Original Article

YTHDC2-mediated m6A mRNA modification of *Id3* suppresses cisplatin resistance in non-small cell lung cancer

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Background: The methylation of adenosines at the N6 position (N6-methyladenosine; m6A) is one of the most conserved internal RNA modifications. m6A can modulate the expression of oncogenes or tumor suppressor genes, as well as m6A levels and the expression and activity of m6A enzymes, thus influencing tumor progression and therapeutic response. This study investigates the role of *YTHDC2*-mediated m6A messenger RNA (mRNA) modification of *Id3* in controlling cisplatin resistance in non-small cell lung cancer (NSCLC).

Methods: The expression of the m6A reader protein *YTHDC2* was detected in an NSCLC cisplatin-resistant cell line (A549/DDP) using real-time fluorescence quantitative polymerase chain reaction (qPCR). *YTHDC2* overexpression plasmids were constructed and transfected into A549/DDP and A549 cells respectively. We performed qPCR and western blot (WB) to detect changes in *YTHDC2* and Id3 expression, and the effects of *YTHDC2* overexpression on proliferation, apoptosis, invasion, and migration of drug-resistant cells were assessed by cell counting kit-8 (CCK-8), flow cytometry, and transwell and scratch assays. The m6A modification of Id3 by *YTHDC2* was clarified by m6A-immunoprecipitation-PCR (m6A-IP-PCR) assay.

Results: The CLIPdb online database predicted that *YTHDC2* might bind to Id3. The results of qPCR showed that *YTHDC2* was downregulated in the NSCLC cisplatin-resistant cell line A549/DDP compared to the cisplatin-sensitive cell line A549. Overexpression of *YTHDC2* increased the expression of *Id3*, and the methylation inhibitor 3-deazaadenosine abrogated the regulatory effect of *YTHDC2* on *Id3*. *YTHDC2* overexpression significantly inhibited A549/DDP cell proliferation, migration, and invasion, and promoted apoptosis by synergistically promoting the effects of *Id3*. m6A-IP-PCR analysis revealed that *YTHDC2* could inhibit the m6A level of *Id3* mRNA.

Conclusions: To regulate the activity of *Id3*, *YTHDC2* requires modifications to m6A, which ultimately inhibit cisplatin resistance in NSCLC.

Keywords: N6-methyladenosine (m6A); *Id3*; *YTHDC2*; RNA modifications; NSCLC

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Introduction

In China and worldwide, lung cancer is the most common cancer, and it accounts for the highest death rate among cancers (1). A major clinical problem is resistance to cisplatin therapy, which contributes to poor patient outcomes. Usually, changing oncogenes and tumor suppressor genes genetically and epigenetically cause lung cancer to develop in a multistage process (2). Epigenetic mechanisms for cisplatin resistance in lung cancer remain largely unexplored.

In lung adenocarcinomas, *Id3* modulates PI3K/Akt to reverse cisplatin resistance (3). There is general agreement that Id3 plays a role in lung cancer proliferation and in resistance to cisplatin, but its exact mechanism remains unclear. Epigenetic regulation at a new level, the post-transcriptional level, is emerging through RNA modifications, in contrast to DNA methylation and histone modification (4). N6-methyladenosine (m6A) is the most common and abundant messenger RNA (mRNA) modification, modulated by 'writers', 'erasers', and 'readers' of this mark (5). The complex of writers, which includes *METTL14*, *METTL3*, and *WTAP*, promotes m6A modification in RNA. Ye *et al.* (6) found decreased IncRNA DBH-AS1 expression in pancreatic cancer was related to the *METTL3*-dependent m6A methylation of the lncRNA, which downregulation was negatively correlated with the malignancy of pancreatic cancer and with patient survival outcomes. Chen *et al.* (7) discovered the mechanism through which m6A methylation modulates autophagy and chemosensitivity in the TCam-2 cell line, and *METTL3* was identified as a possible target to overcome seminoma cisplatin resistance. The mechanism of *METTL3*-mediated autophagy in reversing gefitinib resistance of non-small cell lung cancer (NSCLC) cells by β-elemene was revealed by Liu *et al.* (8). In contrast, erasers in m6A-modified mRNA counteract the effects of writers. The first identified demethylase was *FTO*. *FTO* might play an important role in promoting NSCLC by lowering m6A levels and activating *KRAS* signaling, as described by Shi *et al.* (9), who for the first time identified the genetic changes in m6A regulatory factors in NSCLC. The readers convert instructions of m6A modification into functional signals, including YTH family proteins. In the study by Tsuchiya *et al.* (10), as RNA-binding proteins(readers) that recognize m6A, high expressions of both *YTHDF1* and *YTHDF2* are connected to a favorable prognostic outcome of NSCLC patients, a larger quantity of tumor-infiltrating lymphocytes (TILs), and downregulation of programmed cell death 1 (PD-1).

