European Review for Medical and Pharmacological Sciences 2018; 22: 336-342

miRNA211 induces apoptosis of cervical cancer SiHa cells via down-regulation of inhibitor of apoptosis proteins

Y.-L. LIU[1], G.-Q. WANG[1], H.-X. CUI[1], X.-X. LI[1], Z.-L. XU[1], X.-Y. WANG[2]

[1]Department of Oncology, Binzhou Central hospital, Binzhou, Shandong, China
[2]Department of Obstetrics and Gynecology, Binzhou Central hospital, Binzhou, Shandong, China

Abstract. – OBJECTIVE: Cervical cancer severely threatens patients' lives. MicroRNAs contribute to regulatory function in the growth and apoptosis of cells. The present study investigated the effect of miRNA211 on growth and apoptosis of cervical cancer SiHa cells.

MATERIALS AND METHODS: miRNA211 and control miRNA were synthesized and transfected into cervical cancer SiHa cells. MTT assay and flow cytometry were used to study the effect of miRNA211 on growth and apoptosis of SiHa cells. RT-PCR and Western blot were used to detect the expression of inhibitor of apoptosis proteins (IAPs) at both mRNA and protein levels. Groups of miRNA (NC), miRNA211, miRNA+siRNA IAP, miRNA211+siRNA IAP were established and levels of IAP and caspase 3 from each group were measured after transfection.

RESULTS: After transfection with miRNA211, the growth of SiHa cells was significantly inhibited and apoptosis of SiHa cells was induced, with the reduction of IAPs at both mRNA and protein levels ($p<0.05$). Knockdown of IAPs enhanced the apoptosis of SiHa cells that were induced by miRNA211, while the overexpression of limited the pro-apoptotic effect of miRNA211 on SiHa cells.

CONCLUSIONS: MiRNA211 inhibits the growth of SiHa cells via down-regulation of IAPs.

Key Words:
miRNA211, Inhibitor of apoptosis proteins, Cervical cancer SiHa cells, Apoptosis.

Introduction

The recent incidence of cervical cancer showed an increasing trend, which threatens the health and life of cervical cancer patients[1]. Individualized treatment for cervical cancer has been under clinical practice[2,3]. Different surgical procedures can be chosen according to different stages of cancer, but surgery including hysterectomy is still mainly applied to patients at early phase cervical cancer[4]. Radiotherapy, on the other hand, is generally utilized for patients with advanced cervical cancer but require adjuvant treatment[5], while chemotherapy is adopted for patients with advanced cervical cancer or under the circumstance of recurrent metastasis. Commonly-used chemotherapy drugs include cisplatin and fluorouracil[6,7]. It has been shown that, however, side effects exist in all above-mentioned treatments. Therefore, more effective treatment with fewer side effects is needed in further cervical cancer therapy based on pioneering theoretical study and clinical practice. MicroRNAs (miRNA) belong to the a group of non-coding single-stranded RNA molecules[8,9]. So far, a total of 28645 miRNA molecules have been found[10]. Study showed[11] that miRNA211 regulates the growth and proliferation in a variety of cancer cells, particularly in lung cancer, liver cancer, and colon cancer cells[12,13]. Currently, it is unclear whether miRNA211 can play a regulatory role in cervical cancer cells. Inhibitor of apoptosis proteins (IAPs) are associated to anti-apoptotic factor family[14,15]. IAPs serve the inhibiting function to apoptosis by the suppression of Caspase protein[16-18]. Previous finding demonstrated that IAPs played an important role in the development of colon cancer[19-21]. However, the role of IAPs in cervical cancer remains to be elucidated. Therefore, the purpose of this study is to explore the effect of miRNA211 on the growth and apoptosis of cervical cancer SiHa cells as well as to elucidate the underlying molecular mechanism.

