MicroRNA-410 promotes cell proliferation by targeting BRD7 in non-small cell lung cancer

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1. Introduction

Lung cancer is the most common type of cancers and the leading cause of cancer-related mortality worldwide. Non-small cell lung cancers (NSCLC), including adenocarcinoma and squamous cell carcinoma, account for approximately 85% of all lung cancer cases [1–4]. Despite recent advances in clinical and experimental oncology, the prognosis of NSCLC remains very poor with the 5-year survival rate a dismal 11% [5–8]. Therefore, a better understanding of the detailed mechanisms of NSCLC is urgently needed.

MicroRNAs (miRNAs) are a class of single-stranded, small non-coding RNAs that play important roles in all biological processes through base pairing with the 3′ untranslated region (3′UTR) of target mRNAs [9–13]. Increasing evidences show that aberrant expression of miRNAs plays significant roles in diverse biological processes such as development, differentiation, growth, and metabolism [13–16]. Meanwhile, deregulations of miRNAs are observed in a wide range of cancers, including breast cancer, gastric cancer, hepatocellular carcinoma, bladder cancer, osteosarcoma and glioblastoma [17–23]. MiRNAs can function either as tumor suppressor genes or oncogenes during tumor development and progression [23–25].

Deregulated expression of miR-410 was found in various cancers, suggesting that miR-410 might play an important role in tumor development and progression [26–29]. Previous result showed that the expression of miR-410 was associated with disease free survival of the non-MYCN-amplified favorable neuroblastoma [27]. Furthermore, miR-410 was reported to negatively regulate Rb/E2F pathway by directly targeting CDK1 in breast cancer [30]. However, the role of miR-410 in NSCLC is still unknown. In this study, we found that the expression of miR-410 was increased in NSCLC tissues compared with the matched normal lung tissues. Moreover, overexpression of miR-410 promoted cell proliferation, migration and invasion in NSCLC. Furthermore, bromodomain-containing protein 7 (BRD7) was identified as a new direct target of miR-410. These results suggested that high expression of miR-410 might be involved in NSCLC carcinogenesis.

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2. Materials and methods

2.1. Ethics statement

Written informed consent was obtained from all patients. All experimental procedures were approved by the ethical board of the institute of Chest hospital of Hebei province and complied with the Declaration of Helsinki.

2.2. Tissue specimens and cell lines

NSCLC tissues and their corresponding adjacent non-tumor lung tissues were obtained in our hospital from 2011 and 2013. No patients had received blood transfusion, radiotherapy, or chemotherapy before surgery. Tissue samples were immediately snap-frozen in liquid nitrogen and stored in liquid nitrogen until RNA extraction. Four Human NSCLC cell lines (A549, SPC-A1, H1299, and H1650) and normal bronchial epithelial cell line (16HBE) were obtained from the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in DMEM media (Invitrogen, Carlsbad, CA, USA), which contained 10% FBS at 37 °C with 5% CO2.

2.3. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis

Total RNA was extracted from the cultured cells or tissues using TRIzol reagent (Invitrogen, USA). Relative expression of miR-410 was examined using SYBR green real-time PCR and normalized with U6 snRNA. The primers of miR-410, U6 snRNA and miScript SYBR Green PCR kit were collected from Qiagen for real-time PCR. The real-time PCR reactions were performed on a 7500 Fast System real-time PCR cycler (Applied Biosystems, USA) in accordance with manufacturer’s instructions.

2.4. Western blot analysis

Western blotting was performed as previously described [15]. Membranes were probed with mouse polyclonal antibodies (anti-AKT, anti-p-AKT, and anti-BRD7 antibody) (R&D Systems, USA) at 4 °C overnight or mouse monoclonal anti-GAPDH antibody (Beyotime, China) for 1 h at 37 °C. Then the membranes were incubated with the horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG antibody (ZSGB-BIO, China). Protein bands were visualized using an enhanced chemiluminescence kit (Amersham, Little Chalfont, UK).

2.5. Oligonucleotides and transfection

miR-410 mimics, scramble and inhibitor control were synthesized by Dharmacon and transfected with Lipofectamine 2000 reagent (Invitrogen) following to the manufacturer’s instructions. Transfection complexes were added directly to the cells to a final oligonucleotide concentration of 20 nmol/L. The full-length cDNA of BRD7 (which included the ORF and 3’UTR) was amplified and cloned into pcDNA to generate the pcDNA-BRD7 constructs, which was applied for the rescue experiment. A549 cells were first transfected with miR-410 mimic. Then, these cells were then transfected with either pcDNA-BRD7 or pcDNA-empty.

