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Long noncoding RNA OR3A4 promotes cisplatin resistance of non-small cell lung cancer by upregulating CDK1

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Abstract. – OBJECTIVE: Numerous studies have proved that long non-coding RNAs (lncRNAs) have an important role in malignant tumors, including non-small cell lung cancer (NSCLC). LncRNA olfactory receptor family 3 subfamily A member 4 (OR3A4) was explored to identify how it functions in resistance of NSCLC patients to cisplatin.

MATERIALS AND MATHODS: Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was utilized to detect OR3A4 expression in NSCLC patients. Then, we conducted Cell Counting Kit-8 (CCK-8) assay and flow cytometric analysis to detect the function of OR3A4 on the resistance of NSCLC cells to cisplatin. Furthermore, the potential mechanism was explored by mechanism assays.

RESULTS: Compared with the expression of paired A549 cells, OR3A4 expression of A549/DDP cells was higher. Moreover, the functional assay showed that after OR3A4 was silenced in A549/DDP cells, cell viability and cell apoptosis was induced and resistance to cisplatin was reversed. Furthermore, it was found that CDK1 expression was suppressed in A549/DDP cells by knocking down OR3A4.

CONCLUSIONS: The present work suggests that OR3A4 participates in regulating cell cycle, cell apoptosis of NSCLC cells and the resistance to cisplatin via upregulating CDK1, indicating that OR3A4 could be identified as a potential therapeutic target for NSCLC patients.

Keywords:
LncRNA, OR3A4, NSCLC, CDK1, Cisplatin.

Introduction

More than 80% of all lung cancer cases are non-small cell lung cancer (NSCLC)[1]. The 5-year overall survival rate is less than 15% in the NSCLC patients[2]. Chemotherapy drugs are emerging as the important step of therapeutic strategy, particularly in NSCLC. The resistance to chemotherapy drugs remains an important factor for the prognosis of NSCLC patients. Therefore, there is an urgent need to identify a novel biomarker and therapeutic target for the patient with NSCLC.

Although long noncoding RNAs (lncRNAs) have little potential of protein-coding, they are important regulators in carcinogenesis of cancers. For example, LncRNA H19 is correlated with resistance to cisplatin in lung adenocarcinoma[3]. LncRNA HOTAIR induces cisplatin resistance by up-regulating miR-34a in gastric cancer[4]. LncRNA CCAT1 regulates SOX4 and promotes cisplatin resistance in NSCLC cells[5]. LncRNA UCA1 promotes cell growth and cisplatin resistance of oral squamous cell carcinoma *via* downregulating miR-184[6]. However, the clinical role of olfactory receptor family 3 subfamily A member 4 (OR3A4) in cisplatin-resistance remains unknown.

Therefore, we conducted studies and found that OR3A4 could regulate cell apoptosis, cell cycle and cisplatin resistance in NSCLC. Meanwhile, Cyclin-dependent kinase 1 (CDK1) has been identified as a potential marker in many cancers and participates in many tumors. In this work, we found that CDK1 was upregulated by OR3A4 and is associated with resistance to cisplatin.

Materials and Methods

NSCLC Cell Lines

A549/DDP cells and A549 cells were cultured in Roswell Park Memorial Institute-1640

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(RPMI-1640) medium (Life Technologies, Gaithersburg, MD, USA) added with 10% fetal bovine serum (FBS; Life Technologies, Gaithersburg, MD, USA). Besides, the incubator for cell culture consisted of 5% CO_2 at 37°°C.

Cell Transfection

The complementary deoxyribose nucleic acid (cDNA) oligonucleotides specifically targeting OR3A4 (OR3A4/shRNA) was synthesized by GenePharma (Shanghai, China), and cloned into pGPH1/Neo. Then, OR3A4/shRNA were used for transfection in NSCLC cells. 48 h later, Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was used to measure the transfection efficiency.

RNA Extraction and Real Time-Quantitative Polymerase Chain Reaction

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was utilized for extracting total RNA, which was then reversely transcribed to cDNA through reverse Transcription Kit (TaKaRa Biotechnology, Otsu, Shiga, Japan). Following are the primers used for RT-qPCR: OR3A4, fo d 5'-CCTATCCCTTTCTCTAAGAA-3' a verse 5'-ACTTCTGCAAAAACGTGC ; and for glyceraldehyde 3-phosphate deh genase (GAPDH), forward 5'-CCACATCG CAGACACCAT-3' and revers CCAGG GCCCAATACG-3'. The th was as follows: 95°C for 30 sec, c for 4 ycles at 95°C, 60°C for 35 sec.