Materials and Methods

Reagents and Cell Lines

MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) was purchased

Corresponding Author: Xiaoyan Wang, MD; e-mail: xiaoyanwangt@sohu.com

from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Apoptosis detection kit and flow cytometry kit were provided from Biyuntian Biotechnology (Beijing, China). Cervical cancer cell line SiHa cells were got from ATCC (Manassas, VA, USA). Dulbecco's Modified Eagle's medium (DMEM) high glucose culture medium and fetal bovine serum (FBS) were from Huamei Biological Technology Co., Ltd., (Luoyang, Henan, China). Anti-IAPs antibody and anti-actin antibody were obtained from Sigma-Aldrich (St. Louis, MO, USA). RNA extraction kit was purchased from Beijing Dingguo Biological Technology Co., Ltd (Beijing, China). miRNA211 and control miRNA were synthesized by Jima Biotechnology Co., Ltd (Shanghai, China). Sequences for miRNA211 and control miRNA are as follows: 5 'AATAACAGAACAGATGTAAGTTGTCAACA 3' and 5 'AGAATTAAGTAGATCAGATACAGTCAACA 3' ; 5'ACCTCATTTACTACACCACACAGTGGCCA3' and 5'ACTGACATCTACTTCACCTGCCAACAGCA3'.

Cell Culture

Cervical cancer cells were cultured according to previous published method[9]. Transfection was performed when cells reached 80% confluence.

MTT test

MTT test was performed according to conventional methods[10,11]. SiHa cells were inoculated into 24-well plates and cultured at 37°C incubator with 5% CO_2. MTT solution (5 mg/ml) was added to each well 24 h after inoculation and incubated for 24 h. Dimethyl sulfoxide (DMSO) (100 µl) was added to each well to stop reaction. Optical density at 492 nm was recorded using a microplate reader to draw a growth curve of SiHa cells[11].

Transfection

miRNA211 and control miRNA sequences are as follows:

5 'CAGATGTAAGTAATAACAGAATGTCAACA 3' and

5 'GATCAGATACAGAATTAAGTAAGTCAACA3';

5'ACTACACCACACCTCATTTACAGTGGCCA3' and

5'TTCACCTGCCACTGACATCTACAACAGCA3'.

Transfection was performed according to conventional methods[12]. Briefly, cervical cancer SiHa cells were cultured at 37°C with 5% CO_2. Transfection was performed when cells reached 80% of confluence. 1 µg of miRNA or control miRNA was mixed with Lipo2000 and transfected to cells. Cell viability and apoptosis were measured at 24 h after transfection.

Flow Cytometry

Flow cytometry was performed according to conventional methods and turnover of phosphatidylserine was used as an indicator of apoptosis[13]. SiHa cells were collected and re-suspended in PBS. 250 µl of cells of each treatment group was mixed with fluorescein isothiocyanate (FITC)-Annexin V and reaction solution for flow cytometry.

RT-PCR (Reverse Transcription-Polymerase Chain Reaction)

RNA extraction and RT-PCR were performed according to manufacturer's instructions[15]. Briefly, SiHa cells were collected and TRIzol was added to extract RNA. RNA was then reverse-transcripted into cDNA to perform PCR reaction. PCR product was resolved by electrophoresis and the gel imaging system was used to photograph it. ImageJ 2.0 was used to analyze images and quantify band density. Actin was used as internal reference.

Western Blot

Western blot was used to measure the expression level of IAPs[16]. Briefly, SiHa cells were collected, lysed and resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were then transferred to polyvinylidene difluoride (PVDF) membrane. After being blocked with 5% skim milk, membranes were incubated with anti-IAPs antibodies and anti-actin antibodies. After being washed with phosphate-buffered solution tween-20 (PBST), membranes were incubated with secondary antibodies, developed, and fixed. Expression levels of IAPs were analyzed.

Caspase-3 Activity Assay

Caspase-3 activity was detected according to kit instructions[17]. Briefly, cells were collected, lysed, and mixed with chromogenic substrate Ac-DEVD-pNA. A microplate reader was used to record the absorbance of the sample.