2.6. Luciferase assay

Cells were seeded in 96-well plates and transfected with pLuc-3’-UTR, 10 ng Renilla and miRNA mimic or scramble using the Lipofectamine 2000 reagent. After 48 h of transfection, firefly and Renilla luciferase activities were performed by the Dual-Luciferase Reporter Assay System (Promega). The relative firefly luciferase activities were measured by normalizing to Renilla luciferase activities.

2.7. Cell proliferation, invasion and migration assays

Cell proliferation was detected using a CCK8 (Dojindo Laboratories) according the manufacturer’s instructions. Cell invasion was detected using a transwell chamber (Millipore, Billerica, MA, USA) with Matrigel (BD, San Jose, CA, USA). Cells were cultured in medium with DMEM in the top chamber and added with FBS-DMEM in the lower chamber. After 24 h of incubation, invasive cells on the lower chamber were stained with 0.5% crystal violet and counted. Cell migration was detected using wound-healing assay. An artificial wound was created by a 200-ll pipette tip. Mitomycin C was added to the culture wells. To visualize migrated cells and wound healing, images were taken at 0 and 48 h.

2.8. Statistical analysis

Data was expressed as means ± S.D. (standard deviation). One-way ANOVA was performed for serial analysis while two treatment groups were compared by the unpaired Student’s t test. Differences were considered statistically significant when P-value < 0.05.

3. Results

3.1. MiR-410 was upregulated in NSCLC and cell lines

The expression of miR-410 was upregulated in 26 cases (26/30, 87%) compared with adjacent tissues (Fig. 1A). Moreover, the expression of miR-410 was upregulated in NSCLC tissues compared to that in non-tumor normal tissues (Fig. 1B). The expression of miR-410 in four NSCLC cell lines (A549, SPC-A1, H1299 and H1650) was increased compared to that in 16HBE cells (Fig. 1C).

3.2. MiR-410 promoted the proliferation of NSCLC cell lines

The A549 cells were transfected with miR-410 mimics and inhibitors, scrambled or control oligo, which exhibited high transfection efficiency (Fig. 2A). CCK-8 proliferation assay showed that cell proliferation was increased in miR-410 mimics-transfected A549 cells compared with the scrambled oligo-transfected cells or untreated cells (Fig. 2B). In addition, knockdown of miR-410 repressed cell proliferation of the A549 cells (Fig. 2B).

3.3. MiR-410 promoted the migration and invasion of NSCLC cell

The percentage of migrated cells was higher in cells transfected with miR-410 mimic and lower in cells transfected with miR-410 inhibitor (Fig. 3A). Furthermore, the percentage of invasive cell was higher in cells transfected with miR-410 mimic and lower in cells transfected with miR-410 inhibitor (Fig. 3B).

3.4. MiR-410 targeted BRD7 in NSCLC cell

To identify target genes of miR-410, Targetscan was used to find miR-410 the potential targets. As showed in Fig. 4A, there was complementarity between miR-410 and the BRD7 3’UTR. Overexpression of miR-410 repressed the luciferase activity of the reporter gene with the wild-type construct but not the mutant BRD7 3’UTR construct (Fig. 4B). Overexpression of miR-410
miR-410 is highly expressed in NSCLC and cell lines. (A) qRT-PCR was used to detect miR-410 expression in 30 NSCLC and matched normal tissue samples. U6 snRNA was used as internal control. (B) Relative miR-410 expression levels in NSCLC tissues and adjacent normal tissues were determined by qRT-PCR. U6 snRNA was used as internal control. (C) The expression of miR-410 in four NSCLC cell lines, A549, SPC-A1, H1299 and H1650 was significantly increased compared to that in the 16HBE cells. U6 snRNA was used as internal control. ***P < 0.001.

miR-410 promotes the proliferation of NSCLC cell lines. (A) Expression levels of miR-410 were examined by real-time PCR after transfection of miR-410 mimics, inhibitor or scramble or control in A549 cells. (B) Growth of A549 cells was shown after transfection with miR-410 mimics or scramble or no transfection. The growth index was assessed at 0, 24, 48 and 72 h using CCK8 proliferation assay. **P < 0.01, and ***P < 0.001.

miR-410 promotes the NSCLC cell migration and invasion. (A) Migration analysis of A549 cells after treatment with miR-410 mimics, inhibitors or scramble or control; relative ratio of wound closure per field is shown. (B) Invasion analysis of A549 cells after treatment with miR-410 mimics, inhibitors or scramble or control; the relative ratio of invasive cells per field is shown below, ***P < 0.001.
repressed the protein and mRNA expression of BRD7 in A549 cells (Fig. 4C and D).

