary thyroid carcinoma, head and neck squamous cell carcinoma, and lung cancer tumors[11,12]. Moreover, miR-138 suppresses ovarian cancer cell invasion and metastasis by targeting SOX4 and HIF-1a[13]. However, the biological role of miR-138 in cervical cancer has not been explored. Thus, the current study aimed to investigate the expression pattern and biological function of miR-138 in cervical cancer.

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Materials and Methods

Tissue Samples

A total of 24 fresh cervical cancer tissues and 24 non-cancerous cervix tissues were collected from December 2013 to December 2014 in Obstetrics and Gynecology Hospital of Fudan University, China. Written informed consent was obtained from all participants involved in this study. The study was performed in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Obstetrics and Gynecology Hospital of Fudan University, China.

Cell Culture

Human cervical cancer cell lines HeLa, SiHa, C33A and CaSki were obtained from Chinese Academy of Sciences (Shanghai, China). All cell lines used were cultured in DMEM supplemented with 10% FBS (Gibco, Beijing, China) at 37°C and 5% CO_2.

RNA Extraction and Quantitative Real Time PCR (qRT-PCR)

Total RNA was isolated according to the standard procedure using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The miRNA expression was measured using miRNA-specific TaqMan MiRNA Assay Kit (Applied Biosystems, Foster City, CA, USA). PCR reactions were performed on ABI 7500 Real-Time PCR System (Applied Biosystems, USA) with the following conditions: 95°C, 10 min for 1 cycle, then 95°C, 15 sec, 60°C, 1 min for 40 cycles. The U6 small nuclear RNA was used as a loading control. The mRNA expression of c-Met was measured by qRT-PCR on ABI 7500 Real-Time PCR System with GAPDH used as control.

CCK-8 Assay

Tumor cells were seeded in a 96-well plate at a concentration of 5×10^3 cells/well for 24 hours. Then the cultured cells were transfected with miR-138 mimic or inhibitor for 48 h. Then 10 ml of 5 mg/ml CCK-8 was added and incubated in dark at 37°C for another 2 h. The absorbance was determined with the wavelength of 490 nm.

Luciferase Activity Assay

Luciferase reporters were generated based on the firefly luciferase expressing vector pMIR-REPORT (Ambion, Foster City, CA, USA). Cells were seeded in 24-well plates at the density of $5x10^4$ cells per well the day before transfection. Luciferase reporter (500 ng), 50 pmol (miRNA-138 mimic, inhibitor or negative control) and 40 ng of pRL-TK were added in each well. Cells were collected 48 h after transfection and analyzed using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Western Blot

Cultured cells were harvested, washed twice with PBS, and lysed in RIPA lysis buffer. The cell lysates were centrifuged at 12,000×g for 5 min at 4°C. Samples with equal amounts of protein were separated by SDS-PAGE, and then the proteins were transferred onto a nitrocellulose membrane. The membrane was blocked with 5% not-fat milk in TBST and incubated with primary antibodies overnight. The membrance was washed with TBST for three times and incubated with secondary antibodies in TBST at room temperature for 1 h. The proteins were detected using enhanced chemiluminescence (ECL) detection reagents (Roche, Basel, Switzerland).

Statistical Analysis

Data were expressed as mean ± SD and statistical comparisons were performed using one-way analysis of variance (ANOVA). $p < 0.05$ was considered statistically significant.

Results

Down-Regulation of miR-138 in Cervical Cancer Tissue

We first examined the expression level of miR-138 in 24 human cervical cancer samples and paired non-cancerous cervix tissues. As shown in Figure 1, there was a 5-7 fold decrease in the expression level of miR-138 in the 24 tumors than in their paired normal tissues ($p < 0.001$). These data implied that miR-138 may play an important role in the pathogenesis of cervical cancer.

miR-138 Inhibits the Proliferation of Cervical Cancer Cells

Then we evaluated the *in vitro* effects of miR-138 in cervical cancer by transfection of miR-138 mimic/inhibitor into four tumor cell lines (HeLa, SiHa, C33A and CaSki). Then, CCK-8 assay was performed to determine the cervical cancer cell viability. Data showed that transfection with miR-138 mimics significantly sup-

