MicroRNA-451a promotes cell proliferation, migration and invasion in cervical cancer cell lines

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Abstract: MicroRNAs (miRNAs) are an important class of small non-coding RNA molecules that can regulate gene expression at the transcriptional or post-transcriptional level. In our study, we aimed to investigate the expression of microRNA-451a (miR-451a) and clarify its biological role in cervical cancer development. The expression of miR-451a in human cervical cancer tissues and cell lines was detected by real-time PCR (qPCR). Furthermore, the effects of miR-451a on cell proliferation, migration and invasion were evaluated, using MTT, migration scratch and transwell assays. We found that the expression of miR-451a was up-regulated in cervical cancer tissues, as compared with paired normal cervical tissues (P<0.05). MiR-451a was also up-expressed in cervical cancer cell lines (Hela, C33A, Caski and SiHa) compared with normal cervical epithelial cell (End1/E6E7). Knock-down of miR-451a in SiHa and C33A cells using a synthesized inhibitor can significantly suppress cell proliferation, migration and invasion in vitro, as compared with a negative control (P<0.05). In the present study, it was determined that miR-451a plays an oncogenic role in cervical cancer development. Silencing miR-451a may be a potential novel therapeutic approach for cervical cancer treatment.

Keywords: microRNA-451a, cervical cancer, proliferation, migration and invasion, treatment

Introduction

Cervical cancer is one of the most common cancers in women worldwide, with an estimated more than 529,000 new cases annually in the world [1, 2]. At present, although early stage diagnosis and treatment over high-risk population have decreased the incidence and mortality of cervical cancer, its poor prognosis is a major public health worldwide concern and results in significant healthcare costs [3, 4]. Although previous studies have found that the high-risk HPV, environmental, genetic, and epigenetic factors, are among the etiological causes contributing to cervical carcinogenesis, the molecular pathogenesis of cervical cancer is complicated and poorly understood [5]. Therefore, further investigations on the molecular pathogenesis of cervical cancer and finding effective biomarkers were imperative to better predict the cancer prognosis.

MicroRNAs (miRNAs) are non-coding 19-23 nucleotide RNA molecules, which are involved in post-transcriptional regulation of gene expression [6, 7]. In fact, miRNAs have already been implicated as regulators of many physiological and pathological processes, including proliferation, differentiation, apoptosis, and tumor progression and metastasis [8, 9]. Accumulating evidences demonstrate that microRNA-451a (miR-451a) is widely dysregulated in many human tumors, including melanoma [10], renal cell carcinoma [11] and breast cancer [12], which suggest miR-451a plays an important role in oncogenesis. Recently, miRNA expression profiles revealed the up-regulation of miR-451a in patients with cervical cancer [13], but its biological role in tumor pathogenesis has not been investigated. Therefore, the aim of the present study is to investigate the expression of miR-451a and clarify its biological role in cervical cancer development.

Materials and methods

Tissue samples and cell culture

Total 30 cases of paired cervical cancer tissues and adjacent non-cancerous cervical tissues
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were collected from May 2011 to May 2014 in the Fourth Affiliated Hospital of Harbin Medical University (Harbin, China). The present study was approved by the Institutional Review Board and Ethical Committee of The Fourth Affiliated Hospital of Harbin Medical University and written informed consent was also obtained from all patients involved in this study. The fresh tissue samples were immediately immersed in RNA later (Qiagen, Hilden, Germany) following surgical resection, stored at 4°C over-night and then frozen in liquid nitrogen and stored at -80°C for further use. Human cervical cancer cell lines (HeLa, Caski, SiHa and C33A) and normal cervical epithelial cells (End1/E6E7) were purchased from American Type Culture Collection (ATCC, VA, USA). All cells were cultured in DMEM medium (Invitrogen, CA USA) supplemented with 10% fetal bovine serum (Invitrogen, CA USA), 100 U/mL penicillin and 100 U/mL streptomycin sulfate at 37°C in a humidified incubator with 5% CO2.

Cells in the logarithmic phase of growth were used for experiment in this study.

