Cancer Biol Med 2025. doi: 10.20892/j.issn.2095-3941.2024.0459

ORIGINAL ARTICLE

Carnitine palmitoyltransferase 1C promotes EMT-associated cisplatin resistance in non-small cell lung cancer cells

Renjie Chen[1,2*], Jiahui Wang[1,3*], Shuoyu Huang[1,3*], Xuefeng Hu[1,4], Xinran He[1,5], Tiange Zhang[1,6], Yunhan Hu[1,6,7], Huijun Wei[1,7], Sihui Nian[8], Yushu Huang[1], Zhihao Wu[1,6,9,10]

[1]Research Laboratory of Tumor Microenvironment, Wannan Medical College, Wuhu 241001, China; [2]School of Laboratory Medicine, Wannan Medical College, Wuhu 241001, China; [3]School of Anesthesiology, Wannan Medical College, Wuhu 241001, China; [4]School of Medical Imageology, Wannan Medical College, Wuhu 241001, China; [5]School of Clinical Medicine, Wannan Medical College, Wuhu 241001, China; [6]Anhui Province Key Laboratory of Active Biological Macro-molecules Research, Wannan Medical College, Wuhu 241001, China; [7]Provincial Engineering Laboratory for Screening and Re-evaluation of Active Compounds of Herbal Medicines in Southern Anhui, Wannan Medical College, Wuhu 241001, China; [8]School of Pharmacy, Wannan Medical College, Wuhu 241001, China; [9]Anhui Province Key Laboratory of Non-coding RNA Basic and Clinical Transformation, Wannan Medical College, Wuhu 241001, China; [10]Anhui Provincial Engineering Research Center for Dental Materials and Application, Wannan Medical College, Wuhu 241001, China

ABSTRACT

Objective: Lung cancer is the most common cause of cancer-related deaths worldwide. Platinum-based chemotherapy is one of the main treatment options for patients with non-small cell lung cancer (NSCLC) but the effectiveness of chemotherapy is encumbered by drug resistance. Therefore, understanding the molecular mechanisms underlying chemotherapy resistance is crucial in improving treatment outcomes and prognosis.

Methods: The cell viability assay and apoptosis were used to analyze chemoresistance. Western blot analysis and wound healing testing were used to evaluate the epithelial-to-mesenchymal transition (EMT). Immunoprecipitation was used for analysis of protein modification. Promoter activity was determined using the luciferase reporter assay. Immunofluorescence staining was used to determine reactive oxygen species levels. The expression patterns of EMT markers and carnitine palmitoyltransferase 1C (CPT1C) were determined by Western blot analysis.

Results: CPT1C, which was shown to be highly expressed in lung cancer, is associated with cisplatin resistance in NSCLC cells. CPT1C depletion increased NSCLC cell sensitivity to cisplatin, while overexpression of CPT1C increased NSCLC cell resistance to cisplatin. Induction of EMT mediated CPT1C-induced cisplatin resistance. Ectopic expression of Snail reversed the increase in cisplatin sensitivity triggered by CPT1C knockdown. Moreover, CPT1C was shown to be regulated at the post-translational level and an E3-ubiquitin ligase, NEDD4L, was shown to be a major regulator of CPT1C stability and activity.

Conclusions: These data provide evidence for the first time that the lipid metabolism enzyme, CPT1C, mediates resistance to chemotherapy. Therefore, the use of combination therapy with a CPT1C inhibitor may be a promising new avenue in lung cancer treatment.

KEYWORDS CPT1C; chemoresistance; EMT; NEDD4L; NSCLC

Introduction

Despite the advent of targeted therapies and breakthroughs in immunotherapy, lung cancer remains the most common fatal malignancy worldwide. Although recent treatment advances have provided significant survival benefits for some patients with non-small cell lung cancer (NSCLC), approximately 60% of NSCLC patients do not have targetable driver mutations and anti-PD-1 or anti-PD-L1 treatment only benefits 20% of

*These authors contributed equally to this work.
Correspondence to: Yushu Huang and Zhihao Wu
E-mail: 1575841642@qq.com and zwu2ster@wnmc.edu.cn
ORCID ID: https://orcid.org/0000-0001-8368-5129
Received October 23, 2024; accepted December 31, 2024.
Available at www.cancerbiomed.org
©2025 The Authors. Creative Commons Attribution-NonCommercial 4.0 International License

NSCLC patients[1-3]. Therefore, chemotherapy remains one of the main treatment options for NSCLC patients. However, primary resistance to chemotherapy drugs and the development of acquired resistance during treatment often severely limits the ability to cure lung adenocarcinoma. Therefore, further exploration of the molecular mechanisms underlying chemotherapy resistance is of great significance for improving the prognosis of NSCLC patients.

