



DNAJC12 activated by *HNF1A* enhances aerobic glycolysis and drug resistance in non-small cell lung cancer

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Background: The DnaJ heat shock protein family member C12 (*DNAJC12*) gene has been demonstrated to promote lung cancer cell growth and migration by increasing β -catenin expression. In this study, we aimed to reveal the role of *DNAJC12* in modulating aerobic glycolysis and cisplatin (DDP) resistance in non-small cell lung cancer (NSCLC).

Methods: Bioinformatics analysis was applied to assess the expression levels of *DNAJC12* and hepatocyte nuclear factor 1-alpha (*HNF1A*) in NSCLC tissues and the correlation between *DNAJC12* and *HNF1A* expression levels. Cell Counting Kit-8 (CCK-8), flow cytometry assay, and in vivo tumor formation were used to evaluate cell growth, apoptosis, and tumorigenesis. Luciferase gene reporter assay was adopted to assess the relationship between *HNF1A* and *DNAJC12*.

Results: Both *DNAJC12* and *HNF1A* were overexpressed in NSCLC tissues and cells, and their expressions showed a positive correlation. The *HNF1A* gene could bind to the promoter of *DNAJC12* and promoted its transcription and translation. Overexpression of both *DNAJC12* and *HNF1A* promoted cell growth, aerobic glycolysis, and inhibited cell apoptosis in NCI-H1975 and NCI-H1650 cells, as well as the cell apoptosis induced by DDP. In addition, cell growth and aerobic glycolysis mediated *DNAJC12* were reversed by the silencing of β -catenin, and downregulation of *DNAJC12* abolished the above roles of *HNF1A*.

Conclusions: This study revealed that *DNAJC12*, activated by the transcription factor *HNF1A*, could enhance aerobic glycolysis and drug resistance to DDP in NSCLC through regulating β -catenin expression.

Keywords: Hepatocyte nuclear factor 1-alpha (*HNF1A*); DnaJ heat shock protein family member C12 (*DNAJC12*); cell growth; apoptosis; drug resistance

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Introduction

Lung cancer is one of the leading causes of cancer-related death worldwide (1). It is estimated that more than 7,333,000 patients are diagnosed with lung cancer every year and it causes approximately 6,102,000 deaths annually in China (2). Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for about 80% of reported cases (3). Platinum, especially cisplatin (DDP)-based

chemotherapy is a main treatment method for NSCLC after surgical resection (4). However, prolonged use of DDP often induces chemoresistance in tumor cells, allowing them escape apoptosis (5). Therefore, it is an urgent need to reveal the mechanism underlying DPP resistance in NSCLC.

Cancer cells are characterized by metabolic deregulation, including aerobic glycolysis, which is also known as the “Warburg effect” (6). Through increasing of the uptake and

consumption of glucose, cancer cells gain enough energy to maintain their rapid growth, even in the presence of high oxygen concentration (7). Studies have shown that glycolysis is closely related to the prognosis of NSCLC patients. For instance, Smolle *et al.* (8) showed that the expression of *GLUT1*, the prime glucose transporter, was associated with decreased overall survival of patients with NSCLC. Monaco *et al.* (9) revealed that patients with total lesion glycolysis (TLG) values lower than the median values had improved overall survival compared to patients with higher TLG. Yao *et al.* (10) obtained 200 glycolysis-related genes from Gene Set Enrichment Analysis (GSEA) and found 46 genes were significantly associated with the overall survival of patients with NSCLC. Also, it has demonstrated that the aerobic glycolysis plays crucial roles in accelerating cancer cell growth, migration, and drug resistance (11,12). Targeting of aerobic glycolysis is an innovative idea to overcome drug resistance in cancers (13,14).

The gene DnaJ heat shock protein family member C12 (*DNAJC12*) belongs to the heat shock protein (HSP) family, which act as molecular chaperones (15,16). Recently, studies have demonstrated that *DNAJC12* is overexpressed and implicated in carcinogenesis, including gastric cancer (17) and rectal cancer (18). Using bioinformatics technology, we found that *DNAJC12* is upregulated in lung adenocarcinoma (LUAD). Consistently, Li *et al.* (19) recently reported that *DNAJC12* was overexpressed in lung cancer, and overexpression of *DNAJC12* significantly promoted lung cancer cell growth and migration by increasing β -catenin expression. Noticeably, accumulated evidence has verified that the β -catenin signaling is closely implicated in regulating the aerobic glycolysis and drug resistance in lung cancer (20–23), suggesting that *DNAJC12* may participate in regulating the aerobic glycolysis and drug resistance of lung cancer through activating the β -catenin signaling. However, the role of *DNAJC12* in modulating aerobic glycolysis and DDP resistance in NSCLC still remains unclear.

Hepatocyte nuclear factor 1-alpha (*HNF1A*) is an endoderm-restricted transcription factor and has been reported to induce long non-coding RNA (lncRNA) *HCG18* expression which promoted gastric cancer progression (24). Also, *HNF1A* has been identified to serve as an oncogene in pancreatic cancer to increase cancer stem cell properties (25). Using bioinformatics technology, we found that *HNF1A* expression was positively correlated with *DNAJC12*, and *HNF1A* can bind to the promoter region of *DNAJC12*. Noticeably, it has been reported that

overexpression of *HNF1A* causes significant enhancement in the drug resistance of colorectal cancer (26). Thus, we assumed that *DNAJC12* transcription may be increased by *HNF1A* and then accelerates aerobic glycolysis and enhances drug resistance to DDP in NSCLC.

Although it has been demonstrated that *DNAJC12* was overexpressed and served as an oncogene in lung cancer through regulating β -catenin signaling (19), *DNAJC12* roles in modulating aerobic glycolysis and DDP resistance in NSCLC still remain unclear, as well as the underlying mechanisms. In this study, we explored the role of *DNAJC12* in aerobic glycolysis and DDP resistance of NSCLC, and studied whether *DNAJC12* transcription could be modulated by *HNF1A* for the first time using both *in vitro* (cell lines) and *in vivo* assays through construction of the xenotransplantation model in 6-week-old BALB/c nude mice. We present the following article in accordance with the ARRIVE reporting checklist (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-1475/rc>).

Methods

Tissue samples

A total of 60 NSCLC tissues and normal tissues adjacent to the cancer tissues were obtained from patients with primary NSCLC at the Department of Thoracic Surgery, Shanghai General Hospital between February, 2013 and August, 2015. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethics Committee of Shanghai General Hospital (No. 2018KY086) and informed consent was taken from all the patients.

Bioinformatics analysis

We used the University of Alabama Cancer (UALCAN; <http://ualcan.path.uab.edu/analysis.html>) and Gene Expression Profiling Interactive Analysis (GEPIA; <http://gepia.cancer-pku.cn/>) databases to analyze the expression pattern of *DNAJC12* in NSCLC tissues. The starBase database (<https://starbase.sysu.edu.cn/>) was applied to analyze the expression levels of *HNF1A* in NSCLC samples, and the correlation between the expression levels of *DNAJC12* and *HNF1A* in NSCLC. The JASPAR (<https://jaspar.genereg.net/>) database was used to predict the binding sites of transcription factor *HNF1A* in the *DNAJC12* promoter region.

Table 1 Primer sequences

| Gene | Forward (5'-3') | Reverse (5'-3') |
|------------------|----------------------|-------------------------|
| <i>DNAJC12</i> | CGAAGCTCACTGTGCCTCTT | GGATGCTTGTCTGGGTGACA |
| <i>HNF1A</i> | ACAGCTTGGAGCAGACATCC | CTGCTTGGTGGGCGTGAG |
| <i>β-catenin</i> | CTGAGGAGCAGCTTCAGTCC | CCATCAAATCAGCTTGAGTAGCC |
| <i>β-actin</i> | ACAGAGCCTCGCCTTTGCC | TTCTCCATGTCGTCCCAGTTG |

DNAJC12, DnaJ heat shock protein family member C12; *HNF1A*, hepatocyte nuclear factor 1-alpha.

Cell culture and DDP-resistance cell line construction

A human lung epithelial cell line BEAS-2B and 4 human lung cancer cell lines A549, NCI-H2106, NCI-H1975, and NCI-H1650 were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). Then, NCI-H1975/DDP and NCI-H1650/DDP, 2 cisplatin-resistant cell lines were established by explosion to a series of stepwise-increased concentrations of DDP. The A549 cells were maintained in F-12K medium, while NCI-H2106, NCI-H1975, NCI-H1650, NCH-H1975/DDP, and NCI-H1650/DDP cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium, with 10% fetal bovine serum (FBS). To maintain the resistance, 1 µg/mL DDP was added to cell cultures of NCH-H1975/DDP and NCI-H1650/DDP cells. All cells were maintained in a humid cell incubator with 5% CO₂ at 37 °C. Cell culture medium and FBS were obtained from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). The NCH-H1975/DDP and NCI-H1650/DDP cells were administrated with 10 µM cisplatin (DDP, Sigma Aldrich, St. Louis, MO, USA) for 24 hours.