Since the m6A modification machinery plays a crucial role in normal bioprocesses, it is not surprising that evidence is emerging that dysregulation of m6A modification and the associated proteins also contributes to cancer initiation, progression, and response to drugs (11). In recent studies, *YTHDC2* was identified as an essential member of the m6A reading proteins involved in the development and progression of a variety of tumors. He *et al.* reported that as *YTHDC2* activates the IGF1R/ATK/S6 signaling axis, it may serve as a potential therapeutic target in radiosensitization of neural progenitor cells (12). It was found that alterations in *YTHDC2* were associated with better prognosis in head and neck squamous cell carcinoma (HNSCC), which may serve as a novel prognostic biomarker (13). However, the mechanism of m6A modification in the regulation of cisplatin resistance in lung cancer is unclear.

In this study, the downregulation of *YTHDC2* expression was associated with a poor prognosis of lung cancer in cisplatin-resistant cells, which were expressed at a lower level than in sensitive cells. In addition, we demonstrated that *YTHDC2*, as a specific reader of m6A, inhibited the m6A level of *Id3*, which inhibited the proliferation and migration of A549/DDP cells and ultimately inhibited NSCLC resistance to cisplatin. As a result of our findings, we propose that the RNA methyltransferase *YTHDC2* may serve as a new target for lung cancer cisplatin-resistant therapy. We present the following article in accordance with the MDAR reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-187/rc).

Highlight box

Key findings

- RNA methyltransferase *YTHDC2* might be a new target for cisplatin-resistant lung cancer treatment.

What is known and what is new?

- *Id3* has the potential to reverse cisplatin resistance in lung adenocarcinomas.
- *YTHDC2* inhibited the m6A level of *Id3*, which eventually inhibited NSCLC resistance to cisplatin.

What is the implication, and what should change now?

- We have identified a new tumor suppressor in NSCLC resistant to cisplatin called *YTHDC2*. The information will be useful for developing next-generation therapeutics.

Methods

Clinical samples

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study protocol was reviewed and approved by the Ethics Committee of Jinling Hospital (No. 2020DZGZRZX-096). All participants signed an informed consent form. During June 2020 and September 2021, a total of 9 cases of NSCLC tissues were collected from patients in Jinling Hospital. Among them, 5 cases were in cisplatin-sensitive tissues and 4 cases were in cisplatin-resistant tissues. The following were the inclusion requirements: NSCLC was confirmed by a histopathological examination, and first-line chemotherapy included at least 3 cycles of a cisplatin-based combination therapy. A total of 9 patients enrolled in the study received cisplatin chemotherapy and were evaluated for sensitivity or resistance to cisplatin by computed tomography (CT) before and after cisplatin treatment. In addition, 3 cases of paracancerous tissues were also collected. All tissues were placed in liquid nitrogen for storage immediately after collection.

Cell lines, cell culture, vector construction, and transfection

In A549 (human species, KG007, KeyGen Biotech, Nanjing, China) cells, 10% fetal bovine serum (FBS) was added to Roswell Park Memorial Institute (RPMI)-1640 media, whereas in A549/DDP (human species, KG354, KeyGen Biotech) cells, 1,000 ng/mL DDP and 10% FBS were added to the RPMI-1640 media. After amplifying *YTHDC2*'s full-length coding sequence by polymerase chain reaction (PCR), and cloning it into pCDH vector, the recombinant plasmid was named pCDH-CMV-hYTHDC2(NM_022828.5)-C-3Flag-EF1A-GFP-T2A-Puro. Lipo8000™ transfection reagent was purchased from Beyotime Biotechnology (Shanghai, China). Transfection was carried out when approximately 80% confluency was reached in 96- or 6-well plates. For each transfection of 6-well plate, the following was added to each well: 2.5 ng plasmid DNA, 4 μL Lipo8000™ transfection reagent, and in 125 μL Opti-MEM (Gibco, Grand Island, NY, USA). After 24–48 h, cells were successfully transfected for subsequent analyses.

RNA isolation and quantitative real-time PCR (qPCR)

The RNA was isolated using TRIzol (Takara, Shiga, Japan), and its concentration was determined using a NanoDrop 2000. Using a reverse transcription kit, complementary DNA (cDNA) was created (RR036A; Takara). Using a TB Green Premix Extra kit, the real-time PCR (RT-PCR) reaction was performed (RR420A; Takara). Our qPCR reactions were conducted using Thermo Fisher Scientific's Step One Plus Real-Time PCR System (Thermo Fisher, Waltham, MA, USA). We followed the manufacturer's instructions for all subsequent steps. With glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the housekeeping gene, relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The primers used were as follows: GAPDH: forward 5'-CGCTCTCTGCTCCTCCTGTTC-3', revervse 5'-ATCCGTTGACTCCGACCTTCAC-3'; Id3 forward 5'-GGACGACATGAACCACTGCTACTC-3', reverse 5'-GCTGTAGGATTTCCACCTGGCTAAG-3'; YTHDC2 forward: 5'-CAAAACATGCTGTTAGGAGCCT-3', reverse 5'-CCACTTGTCTTGCTCATTTCCC-3'.