The epithelial-to-mesenchymal transition (EMT) is a cellular process in which epithelial cells lose apical-basal polarity and cell-cell junctions, undergo changes in cell shape and cytoskeletal organization, and acquire mesenchymal features, such as fibroblast-like cell morphology, to increase cell migration and invasiveness[4]. Classical EMT, first described in embryonic development, is crucial for many stages of embryonic development. However, in addition to activating classical EMT-associated properties, additional pleiotropic functions also underscore an even more important role of EMT in cancer biology. EMT has been shown to maintain stemness properties and increase tumorigenicity. Additionally, EMT is involved in the escape from senescence and therapy resistance[5,6].

EMT is executed by so-called EMT-activating transcription factors (EMT-TFs), primarily from the SNAIL, TWIST, and ZEB families. EMT-TFs have crucial roles in cancer progression, from initiation and primary tumor growth to invasion, metastasis, and resistance to therapy. One major form of chemotherapy resistance has been associated with the presence of molecular pumps that transport drugs out of cells[2]. The most common transport proteins belong to the ATP-binding cassette (ABC) membrane protein family, such as multidrug resistance protein 1 (MDR1) and multidrug resistance-associated protein 1 (MRP1). Our previous work showed that the complex formed by Snail and activated by TGF-β1 and TAZ/YAP-1 upregulates expression of the multidrug resistance protein, MRP1 (ABCC1), thereby conferring chemotherapy resistance in NSCLC[7].

Tumor cells require higher amounts of substances and energy compared to other tissues. To adapt to the metabolic demands of rapid proliferation and division, tumor cells undergo metabolic reprogramming. The most common metabolic reprogramming phenomenon is the Warburg effect. While many studies on tumor metabolism have focused on changes in glucose and glutamine metabolism, another widely described metabolic feature that has long been associated with tumor cells is fatty acid metabolism[8]. Some tumor cells exhibit increased fatty acid oxidation (FAO) under metabolic stress conditions to maintain cellular energy homeostasis. FAO is tightly regulated at the step of fatty acid entry into the mitochondria, which is executed by tissue-specific isoforms of carnitine palmitoyltransferase 1 (CPT1). The CPT fatty acid family mediates the transport of long-chain fatty acids (LCFAs) across the mitochondrial double membrane for β-oxidation, providing energy to the cells. There are two known isoforms of CPT1 (CPT1a and CPT1b). CPT1a is the hepatic isoform, while CPT1b is predominantly found in muscle cells. Recently, a new third isoform of CPT1, CPT1C, has been described. CPT1C is specifically expressed in brain tissue and tumors, making CPT1C a subject of great interest in tumor research[9].

The specific role of CPT1C in cancer cells has not been established. Compared to other members of the CPT1 family, the catalytic activity of CPT1C is extremely low. Therefore, CPT1C is not thought to be involved in regulation of mitochondrial FAO. However, Zaugg et al.[10] reported that MCF-7 breast cancer cells with sustained overexpression of CPT1C increase FAO, ATP production, and resistance to glucose deprivation. Conversely, suppression of CPT1C activity through specific shRNA targeting CPT1C produced the opposite effects[10]. These findings suggest that CPT1C may be a regulator of FA homeostasis and may be involved in the biological energy regulation of tumor cells under metabolic stress. It was recently shown that high expression of CPT1C is negatively correlated with 5-year overall survival (OS) and disease-free survival (DFS) in gastric cancer (GC) patients[11]. Further functional studies revealed that HIF1α, which is produced by hypoxia in GC cells, upregulates CPT1C expression in GC cells, promoting proliferative effects. However, further studies are warranted to delve deeper into the mechanism underlying the CPT1Cs role in tumor biology. In the current study CPT1C was shown to be highly expressed in lung cancer and positively correlated with resistance to cisplatin. CPT1C-induced EMT was also shown to be involved in chemoresistance. Furthermore, the E3 ubiquitin ligase, NEDD4L, is functionally relevant to regulation of CPT1C expression.