Cell transfection

The overexpressing plasmid used to upregulate *DNAJC12* (OE-DNAJC12) and *HNF1A* (OE-HNF1A) expression in NSCLC cells were purchased from Origene (cat. RC201931 and RC211201; Beijing, China). The cells were plated into 6-well plates and transfected with 2 µg of OE-DNAJC12 or OE-HNF1A with the help of Lipofectamine 3000 (Invitrogen, Thermo Fisher Scientific) when cell confluence reached about 50%. The lentivirus short hairpin RNA (shRNA) vectors used to downregulate *DNAJC12* (sh-DNAJC12), *HNF1A* (sh-HNF1A) and *β-catenin* (sh-β-catenin) were synthesized by GenePharma (Shanghai, China) and introduced into cells using polybrene (5 µg/mL). The infected cells were cultured in RPMI-1640 medium

with puromycin (4 µg/mL) for 14 days to establish stable cell lines to be used in the animal assay.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

TRIzol reagent (Invitrogen, USA) was used for the total RNA extraction. After that, the complementary DNA (cDNA) was synthesized using PrimeScript RT Master Mix kit (RR036A; Takara, Shiga, Japan). Then, the PCR detection was carried out with 2× SYBR Green PCR Mastermix (Solarbio, Beijing, China) in 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with the following reactions: 95 °C for 1 minute, 39 cycles of 95 °C for 15 seconds and 60 °C for 1 minute. The relative expressions of messenger RNA (mRNA) were calculated in the light of the 2^{-ΔΔC_q} method (27). The primers are listed in Table 1.

Western blotting

Lysis buffer (Solarbio, Beijing, China) with 1% protease inhibitor (Solarbio) were used for total protein isolation. After centrifugation at 4 °C for 30 minutes, protein concentrations was tested using bicinchoninic acid (BCA) Protein Assay kits (Thermo Fisher Scientific). Then, the proteins were loaded to 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel and separated by electrophoresis. Then, transformation to polyvinylidene difluoride membranes (PVDF; Millipore, Billerica, MA, USA) was performed. After that, the membranes were blocked in 5% non-fat milk for 1 hour at room temperature and immersed in primary antibodies overnight at 4 °C [anti-β-actin antibody (1:5,000 dilution; cat. ab8226, Abcam, Cambridge, MA, USA) and anti-DNAJC12 antibody (1:2,000 dilution; cat. ab167425, Abcam)]. The membranes were then incubated with the horseradish peroxidase (HRP)-conjugated secondary antibodies at room temperature

for 1 hour. Protein signaling was measured by using the ProfiBlot-48 (Tecan, Zurich, Switzerland) with the help of electrochemiluminescence (ECL) reagent (Millipore, USA) and quantified using Image J v2.1.4.7 (National Institutes of Health, Bethesda, MD, USA).

Double luciferase gene reporter measurement

Sequences of *DNAJC12* promoter regions were inserted into the luciferase reporter pGL3 vectors. Then, the cells were co-transfected with the pGL3 vectors and OE-NC, OE-HNF1A, sh-NC, or sh-HNF1A. After 48 hours, cells in each group were collected for luciferase activity detection based on the Dual-Luciferase Reporter Assay protocol (Promega, Madison, WI, USA).

Detection of lactate production, glucose consumption, and adenosine triphosphate (ATP) levels

The lactate production, glucose consumption, and ATP levels were evaluated as previously reported (28). Cells were placed into 6-well plates at a density of 2×10^5 /well and cultured at 37 °C for 48 hours, then the lactate production of cells was examined using Lactate Colorimetric Assay Kits (cat. no. K627, Biovision, Milpitas, CA, USA). Supernatant was collected from cells kept in FBS-free medium for 1 hour, and used for the measurement of lactate production. The reaction mixture was incubated for 30 minutes at room temperature in the dark. The lactate levels were measured at 450 nm in a microplate reader.

Glucose consumption was examined using Glucose Uptake Colorimetric Assay Kit (cat. no. K676, Biovision, Milpitas, CA, USA). Briefly, cells with lentivirus infection were collected and placed into 96-well plates at a density of 1×10^4 cells/well. Then, the cells were incubated with Krebs-Ringer-Phosphate-HEPES (KRPH) buffer (100 μ L) for 40 minutes, and 10 μ L 2-deoxyglucose (10 mM) for 20 minutes, and Reaction Mix A for 1 hour at 37 °C to deprive glucose. Then, the cells were probed with 90 μ L extraction buffer for 40 minutes at 90 °C, followed by an ice bath for 5 minutes. Each well was then added reaction Mix B, and centrifugation at 12,000 rpm at 4 °C for 2 minutes was performed. The optical density (OD) value at 412 nm was measured using a microplate analyzer for the supernatant.

An ATP Colorimetric Assay Kit purchased from Sigma-Aldrich (cat. no. MAK1900) was used for ATP level detection based on the manufacturer's protocols. Cells

(5×10^5) were harvested and extracted in 100 μ L of the ATP Assay Buffer. The cells were centrifuged at 12,000 rpm for 5 minutes and the supernatant was used for ATP measurement. The reaction mixture was incubated for 30 minutes at room temperature, protected from light, and measured at 570 nm in a microplate reader.

Cell Counting Kit-8 assay

The NSCLC cells at logarithmic growth phase were adjusted to a density of 2×10^4 cells/mL and placed into 96-well plates (100 μ L per well). Then, 10 μ L of CCK-8 (Abcam) was added and the cells were cultured at 37 °C for another 4 hours after cell transfection. The OD at 450 nm was measured on microplate reader (Tecan Infinite M200 Micro Plate Reader; LabX, Männedorf, Switzerland).

Flow cytometry assay

An FITC-Annexin V Apoptosis Detection Kit (Becton, Dickinson, and Co. Biosciences, San Diego, CA, USA) was used to determine cell apoptosis rates according to the manufacturer's instructions. The cells were analyzed by a Beckman FC500 flow cytometer (Beckman Coulter, Inc., Brea, CA, USA). The data were analyzed by FlowJo 7.6 software (<https://www.flowjo.com/>).

Animal assay

Animal experiments were performed under a project license (No. 2018AWS0095) granted by the Ethics Committee of Shanghai General Hospital, in compliance with the institutional guidelines for the care and use of animals. A protocol was prepared before the study without registration. The 6-week-old BALB/c nude mice with specific-pathogen-free (SPF) grade were obtained from the Animal Center of Air Force Medical University (Shanghai, China). The mice were housed at 25 °C with 55% humidity in a 12 light/12 dark cycle with ad libitum access to water and food. The weight of the included mice were 20 ± 2 g and the mice were fed in same condition to minimize potential confounders. A total of 10 mice were randomly divided into the sh-NC and sh-DNAJC12 group, with each group containing 5 mice. The NCI-H1650 cells with sh-NC or sh-DNAJC12 stable transfection were used to build the model. The mice armpits were injected with 1×10^6 cells following 7 days of accommodation. Tumor volume was measured every 3 days. At 28 days after injection, or when the diameter

of tumors reached over 1 cm, mice were sacrificed by cervical dislocation. Mice were considered dead when their breathing and heartbeat stopped, they had no reflexes, and the body became cold. Then, the tumors were harvested and weighed, and the tissues were used for western blotting, qRT-PCR and hematoxylin and eosin staining.

In addition, hematoxylin and eosin staining was used to assess the morphological changes of the tumor tissues. In brief, tumor samples were washed, dehydrated in 70%, 80% and 90% ethanol solutions, immersed in equal volumes of absolute alcohol and xylene for 15 min, transparentized in xylene and immersed in paraffin and xylene for 15 min. Following being embedded in paraffin, the tissues were cut into 4 μm -thick sections, and the sections were dewaxed and hydrated, following by staining with hematoxylin solution for 3 min. The sections were then washed with water and immersed in eosin solution for 3 min. The staining was assessed under a light microscope (CKX41; Olympus Corporation).