Western blot (WB) assay

The cells were treated with *YTHDC2* or NC vector or *YTHDC2* + m6A methylation inhibitor 3-deazaadenosine, after that, the cells were collected for WB. PBS was used to wash the cells twice. Proteins were extracted using radioimmunoprecipitation assay (RIPA) buffer. A bicinchoninic acid (BCA) assay was performed on the supernatants after centrifugation to determine the concentration of protein. The protein concentration was determined using the standard curve. For electrophoresis, samples were prepared by adding 5× loading buffer and boiling for 5 min. Each protein was given a special polyacrylamide gel electrophoresis (PAGE) glue based on its molecular weight, and the samples were electrophoretically separated after adding the glue. Polyvinylidene fluoride (PVDF) membranes were applied to the electrophoresed products. After blocking the membrane for an hour at room temperature with 5% non-fat milk, the membrane was incubated with a primary antibody (Ab) then secondary Ab. Using electrochemiluminescence (ECL), we measured the intensity of protein expression. Antibodies used in this study were Id3 (Abcam, ab236505), β-Actin (Abcam, ab8226).

Cell proliferation assay

We seeded cells in 96-well plates, incubated them at 37 ℃ for 24 h, then transfected them with plasmids. Using the cell counting kit-8 (CCK-8; APExBIO, Houston, TX, USA), we performed an assay to determine cell proliferation. The

CCK-8 assays were conducted at 24, 48, and 72 h. We used a microplate reader to measure the degree of yellow in the medium at an absorbance of 450 nm following the addition of CCK-8 and incubation for 2 h at 37 ℃ in a humidified CO_2 incubator (Bio-Rad, Hercules, CA, USA).

Cell apoptosis analyses

An apoptosis detection Kit (KGA107, KeyGen) featuring FITC Annexin V and propidium iodide (PI) was used in this study. In 6-well plates, cells were cultivated before trypsinization. Cells were resuspended in 500 µL of binding buffer after being washed twice with PBS. A549/DDP cells were then stained for 15 min with FITC Annexin V and PI. Samples were run on a Beckman Coulter FC500 (Beckman Coulter, Brea, CA, USA). Positive Annexin V staining indicated apoptotic cells.

Migration and invasion assays

Cell migration was assessed using transwell chambers with pores of 8 µm (Corning-Costar, Corning, NY, USA). After 12 h of transfection, A549/DDP cells were digested with trypsin and suspended in appropriate amounts of serum-free 1640 medium. We then seeded 1×10^5 cells in 1640 basal medium in the upper chamber and in RPMI 1640 medium with 15% fetal calf serum in the lower chamber. Cells that did not migrate were removed from the transwell surface after 48 h with a cotton swab. For 20 min, we fixed the transwell membranes in paraformaldehyde at 4%, dried them, and stained them with crystal violet (0.1%, Beyotime) for 30 min. The number of cells that had passed through the cell membrane to the lower surface was determined by optical microscopy and counted using ImageJ (version 4.0.4; National Institutes of Health, Bethesda, MD, USA). An invasion assay was performed in the same manner as a cell migration assay, however, a diluted layer of Matrigel (Biozellen, Ord, NE, USA) was precoated on the membrane filter prior to the assay.

Wound healing migration assay

The 6-well plates were seeded with 5×10^5 cells per well 12 h prior to wound cutting. Cell monolayers were scratched in a straight line using a 200 µL pipette tip. With phase contrast microscopy, wounds were photographed immediately after wound formation and 24 h later. All optical images were analyzed using ImageJ software.

Quantification of the m6A modification

Similar to the experiment above, RNA was extracted and detected. The m6A levels of tumor/normal lung tissues and DDP-sensitive or -resistant A549 cells were measured using the EpiQuik m6A RNA Methylation Quantification Kit (P-9005-96; Epigentek, Farmingdale, NY, USA) following the manufacturer's protocol. Assay cells were bound with 200 ng (1–8 µL) of RNA samples. After that, the capture antibody solution and detection antibody solution were added separately to the assay wells at a diluted concentration that was suitable for each. We measured each sample's absorbance at 450 nm, using a standard curve to quantify RNA m6A.

m6A RNA immunoprecipitation

According to previously published protocols, the immunoprecipitation of m6A RNA (m6A RIP) was performed. The primers for methylated RNA immunoprecipitation (meRIP) analysis were designed based on the high-confidence fragments. For the analysis of m6A modifications, the Sangon Biotech Immunoprecipitation Kit was used (C600689; Sangon Biotech, Shanghai, China) in cisplatin-resistant and cisplatin-sensitive lung cancer patients' tissues plus *YTHDC2* overexpressing A549/DDP cells and common A549/DDP cells based on manufacturer's recommendations. Isolated RNA was fragmented, and validated with 1.5% agarose gel electrophoresis, and RNA samples were then immunoprecipitated with anti-m6A or anti-mouse IgG (for control experiments) antibody-coated magnetic beads. The m6A enrichment was determined by qPCR analysis. The relative m6A level in *Id3* was calculated by the m6A levels (m6A IP) normalized using the expression of itself (Input).

