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MiR-26a-5p potentiates metastasis of human lung cancer cells by regulating ITGβ8- JAK2/STAT3 axis

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ABSTRACT

Most lung cancer patients die of metastasis. Recent studies have indicated that dysregulated microRNAs (miRNAs) are involved not only in tumorigenesis, but also in metastasis. In the present study, we found that over-expression of miR-26a-5p potentiated the migration and invasion of lung cancer cells evidenced by wound healing assay and transwell assay, and metastasis-related genes MMP-9 and CD44 were up-regulated. We identified integrin-beta8 (ITGβ8) as a novel target of miR-26a, and ITGβ8 expression was negatively correlated with miR-26a-5p expression in lung cancer specimens. Mechanism study showed that miR-26a-5p enhanced lung cancer cell metastasis via activation of Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway, and ITGβ8 mediated the activation of JAK2/STAT3 pathway by miR-26a-5p. By using *in vivo* imaging technology, we found that miR-26a-5p enhanced both tumor growth and metastasis *in vivo*; and activated JAK2/STAT3 pathway. Taken together, our results demonstrated that miR-26a-5p potentiated lung cancer cell metastasis via JAK2/STAT3 pathway by targeting ITGβ8. This finding provides insights into the mechanism underlying miRNAs regulation on lung cancer metastasis; and suggests miR-26a-5p as a therapeutic target for lung cancer treatment.

1. Introduction

Lung cancer is the most frequently diagnosed cancer [1]. There are 1.8 million new cancer cases (18.9% of total) and 1.6 million lung cancer death (19.4% of total) [2]. Approximately 80% of lung cancer are non-small cell lung cancer (NSCLC). Nearly 90% of lung cancer patients die of metastasis. There are several steps are involved in metastasis cascades [3], however, the mechanism of cancer metastasis is still poorly understood.

MiRNAs are an abundant class of endogenous small non-coding RNAs of 20–22 nucleotides, which post-transcriptionally regulate gene expression. MiRNAs play important regulatory roles in biological processes through interacting with 3′-untranslated regions (3′-UTR) of mRNAs for cleavage and translational repression. MiRNAs are predicted to regulate more than 60% gene expression and play important roles in most biological process in cells, including cell proliferation, differentiation and apoptosis [4].

Numerous studies reported that abnormal expression of miRNAs occurs in almost every type of cancers, and miRNAs play different roles in different types of cancer as oncogenes or tumor suppressors. Recent evidences have indicated that miRNAs are involved in tumor metastasis. For example, miR-367 promotes proliferation and metastasis of clear-cell renal cell carcinoma by targeting MTA3 [5]. In contrast, miR-133 b suppresses metastasis of human colorectal cancer by targeting HOXA9 [6].

In this study, we investigated the effect of miR-26a-5p on cell migration, invasion and expression of metastasis-related genes. We also identified ITGβ8 as a direct target of miR-26a-5p. Furthermore, we examined its effect on tumor growth in mice model.

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2. Materials and methods

2.1. Materials

The miR-26a mimics, inhibitor, and the siRNA for ITGβ8 were purchased from Genepharma (Shanghai, China). The antibody against ITGB8 was purchased from Abcam (Cambridge, UK), the antibody against p-JAK2, STAT3, p- STAT 3, MMP-2, C-myc, β-actin were purchased from Cell Signaling (Beverly, MA). The secondary antibodies coupled to HRP were purchased from ZSGB-BIO (Beijing, China). Reverse transcription and real-time PCR kit were purchased from TaKaRa Biotechnology (Dalian, China).

2.2. Cell lines and clinical specimens

The human lung cancer cell lines A549, H1299 and H661 were purchased from the American Type Culture Collection (Manassas, VA). The A549 cells were grown and maintained in DMEM medium, the H1299 and H661 cells were grown and maintained in RPMI-1640 medium at 37 °C, 5% CO_2. The culture medium was supplemented with 10% fetal bovine serum.

Lung cancer specimens were obtained from the Tianjin Medical University General Hospital. The informed consents were obtained from patients. The study has been approved by hospital's Ethical Review Committee.

2.3. Cell proliferation assay

The cells were seeded in 96-well plates at an initial density of 1×10^4 cells/mL. Cells were incubated with miR-26a-5p mimics or inhibitor for 48 h. The cell viability was determined by the Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) following the manufacturer's instruction.

