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microRNA-381 suppresses the growth and increases cisplatin sensitivity in non-small cell lung cancer cells through inhibition of nuclear factor-κB signaling

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ABSTRACT

microRNA (miR)-381 is downregulated and exhibits anti-invasive activity in non-small cell lung cancer (NSCLC). In this study, we investigated the role of miR-381 in proliferation, tumorigenesis, and cisplatin resistance of NSCLC cells. The effects of miR-381 overexpression on proliferation, tumorigenesis, cell cycle progression, and cisplatin sensitivity were examined. Overexpression of miR-381 significantly inhibited cell proliferation and colony formation *in vitro* and tumorigenesis *in vivo*. Ectopic expression of miR-381 arrested NSCLC cells at G0/G1 phase, which was accompanied by increased expression of p21 and p27 and decreased expression of cyclin D1 and CDK4. Compared to A549 parental cells, cisplatin-resistant equivalents (A549/CDDP) had reduced levels of miR-381. miR-381 re-sensitized A549/CDDP cells to cisplatin and potentiated cisplatin-induced apoptosis. Mechanistically, miR-381 interfered with the activation of nuclear factor (NF)-κB through repression of inhibitor of differentiation 1 (ID1). Co-expression of ID1 reversed the suppression of proliferation and enhancement of cisplatin cytotoxicity by miR-381. Taken together, miR-381 can induce growth suppression and chemosensitization in NSCLC, largely through inactivation of NF-κB via downregulation of ID1. Restoration of miR-381 represents a potential therapeutic strategy for NSCLC.

1. Introduction

Non-small cell lung cancer (NSCLC) accounts for ~85% of all lung cancers [1]. Despite treatment improvement in the past decades, the 5-year overall survival rate for NSCLC is only about 17% [2]. Platinum-based chemotherapy is an important treatment option for non-resectable or advanced NSCLC [3]. However, the therapeutic efficacy of chemotherapy is limited due to development of drug resistance. Many signaling pathways including nuclear factor (NF)-κB signaling are implicated in the resistance of tumor cells to cisplatin [4]. In an inactive status, NF-κB as a heterodimer is retained in the cytoplasm by inhibitory IκB proteins. In the canonical activation pathway, IκB undergoes phosphorylation, ubiquitination and proteolytic degradation in response to stimuli. The released NF-κB dimer is subsequently translocated into the nucleus where it transactivates NF-κB-responsive genes [5]. Activation of NF-κB signaling secondary to TAB3 overexpression contributes to cisplatin chemoresistance in A549 NSCLC cells [6]. Pharmacological inhibition of NF-κB was reported to enhance cisplatin cytotoxicity to NSCLC cells [7]. Further exploration of the molecular mechanisms for NSCLC progression is of significance in developing novel treatment strategies.

microRNAs (miRs) are a large class of endogenous, non-coding regulatory RNAs of ~22 nucleotides. They have an ability to suppress the expression of multiple target genes, typically via cleavage of target mRNA or repression of protein translation [8]. Several miRs have been found to modulate the chemosensitivity of NSCLC cells to cisplatin [9–11]. For instance, miR-451 can overcome cisplatin resistance in NSCLC cells by targeting Mcl-1 [9]. Another study reported that miR-185-5p modulates the sensitivity of NSCLC cells to cisplatin via downregulation of ABCC1 [11].

miR-381 acts as a tumor suppressor in multiple cancer types such as gastric cancer [12] and breast cancer [13]. A previous study has shown that miR-381 is downregulated in human lung adenocarcinoma and overexpression of miR-381 significantly decreases cell migration and invasion through repression of inhibitor of differentiation 1 (ID1) [14]. However, the role of miR-381 in the growth and survival of NSCLC cells remains unknown. In this study, we explored the effects of restoration of miR-381 on the proliferation and apoptosis of NSCLC cells and examined its impact on cisplatin sensitivity. The molecular mechanism underlying the action of miR-381 was also checked.