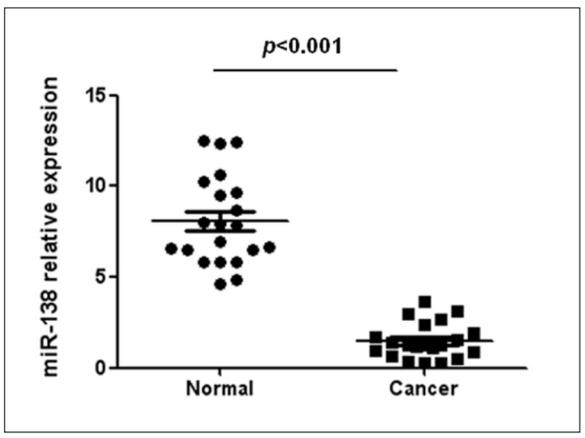

Figure 1. Down-regulation of miR-138 in cervical cancer tissues. Total RNAs were isolated from human cervical cancer tissues and adjacent non-tumor tissues. qRT-PCR was performed to determine the miR-138 expression in human samples.

pressed the proliferation of HeLa cells. On the contrary, miR-138 inhibitor transfection into HeLa cells remarkably enhanced cell viability compared to the control (Figure 2A). In addition, we found that up-regulation of miR-138 inhibited SiHa cell growth, whereas miR-138 down-regulation promoted the proliferation of SiHa cells (Figure 2B). Similar results were also confirmed in C33A (Figure 2C) and CaSki (Figure 2D) cells. These data suggested that miR-138 served as a tumor suppressive miRNA in cervical cancer cells.

miRNA-138 Represses the Expression of c-Met in Cervical Cancer Cells

It is well known that miRNAs is involved in biological activities through regulating genes expression. Therefore, we used the computer-aided Target-Scan database to predict the potential tar-