Statistical Analysis

SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All data were expressed as mean ± standard deviation. $p<0.05$ was considered statistically significant.

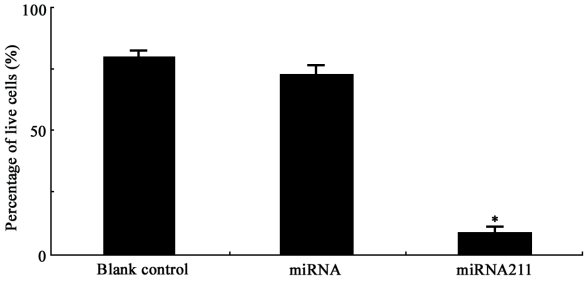

Figure 1. Transfection of miRNA211 inhibited the growth of SiHa cells. *p<0.05, compared with control miRNA group.

Results

Transfection of miRNA211 Inhibited the Growth of SiHa Cells

MTT assay results showed that transfection of miRNA211 significantly inhibited the growth of SiHa cells, compared with that of SiHa cells transfected control miRNA (p = 0.017) (Figure 1).

Transfection of miRNA211 Induced Apoptosis of SiHa Cells

The apoptosis of SiHa cells in-group of miRNA211 was significantly induced, with the increase of phosphatidylserine level, compared to that in miRNA control group (p = 0.0064) (Figure 2). Moreover, under the transfection of miRNA211, the activity of Caspase-3 in SiHa cells was significantly enhanced, compared with that of SiHa cells transfected control miRNA (p = 0.015).

Transfection of miRNA211 Reduced IAPs Expression at both mRNA and Protein Levels

We detected the IAPs level after the transfection of miRNA211. Our data showed that the overexpression of miRNA211 significantly reduced IAPs expression at both mRNA and protein levels, compared with that of SiHa cells transfected control miRNA (Figures 4 and 5).