Cell Counting Ki (CCK8) ay

0, 1, 5, 8, 10, 20, 22 o g/mL cisplatin were us the cells in d status, respectively. Then, cel ility was detected by Cell Cou Kit-8 (Doji olecular Technologies, K amoto, Japan) at 0, 48, 72 and 96 h. The rbance measured at 450 nm.

Cell alysis

×10⁵/ ells diluted by RNase A in e-co overnight, and these cells we tained w Propidium Iodide (PI; 50 mg/m time, Shanghai, China) in the dark for . Then, they were measured with w cytometer (FACScan, BD Biosciences, klin Lakes, NJ, USA).

Cell Apoptosis Analysis

Harvested cells were washed twice using ice-cold. Then 100 µL of flow cytometry binding buffer was added. After Annexin V/fluorescein isothiocyanate (FITC; 5 µL; Beyotime, Shanghai, China) and PI (5 µL) were mixed, these cells were stained for 15 min in the dark. Each tube w d ed with four hundred microliters bind FACSCalibur flow cytometer (BD sciences, Franklin Lakes, NJ, USA) was pe ed to analyze cell apoptosis.

Western Blotting Ana

After being separate th 12 sodium decyl sulphate-poly amide electrophoresis (SDS-PAGE) th as re d to polyvinylidene orid DF) mbrane (Millipore, B ca, MA, bbit anti-CDK1 (Cambridge, USA) and anti-GAP (Ab Cambridge, MA, USA) were used as the pri ntibodies, which were utili incubating t mbranes overnight. G anti-rabbit secondar antibody (Abcam, bridge, MA SA) was then applied for incu tion. Image ftware (NIH, Bethesda, MD, U was perfo d for data analysis.

Statis alysis

Statistical Product and Service Solutions 17.0 (SPSS, Chicago, IL, USA) was utilized perform statistical analysis. The method of $2^{-\Delta\Delta CT}$ and two-tailed Student's *t*-test were used. It was considered statistically significant when $p<0.05$.

Results

The Expression of OR3A4 in A549/DDP and A549 Cells

As half of the maximal inhibitory concentration (IC50) of cisplatin was an important factor in resistance to cisplatin, it was detected through the CCK-8 assay in NSCLC cells. As a result, the IC_{50} of cisplatin in A549/DDP cells was remarkably increased compared with A549 cells (Figure 1A). Furthermore, OR3A4 was lower-expressed in A549 cells than A549/DDP cell line (Figure 1B).

OR3A4 Was Upregulated in A549 Cells Treated With Cisplatin

Then, A549 cells were treated with 0.0, 0.5, 1.0, 1.5, 2.0 or 2.5 µg/ml cisplatin. RT-qPCR was utilized to monitor OR3A4 expression in these treated cells. After cisplatin concentrations were increased, the OR3A4 expression of these treated cells was increased (Figure 2).

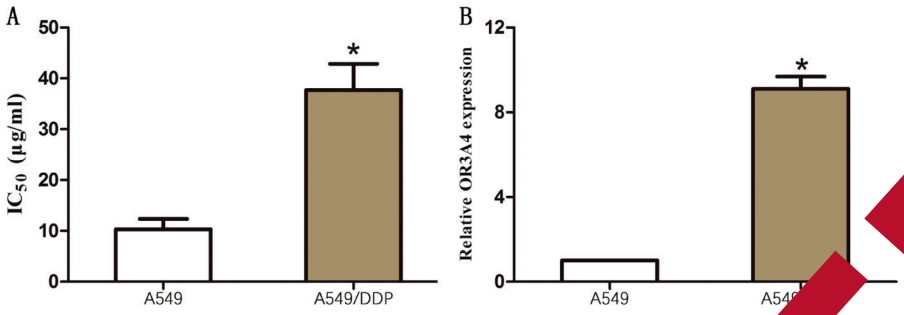


Figure 1. The expression levels of OR3A4 were increased in A549/DDP cells. ***A,*** IC_{50} value[illegible]platin w[illegible]gher in A[illegible] DDP cells, compared with that of the A549 cells. ***B,*** The expression levels of OR3A4 relati[illegible]GAPD[illegible]e determined in the A549/DDP and A549 cells by RT-qPCR. GAPDH was used as an internal control. *[illegible]0.0[illegible]