Bioinformatics prediction tools

The tools used for bioinformatics prediction included the following: POSTAR3 (CLIPdb) (http://111.198.139.65/RBP.html), RMVar (https://rmvar.renlab.org/) and R programming language.

Statistical analysis

Experiments were repeated 3 times with biological replicates. All statistical analyses were performed using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). Comparisons between 2 groups were analyzed using

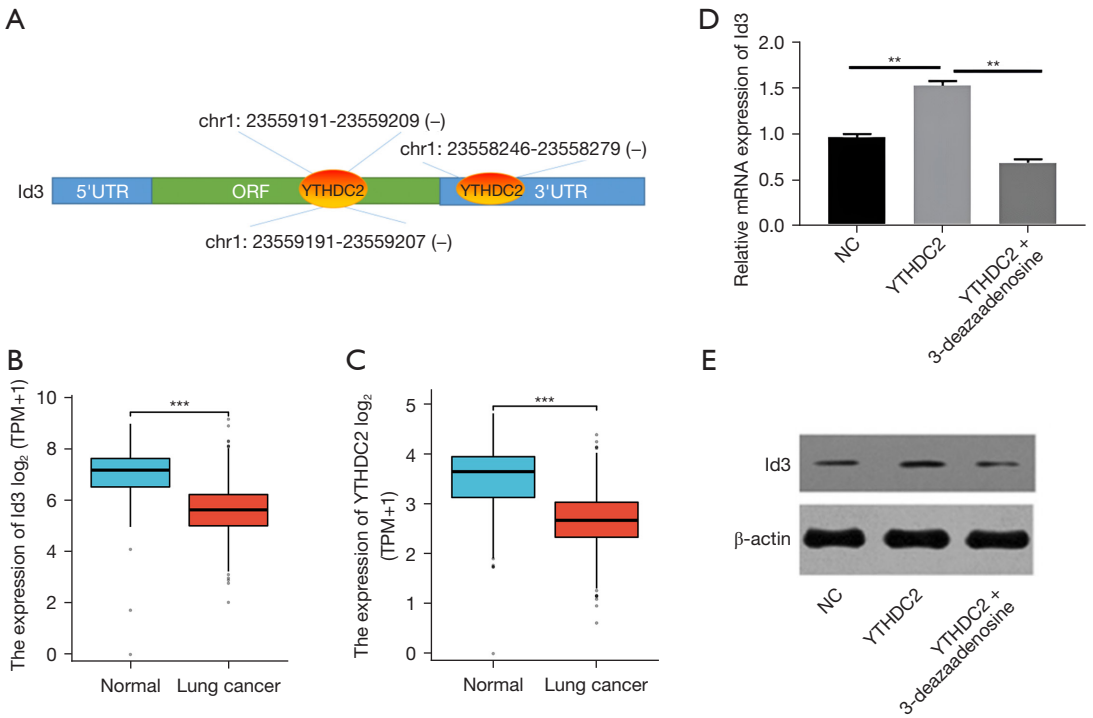

Figure 1 There is a positive correlation between *YTHDC2* and *Id3* expression in A549/DDP cells. Id3 may be modified by m6A methylation of *YTHDC2*. (A) Online analysis of potential binding sites for *YTHDC2* to Id3 via the CLIPdb database; (B) expressions of Id3 in LUAD and LUSC and normal tissues analyzed by R package using TCGA & GTEx database, ***P<0.001; (C) expressions of *YTHDC2* in LUAD and LUSC and normal tissues analyzed by R package using TCGA & GTEx database, ***P<0.001; (D) relative expression of *Id3* mRNA in the NC group, *YTHDC2*-overexpression group, and *YTHDC2*-overexpression + 3-deazaadenosine group in A549/DDP cells, detected by qRT-PCR. **P<0.01. (E) Western blot analysis of Id3 protein expression in the NC group, *YTHDC2*-overexpression group and *YTHDC2*-overexpression + 3-deazaadenosine group in A549/DDP cell line, β-actin is used as a loading control. TPM, transcripts per million; m6A, N6-methyladenosine; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; TCGA, The Cancer Genome Atlas; mRNA, messenger RNA; qRT-PCR, quantitative real-time polymerase chain reaction; NC, cells transfected with blank control plasmids.

Student's *t*-tests or analysis of variance (ANOVA). Statistical significances were considered when P values were less than 0.05. As a means of determining significance, P<0.05 was highlighted using 1 asterisk, whereas P<0.01 and P<0.001 were highlighted using 2 asterisks and 3 asterisks, separately.