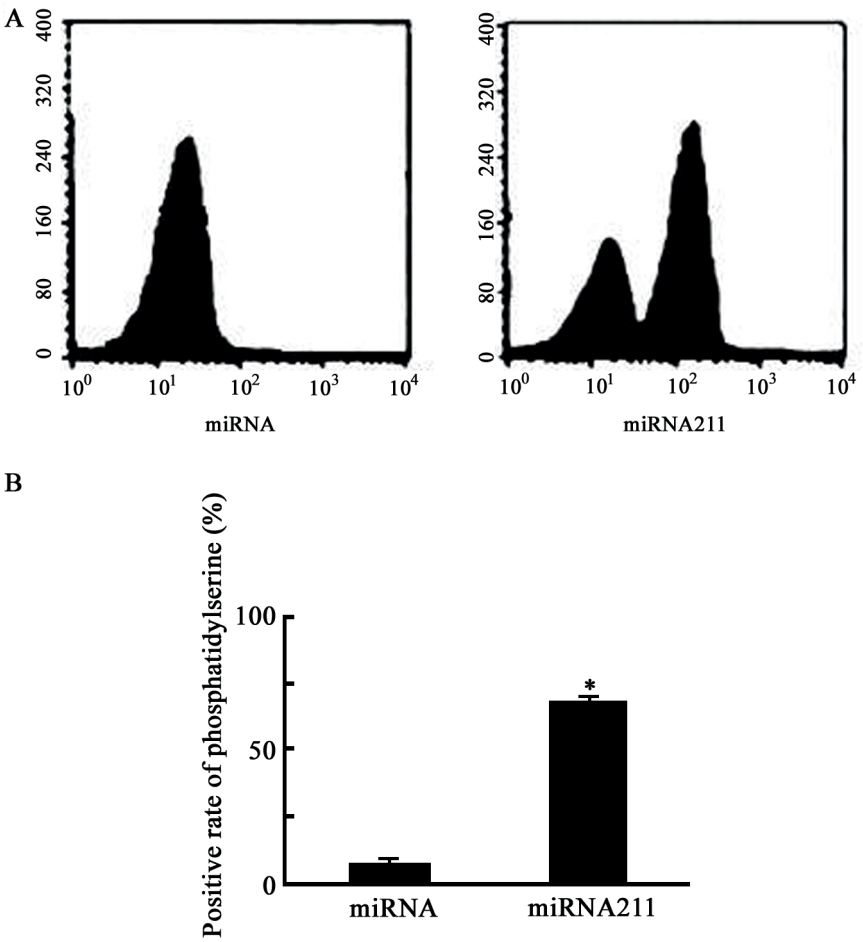

Figure 2. Transfection of miRNA211 induced apoptosis of SiHa cells. *p<0.05, compared with control miRNA group.

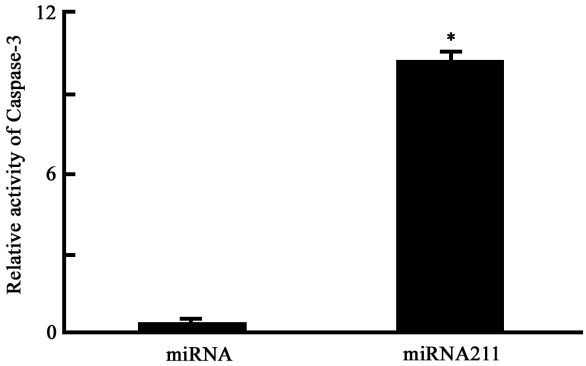

Figure 3. Transfection of miRNA211 activated Caspase-3 in SiHa cells. *p<0.05, compared with control miRNA group.

Silence of IAPs enhanced miRNA211-induced Apoptosis of SiHa Cells

Caspase-3 activity assay result indicated that the decrease of IAPs expression by siRNA transfection significantly increased the pro-apoptoic effect of miRNA211 on SiHa cells, compared to that of SiHa cells transfected with control siRNA + miRNA211 (p = 0.013).

Overexpression of IAPs inhibits miRNA211-induced apoptosis of SiHa cells

Our results also revealed that, in contrast to the effect of siRNA of IAPs, the overexpression of IAPs significantly inhibited the apoptosis of SiHa cells, compared to that of SiHa cells transfected with control miRNA211 (poverexpression 0.0037).

Discussion

Cervical cancer represents a female reproductive system disease[22]. Chemotherapy, radiotherapy and surgery therapy have played a key role in the treatment of cervical cancer[23]. However, it is regrettable that limitations still exist in all the above-mentioned methods[18]. A variety of side effects restrict the extensive application[19]. Emerging molecular targeted therapies shed new lights on the treatment of cancers. Since the pathogenesis and development of cervical cancer are very complicated, it is difficult to screen up a universal target for all types of cervical cancer[24]. The present study attempts to determine potential targets of cervical cancer at cellular levels.

It has been demonstrated that miRNAs conduct to regulatory effect during the development of

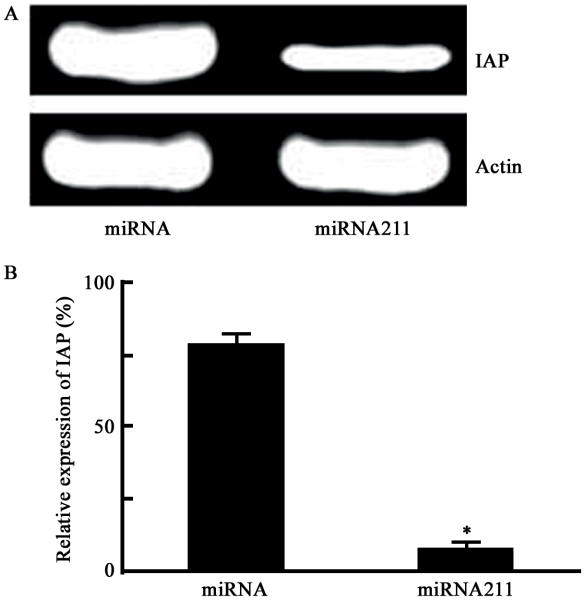

Figure 4. Transfection of miRNA211 reduced IAPs expression at mRNA level. *p<0.05, compared with control miRNA group.