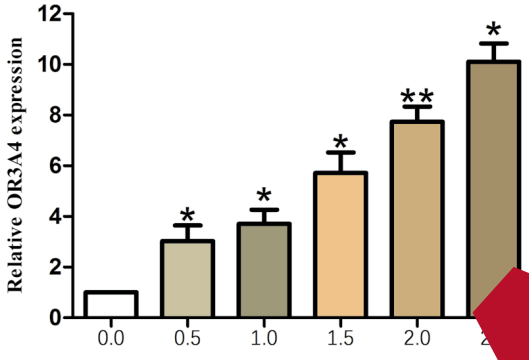


Figure 2. OR3A4 was upregulated in A549 cells tr[illegible] with cisplatin. A549 cells were cultured in various conc[illegible] trations of cisplatin (0.0, 0.5, 1.0, 1.5, 2[illegible]5 μg/mL) f[illegible] 24 h. OR3A4 expression was evalua[illegible]CR. GAP[illegible] DH was used as an internal contr[illegible]<0.05.

monitore[illegible]y R[illegible]R (Figure 3A). We conducted the CCK-8 a[illegible]nd found that IC_{50} of cisp[illegible]s decreased[illegible]ugh knockdown of O[illegible]4 in A549/DDP cel[illegible] (Figure 3B). Moreo[illegible], after these c[illegible]ls were treated with cisplatin ([illegible]1.0 or 2.0 μ[illegible]l), cell apoptosis of OR3A4/s[illegible]A group w[illegible]promoted compared with the co[illegible]rou[illegible]gure 4A). In addition, these treated [illegible]subG0/G1 phases in the OR3A4/hRNA group was increased compared with that [illegible]ontrol group (Figure 4B).

Knockdown of OR3A4 [illegible] Cispl[illegible]n Resistance in A5[illegible]DPP C[illegible]ne

After A549/D[illegible] were sile[illegible] through OR3A4/shRN[illegible]t[illegible]ction effi[illegible]cy was

Knockdown of OR3A4 Decreased Cisplatin Resistance in A549/DDP Cells by Downregulating CDK1

RT-qPCR results revealed that CDK1 of A549/DDP cells was remarkably lower-expressed in the OR3A4/shRNA group compared with that in the control group (Figure 5A). Western blot analysis results also revealed that CDK1 of

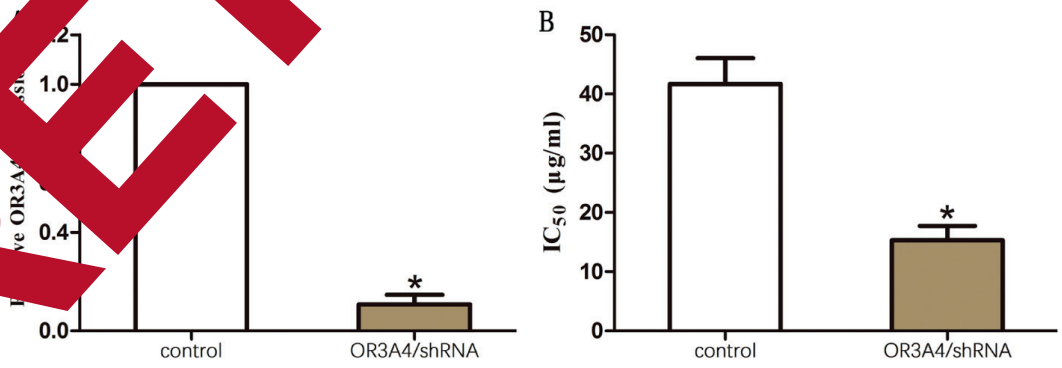


Figure 3. The knockdown of OR3A4 reduced IC50 values of cisplatin in A549/DDP. ***A,*** 48 h after A549/DDP cells were transfected with OR3A4/shRNA, the inhibition efficiency was detected by RT-qPCR. GAPDH was used as an internal control. ***B,*** IC_{50} values of cisplatin in A549/DDP cells transfected with control and OR3A4/shRNA were analyzed by CCK-8 assay. The results represent the average of three independent experiments. *$p<0.05$.