RNA extraction and real-time PCR (qPCR)

Total RNA was extracted from cervical cancer tissues and cell lines using TRizol® (Invitrogen, CA USA), according to the manufacturer's instructions. qPCR reaction was performed on the Roche Light-cycler 480 Real-Time PCR System (Roche, AL, USA) with the following conditions: 95°C, 10 min; 95°C, 15 s; and 60°C, 15 s for 40 cycles. The U6 small nuclear RNA was used as a control. The specific primers for miR-451a and U6 are as follows: miR-451a primers forward: 5’-AAACCGTTACCATTACTGAGTT-3’; reverse: miScript SYBR Green PCR kit Universal Primer; U6 primers forward: 5’-CTCGCTTCGGCAGCACA-3’; reverse: 5’-ACGC- TTCAGGAATTTCGCT-3’.

Cell transfection

The inhibitor and negative control of miR-451a were chemically synthesized by GeneCopoeia (Rockville, MD, USA). The sequences were as follows: inhibitor: 5’-AGUAAUGGUAAUGGUUCUCUUGCUA-3’; negative control: 5’-CAGUACUUUGUUGUAACAA-3’. Cells were counted and seeded in plates on the day before transfection to ensure suitable cell confluance. Transfection with the miR-451a inhibitor or negative control was performed using lipofectamine 2000 reagent (Invitrogen, CA, USA) following manufacturer’s protocols.

Cell proliferation assay

The cellular proliferation potency was determined by MTT assay. SiHa and C33A cells were seeded into 96-well plates and transfected with 10 pmol of miR-451a inhibitor or a negative control. After 48 h, 10 µl of MTT reagent (5 mg/ml, Sigma-Aldrich, MO, USA) was added to each well. The cells were incubated at 37°C for another 4 h and solubilized in 150 µl of dimethyl sulfoxide. The optical density (OD) values were measured at 450 nm with a microplate reader (Bio-Rad, CA, USA). The proliferation assay was performed for three days and the cell growth was measured at 24 h intervals. Each experiment was performed in triplicate and repeated three times.

Cell migration assay

The migration scratch assay was used to assess the migratory ability of SiHa and C33A cells. SiHa and C33A cells were seeded into 6-well plates and transfected with miR-451a inhibitor or a negative control. Following 6 h of transfection, confluent cells monolayer were then scraped with a yellow pipette tip to generate scratch wounds and washed twice with media to remove cell debris. Time lapse images were captured after 12 h. Images were captured from five randomly selected fields in each sample, and the wound areas were calculated by NIH ImageJ software (Biocompare, CA, USA). The experiments were performed in triplicate and repeated 3 times.

Cell invasion assay

Cell invasion were examined using transwell assay. 24 h after transfection with the miR-451a inhibitor or a negative control, transfected cells were plated into the top side of polycarbonate transwell filter coated with Matrigel in the upper chamber of the BioCoat™ Matrigel™ Invasion Chambers (Becton-Dickinson Biosciences, MA, USA) and incubated at 37°C for 24 h. The cells inside the upper chamber with cotton swabs were then removed. The cells that had migrated through the matrix to the other side of the insert were fixed, stained with hematoxylin, and counted for 10 random 100× fields per well. Cell counts are expressed as the mean number of cells per field of view. All experiments were performed in triplicate.
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Statistical analysis

SPSS17.0 statistical software package (SPSS, Chicago, USA) was used for statistical analysis. Each experiment was repeated independently at least three times, and the results are expressed as mean ± SD. For comparison of differences, student’s t test or analysis of variance (ANOVA) was used. P<0.05 was considered to be statistically significant.

Results

**MiR-451a is significantly up-regulated in cervical cancer tissues and cell lines**

To further confirm the altered expression of miR-451a in cervical cancer, we detected its expression in 30 pairs of cervical cancer tissues and adjacent normal cervical tissues using qPCR. Consistent with our speculation,
the expression level of miR-451a was significantly up-regulated in human cervical cancers than adjacent normal cervical tissues (Figure 1A). Furthermore, as showed in Figure 1B, miR-451a expression was also increased in cervical cancer cell lines (HeLa, Caski, SiHa and C33A) compared with that of normal cervical epithelial cell (End1/E6E7).