tumors and previous data unraveled that mRNA-338-3p regulated cervical cancer cells proliferation by targeting MACC1 through MAPK signaling pathway[25]. The study on the role of miRNA211 indicated that it regulated the growth and survival of cancer cells including breast cancer and intes-

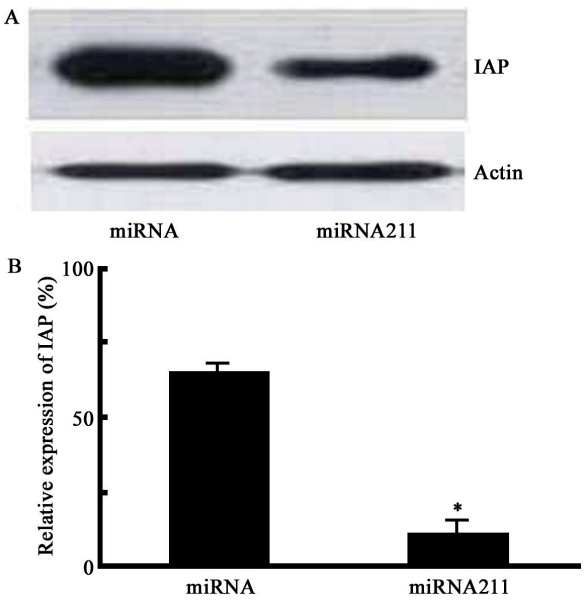

Figure 5. Transfection of miRNA211 reduced IAPs expression at protein level. *p<0.05, compared with control miRNA group.

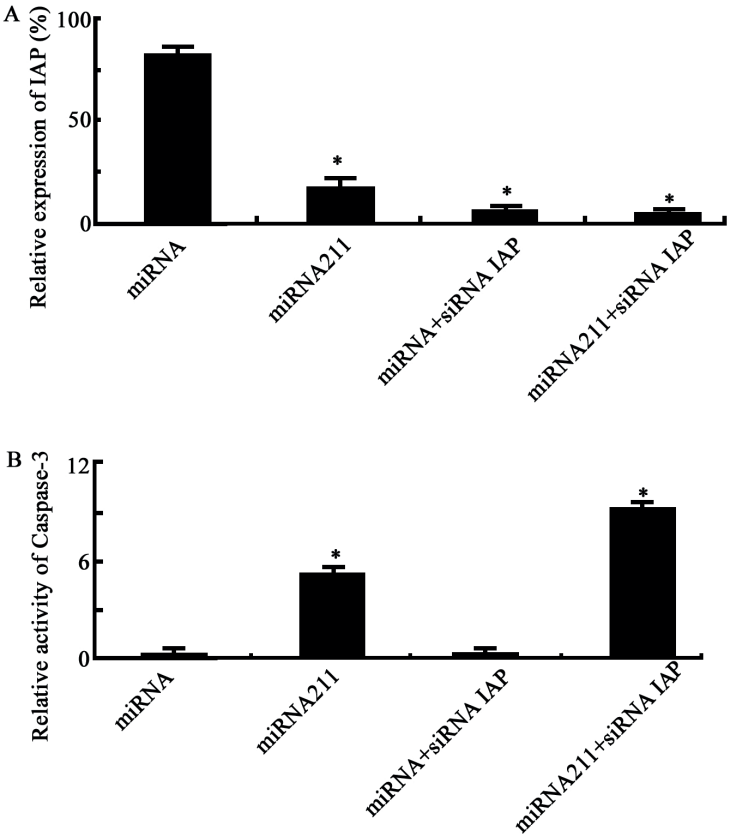

Figure 6. Silence of IAPs enhanced miRNA211-induced apoptosis of SiHa cells. *$p<0.05$, compared with control miRNA group.