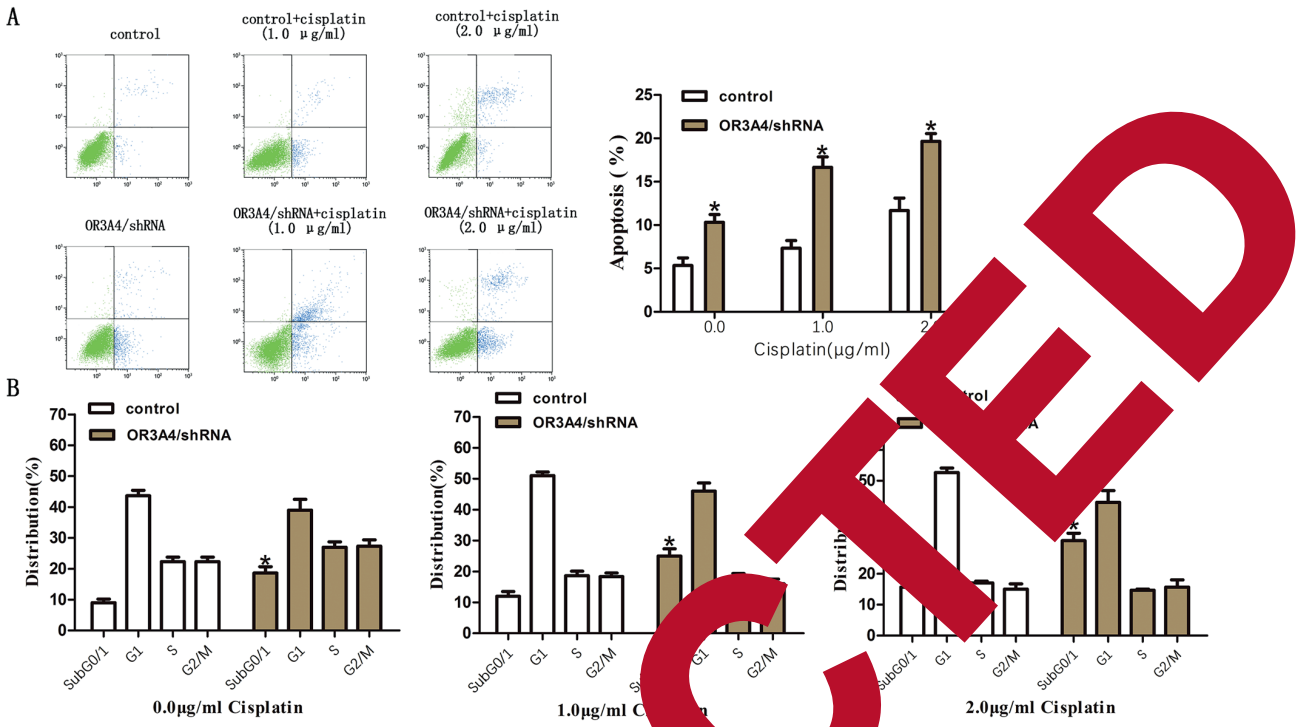
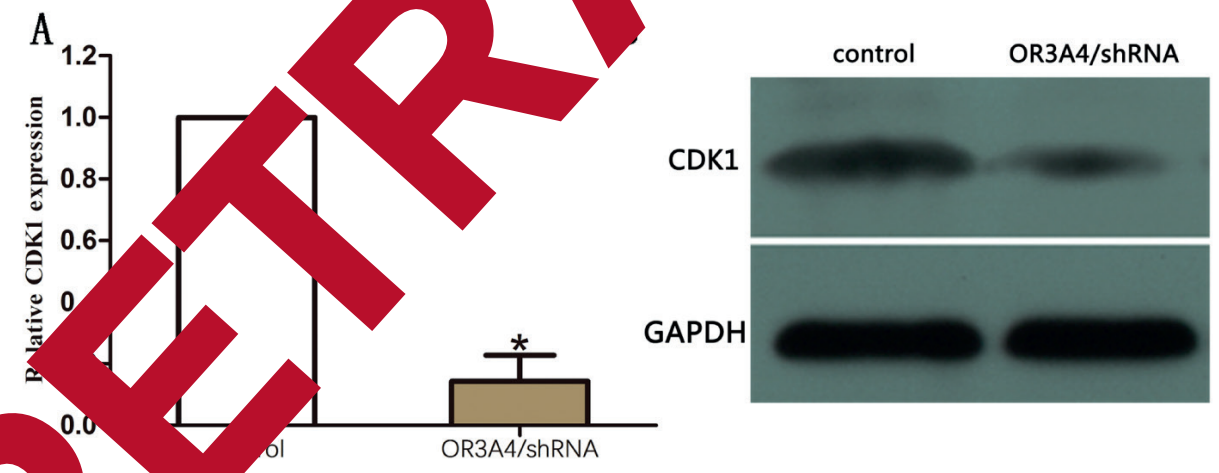