Results

Id3 may be modified by m6A methylation of YTHDC2

The RMVar database predicted the m6A modification of *Id3*, and the results showed the presence of multiple m6A modification sites in the coding sequence (CDS) region and 3' untranslated region (3'-UTR) of *Id3*. The CLIPdb database found that m6A reader protein *YTHDC2* can bind to *Id3* (*Figure 1A*). The 1410 public TCGA and GTEx lung cancer cases showed that in lung cancer tissues, the expression of *YTHDC2* was lower than in adjacent tissues, in accordance with *Id3* (*Figure 1B,1C*). The above results suggested that *Id3* may be modified by m6A methylation of *YTHDC2*, and our experimental observations confirmed this conjecture. The qPCR and WB results showed that *Id3* expression was significantly up-regulated after overexpression of *YTHDC2* in the cisplatin-resistant lung cancer A549/DDP cells, whereas the methylation inhibitor 3-deazaadenosine abolished the regulatory effect of *YTHDC2* on *Id3* (*Figure 1D,1E*).

YTHDC2 expression is reduced in cisplatin resistance

We used the A549/DDP cells and A549 cells to explore cisplatin-resistance in NSCLC. Our results showed that the

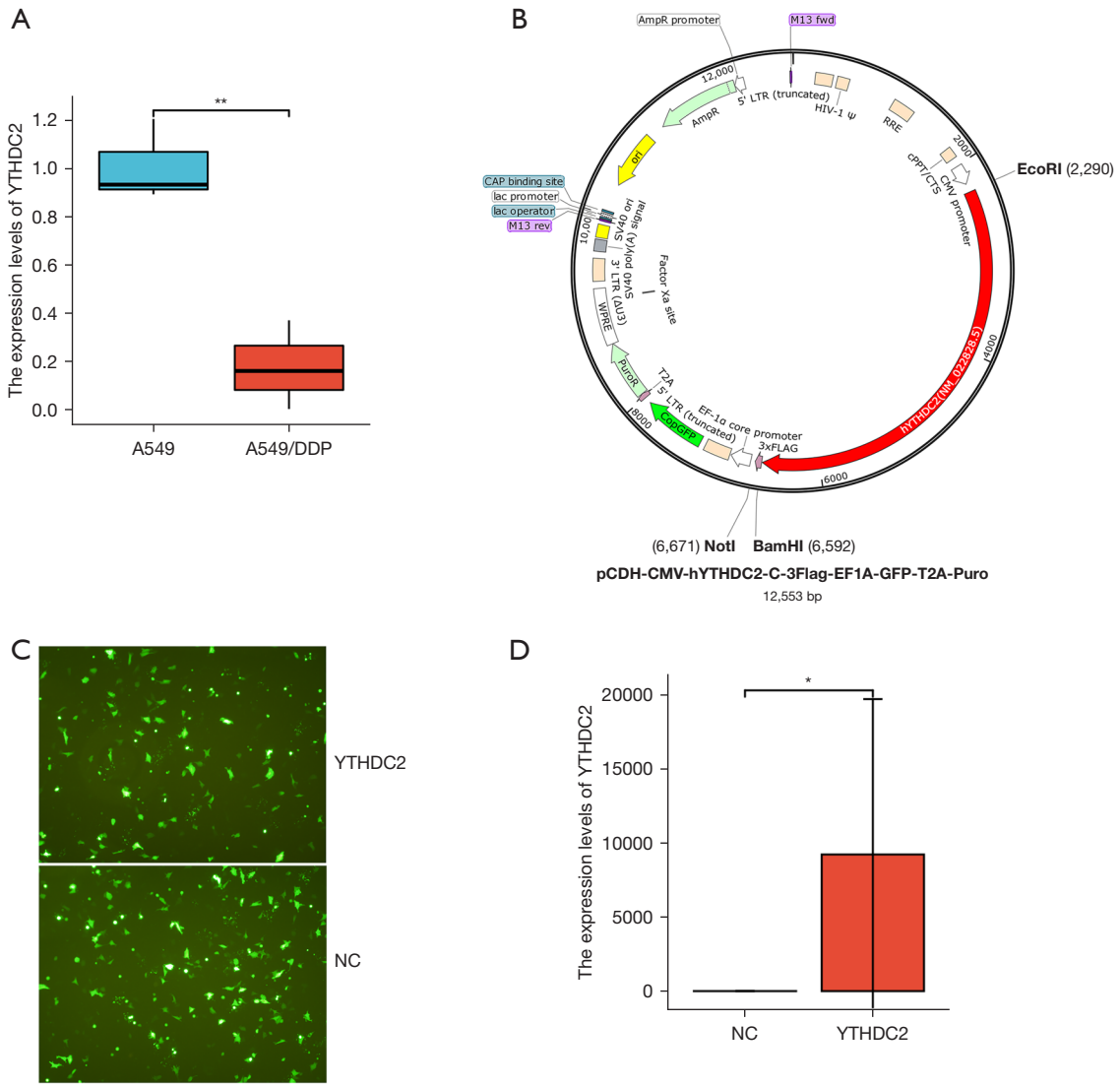

Figure 2 *YTHDC2* is lowly expressed in cisplatin-resistant cells and *YTHDC2* promotes survival in NSCLC patients. *YTHDC2* was successfully transfected into cisplatin-resistant cells. (A) Relative expression of *YTHDC2* mRNA in A549/DDP cell line *vs.* A549 cell line, detected by qRT-PCR. **P<0.01. (B) Structure of *YTHDC2* overexpression vector pCDH-CMV-hYTHDC2-C-3Flag-EF1A-GFP-T2A-Puro 12,553 bp in length and contains EcoRI and NotI cleavage sites. (C) The efficiency of transfection was monitored by fluorescence microscopy (×100 magnification). Greater than 70% cells were transfected successfully. (D) qRT-PCR analysis of *YTHDC2* in A549/DDP cells transfected with NC plasmid or *YTHDC2* overexpression plasmid. *P<0.05 compared with the corresponding Ctrl. NSCLC, non-small cell lung cancer; mRNA, messenger RNA; qRT-PCR, quantitative real-time polymerase chain reaction; NC, cells transfected with blank control plasmids.

