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Circular RNA circMAN2B2 facilitates lung cancer cell proliferation and invasion via miR-1275/FOXK1 axis

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ABSTRACT

Lung cancer remains a leading cause of cancer-related deaths worldwide. In the past years, increasing reports indicate that circular RNAs (circRNAs) exert a great important role in human cancers, including lung cancer. However, the knowledge about circRNA in lung cancer remains very little so far. In the present study, we screened out a highly expressed novel circRNA named circMAN2B2 in lung cancer tissues. We investigated the function of circMAN2B2 and found that circMAN2B2 knockdown significantly inhibited cell proliferation and invasion in both H1299 and A549 lung cancer cells. Mechanistically, we found that circMAN2B2 could sponge miR-1275 to inhibit its level. Through a series of functional experiments, we dissected the role of miR-1275 in lung cancer and proved the anti-tumor role of miR-1275. Furthermore, we found that miR-1275 exerted its role in lung cancer by regulating FOXK1 expression. In addition, we demonstrated that restoration of FOXK1 could rescue circMAN2B2 knockdown-induced repression of cell proliferation and invasion. Taken together, our study demonstrated that circMAN2B2 acts as an oncogenic role in lung cancer through promoting FOXK1 expression by sponging miR-1275.

1. Introduction

Lung cancer is one of the most common cancers and leads to the most cancer-related deaths around the world [1]. To date, the major approach for lung cancer treatment is chemotherapy. However, because of the aggressive nature and lack of effective diagnostic biomarker, the outcomes of lung cancer patients remain very unsatisfactory [2]. And the five-year survival rate of lung cancer patients is quite low [3]. Hence, in order to achieve more improvements on lung cancer intervention, it is momentous to determine the underlying molecular mechanism of lung cancer development and progression.

In the past decades, due to the rapid development of high-throughput sequencing technology, more and more noncoding RNAs (ncRNAs), such as long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs), are identified in various tissues or cells [4]. Although the functions of lncRNAs in tumors have been widely explored [5,6], the roles of circRNAs still remain largely uncovered. Emerging evidence implies that circRNAs are closely related with human cancers [7]. Several reports show that many circRNAs are aberrantly expressed in cancer tissues and regulate tumor progression [4,8,9]. A study suggests that circRNAs might be promising diagnostic markers and therapeutic targets for cancer treatment [9]. Thus, it is necessary to explore the physiological role of circRNAs in cancer. In lung cancer, a few of circRNAs has been identified as oncogenes, such as hsa_circ_0014130 [10], hsa_circ_0007385 [11] and circRNA_100876 [12]. However, most circRNAs in lung cancer need to be identified and their functions require to be investigated.

circMAN2B2 (circRNA ID: hsa_circRNA_103595) is a product of MAN2B2 mRNA splicing and consists of two exons of 291 nucleotides in length. The function of circMAN2B2 has not been defined. In our study, we found that circMAN2B2 expression was significantly upregulated in lung cancer tissues compared to normal lung tissues by bioinformatics analysis and qRT-PCR. Moreover, we found that circMAN2B2 knockdown suppressed the proliferation

Abbreviations: CircRNAs, Circular RNAs; miRNAs, microRNAs; 3′-UTR, 3′-Untranslated Regions.

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and invasion of lung cancer cells. In mechanism, we found that circMAN2B2 promoted FOXK1 expression by sponging miR-1275 in lung cancer cells. In conclusion, our findings demonstrated the key function and mechanism of circMAN2B2/miR-1275/FOXK1 signaling in lung cancer cell progression.

2. Materials and methods

2.1. Patient samples

Tissue samples of lung cancer and paired normal tissues were obtained from patients who received surgical treatment at First Affiliated Hospital of Jiamusi University. All of the patients enrolled in this study had not undergone previous surgery, radiotherapy and chemotherapy. This study was approved by the Human Research Ethics Committee from First Affiliated Hospital of Jiamusi University. Informed consent was obtained from all patients.

2.2. Cell culture and transfection

The human lung cell line BESA-2B and four lung cancer cell lines (A549, H226, H1299 and H446) were purchased from American Type Culture Collection (ATCC) and were cultured in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco). Cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2.

The small interfering RNAs (siRNAs), miR-1275 mimics, miR-1275 inhibitors and negative controls used for cell transfection were synthesized by GenePharma Co. Ltd. (Shanghai, China). The siRNAs and miRNAs were transfected into lung cancer cells using Lipofectamine 2000 Reagent (Life Technologies).