Statistical analysis

The data from three independent assays were presented as the mean \pm standard deviation (SD), and $P < 0.05$ was considered statistically significant. The statistical analyses were carried out using the software SPSS 21.0 (IBM Corp., Armonk, NY, USA). Student's *t*-test was used to analyze the difference between two groups. One-way analysis of variance (ANOVA) followed by Dunnett's test was adopted to compare more than two groups. Pearson correlation coefficient was adopted to analyze the correlation between *DNAJC12* expression and *HNF1A* expression in NSCLC tissues.

Results

DNAJC12 is overexpressed in NSCLC tissues and cells

First, we assessed the expression levels of *DNAJC12* in NSCLC tissues and cells. The UALCAN database showed that *DNAJC12* was among the top 25 upregulated genes in LUAD (Figure 1A), which was confirmed by the GEPIA database (Figure 1B). To verify this, we detected *DNAJC12* expression in 60 paired NSCLC tissues and normal tissues using qRT-PCR assay. The results showed that the level of *DNAJC12* was significantly higher in NSCLC tissues than in normal tissues (Figure 1C). We also detected *DNAJC12*

protein levels in 4 matched cancer tissues and normal tissues, randomly selected from the 60 paired tissues, using western blotting assay. Consistently, *DNAJC12* level was elevated in cancer tissues as compared with the normal tissues (Figure 1D). In addition, *DNAJC12* levels in NSCLC cell lines, including NCI-H1975, A549, NCI-H2106, and NCI-H1650 cells were obviously higher than that of BEAS-2B cells (Figure 1E,1F). These results demonstrated that *DNAJC12* was overexpressed in NSCLC.

DNAJC12 accelerates aerobic glycolysis in NSCLC cells

Then, we explored the role of *DNAJC12* in regulating aerobic glycolysis in NSCLC cells using the gain- and loss-of-function assays. As NCI-H1975 and NCI-H1650 cells showed medium expression levels of *DNAJC12* among the 4 detected cell lines, NCI-H1975 and NCI-H1650 cells were chosen for the following studies. The level of *DNAJC12* was significantly increased following cell transfection with OE-*DNAJC12*, while decreased when cells were infected with sh-*DNAJC12* (Figure 2A,2B). Compared with the control group, *DNAJC12* overexpression significantly increased ATP levels, lactate production, and glucose consumption, while silencing of *DNAJC12* caused the opposite results (Figure 2C-2E). These results demonstrated that *DNAJC12* promoted aerobic glycolysis in NSCLC cells.

DNAJC12 promotes cell growth and enhances DDP resistance in NSCLC

We assessed the role of *DNAJC12* in modulating cell growth and drug resistance. Cell growth was significantly enhanced when *DNAJC12* was overexpressed in NCI-H1975 and NCI-H1650 cells (Figure 3A,3B), while cell apoptosis was reduced (Figure 3C,3D). However, downregulation of *DNAJC12* inhibited cell growth and increased cell apoptosis (Figure 3A-3D). In addition, *DNAJC12* levels in NCI-H1975/DDP and NCI-H1650/DDP cells were higher than those in their parental cells (Figure 3E-3H). Moreover, overexpression of *DNAJC12* reversed the inhibition of cell growth induced by DDP and reduced cell apoptosis, while depletion of *DNAJC12* enhanced the role of DDP in inhibiting cell growth (Figure 3I,3J) and inducing cell apoptosis (Figure 3K,3L). These results demonstrated that *DNAJC12* promoted NSCLC cell growth and enhanced drug resistance to DDP.

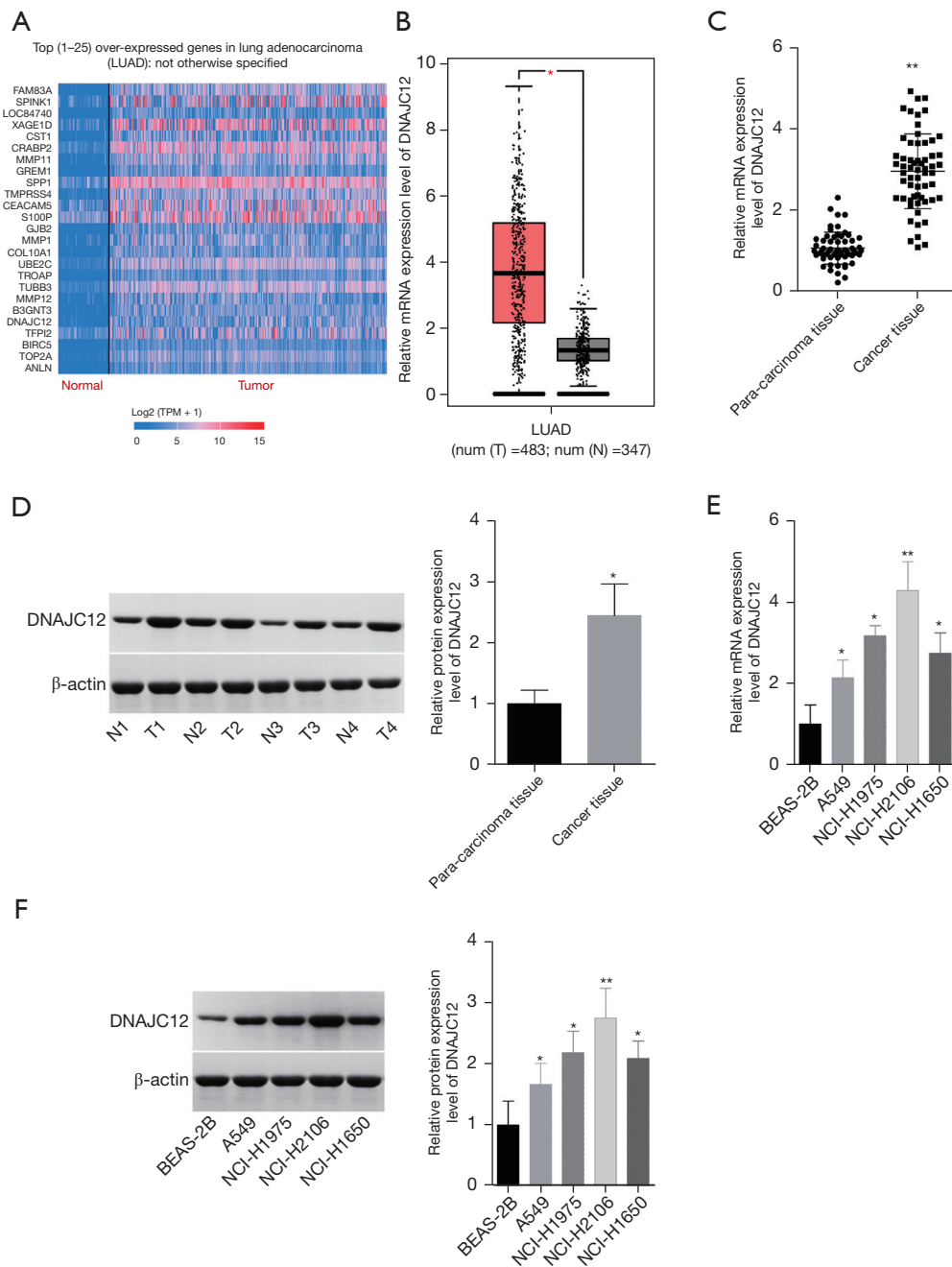


Figure 1 *DNAJC12* is overexpressed in NSCLC tissues and cells. (A,B) bioinformatics database UALCAN and GEPIA showed that *DNAJC12* expression was increased in lung cancer tissues. (C,D) qRT-PCR and western blotting were adopted to detect the mRNA and protein levels of *DNAJC12* in NSCLC tissues and normal tissues. (E,F) qRT-PCR and western blotting were adopted to detect the mRNA and protein levels of *DNAJC12* in BEAS-2B, NCI-H1975, A549, NCI-H2106, and NCI-H1650 cells. (*, $P < 0.05$, **, $P < 0.01$). LUAD, lung adenocarcinoma; *DNAJC12*, DnaJ heat shock protein family member C12; N, normal; T, tumor; NSCLC, non-small cell lung cancer; UALCAN, University of Alabama Cancer; GEPIA, Gene Expression Profiling Interactive Analysis; qRT-PCR, quantitative reverse transcription polymerase chain reaction; mRNA, messenger RNA.