levels of *YTHDC2* mRNA was lower expressed in A549/DDP cells than in A549 cells (*Figure 2A*).

Construction and transfection of YTHDC2 vector

To validate the biological effects of *YTHDC2* mimics, we constructed lentiviral vector to overexpress *YTHDC2* (*Figure 2B*). First, we qualitatively evaluated the transfection rates of the different vectors using fluorescent microscopy and found no significant differences among them (*Figure 2C*). qPCR analysis confirmed that the transfection was effective (*Figure 2D*).

YTHDC2 overexpression suppressed the proliferation, invasion, and migration and A549/DDP cells and promoted their apoptosis

As shown in *Figure 3A*, the proliferation capacity of A549/DDP cells were determined with CCK-8 proliferation curves, which clearly showed that the overexpression of *YTHDC2* suppressed cell proliferation. Flow cytometry assay showed a significant promotion in A549/DDP cell apoptosis after *YTHDC2* overexpression at 24 and 48 h (*Figure 3B*,*3C*). Furthermore, tumor migration and invasion ability can also be used to assess tumor malignancy and influence tumor progression. The ability of tumor invasion and migration was measured using transwell assays. As well, overexpression of *YTHDC2* significantly inhibited A549/DDP cells migration and invasion (*Figure 3D*). The above experimental results were further verified by performing wound healing assays. In *Figure 3E*, A549/DDP cells with overexpressed *YTHDC2* displayed a significant reduction in migration capacity, as expected. The above experimental results indicated that *YTHDC2* overexpression suppressed the proliferation, invasion, and migration of A549/DDP cells and promoted their apoptosis.

YTHDC2 positively regulates Id3 in an m6A-dependent manner

We affirmed m6A expression profiles between lung cancer and paracancerous tissues, plus between A549 and A549/DDP cells by RNA m6A colorimetry, which confirmed that m6A levels were higher in lung cancer tissues compared with normal tissues, higher in A549/DDP cells compared with A549 cells (*Figure 4A*,*4B*). The data suggested that m6A modification could contribute to cisplatin resistance in lung cancer. To determine whether *YTHDC2* regulates *Id3* m6A levels, immunoprecipitation of the methylated RNA was performed. Cisplatin-resistant lung cancer tissues had a lower relative methylated-*Id3* level compared to cisplatin-sensitive tissues; *YTHDC2* overexpressing resistant cells A549/DDP had a higher relative methylated-*Id3* level compared to normal resistant cells A549/DDP (*Figure 4C*,*4D*). Based on these results, *Id3*'s m6A modification could be transferred in large extent by *YTHDC2*. In summary, *YTHDC2* inhibits NSCLC resistance to cisplatin by upregulating *Id3* through inhibition of m6A methylation.

Discussion

It has long been known that the development of NSCLC is a complex, multi-stage process involving the progressive adoption of genetic and epigenetic alterations, leading to unlimited growth and proliferation of tumor cells. Hence, understanding the molecular mechanisms behind NSCLC is crucial, especially in the pathogenesis of chemotherapy resistance. A number of studies have implicated m6A modification in diseases such as acute myeloid leukemia (14), hepatocellular carcinoma (15), type 2 diabetes (16), rheumatoid arthritis (17), and pulmonary hypertension (18). Recently, further studies have reported on m6A modification related protein's biological function in lung cancer. For example, through m6A modification, *METTL3* (m6A writer) promoted Bcl2 translation, increasing viability and enhancing migration in NSCLC cells (19). *ALKBH5* (m6A eraser) is another m6A demethylase that promotes NSCLC progression as a result of regulating the stability of *TIMP3* (20). In addition, Wang *et al.* demonstrated that *NELL2* expression is induced by m6A demethylase *FTO* (m6A eraser) by inhibiting *E2F1* m6A modification, leading to NSCLC metastasis (21). However, m6A binding proteins (m6A readers) remain unexplored in NSCLC.

Although *YTHDC2*, an m6A reader protein, has been linked to multiple cancers, its role in NSCLC is unclear. Several studies have demonstrated that the expression of *YTHDC2* was significantly reduced in different cell lines of NSCLC (22). Our study found that *YTHDC2* was deregulated in drug-resistant cells. However, some studies have found that *YTHDC2* has high levels of expression in various cancers, including gastric cancer (23), nasopharyngeal carcinoma (12), and colon cancer (24). In future studies, we plan to investigate this mechanism further.