2.3. Cell proliferation assay

Cell proliferation was detected by using the Cell Counting Kit-8 (7 sea biotech, Shanghai, China). Cells were grown in 96-well plates with 1×10^4 cells per well and incubated at 37 °C in 5% CO_2 until cell confluence reached 70%. After 48 h of plasmid transfection, cells were incubated for an additional 48 h. A volume of 10 μL of CCK8 solution was added into each well. The absorbance at 450 nm was measured with the SUNRISE Microplate Reader (Tecan, Switzerland).

2.4. Transwell invasion assay

Transwell invasion assay was evaluated using Matrigel invasion chambers (BD Biosciences, Franklin Lakes, New Jersey, USA) based on the manufacturer's specifications. Transfected cells (5×10^4 cells/well) suspended in serum-free RPMI-1640 medium was seeded into the upper chamber, while the complete RPMI-1640 medium with 5% FBS was placed into the lower chamber. After 24 h of incubation, cells remaining on the upper membrane were removed carefully, and adherent to underside of the membrane were fixed and stained with 0.5% crystal violet (Sigma, Santa Clara, CA, USA), followed by photographed and counted under a microscopy.

2.5. Reverse transcription and quantitative real-time PCR (qRT-PCR)

The RNA was extracted from tissues or cultured cells with Trizol reagent according to the manufacturer's protocol (Life Technologies, Scotland, UK). Then, 500 ng of total RNA was reverse transcribed in a final volume of 10 μl using random primers and standard conditions with the Prime Script RT Master Mix. Then, we performed quantitative real-time polymerase chain reaction (qRT-PCR) using the SYBR Select Master Mix (Applied Biosystems) with 0.5 μl complementary DNA (cDNA) on the ABI7300 system (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. We used GAPDH and U6 as internal controls and tested circMAN2B2, miR-1275 and FOXK1 expression levels by qRT-PCR.

2.6. Luciferase reporter assay

Partial sequences of circMAN2B2 and FOXK1 3′ untranslated region (3′UTR) containing wide-type or mutant-type miR-1275 binding sites were cloned into the pGL3-Basic luciferase vector (Promega, Madison, WI, USA) to generate circMAN2B2-WT, circMAN2B2-Mut and FOXK1-3′UTR-WT (WT), FOXK1-3′UTR-Mut (Mut). Then the constructed luciferase vectors were respectively transfected into lung cancer cells along with pRL-TK vector (Promega) and NC mimic or miR-1275 mimics. Then dual-luciferase Reporter assay system (Promega) was used to detect luciferase activity in the cells lysates 48 h post-transfection.

2.7. Statistical analysis

All data are shown as mean ± standard deviation (SD). Student's t-test and one-way ANOVA analysis were used to estimate significant difference of different groups. $P < 0.05$ represented the difference was statistically significant.

3. Results

3.1. CircMAN2B2 knockdown suppresses lung cancer cell proliferation and invasion

In order to identify important circRNAs in lung cancer development and progression, we first analyzed an online microarray data (GSE101586) and screened out the most upregulated has_circRNA_103595 in lung cancer tissues compared to normal lung tissues (Fig. 1A). We named has_circRNA_103595 as circMAN2B2 because it comes from MAN2B2 mRNA splicing. As shown in Fig. 1B, circMAN2B2 expression was significantly upregulated in lung cancer tissues according to this dataset (GSE101586). To validate it, we further analyzed circMAN2B2 expression in 41 pairs of lung cancer tissues and adjacent normal tissues. We found that circMAN2B2 expression was markedly upregulated in lung cancer tissues compared to adjacent normal tissues (Fig. 1C). Consistently, qRT-PCR analysis also showed that circMAN2B2 possessed higher levels in lung cancer cell lines (H1299, H446, H226 and A549) than in human lung cell line BESA-2B (Fig. 1D).

To explore the function of circMAN2B2 in lung cancer, we knocked down circMAN2B2 in H1299 and A549 cells by transfection with specific siRNA against circMAN2B2. qRT-PCR results indicated that circMAN2B2 expression was significantly decreased in H1299 and A549 cells after transfection (Fig. 1E). We then used CCK8 and transwell invasion assays to analyze whether circMAN2B2 knockdown affects lung cancer cell proliferation and invasion. CCK8 assay showed that reduced expression of circMAN2B2 markedly suppressed cell proliferation in both H1299 and A549 cells (Fig. 1F). Moreover, transwell invasion assay indicated that circMAN2B2 depletion strikingly repressed H1299 and A549 cell invasion (Fig. 1G). In collection, these findings implied that circMAN2B2 silence inhibited the proliferation and invasion of lung cancer cells.

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