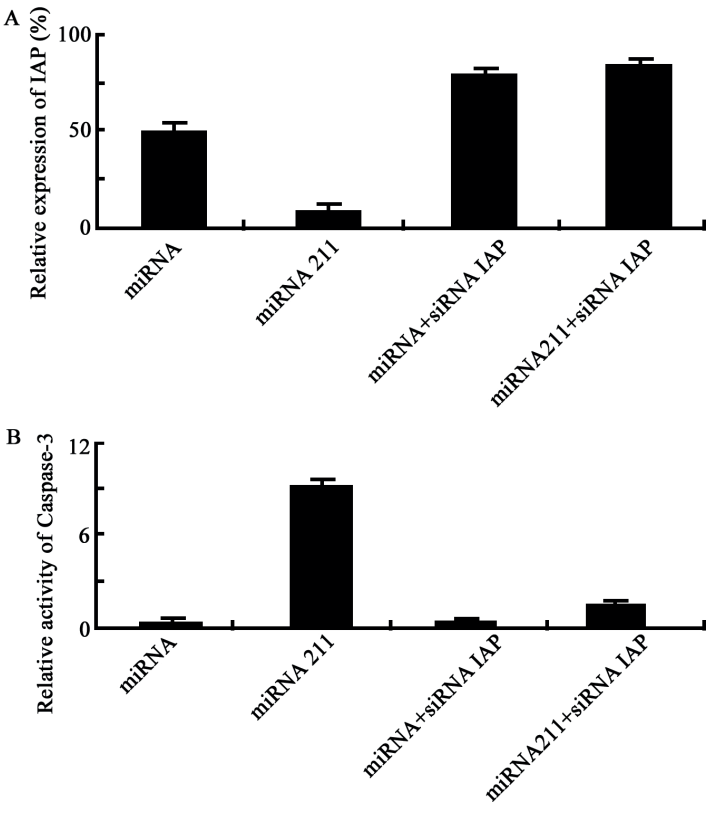

Figure 7. Overexpression of IAPs inhibited miRNA211-induced apoptosis of SiHa cells. *$p<0.05$, compared with control miRNA group.

tinal cancer[26]. However, whether miRNA211 regulates cell growth and apoptosis of cervical cancer, it remains to be further explored. This study explored the possible role of miRNA211 in regulating cervical cancer SiHa cells and found that transfection of miRNA211 inhibited the growth of SiHa cells and promoted apoptosis of SiHa cells, suggesting that miRNA211 also play an important role in regulation of growth and apoptosis of cervical cancer cells, which is consistent with previous study[27,28].

The present study showed that the overexpression of miRNA211 inhibited growth of SiHa cells, decreased IAPs levels, and promoted apoptosis of SiHa cells. By contrast, the down regulation of IAPs promoted the apoptosis of SiHa cells induced by miRNA211. The increase of IAPs level reversed the pro-apoptotic role of miRNA211. All the data suggested that miRNA211 inhibited growth and promoted apoptosis of SiHa cells via down regulation of IAPs levels. IAP is characterized as a new member of the MMP family, which plays a critical role in tumor invasion and metastasis. To our surprise, transfection of miRNA211 caused reduction of IAPs in SiHa cells, suggesting that the apoptosis of SiHa cells induced by miRNA211 is related with IAPs. However, further investigation is required since the role of miRNA211 in cancer tissues and adjacent tissues from patients with different types of cervical cancer ought to be identified.

Conclusions

Our data showed that miRNA211 inhibited the growth of cervical cancer SiHa cells via inhibiting the expression of IAPs.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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