Uncertainty exists regarding whether cisplatin resistance in NSCLC is regulated by functional proteins involved in m6A modification. Herein, based on our functional experiments, we discovered that *YTHDC2* was able to inhibit lung cancer cisplatin-resistance cells proliferation and migration, and promoted their apoptosis through m6A-dependent increases in *Id3*, which ultimately inhibited cisplatin resistance in lung cancer. Similarly, as Zhao *et al.* discovered, the expression level of *YTHDC2* was positively associated with HNSCC prognoses, indicating *YTHDC2* might play a HNSCC suppressor role (25). In line with our findings, Sun *et al.* (26) found that low expression of *YTHDC2* was significantly associated with poor differentiation, lymph node metastasis, tumor size and stage by immunohistochemistry (IHC) performed in 96 NSCLC tissues and 31 adjacent normal tissues. However, a study has shown that *YTHDC2* promotes

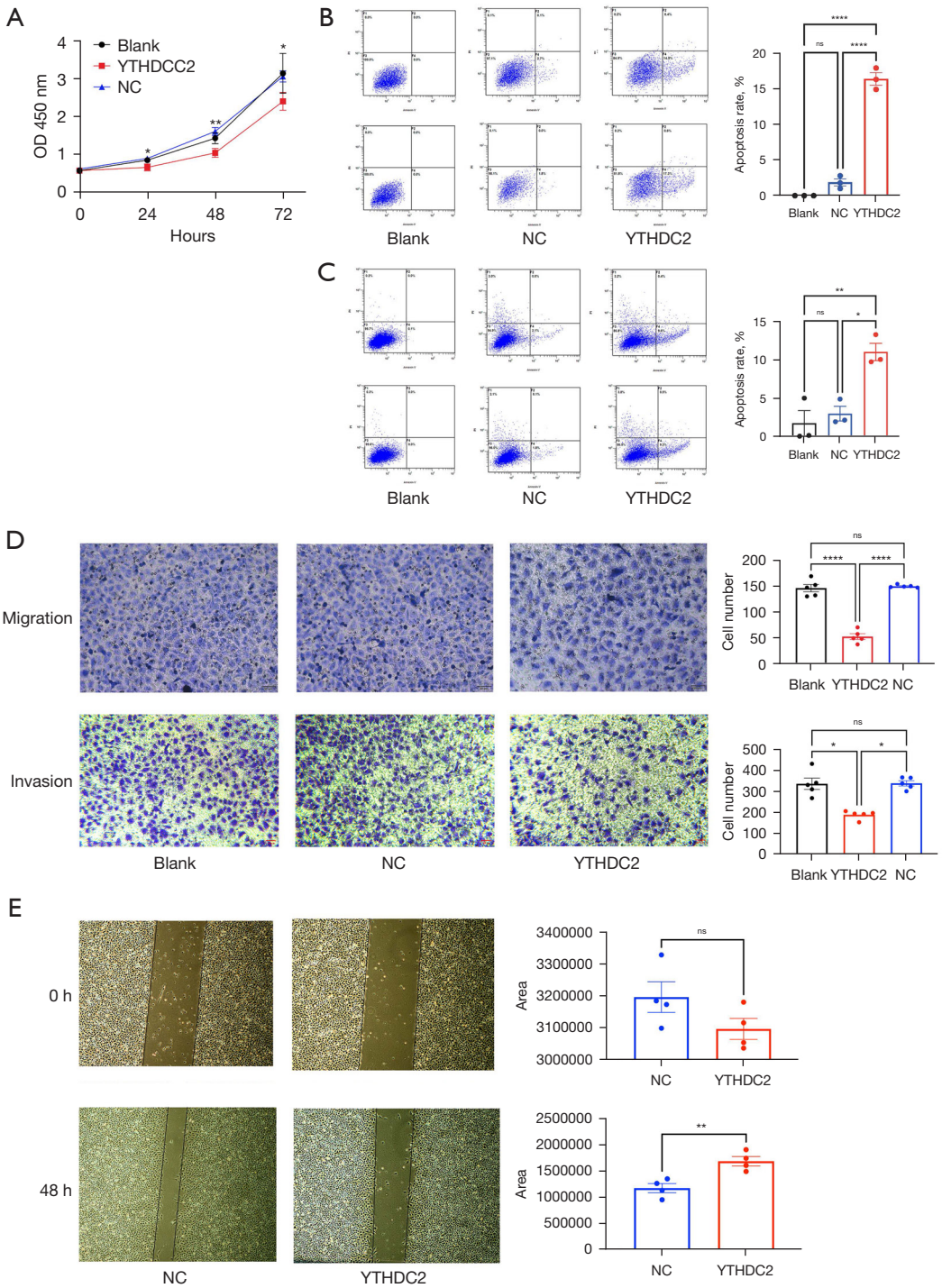

Figure 3 The overexpression of *YTHDC2* inhibits the proliferation, migration, and invasion of A549/DDP cells *in vitro* and promotes their apoptosis, which in turn inhibits NSCLC cisplatin resistance. CCK-8 assay (A), flow cytometry 24 h (B) and 48 h (C), transwell migration and invasion assays (the migrated or invaded cells were stained with crystal violet. Number of migrating cells was counted under ×100 magnification.) (D) and cell scratch test (×40) (E) of A549/DDP cells transfected with *YTHDC2* overexpression or control. Error bars represent mean ± SD of at least 3 independent experiments. Two-way ANOVA or Student's *t* test were used. *P<0.05; **P<0.01; ****P<0.0001. NSCLC, non-small cell lung cancer; NC, cells transfected with blank control plasmids; OD, the optical density value, the value of OD 450 nm represents the number of viable cells; ns, non-significant difference. CCK-8, cell counting kit-8; SD, standard deviation; ANOVA, analysis of variance.

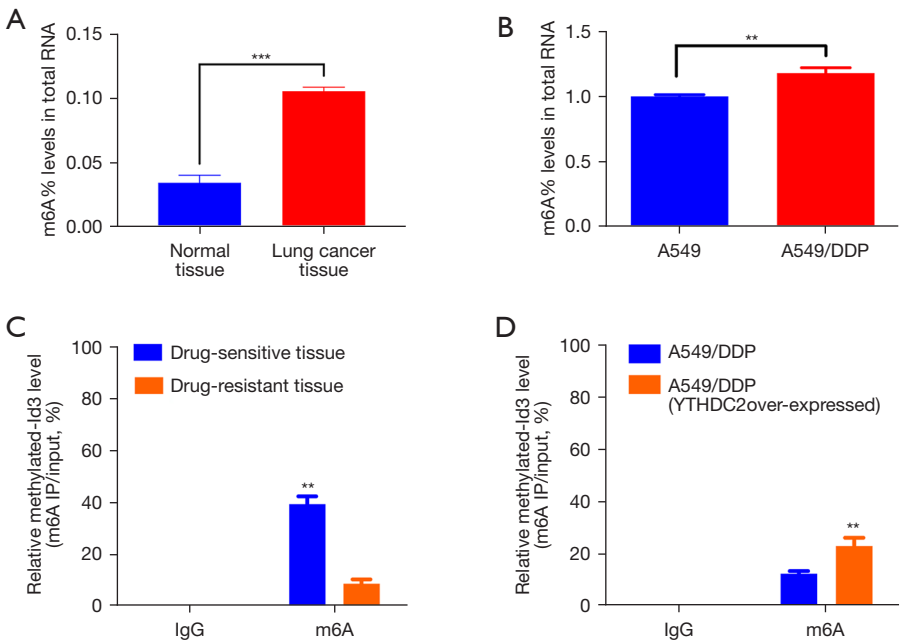

