Biomedicine & Pharmacotherapy 99 (2018) 523–529

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

MiR-760 enhances TRAIL sensitivity in non-small cell lung cancer via targeting the protein FOXA1

Xiang Zhang[1], Lei Wang[1], Yu Liu, Weicong Huang, Dezhi Cheng*

Department of Thoracic, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, 325000, China

ARTICLE INFO

Keywords:
miR-760
TRAIL
FOXA1
NSCLC
Apoptosis

ABSTRACT

Non-small cell lung cancer (NSCLC) is one of the leading cause of death worldwide. TNF-related apoptosis-inducing ligand (TRAIL) is a promising anti-tumor agent with the ability to kill tumor cells while spare normal ones. MicroRNAs (miRNAs) are small, non-coding RNAs that play vital roles in carcinogenesis. Although miR-760 has been reported to be dysregulated in a variety of cancers, the role of miR-760 in NSCLC is not fully understood, and the relationship between miR-760 dysregulation and TRAIL sensitivity is still elusive. In the current study, we found that miR-760 is significantly downregulated in NSCLC tissues and cell lines. We also found that ectopic expression of miR-760, by targeting the FOXA1, enhanced TRAIL sensitivity in NSCLC cells. Correspondingly, silencing of FOXA1 also sensitized NSCLC cell to TRAIL-induced apoptosis and proliferation inhibition. In summary, these findings suggest that miR-760 should be considered as a tumor suppressor since it negatively regulates the oncogene protein FOXA1 and regulated TRAIL sensitivity in NSCLC cells.

1. Introduction

Lung cancer is one of the most common causes of cancer-related deaths worldwide. Non-small cell lung cancer (NSCLC) is the most common frequent type of lung cancer, accounts for more than 85% of all cases [1]. Despite the progress in therapeutic methods, the prognosis of NSCLC still remains poor with the 5-year survival rate of less than 15% [2]. Therefore, identification of the molecular mechanisms relevant to NSCLC development and progression is urgently needed.

MicroRNAs (miRNAs) are endogenously expressed small, non-coding RNAs contains 20–24 nucleotides [3]. miRNAs can induce mRNA degradation thereby inhibit protein synthesis via partially binding with the complementary sequence of the 3′-untranslated region (UTR) of their targets mRNA [4]. To date, amounting evidence indicate that miRNAs play an essential role in proliferation, metastasis, apoptosis and carcinogenesis of various cancers [5]. Aberrant expression of miRNAs has been identified in pathogenesis of NSCLC. For example, overexpression of miRNA let-7 g could induce cell cycle arrest and apoptosis of NSCLC cells [6]. miR-210 has been found overexpressed in late stage of NSCLC and associated with poor prognosis of NSCLC patients [7]. Moreover, miRNA has also been found associated with chemo-resistance in NSCLC cells. For example, miR-181a and miR-630 have been identified as regulators of NSCLC cells response to cisplatin [8]. In a recent study, overexpression of miR-107 could enhance chemosensitivity to paclitaxel in NSCLC cells via inhibition of Bcl-w [9]. However, the role of miR-760 in NSCLC has not been described yet.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF family, has the ability to induce cell death in cancer cells but not normal cells. TRAIL can bind to five different death receptors (DRs), of which DR4 and DR5 mediate the induction of apoptosis [10]. After binding of TRAIL to DR4 and/or DR5, several proteins are recruited to the death domains of the receptors facilitating the formation of the death-inducing signaling complex (DISC) [36]. Within the DISC, FADD and caspase-8 are recruited and lead to the activation of caspase-8 which can subsequently activate downstream effector caspases and lead to the execution of apoptosis [11]. However, a number of cancer cells are resistant to TRAIL, especially highly malignant tumors including NSCLC [12]. Therefore, it is of great importance to unveil the resistance mechanisms of TRAIL-induced apoptosis, as well as to find molecules that may play critical roles in resistance process in order to enhance the anti-tumor effects of TRAIL. Many studies have found that miRNAs are involved in TRAIL-induced apoptosis and are able to regulate sensitivity to TRAIL in many different cancer cells [13–15]. In NSCLC, miR-148a could reduce tumorigenesis and increase apoptosis via inhibition of MMP15 and ROCK1 proteins [16].

Abbreviation: TRAIL, tumor necrosis factor (TNF-related apoptosis-inducing ligand; FOXA1, Forkhead box protein A1; NSCLL, Non-small cell lung cancer

* Corresponding author.
E-mail address: m15868755708_1@163.com (D. Cheng).

[1] These authors contribute equally.

https://doi.org/10.1016/j.biopha.2018.01.076
Received 13 November 2017; Received in revised form 29 December 2017; Accepted 12 January 2018

Recently, miR-760 is identified as a potential anti-tumor miRNA with altered expression levels in colorectal cancer [17]. Moreover, miR-760 could suppress breast cancer cell proliferation and metastasis via repression of NANOG [18]. In contrast, overexpression of miR-760 promotes proliferation in ovarian cancer cells by downregulation of PHLPP2 expression [19]. However, the role of miR-760 in NSCLC remains elusive. In this study, we demonstrated that miR-760 was downregulated in NSCLC tissues and cell lines. Ectopic expression of miR-760 could enhance sensitivity to TRAIL in NSCLC cells. Further investigations revealed that FOXA1 is the target of miR-760 and silencing of FOXA1 could enhance the cytotoxicity of TRAIL in NSCLC as well. Our findings may improve the understanding to the pathology of TRAIL resistance and potentially reveal a promising therapeutic target for TRAIL-resistant NSCLC patients.