**Up-regulated of miR-451a promotes cell proliferation in cervical cancer**

Considering a significantly increase of miR-451a expression in cervical cancer tissues and cell lines, we hypothesized that a high level of miR-451a might be involved in cancer cell growth. To explore the proliferation of miR-451a in cervical cancer, miR-451a inhibitor or a negative control was transfected into SiHa and C33A cell lines for MTT assays. MTT assay revealed that transfection with miR-451a inhibitor markedly reduced cell growth compared to the control of SiHa and C33A cells (Figure 2). These data indicates that increase of miR-451a promotes cell proliferation in cervical cancer.

**Up-regulated of miR-451a increases cell migration in cervical cancer**

Migration scratch assay was performed to observe the function of miR-451a in cell migration, images of the scratches were captured at 0 and 12 h after transfection. The results demonstrated that the migration of SiHa and C33A cells treated with miR-451a inhibitor group were significantly reduced as compared with
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Figure 3. Up-regulated of miR-451a increases cell migration in cervical cancer. A. MiR-451a inhibitor or negative control-treated SiHa cell was subjected to transwell assays. B. MiR-451a inhibitor or negative control-treated C33A cell was subjected to transwell assays. Data are presented as the mean of three measurements and the bars present the standard deviation of the mean (*P<0.05).

Figure 4. Up-regulated of miR-451a accelerates cell invasion in cervical cancer. A. MiR-451a inhibitor or negative control-treated SiHa cell was subjected to transwell assays. B. MiR-451a inhibitor or negative control-treated C33A cell was subjected to transwell assays. Data are presented as the mean of three measurements and the bars present the standard deviation of the mean (*P<0.05).

Discussion

Cervical cancer is one of the most common cancers and a leading cause of mortality in women worldwide [1, 2]. Despite remarkable advances in diagnostics and treatments, the prognosis of patients with cervical cancer has not improved significantly over the past decades. Therefore, it is imperative to investigate the molecular mechanisms involved in cervical cancer pathogenesis and develop novel potential therapeutic strategy. In the current study, we examined the miR-451a expression and found that miR-451a function as an oncogene in cervical cancer.

MiRNAs function as classical oncogenes or tumor suppressor genes have been identified...
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in many cancers [14-17]. MiR-451a is located on the 17q11.2 chromosome in human genome. MiR-451a has been reported to be up-regulated in colon cancer [18] and lung cancer [19], while down-regulated in osteosarcoma [20] and hepatocellular carcinoma [21]. These dates suggest that miR-451a can function as either a tumor suppressor or an oncogene, which mainly be dependent on the cancer type. However, the expression and function of miR-451a in cervical cancer is still unknown. An understanding of the role of miR-451a in cervical cancer development may provide new insights into the underlying value of diagnosis and therapy for patients in the future.

In this study, qPCR was used to detect the relative expression levels of miR-451a in cervical cancer tissues and cell lines. We found that miR-451a is remarkably up-regulated in cervical cancer tissues and cell lines, which were consistent with previous miRNA microarray chip analysis in cervical cancer [13]. Furthermore, the functions of miR-451a on cell proliferation, migration and invasion were analyzed by using miR-451a inhibitor or negative control into SiHa and C33A cells. The results demonstrated that knock-down of miR-451a expression could inhibit cell proliferation, migration and invasion in cervical cancer. To the best of our knowledge, the results of the present study provide a novel insight into the role of miR-451a in the occurrence and development of cervical cancer.

In summary, our present study suggested that miR-451a was up-regulated in cervical cancer. Moreover, down-regulation of miR-451a could inhibit cell proliferation, migration and invasion in cervical cancer. These finds may provide the therapeutic potential of miR-451a in the treatment of cervical cancer. Further research should be devoted to illustrate the target genes of miR-451a in cervical cancer.

Disclosure of conflict of interest

None.

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References


[14] Li J, Fang L, Yu W and Wang Y. MicroRNA-125b suppresses the migration and invasion of he-