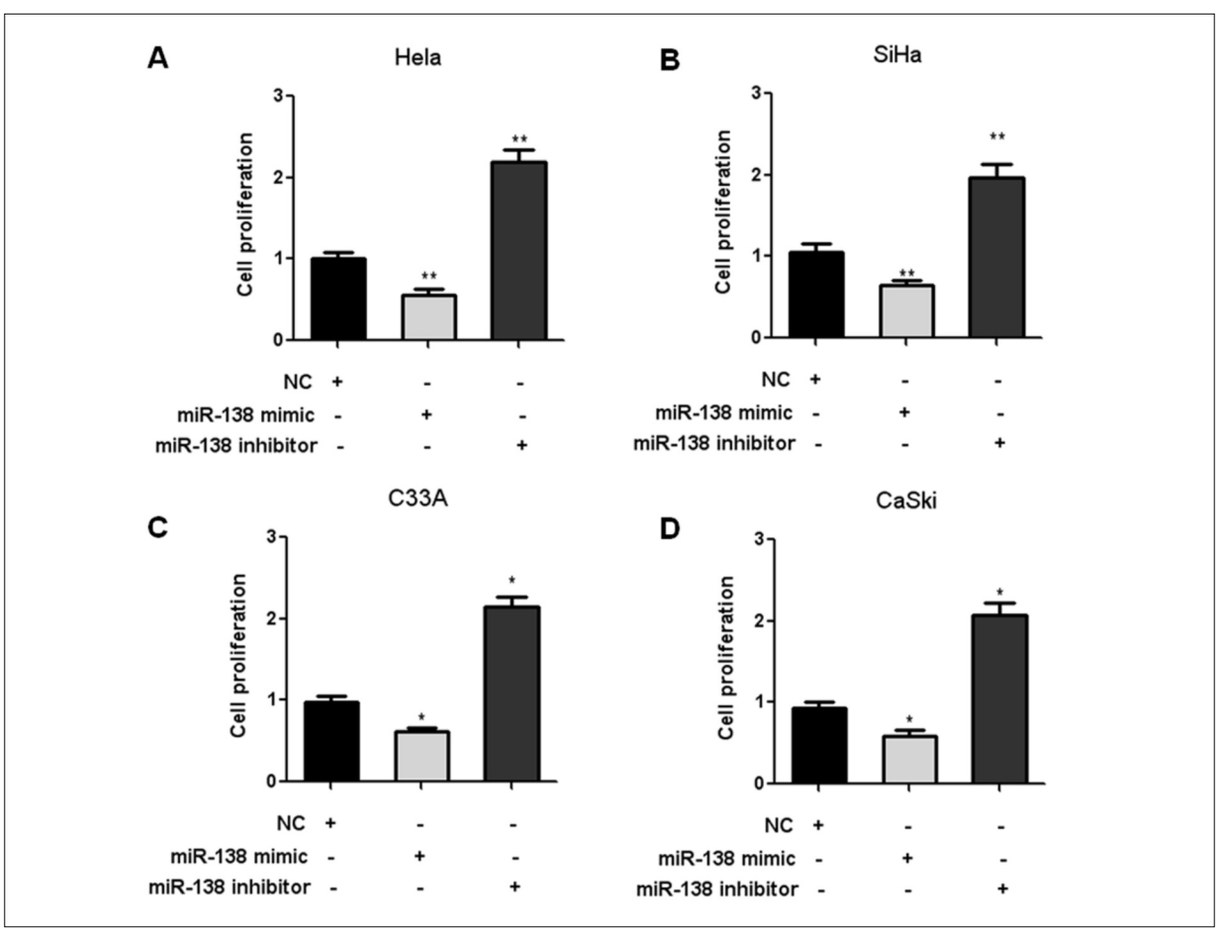

Figure 2. miR-138 inhibited cervical cancer cell proliferation. Cervical cancer cells were transfected with miR-138 mimics and inhibitor using Lipofectamine 2000. CCK-8 assay was applied to measure the proliferation of Hela ***(A)***, SiHa ***(B)***, C33A ***(C)*** and CaSki ***(D)*** cells. *$p < 0.05$; **$p < 0.01$.

gets of miR-138 in humans, and found 3'UTR of c-Met containing the conserved putative miRNA-138 binding sites. Consistent with previous reports, the expression of c-Met was obviously increased in cervical cancer tissues as determined by real time PCR (Figure 3A) and Western blot (Figure 3B and C). To confirm whether miR-138 targeted c-Met *in vitro*, Hela cells were transfected with miR-138 mimic or inhibitor and the mRNA expression of c-Met was measured by qRT-PCR. We found that up-regulation of miR-138 inhibited c-Met expression both at mRNA and protein levels (Figure 4A and B). By contrast, miR-138 inhibitor transfection markedly enhanced c-Met expression in Hela cells (Figure 4C and D). In addition, the firefly luciferase activity was reduced in Hela cells after ectopic expression of miR-138 (Figure 4E). In addition, the miR-138 inhibitor treatment of Hela cells caused a significant increase in luciferase activity (Figure 4F), suggesting that c-Met is a direct target of miR-138 in cervical cancer cells.

Discussion

Cervical cancer is one of the most common diseases and a leading cause of mortality in women worldwide[1]. Exploration of the molecular mechanisms underlying tumorigenesis helps to develop novel therapeutics for cervical carcinoma. In this work, we for the first time explored the expression pattern and biological function of miR-138 in cervical cancer.

miRNAs are a group of small non-coding RNAs that regulate genes expression by targeting mRNAs for translational repression[14,15]. miR-138 has been known to be down-regulated in head and neck, thyroid and lung cancers[11,12]. A number of studies have implicated the functions of miR-138 sensitizing tumor cells to anti-cancer drugs[16,17]. Functional study suggested that miR-138 could inhibit cell migration and invasion by targeting RhoC and ROCK2 in tongue squamous cell carcinoma cell lines[18]. Moreover, a recent study demonstrated that ectopic expression of miR-138 attenuated the Src, FAK,and Erk1/2 signaling pathways through inhibition of RhoC in head and neck squamous cell carcinoma[19]. In the current work, we found that miR-138 was down-regulated in cervical cancer tissues compared to the non-cancerous cervix tissues. In addition, ectopic expression of miR-138 suppressed the proliferation of four cervical cancer cell lines including HeLa, SiHa, C33A and CaSki. By contrast, miR-138 overexpression promoted tumor