2. Materials and methods

2.1. Clinical samples

After informed consent, a total of 35 patients with NSCLC undergoing surgical resection at Department of Thoracic, The First Affiliated Hospital of Wenzhou Medical University from 2015 to 2017 were enrolled in this study. This study was approved by the Ethics Committee of Wenzhou Medical University.

2.2. Cell culture and chemicals

BEAS-2B, which are human bronchial epithelial cells, H1650, NCI1460, H1299, and A549 cells were purchased from ATCC. All these cells were cultured according to the guidance provided by ATCC. Media were supplemented with 10% FBS, 2 mmol/L L-glutamine, and 100 units/ml penicillin/streptomycin. The recombinant TRAIL was purchased from Solarbio Ltd (Beijing, China). The miR-760 mimics, miR-NC mimics, miR-760 inhibitors, miR-NC inhibitos, si-NC and si-FOXA1 were purchased from RiboBio Company (Guangzhou, China).

2.3. Transfection

For transient transfection, cells at 50–70% confluency were transfected with miRNAs or siRNAs at a final concentration of 50 nM using Lipofectamine 2000 (Life technologies, Gaithersburg, USA) according to the manufacturer's guide. pcDNA3.1-FOXA1 was generated as described earlier [20]. Transfection of vectors was carried out using Lipofectamine 2000 (Life technologies) according to the manufacturer's guide.

2.4. Cell viability assay

Briefly, cells with or without transfection of miRNAs or siRNAs were cultured in 96-well plates and treated with various concentrations of TRAIL for 48 h. Cell viability was measured by using CCK-8 kit (Beyotime, Shanghai, China) according to the manufacturer's guide. Absorbance of each well was measured at 450 nm using a microplate reader. The IC50 values were calculated as the concentration of drug at which 50% of cell proliferation was inhibited. Each test was carried out in triplicates.

2.5. Apoptosis assay

For the quantification of apoptotic cells, we used the Nucleosome ELISA kit from Oncogene (Cambridge, USA). Briefly, Cells ($2 \times l06$) were seeded into 24-well plates in the presence or absence of treatment for 48 h. During this assay, anti-histone 3 biotinlabeled antibody binds to the histone component of captured nucleosomes and is detected following incubation with streptavidin-linked horseradish peroxidase conjugate. HRP catalyzes the conversion of colorless tetramethylbenzidine to blue and addition of a stop solution changes the color to yellow. Absorbance was measured on an ELISA reader at a test wavelength of 450 nm. Each test was carried out in triplicates.

2.6. Western blot assay

Cells were collected and lysed with CHAPS lysis buffer (Cell Signaling Technology, USA). Equal amounts of protein lysates were subjected to 12% SDS-PAGE gels and then transferred to PVDF membrane (Millipore, Billerica, USA). The membranes were incubated with the primary antibodies at 4 °C overnight. The following primary antibodies were used: Caspase-3 (Cell Signaling, USA), Cleaved PARP (Cell Signaling, USA), Caspase-8 (Cell Signaling, USA), Bcl-2 (Cell Signaling, USA), Mcl-1 (Cell Signaling, USA), Bax (Cell Signaling, USA), AKT (Cell Signaling, USA), Mcl-1 (Cell Signaling, USA), XIAP (Cell Signaling, USA), DR4 (Cell Signaling, USA), DR5 (Cell Signaling, USA), FOXA1 (Abcam, USA), GAPDH (Cell Signaling, USA). After incubating with secondary horseradish peroxidase-conjugated antibodies (Cell Signaling, USA) for 2 h at room temperature, blots were visualized through an enhanced chemiluminescence system (Thermo Fisher Scientific, USA). Protein bands were quantified by densitometric analysis using Quantity One software (Bio-Rad Laboratories, San Diego, CA, USA).

2.7. Quantitative real-time PCR assay

The total RNA was extracted using the TRIzol reagent (Life Technologies). For the miRNA expression assay, the total RNA was reversely transcribed using the stem-loop RT primer and the PrimeScript RT reagent Kit (Takara, Dalian, China) according to the manufacturer's guide. The miR-760 primers were synthesized by RiboBio Ltd with the following sequences:

Forward: 5′-AGCCGCGGCTCTGGOTCTG-3′; Reverse: 5′-GTGCAGG GTCCGAGGT-3′. qRT-PCR was performed in triplicate using SYBR Premix ExTaq (TaKaRa), according to the manufacturer's instructions. All reactions were done in triplicate. The expression of miR-760 was defined based on the threshold cycle (Ct), and relative expression levels were calculated as $2^{-\Delta\Delta CT}$ after normalization with reference to expression of snRNAU6.

2.8. Luciferase activity assay

For the luciferase assay, the putative binding sites of miR-760 in the 3′UTR of the human FOXA1 gene were amplified and inserted into the luciferase reporter pMIR-Report-plasmid (Life Technologies). To investigate whether this UTR was directly targeted by miR-760, the predicted target site was mutated by site-directed mutagenesis. Cells were contransfected with reporter plasmids and miR-760 or miR-NC mimics using Lipofectamine 2000 (Life Technologies). Then firefly and renilla luciferase activities were measured by the dual-luciferase reporter assay system (Promega) according to the manufacturer's guide.

2.9. Caspase-3/-7 and caspase-8 activities assay

For detection of caspase-8 and caspase-3/7 activity, cells were cultured in 96-well plates and treated with TRAIL and analyzed by using Caspase-Glo Assay kits (Promega) according to the manufacturer's instructions.

2.10. Statistical analysis

All data are expressed as the mean ± SD and were carried out by three independent experiments.