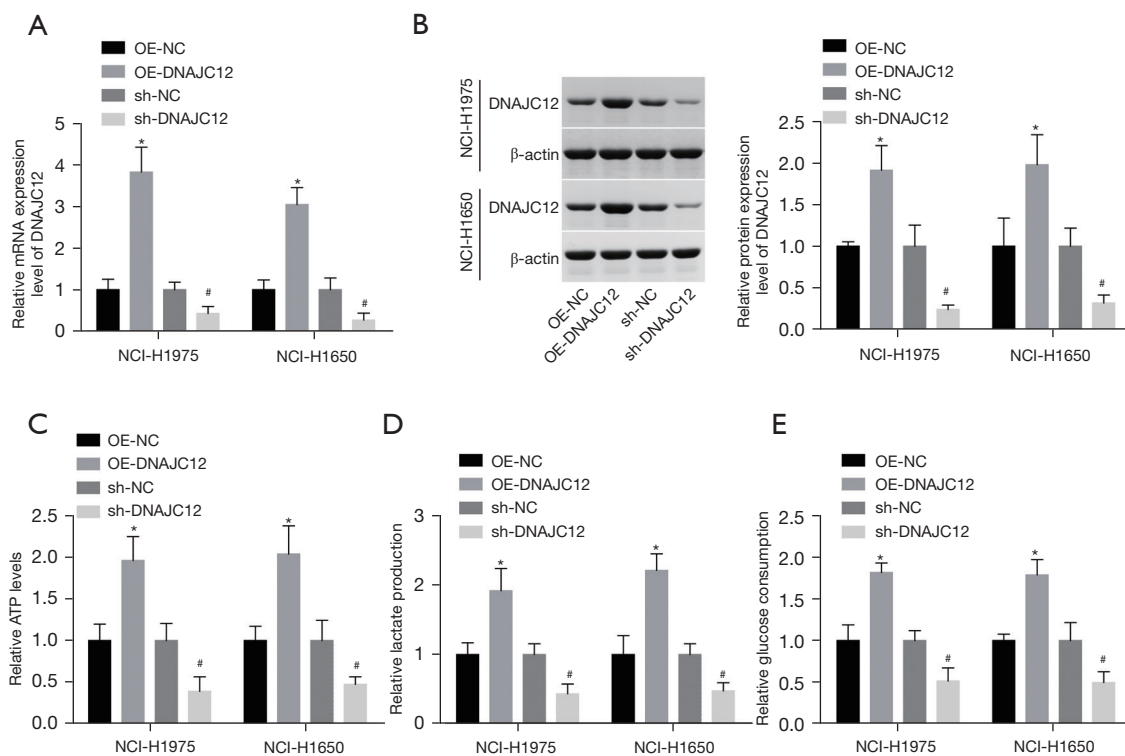


Figure 2 *DNAJC12* promoted cell aerobic glycolysis in NSCLC. (A,B) *DNAJC12* levels were measured by qRT-PCR and western blotting assay in NCI-H1975 and NCI-H1650 cells from OE-NC, OE-DNAJC12, sh-NC and sh-DNAJC12 groups. (C) ATP levels, (D) lactate production and (E) glucose consumption in NCI-H1975 and NCI-H1650 cells from OE-NC, OE-DNAJC12, sh-NC, and sh-DNAJC12 groups were tested. (*, $P < 0.05$ vs. OE-NC group, #, $P < 0.05$ vs. sh-NC group). mRNA, messenger RNA; *DNAJC12*, DnaJ heat shock protein family member C12; OE, overexpressing; sh, short hairpin; NC, normal control; ATP, adenosine triphosphate; NSCLC, non-small cell lung cancer; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

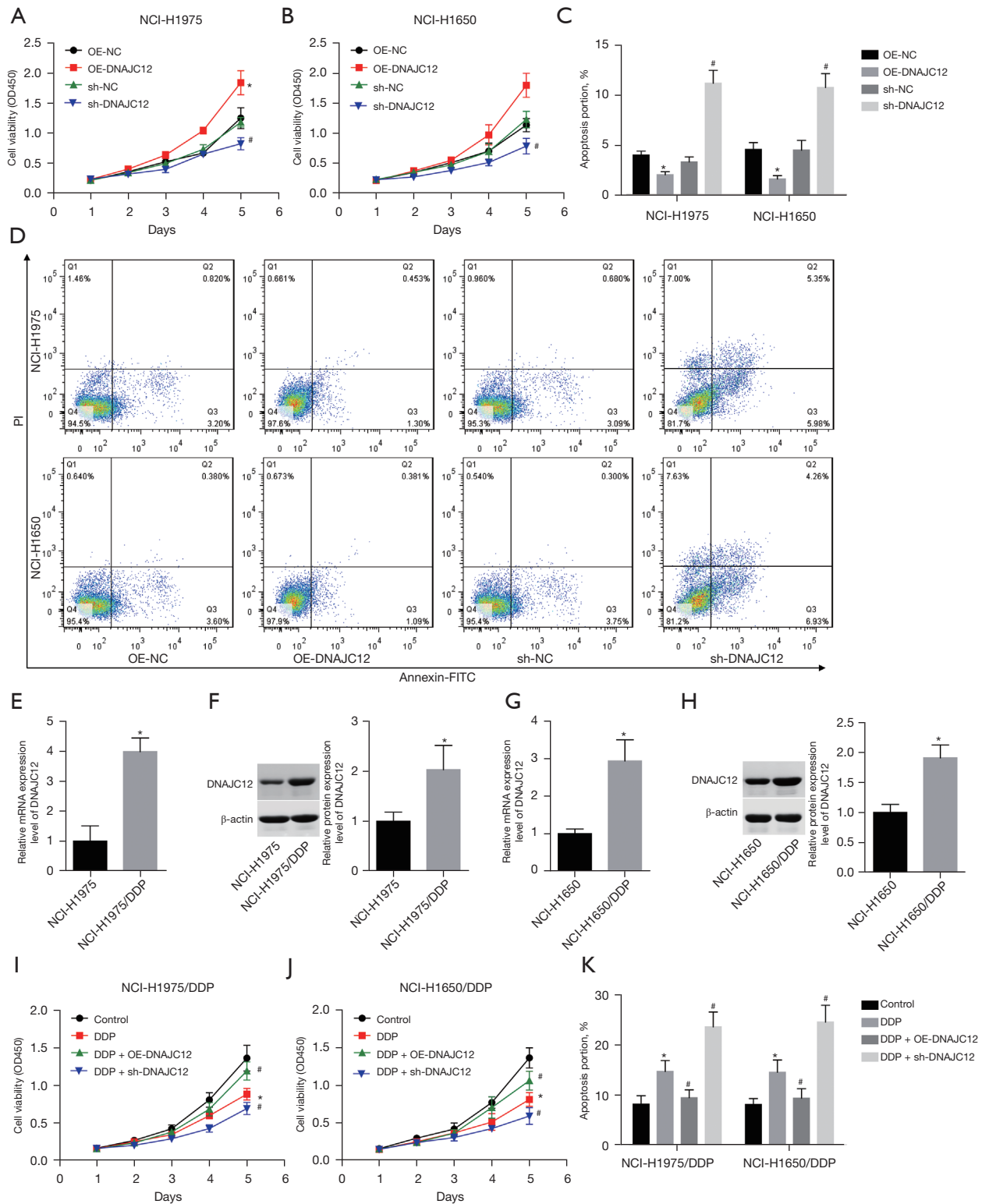
HNF1A promotes *DNAJC12* expression

Through bioinformatics, we found that *DNAJC12* expression was positively correlated with the expression level of *HNF1A*, which was also upregulated in lung cancer tissues (Figure 4A,4B). We then used qRT-PCR to detect *HNF1A* expression in 60 paired cancer tissues and normal tissues. The expression level of *HNF1A* was found to be higher in cancer tissues compared with the normal tissues (Figure 4C) and its expression showed a positive correlation with *DNAJC12* expression in NSCLC tissues (Figure 4D). As *HNF1A* is a transcription factor, we then applied JASPAR databases to determine whether *HNF1A* may regulate *DNAJC12*. The DNA motif of *HNF1A* and 2 predicted binding sites of *HNF1A* in *DNAJC12* promoter are illustrated in Figure 4E,4F. Luciferase gene reporter assay was then applied to verify these findings. The expression of *HNF1A* was significantly increased when the

cells were transfected with OE-*HNF1A*, while decreased when cells were infected with sh-*HNF1A* (Figure 4G). Overexpression of *HNF1A* induced a significant increase in the luciferase activity and vice versa (Figure 4H), indicating that *HNF1A* positively modulated *DNAJC12* expression in NSCLC cells. This was further confirmed by the qRT-PCR and western blot assays (Figure 4I,4J). These results illustrated that *HNF1A* was overexpressed in NSCLC and positively promoted *DNAJC12* expression.