3.5. MiR-410 induced cell proliferation in the AKT-dependent pathway

Overexpression of miR-410 increased the expression of p-AKT in A549 cells and knockdown of miR-410 decreased the expression of p-AKT in the A549 cells (Fig. 5A). Moreover, downregulation of BRD7 led high AKT phosphorylation (Fig. 5B). Furthermore, knockdown of BRD7 by siRNA partially abrogated the function of BRD7 induced by miR-410 inhibitor (Fig. 5C). Importantly, knockdown of Akt expression repressed miR-410 mimic induced A549 cell proliferation (Fig. 5D). Inhibition of BRD7 enhanced cell proliferation in A549 cells (Fig. 5E). In addition, knockdown of BRD7 could reverse the proliferation inhibition imposed by miR-410 inhibitor (Fig. 5E).

3.6. Re-expression of BRD7 reversed the miR-410-induced cell proliferation

The expression of BRD7 was increased using pcDNA-BRD7 (Fig. 6A). We rescued the expression of BRD7 in cells overexpressing miR-410. Overexpression of BRD7 decreased the cells proliferation induced by miR-410 (Fig. 6B).

3.7. The expression of BRD7 was downregulated in NSCLC and was inversely expressed with miR-410 in NSCLC

The expression of BRD7 was downregulated in 26 NSCLC cases (26/30, 87%) compared with adjacent tissues (Fig. 7A). Moreover, the expression of BRD7 was decreased in NSCLC tissues compared to that in non-tumor normal tissues (Fig. 7B). Moreover, the expression of BRD7 was inversely correlated with the expression of miR-410 in NSCLC tissues (Fig. 7C).

4. Discussion

Aberrantly expressed miRNAs play a crucial role in carcinogenesis [31–34]. In recent years increasing studies have indicated that deregulation of miRNAs expression is implicated in many aspects of cancers, functioning as either tumor suppressors or oncogenes [33,35–38]. In this study, the expression of miR-410 was higher in NSCLC tissues compared with the matched normal lung tissues. Moreover, overexpression of miR-410 increased cell proliferation, migration and invasion in NSCLC cells. Furthermore, we have identified BRD7 as a novel direct target of miR-410. Additionally, overexpression of miR-410 led to increased Akt signaling by directly targeting BRD7. Re-expression of BRD7 reversed the miR-410-induced cell proliferation. The expression of BRD7 was downregulated in 26 NSCLC cases (26/30, 87%) compared with adjacent tissues. The expression level of BRD7 was inversely correlated with the expression level of miR-410 in NSCLC tissues. These findings suggested that high expression of miR-410 might be involved in NSCLC carcinogenesis and might be potential novel therapeutic targets in treatment of NSCLC.

Using the bioinformatics tool (Targetscan), we demonstrated that miR-410 bound to the 3′-UTR of BRD7. BRD7 is downregulated in various malignancies, which encourage us to believe that BRD7 might be a direct target in NSCLC [39–41]. In addition, miR-410 inhibited BRD7 mRNA and protein expression. We also
observed that the BRD7 could mediate the function of miR-410 in the proliferation of NSCLC cells. BRD7 is a subunit of SWI/SNF complex and is suggested as a novel tumor suppressor in several cancers such as epithelial ovarian cancer, Nasopharyngeal Cancer, Colorectal Cancer and Endometrial Cancer [39,40,42–44].

Moreover, BRD7 plays critical roles in diverse biological processes such as cell development, differentiation, growth, and metabolism [45–47]. Furthermore, Chiu et al. indicated that BRD7 could serve as a tumor suppressor not only by promoting p53 function, but also by reducing the survival signaling mediated by PI3K/Akt activation.
In this study, downexpression of BRD7 increased AKT phosphorylation and cell proliferation in NSCLC cells. However, the underlying mechanisms were still unclear. In NSCLC cells, miR-410 overexpression induced AKT phosphorylation while knockdown of miR-410 attenuated such induction. Importantly, inhibition of AKT activation partially blocked miR-410-induced cell proliferation. Moreover, we demonstrated that the expression of BRD7 was downregulated in 26 NSCLC cases (26/30, 87%) compared with adjacent tissues. Furthermore, the expression of BRD7 regulator of BRD7 in NSCLC cells, which provided one possible mechanism for the role of miR-410 in NSCLC proliferation, invasion and migration.

In conclusion, we demonstrated for the first time that miR-410 acted as an oncogene in NSCLC through inhibiting the expression of BRD7. These data suggest a novel therapeutic application of miR-410 in NSCLC.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2015.06.031.

References