Figure 4. Knockdown of OR3A4 promoted the sensitivity of A549/D… Cell apoptosis induced by different concentrations (0.0, 1.0 or 2.0 μg/mL) of cisplatin … detected in A54… transfected with control and OR3A4/shRNA by flow cytometric analysis. ***B,*** Cell cycle … duced by different concentrations (0.0, 1.0 or 2.0 μg/mL) of cisplatin was detected in A549/DDP cells transfecte… co… R3A4/shRNA by flow cytometric analysis. The results represent the average of three independent experimen… $p<0.05$



Fig… 5. Knock… n of OR3A4 downregulated CDK1 expression in A549/DDP cells. ***A,*** The mRNA expression levels of CD… 549/DDP cells transfected with control and OR3A4/shRNA were analyzed by RT-qPCR. ***B,*** Protein expression … A549/DDP cells transfected with control and OR3A4/shRNA were analyzed by Western blot assay. GAPDH … used as a control. *$p<0.05$.

A549/DDP cells was markedly downregulated in the OR3A4/shRNA group compared with that in the control group (Figure 5B).

Discussion

The resistance to chemotherapy drugs remains a vital factor of patients' prognosis de-

spite the fact that various chemotherapy drugs are available for lung cancers[7,8]. Recently, many studies have been conducted to explain the mechanism of cisplatin resistance, among which the regulation of cell apoptosis is a vital progression in drug resistance for cancers. Moreover, lncRNAs could modulate cell apoptosis and further regulate drug resistance in cancers[9-12].

Recent studies[13] revealed that olfactory receptor family 3 subfamily A member 4 (OR3A4) is abnormally expressed and participates in the progression of many cancers. For instance, OR3A4 promotes cell proliferation and acts as an oncogene in gastric cancer, and cell growth ability and invaded ability are promoted in breast cancer by upregulating OR3A4[14]. However, the role of OR3A4 in resistance to cisplatin in cancers has not been studied so far. In this work, OR3A4 was upregulated in A549/DDP cells compared with A549 cells. Besides, the OR3A4 was upregulated after the dose of cisplatin for treating A549 cells increased. In addition, cisplatin-induced apoptosis of A549/DDP cells was promoted *via* knockdow of OR3A4. The percent of A549/DDP c subG0/G1 phases was increased in OR 4/shRNA group after treating with different es of cisplatin.

Cyclin-dependent kinase 1 (has bee identified as potential marke in cancers. For example, CDK1 expr n is c lated to clinical stage and treat effica cancer[13]. CDK1 is hi exp colo cancer and inhibits apopt *via* p53 apoptosis pathway[14]. protein is iated with cell growth ar tosis in ov cancer by regulating nk1-C and P53-P21WAF1 signaling away[15]. CDK been proved to functio the regulation o cycle induced by ci in[16]. paper showed that CDK1 was do ulate A549/DDP cells *via* knockdown 4.

Conclusions

We observed that OR3A4 regulated cell apopto cell cycle, and enhanced the resistance of NSCLC cells to cisplatin *via* upregulating CDK1. These findings imply that OR3A4 can be served as a promising mark for NSCLC.

Conflict of interest

The authors declare no conflicts of interest.

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