HNF1A promotes aerobic glycolysis and drug resistance through increasing *DNAJC12* expression in NSCLC cells

Next, we explored whether *HNF1A* promoted aerobic glycolysis and drug resistance by increasing *DNAJC12* expression in NSCLC cells *in vitro*. The ATP levels, lactate production, and glucose consumption were significantly



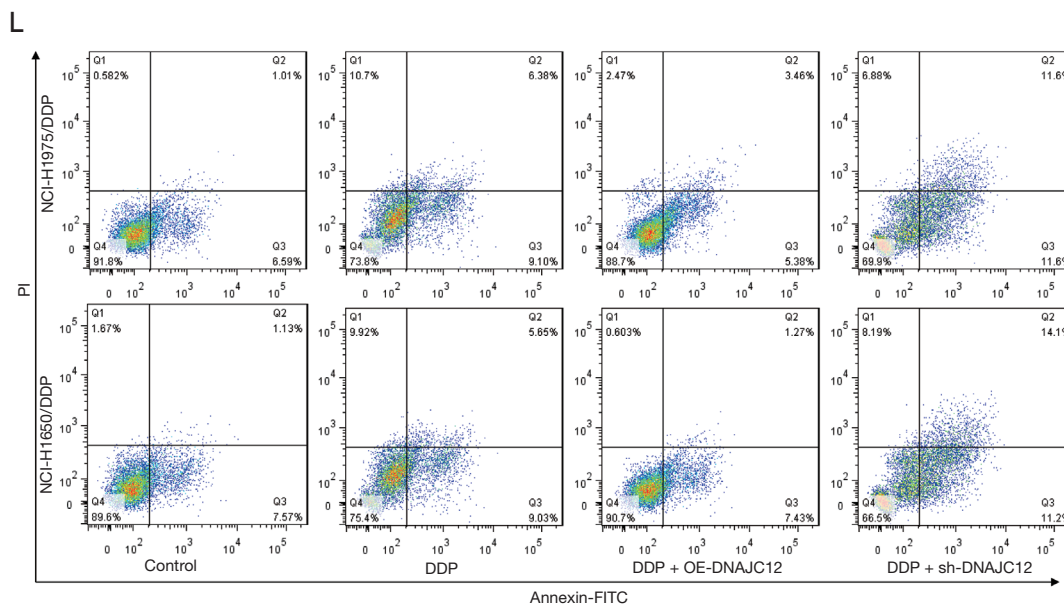


Figure 3 *DNAJ12* promoted cell growth and enhanced DDP resistance in NSCLC. (A,B) CCK-8 and (C,D) flow cytometry assays were adopted to detect cell growth and apoptosis in NCI-H1975 and NCI-H1650 cells from OE-NC, OE-DNAJ12, sh-NC, and sh-DNAJ12 groups. (E-H) The mRNA and protein levels of *DNAJ12* in NCI-H1975, NCI-H1975/DDP, NCI-H1650 and NCI-H1650/DDP cells were detected by qRT-PCR and western blotting experiments. (I,J) CCK-8 was applied to detect *DNAJ12* role in DDP-induced cell growth inhibition. (K,L) Flow cytometry was applied to detect *DNAJ12* role in DDP-induced cell apoptosis. [(A-D) *, $P < 0.05$ vs. OE-NC group, #, $P < 0.05$ vs. sh-NC group; (E-H) *, $P < 0.05$; (I-L) *, $P < 0.05$ vs. control group, #, $P < 0.05$ vs. DDP group]. OD, optical density; OE, overexpressing; *DNAJ12*, DnaJ heat shock protein family member C12; NC, normal control; sh, short hairpin; mRNA, messenger RNA; DDP, cisplatin; NSCLC, non-small cell lung cancer; CCK-8, Cell Counting Kit-8; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

increased when *HNF1A* expression was upregulated, whereas this tendency was abolished with silencing of *DNAJ12* (Figure 5A-5C). In addition, *HNF1A* expression was elevated in NCI-H1975/DDP and NCI-H1650/DDP cells compared to their parental cells (Figure 6A,6B). Overexpression of *HNF1A* enhanced cell growth which had been inhibited by DDP (Figure 6C,6D) and reduced DDP-induced cell apoptosis (Figure 6E,6F), while depletion of *DNAJ12* rescued this. These findings suggested that *HNF1A* facilitated aerobic glycolysis and drug resistance through increasing *DNAJ12* expression in NSCLC cells.

Silencing of β -catenin reverses *DNAJ12*-mediated cell growth and aerobic glycolysis

Li *et al.* (19) recently reported that *DNAJ12* was overexpressed in lung cancer and significantly promoted lung cancer cell growth and migration by increasing

β -catenin expression. And, evidence has verified that the β -catenin signaling is closely implicated in regulating the aerobic glycolysis and drug resistance in lung cancer (20-23). Thus, we conjectured β -catenin may play a role in *DNAJ12*-mediated cell growth and aerobic glycolysis. To this end, the rescue experiments were carried out. Upregulation of *DNAJ12* significantly increased β -catenin expression in NCI-H1650 cells as compared with the control group (Figure 7A), while β -catenin expression were significantly decreased when the cells were infected with the sh- β -catenin (Figure 7B,7C). In addition, downregulation of β -catenin significantly inhibited cell growth mediated by *DNAJ12* under DDP treatment (Figure 7D), and decreased the ATP level (Figure 7E), lactate production (Figure 7F) and glucose consumption (Figure 7G). These results revealed that *DNAJ12* promoted cell growth and aerobic glycolysis in NSCLC through upregulating β -catenin expression.