Figure 4 Id3 is positively regulated by YTHDC2 in an m6A-dependent manner. (A) RNA m6A colorimetry measured the levels of total RNA in tumor and matched normal tissues; (B) RNA m6A colorimetry measured the levels of total RNA in cisplatin-resistant and cisplatin-sensitive cells; (C) meRIP assay-qPCR analysis of Id3 in cisplatin-resistant and cisplatin-sensitive tissues; (D) meRIP-qPCR analysis of Id3 in YTHDC2 overexpressing (OE-YTHDC2) A549/DDP cells and the control (OE-NC). Student's *t*-test was used. **P<0.01, ***P<0.001. m6A, N6-methyladenosine; meRIP, methylated-RNA immunoprecipitation; qPCR, quantitative polymerase chain reaction.

colon cancer metastasis via enhancing HIF-1α mRNA translation (24). The results from these studies might seem contradictory, suggesting that as tumor types vary, *YTHDC2*-mediated tumor progression may act differently, which should be further investigated. Furthermore, *YTHDC2* seems to affect mRNA stability in some studies. In this study, m6A-IP-PCR analysis identified *YTHDC2* specifically targeted *Id3*, hence, we hypothesized that *YTHDC2* would remove the methylation on *Id3* at the transcript level. This suggested that *YTHDC2* exerted a catalytic effect on *Id3* protein via uninstalling the methylation and improving *Id3* mRNA transcript stability. The biological roles of *Id3* have been illustrated in our previous study (27,28). Yuan *et al.* found that *YTHDC2* enhances the translation of *YAP* and activates oncogenic *YAP* signaling through its binding to the m6A coding sequence on *YAP* mRNA 5'-UTR (23). Our observations revealed that *YTHDC2* is a new tumor suppressor in NSCLC resistant to cisplatin.

Conclusions

We have demonstrated that *YTHDC2* relies on m6A modification to regulate *Id3* action and ultimately inhibit cisplatin resistance in NSCLC. Hopefully, this will shed light on next-generation therapeutic strategies of NSCLC, and more studies are required in order to reveal the particular m6A regulatory mechanism.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-187/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-187/dss

Peer Review File: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-187/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-187/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All participants signed an informed consent form, and this study was approved by Ethics Committee of Jinling Hospital (No. 2020DZGZRZX-096). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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