2.4. Wound healing assay

Cell migration ability was assessed by the wound healing assay as previously described [7]. 2×10^5 cells were added to the 6-well plate, cultured to 60–80% confluence A plastic pipette tip was used to generate a clean wound area across the well when cells grown to 100% confluence. Cells were allowed to migrate in the medium. The wound was examined by a microscope (Nikon, Tokyo, Japan) at 40 × magnification after 48 h.

2.5. Cell invasion assay

Quantitative cell invasion assays were performed using a chamber (Corning, Tewksbury, MA) with 8.0 μm polycarbonate filter inserts in 24-well plates as described before [8]. Briefly, 1×10^5 cells suspended in culture medium with 5% fetal bovine serum were added to the upper chamber, and the lower chamber was filled with cell medium with 10% fetal bovine serum. Cells were cultured for 48 h. The invaded cells were stained with crystal violet, photographed under a microscope and counted.

2.6. Quantitative PCR

Total RNA was extracted from cells using Trizol (Invitrogen, Carlsbad, CA). Quantitative PCR reagents were purchased from TaKaRa Biotechnology Co. PCR reactions were performed as preciously described [9]. The primers for CD44: forward GACTCTGCCTCGTGCCG, reverse GGTCTAAATCCGATGCTCA. For MMP-9: forward CGGCATTCAGGGAGA, reverse CGAGTTGGAACCACGAC. For GAPDH: forward TGCACCACCAACTGCTTAGC, reverse GGCATGGACTGTGGTCATGAG. The miR-26a-5p expression was detected using a Hairpin-it miRNAs qPCR Quantitation kit (GenePharma), following manufacturer's instruction. U6 was used for normalization.

2.7. Western blotting analysis

Western blotting analysis were performed as previously described [10]. Briefly, cell pellets were prepared in RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China). Lysates were separated on SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked for 1 h at room temperature with 5% milk in 0.1% Tween 20 in PBS, then were probed with primary antibodies at 4 °C overnight. After washing, the membranes were probed with HRP-conjugated secondary antibody. The blots were developed with the Phototope HRP Western Blot Detection system (Cell Signaling).

2.8. Transfection and luciferase assay

Wild-type and mutant 3′-UTR of human ITGβ8 were amplified and cloned into pGL3 luciferase vector (Promega, Madison, WI). ITGβ8 were amplified and cloned into the pcDNA3.1 (+) vector (Agilent Technologies, Santa Clara, CA). Lipofectamine 2000 (Invitrogen, Carlsbad, CA) was used for cell transfection. Luciferase assay were performed using the Dual-luciferase Reporter Assay System (Promega). For the RNA interference, cells were transfected with 100 nM siRNA duplex for ITGβ8: sense 5′-CACCAUUGCUGGUGAAAUATT-3′, anti-sense: 5′-UAUUUCACCAGCAAUGGUGTT-3' (GenePharma).

2.9. Xenograft mouse model

Female BALB/c nude mice (4–5 weeks old) were maintained in a climate control environment with food and water. The experiments were conducted in accordance with the Tianjin Medical University Institutional Animal Care and Use Committee guidelines. Mice were inoculated with injection of 3×10^6 A549 cells (control group) or A549-miR-26a cells (experiment group). The experiment had been performed for 8 weeks. Tumor growth was detected using IVIS Imaging System (Xenogen Corporation, Alameda, CA). Tumor tissues were dissected and used for PCR and Western blot analysis.

2.10. Statistical analysis

All data were presented as the mean ± SD. The variance analysis between groups was performed using a one-way analysis of variance (ANOVA). The significance of differences between the experiment and control groups was analyzed using Dunnett's multiple comparison test. Statistically significant differences were indicated as $^{*}p < 0.05$, $^{**}p < 0.01$.

3. Results

3.1. MiR-26a-5p had no effect on lung cancer cell proliferation

In order to evaluate the role of miR-26a in lung cancer tumorigenesis, A549 (adenocarcinoma), H1299 (carcinoma) and H661 (large cell carcinoma) cells were transfected with miR-26a-5p (100 nM and 200 nM). The effect of miR-26a on cell growth was assessed. Fig. 1A–B showed that both miR-26a-5p and its inhibitor have no effect on lung cancer cell growth.

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