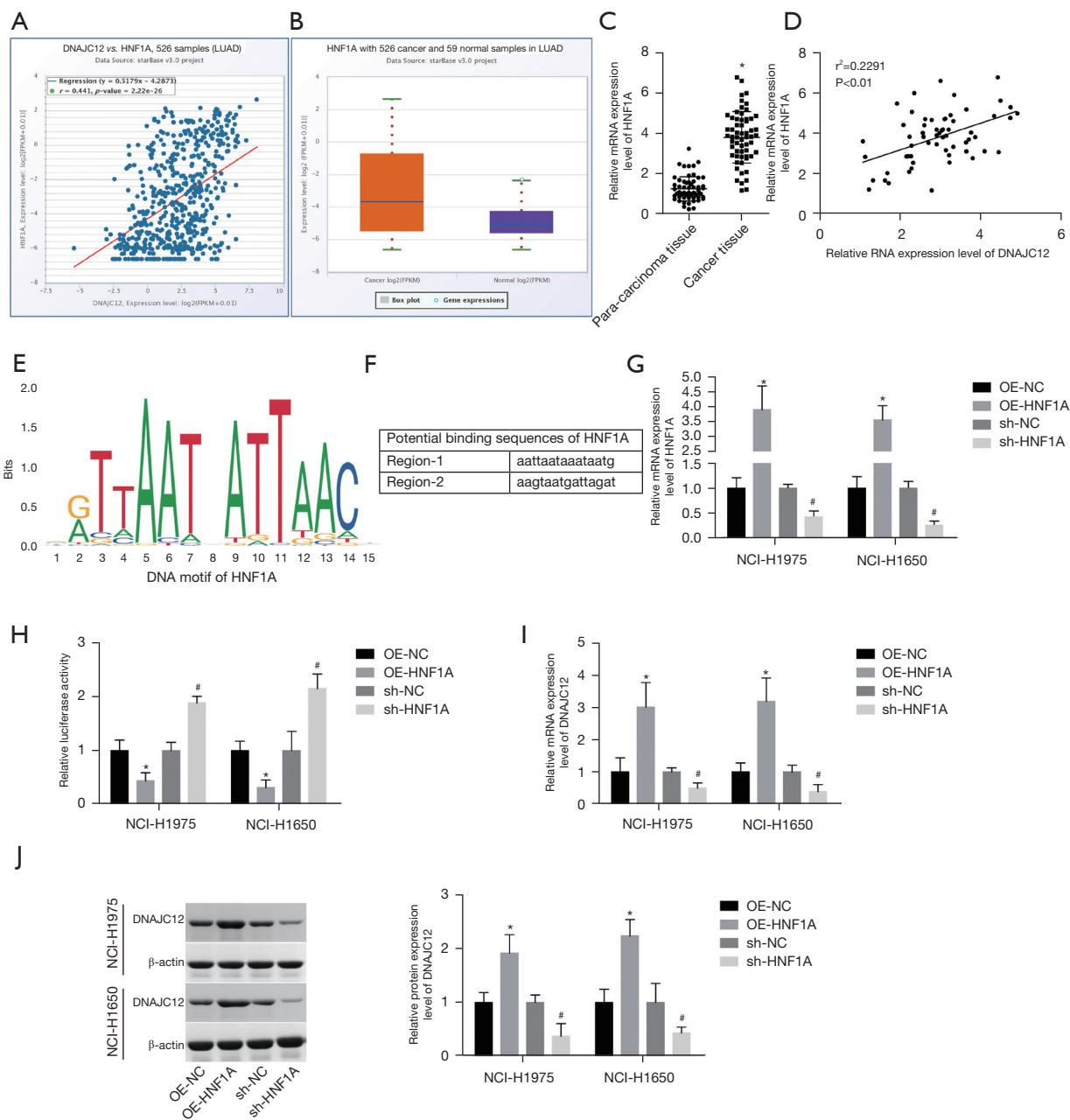


Figure 4 *HNF1A* promoted *DNAJC12* expression in NSCLC cells. (A) starBase was applied to analyze the correlation between the expression level of *HNF1A* and *DNAJC12* expression in lung cancer samples. (B) starBase was applied to analyze the expression level of *HNF1A* in lung cancer samples. (C) qRT-PCR was adopted to detect *HNF1A* levels in 60 paired NSCLC tissues and normal tissues (*, $P<0.05$). (D) Pearson correlation coefficient was adopted to analyze the correlation between *DNAJC12* expression and *HNF1A* expression in NSCLC tissues. (E,F) The DNA motif of *HNF1A* and predicted two binding sites of *HNF1A* in *DNAJC12* promoter. (G) qRT-PCR was done to detect the mRNA levels of *HNF1A* following cell transfection with OE-NC, OE-HNF1A, sh-NC and sh-HNF1A. (H) Luciferase gene reporter assay was used to detect *HNF1A* effect on *DNAJC12* transcription. (I,J) mRNA and protein levels of *DNAJC12* were detected using qRT-PCR and western blot. [(G-J) *, $P<0.05$ vs. OE-NC group, #, $P<0.05$ vs. sh-NC group]. *DNAJC12*, DnaJ heat shock protein family member C12; *HNF1A*, hepatocyte nuclear factor 1-alpha; LUAD, lung adenocarcinoma; mRNA, messenger RNA; OE, overexpressing; NC, normal control; sh, short hairpin; NSCLC, non-small cell lung cancer; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

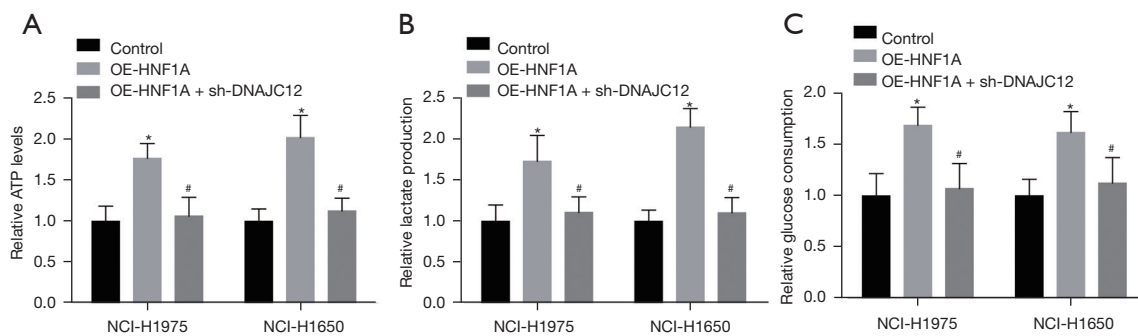


Figure 5 *HNF1A* enhanced aerobic glycolysis through upregulation of the expression of *DNAJ C12* in NSCLC. (A) ATP levels, (B) lactate production and (C) glucose consumption in NCI-H1975 and NCI-H1650 cells from control, OE-HNF1A, OE-HNF1A + sh-DNAJ C12 groups were tested. (*, $P < 0.05$ vs. control group, #, $P < 0.05$ vs. OE-HNF1A group). ATP, adenosine triphosphate; OE, overexpressing; *HNF1A*, hepatocyte nuclear factor 1- α ; sh, short hairpin; *DNAJ C12*, DnaJ heat shock protein family member C12; NSCLC, non-small cell lung cancer.

Downregulation of *DNAJ C12* inhibits tumor growth *in vivo*

We assessed the role of *DNAJ C12* in modulating the tumorigenesis of NSCLC cells. *Figure 8A* showed the tumor pictures of the sh-DNAJ C12 and sh-NC group. The sh-NC group exhibited a relative uniform distribution of tumor cells and less necrotic areas, while large areas of necrosis were observed in the sh-DNAJ C12 group (*Figure 8B*). Also, compared with the sh-NC group, both tumor weight and volume were decreased in the sh-DNAJ C12 group (*Figure 8C, 8D*), with a decrease expression level of *DNAJ C12* in tumor tissues (*Figure 8E, 8F*). This result demonstrated that downregulation of *DNAJ C12* inhibited tumor growth *in vivo*.

Discussion

In the present study, we explored the role of *DNAJ C12*, a member of the HSP family, in modulating cell aerobic glycolysis and drug resistance for the first time. Our results demonstrated that *DNAJ C12* overexpression promoted cell aerobic glycolysis with increased levels of ATP, lactate production, and glucose consumption in NSCLC cells. In addition, *DNAJ C12* expression was upregulated in DDP-resistant cells, and overexpression of *DNAJ C12* significantly enhanced cell resistance to DDP.

The role of *DNAJ C12* in several kinds of cancers has been gradually revealed. Uno *et al.* (17) found that *DNAJ C12* was overexpressed in gastric cancer tissues, which was closely linked to lymphatic involvement, lymph

node metastasis, infiltrative growth type, and advanced stage and shorter overall survival. In addition, knockdown of *DNAJ C12* significantly repressed cell growth and invasiveness in gastric cancer. He *et al.* (18) identified that high expression of *DNAJ C12* served as a negative predictive factor for the response to neoadjuvant concurrent chemoradiotherapy (CCRT) and was strongly linked to shorter survival time in patients with rectal cancers receiving neoadjuvant CCRT and surgery. De Bessa *et al.* (29) reported that *DNAJ C12* mRNA expression was significantly associated with estrogen receptor-positive status in breast cancer, and 17 β -estradiol treatment could increase *DNAJ C12* expression in breast cancer MCF-7 cells. It has also been reported recently that when *DNAJ C12* was overexpressed in lung cancer, knockdown of *DNAJ C12* inhibited the proliferation, colony formation, migration, invasion *in vitro*, and tumorigenesis *in vivo* of lung cancer cells through inhibiting the expression of β -catenin (19). Consistently, we found that *DNAJ C12* showed a higher expression pattern in NSCLC tissues and cells, and overexpression of *DNAJ C12* significantly promoted NSCLC cell growth, and inhibited apoptosis.

It has been verified that *DNAJ C12* is a positive regulator of β -catenin in lung cancer (19), and the β -catenin is closely implicated in regulating the aerobic glycolysis in lung cancer (20,21). These results indicate that *DNAJ C12* may participate in regulating the aerobic glycolysis of lung cancer through activating the β -catenin signaling. As respected, we found that *DNAJ C12* overexpression promoted aerobic glycolysis in NSCLC cells, which was