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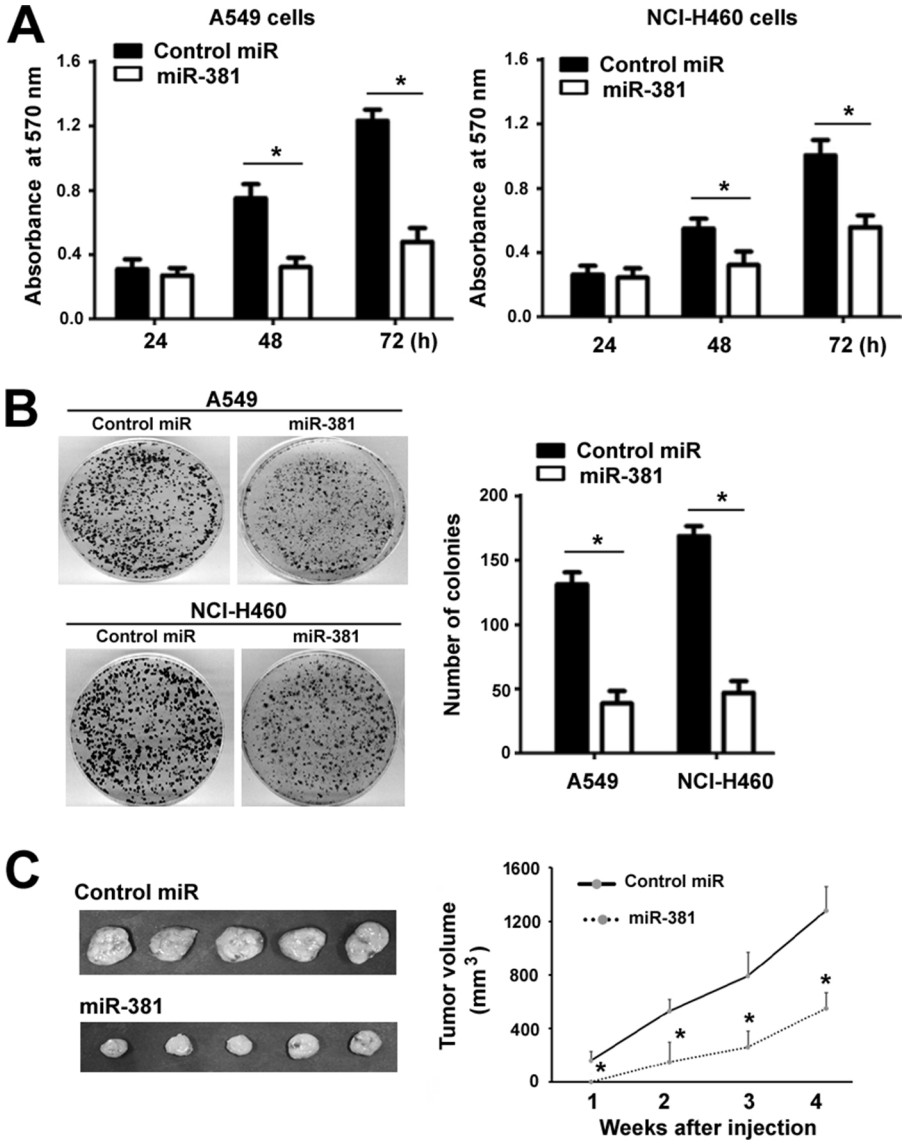

Fig. 1. Restoration of miR-381 inhibits the proliferation, colony formation, and tumorigenesis of NSCLC cells. (A) Cell proliferation determined by MTT assay. A549 and NCI-H460 cells transfected with control miR or miR-381 precursor were cultured for 24–72 h and tested for proliferation. (B) Colony formation assay. A549 and NCI-H460 cells stably transfected with control miR or miR-381 precursor were cultured for 2 weeks and colonies were counted. Bar graphs represent data from three independent experiments (n = 3). (C)*In vivo* tumorigenic experiments. A549 cells stably expressing miR-381 or control miR were injected s.c. into nude mice (n = 5), and tumor volumes were measured weekly for 4 weeks. $^{*}P < .05$ *vs.* the control group.

2. Materials and methods

2.1. Antibodies

Mouse monoclonal antibodies recognizing p21 (sc-271610), p27 (sc-1641), cyclin D1 (sc-20044), cyclin-dependent kinase 4 (CDK4; sc-393653), ID1 (sc-133104), and Lamin B (sc-365962) and rabbit anti-β-actin polyclonal antibody (sc-130656) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse anti-IκBα (#4814) and anti-Bcl-2 (#15071) monoclonal antibodies and rabbit anti-Bcl-xL (#2762), anti-poly (ADP-ribose) polymerase (PARP; #9541), and anti-NF-κB p65 (#3034) polyclonal antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA).

2.2. Cell culture

Two human NSCLC cell lines (A549 and NCI-H460) were purchased from American Type Culture Collection (Gaithersburg, MD, USA) and cultured in Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 U/mL). Cells were allowed to grow in a humidified incubator containing 5% CO_2 at 37 °C. A cisplatin-resistant A549 cell line (A549/CDDP) was generated via exposure to increasing concentrations (0.5, 1, 2, 4, and 10 μM) of cisplatin (Sigma-Aldrich, St. Louis, MO, USA) [15]. Briefly, A549 cells were exposed to 0.5 μM cisplatin for 3 days and recovered for 4 days. This treatment was repeated one more cycle at each concentration of cisplatin. The cells were then treated with increasing concentrations of cisplatin up to 10 μM. The cisplatin-resistant cell lines were maintained in culture medium supplemented with 4 μM cisplatin.

2.3. Plasmid transfection

Human miR-381 precursor-expressing plasmid and negative control miR were purchased from GenePharma (Shanghai, China). ID1-expressing plasmid and empty vector pCMV-Entry were purchased from Origene (Rockville, MD, USA). Plasmid transfection was carried out using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. In brief, cells at 80% confluence were added with DNA-Lipofectamine 2000 complexes. At 6 h after transfection, the medium was replaced with fresh medium containing 10% FBS. For stable expression of miR-381, A549 and NCI-H460 cells transfected with the miR-381-expressing plasmid or control miR were selected in the presence of G418 (800 μg/mL; Sigma-Aldrich) for 2 weeks.