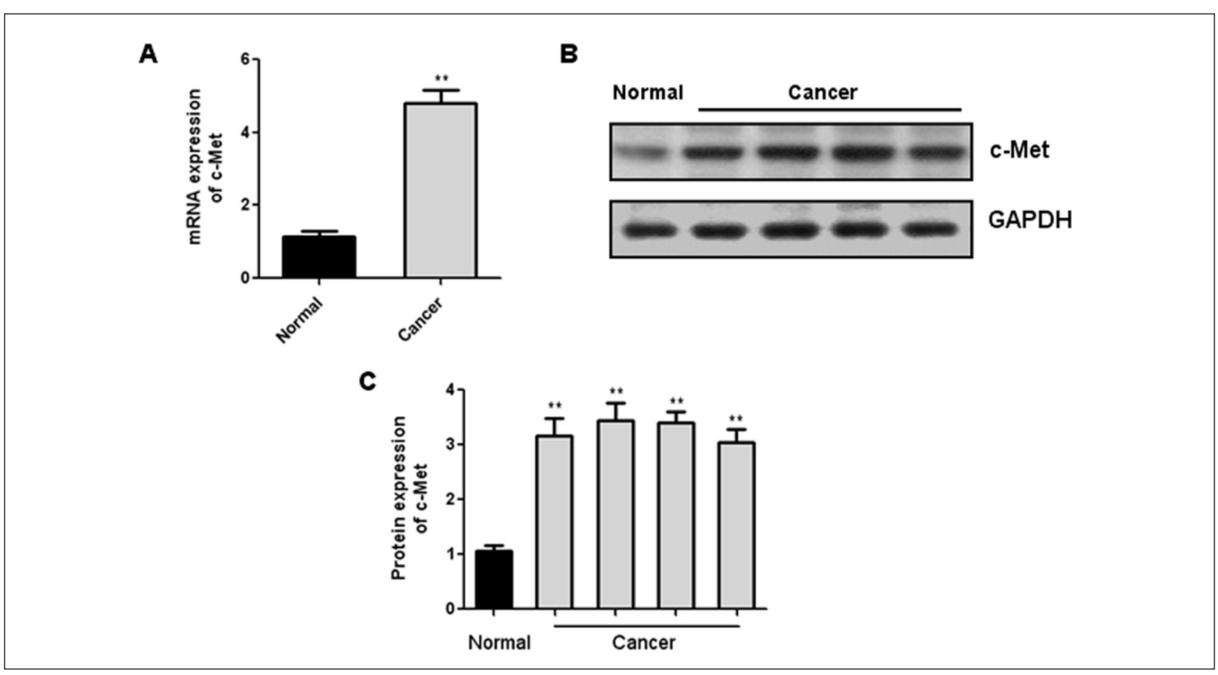

Figure 3. Up-regulation of c-Met in cervical cancer tissues. Total RNAs and proteins were isolated from human cervical cancer tissues and adjacent non-tumor tissues. C-Met expression in cervical cancer and adjacent non-tumor tissues was determined by qRT-PCR ***(A)*** and Western blot ***(B-C)***. $^{**}p < 0.01$.