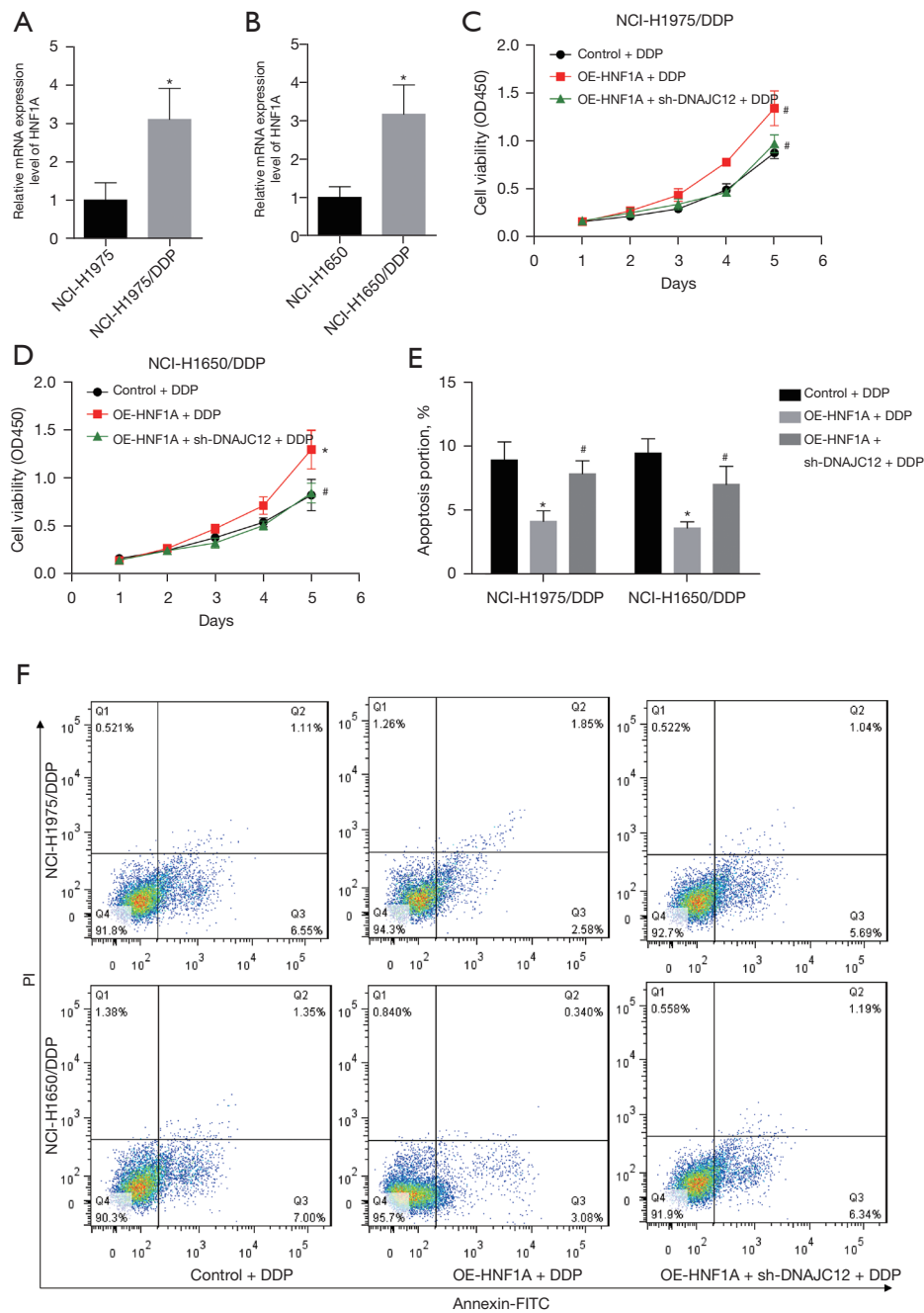


Figure 6 *HNF1A* enhanced cell resistance to DDP through upregulation of the expression of *DNAJC12* in NSCLC. (A,B) The mRNA levels of *HNF1A* in NCI-H1975, NCI-H1975/DDP, NCI-H1650 and NCI-H1650/DDP cells were detected by qRT-PCR (* $P < 0.05$). (C,D) Cell growth in control + DDP, OE-HNF1A + DDP, OE-HNF1A + sh-DNAJC12 + DDP groups were detected using CCK-8 kit. (E,F) Cell apoptosis rates in control + DDP, OE-HNF1A + DDP, OE-HNF1A + sh-DNAJC12 + DDP groups were detected using flow cytometry. [(C-F) *, $P < 0.05$ vs. control + DDP group, #, $P < 0.05$ vs. OE-HNF1A + DDP group]. mRNA, messenger RNA; *HNF1A*, hepatocyte nuclear factor 1-alpha; DDP, cisplatin; OE, overexpressing; sh, short hairpin; *DNAJC12*, DnaJ heat shock protein family member C12; OD, optical density; NSCLC, non-small cell lung cancer; qRT-PCR, quantitative reverse transcription polymerase chain reaction; CCK-8, Cell Counting Kit-8.

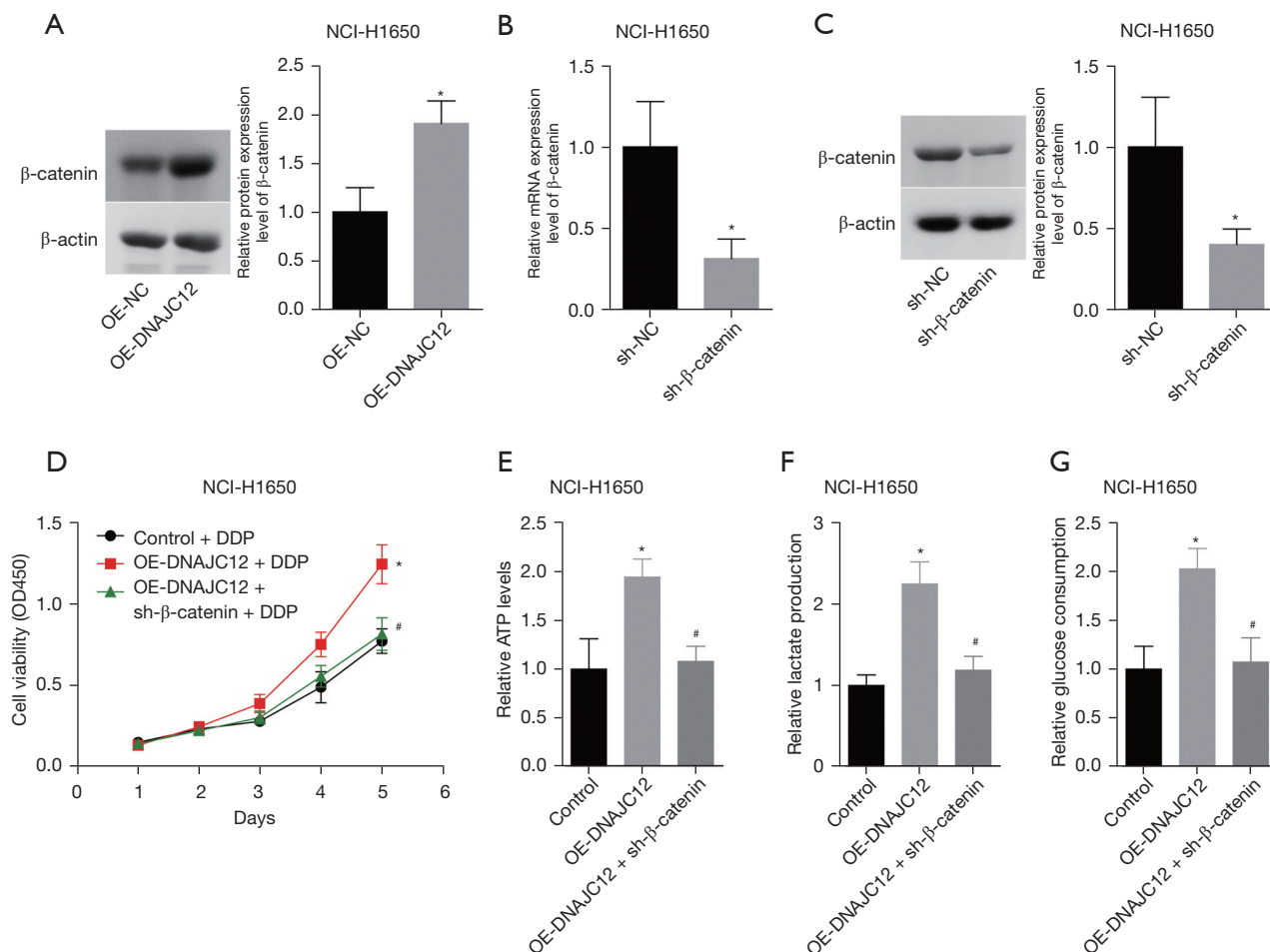


Figure 7 Silencing of β -catenin reverses *DNAJC12*-mediated cell growth and aerobic glycolysis. (A) β -catenin expression was detected by western blotting in OE-NC and OE-DNAJC12 groups (*, $P < 0.05$). (B,C) The knockdown efficiency of sh- β -catenin was detected by qRT-PCR and western blotting (* $P < 0.05$). (D) CCK-8 kit was used to detect cell growth in control + DDP, OE-DNAJC12 + DDP, OE-DNAJC12 + sh- β -catenin + DDP groups (*, $P < 0.05$ vs. control + DDP group, #, $P < 0.05$ vs. OE-DNAJC12 + DDP group). (E) ATP levels, (F) lactate production and (G) glucose consumption in control, OE-DNAJC12, OE-DNAJC12 + sh- β -catenin groups were detected. [(E-G) *, $P < 0.05$ vs. control group, #, $P < 0.05$ vs. OE-DNAJC12 group]. OE, overexpressing; NC, normal control; *DNAJC12*, DnaJ heat shock protein family member C12; sh, short hairpin; OD, optical density; DDP, cisplatin; ATP, adenosine triphosphate; qRT-PCR, quantitative reverse transcription polymerase chain reaction; CCK-8, Cell Counting Kit-8.

rescued by β -catenin downregulation. Consistently, it has been demonstrated that other members of the HSP family are also implicated in aerobic glycolysis. For instance, Xu *et al.* (30) revealed that HSP90 promoted the glycolysis and proliferation of hepatocellular carcinoma cells in a PKM2 dependent manner. Also known as HSPD1, HSP60 was found to be upregulated in myeloma cells, and silencing of HSP60 inhibited glycolysis (31). The novel C-terminal HSP90 inhibitor KU757 could overcome the increase in aerobic glycolysis induced by lenvatinib treatment in

lenvatinib-resistant follicular thyroid cancer cells (32).