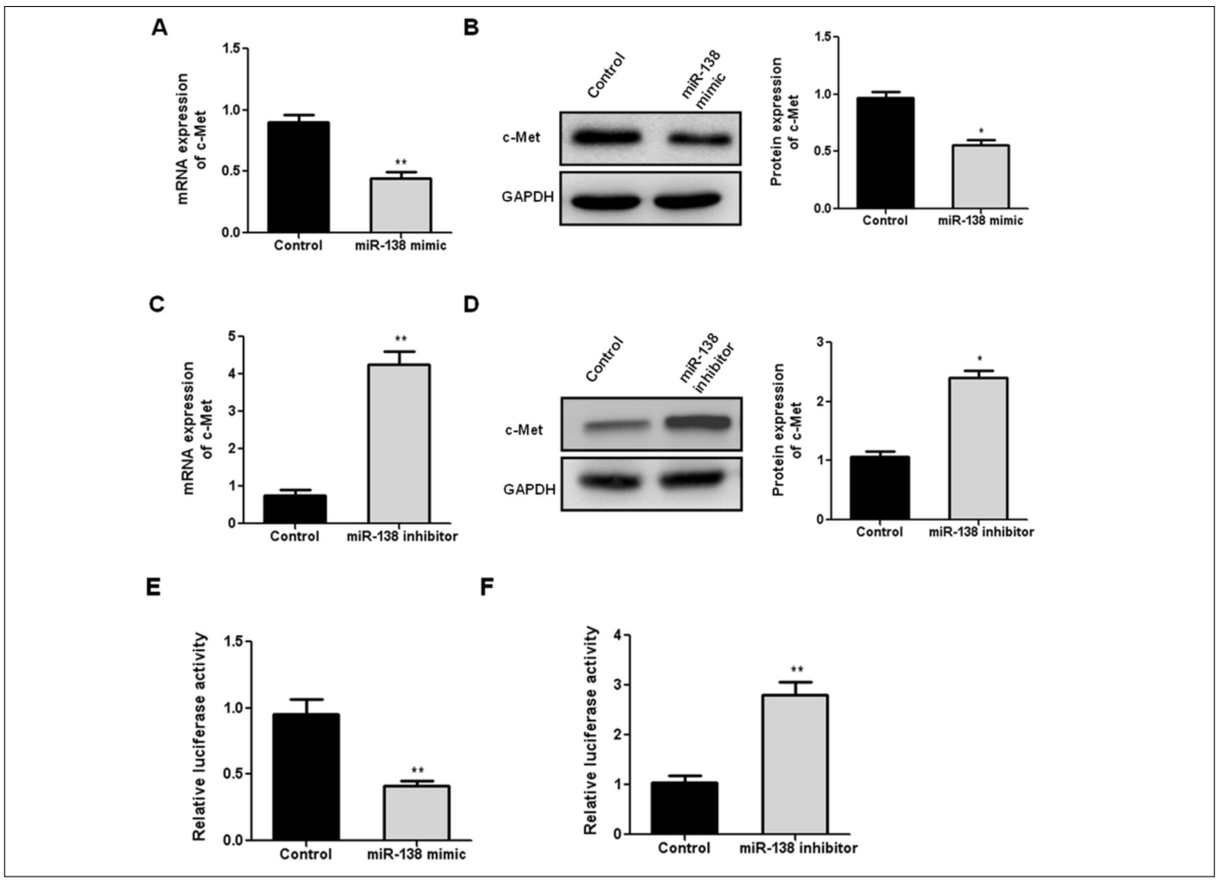

Figure 4. c-Met was a target of miRNA-138 in cervical cancer. Hela cells were transfected with miR-138 mimic ***(A*** and ***B)*** or inhibitor ***(C*** and ***D)*** and the mRNA and protein expression of c-Met was determined by qRT-PCR and western blot. Luciferase activity was measured in Hela cells after transfection with miR-138 mimic ***(E)*** or inhibitor (F). *$p < 0.05$; **$p < 0.01$.

cell growth. Taken together, these data implied that miR-138 functioned as a tumor suppressor miRNA in cervical cancer.

In order to elucidate the molecular mechanism underlying the anti-carcinogenic effect of miR-138 on cervical cancer, we identified c-Met as a potential target of miR-138 with bioinformatics analysis. It has been reported that c-Met is up-regulated in a variety of malignancies such as lung carcinoma, gastric cancer and cervical cancer[20-22]. Overexpression of c-Met could be a potential predictive marker and therapeutic target for patients with advanced cervical cancer[23]. In addition, a large body of research has shown that c-Met promotes the growth and migration of tumor cells via several pathways such as extracellular signal-regulated kinase (ERK) pathways, phosphatidyl inositol 3-kinase (PI3K), and focal adhesion kinase (FAK)[24-26].

Consistent with previous study, we found that the expression of c-Met was significant increased in cervical cancer tissues. In addition, ectopic expression of miR-138 led to a reduction in c-Met expression both at mRNA and protein levels. Moreover, down-regulation of miR-138 obviously enhanced the expression of c-Met in cervical cancer cells. In addition, the firefly luciferase activity was remarkably reduced following transfection with miR-138 mimic. While cells transfected with miR-138 inhibitor exhibited enhanced luciferase activity, suggesting that c-Met is a direct target of miR-138 in cervical cancer cells.

Conclusions

Our study demonstrated that miR-138 inhibited the proliferation of cervical cancer cells by targeting c-Met. These findings indicate that miR-138 may represent a valuable therapeutic target for cervical cancer therapy.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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