In addition, hyper-activation of the β -catenin signaling plays an important role in the drug resistance of lung cancer (22,23), suggesting that *DNAJC12* may participate in regulating the DDP resistance of lung cancer through activating the β -catenin signaling. Moreover, evidence has demonstrated that aerobic glycolysis deregulation can help cancer cells to increase drug resistance. Targeting metabolic disorder is a promising method for cancer intervention (33,34). As *DNAJC12* could promote aerobic glycolysis,

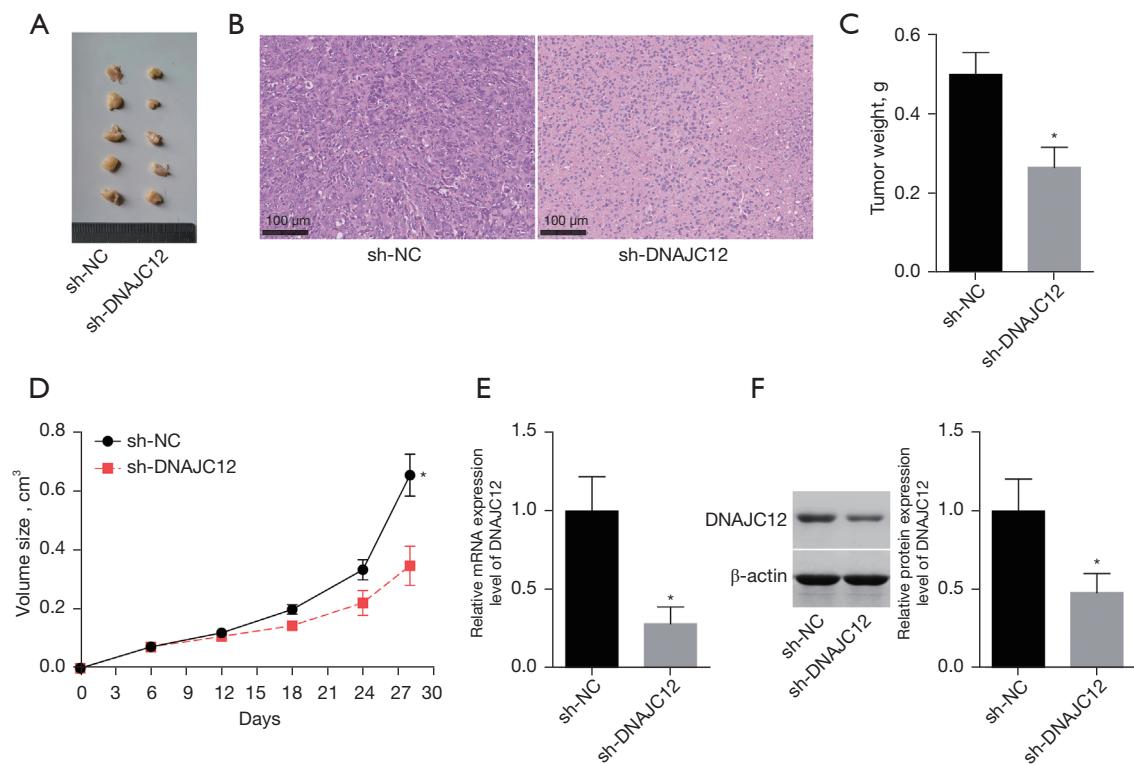


Figure 8 Downregulation of *DNAJC12* inhibits tumor growth *in vivo*. (A) Tumor pictures of the sh-NC and sh-DNAJC12 groups. (B) Hematoxylin and eosin staining was used to assess the morphological features of the sh-NC and sh-DNAJC12 groups. (C,D) Tumor volume and weight were assessed in sh-NC and sh-DNAJC12 groups. (E,F) *DNAJC12* expressions at mRNA and protein levels in mice tumor tissues were detected using qRT-PCR and western blot assays. (*, $P < 0.05$). sh, short hairpin; NC, normal control; *DNAJC12*, DnaJ heat shock protein family member C12; mRNA, messenger RNA; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

we conjectured that *DNAJC12* maybe also implicated in modulating cell drug resistance in NSCLC through regulating β -catenin signaling-mediated aerobic glycolysis. As expected, we observed that *DNAJC12* expression was upregulated in DDP-resistant cells, and overexpression of *DNAJC12* significantly increased cell growth and reduced cell apoptosis in the presence of DDP. Besides, we found that downregulation of β -catenin reversed *DNAJC12*-mediated enhancement of cell viability under DDP treatment, indicating that *DNAJC12* accelerated cellular drug resistance in NSCLC through regulating β -catenin. Also, other HSPs, such as HSP27/HSPB1, HSP90/HSPC, HSP70/HSPA1, and GRP78/HSPA5 were also identified to be implicated in DDP resistance (35).

In exploration of the mechanism, we found that *HNF1A* could bind to the promoter region of *DNAJC12* and served as a transcriptional activator to promote *DNAJC12*. Moreover, we found that *HNF1A* was upregulated in

NSCLC tissues and showed a positive correlation with *DNAJC12* expression. While *HNF1A* promoted aerobic glycolysis and DDP resistance in NSCLC cells, these were rescued by *DNAJC12* downregulation, indicating that *HNF1A* promoted glycolysis and drug resistance in NSCLC cells through upregulating *DNAJC12* expression. Evidence has demonstrated that *HNF1A* is also involved in cancer progression and drug resistance. Overexpression of *HNF1A* has been shown to increase the expression of stem cell markers and tumor sphere formation in pancreatic cancer cells and accelerated cell growth in primary pancreatic ductal adenocarcinoma cells (25). By inhibiting *MMP14* and *HNF1A* expression in cervical cancer, *miR-484* serves as a tumor suppressor (36). These results demonstrated that *HNF1A* serves as an oncogene. However, Gong *et al.* (37) reported that *HNF1A* overexpression inhibited cell growth and migration, and induced apoptosis in pancreatic cancer CanPan-1 cells. Overexpression of *HNF1A* enhances

gemcitabine sensitivity in pancreatic ductal adenocarcinoma both *in vitro* and *in vivo* (38). The different roles of *HNF1A* in carcinogenesis may be caused by different cell contents.

Several limitations of this study should be clarified: the small human sample size, and we did not explore whether *DNAJC12* could accelerate NSCLC progression through other mechanisms. In addition, Fang *et al.* (39) showed that β -catenin overexpression was closely associated with the resistance of NSCLC cells to gefitinib, an epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) used for the targeted therapy of NSCLC, indicating that *DNAJC12* may also cause the resistance of NSCLC to targeted therapy. We intend to continue to explore these aspects in our next work.

In conclusion, this study revealed that *DNAJC12*, activated by the transcription factor *HNF1A*, could enhance aerobic glycolysis and drug resistance to DDP in NSCLC. It is possible that *DNAJC12* is a promising treatment target for overcoming drug resistance in NSCLC. *DNAJC12* is a potential candidate target for the treatment of NSCLC, and overcoming the DDP resistance.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-1475/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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethics Committee of Shanghai General Hospital (No. 2018KY086) and informed consent was taken from all

the patients. Animal experiments were performed under a project license (No. 2018AWS0095) granted by the Ethics Committee of Shanghai General Hospital, in compliance with the institutional guidelines for the care and use of animals.

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