Materials and methods

Cell culture, antibodies, reagents, and plasmids

H1299 (NSCLC cells), A549 (NSCLC cells), H446 (small cell lung cancer cells), HBE (human bronchial epithelial cells),

P-C9 (human lung adenocarcinoma cells), and H1688 cell lines (small cell lung cancer cells) were cultured with DMEM (GibcoThermo Fisher Scientific, Waltham, MA, USA) and 10% fetal bovine serum (FBS) (GibcoBRL, Grand Island, NY, USA) at 37°C in a humidified 5% CO_2 atmosphere. Anti-ubiquitin (3933S), anti-Snail (3895S), anti-EZH2 (5246S), anti-p-AKT (4060T) were purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-CPT1C (A13849), anti-NEDD4L (A9078), anti-E-cadherin (A3044), anti-N-cadherin (A3045) were purchased from Abclonal (Wuhan, China). Anti-β-actin (A1978), anti-FLAG (F1804-1MG), N-acety1-cysteine (NAC, AT250), LY294002 (440202), CHX (Cycloheximide, 239763) were purchased from Sigma-Aldrich (St. Louis, USA). Anti-fibronectin (ab299) was purchased from Abcam (Cambridge, UK). Cisplatin (cat. no. ST1164-10mg) was purchased from Beyotime (Shanghai, China), 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyl tetrazolium bromide (MTT, M8180, Solarbio, Beijing, China), Etomoxir (cat. no. E7740, Solarbio, Beijing, China). The Snail promoter reporter (no. 31694), EZH2 cDNA (pCMVHA hEZH2) (no. 24230), and NEDD4L cDNA plasmids (pCI HA NEDD4L) (no. 27000) were purchased from Addgene (Cambridge, MA, USA). CPT1C cDNA (pCMV-SPORT6-CPT1C) (no. P4976) was purchased from MiaoLingBio (Wuhan, China).

Reactive oxygen species (ROS) level determination

Cells (2×10^5) were placed in 6-well plates, transfected with CPT1C cDNA or CPT1C siRNA for 36 h, then subjected to a Reactive Oxygen Species Assay Kit (Beyotime, Shanghai, China). The positive control group was treated with ROS activator for 30 min, while the negative control group was not treated. Diluted DCFH-DA was added, incubated at 37°C for 20 min, washed 3 times with DMEM medium, and finally measured using an enzyme microplate reader. The fluorescence intensity was measured and photographed.

Western blot analysis

Cells treated as indicated were harvested and lysed using the heated laemmli sample buffer (S3401; Sigma-Aldrich, St. Louis, USA). Proteins were extracted and transferred to nitrocellulose membranes (GE Healthcare, Piscataway, NJ, USA) *via* SDS-PAGE. Then, membranes were blocked with 5% skim milk for 1 h at room temperature prior to incubation with antibodies for 12 h at 4°C incubation.

Annexin V-FITC/PI double-staining flow cytometry for apoptosis detection

Cells were cultured in 6-well plates (2×10^5), transfected for 48 h, starved overnight, and treated with cisplatin for 24 h. Cells were collected, washed twice with PBS, and the supernatant was removed to collect cell pellets. Using the BD Pharmingen FITC AnnexinV Apoptosis Detection Kit (556547; Becton Dickinson, city, state, USA), 500 μL of binding buffer was evenly added with 5 μL of PI staining solution, and 5 μL of FITC staining solution. The solution was incubated for 15 min in the dark and the cells were filtered into a sampling tube using a 0.22-μm filter membrane. Apoptosis was detected within 1 h using a flow cytometer (Becton Dickinson) and analyzed using Flow jo 7.6 software.

Cell viability assay

After culturing cells on a 48-well plate to a confluence of approximately 80%, the cells underwent the specified treatment and 300 μL of MTT solution [5 mg/mL (MTT powder dissolved in PBS solution)] were added to each well, then incubation was continued for 4 h in CO_2. The cell suspension was centrifuged, and the supernatant was aspirated, 150 μL of DMSO was added to each well and shaken for 10 min. The absorbance was measured at a wavelength of 570 nm using a BioTek microplate reader (BioTek, Winooski, VT, USA). GraphPad Prism software was used to draw dose- and time-effect curves. Each experiment was repeated three times.

Wound healing test

Cells were seeded in a 6-well plate, and after growing to 90%, a sterile 200-μL pipette tip was used to draw a uniform straight line. The line was washed five-to-six times with PBS and after the designated treatment the cells were observed using an inverted fluorescence microscope (Olympus, Shinjuku-ku, Tokyo, Japan) and pictures were obtained at 0 h. The cells were returned to the incubator for further cultivation and the migration of the marked sites was photographed at 24 and 48 h. The scratch area was analyzed using software Image Pro and the migration ability of the cells was determined based on the wound healing area.

Dual luciferase reporter assays

H1299 and A549 cells were seeded in 12-well plates at a density of 1×10^5 cells/well. When cell densities reached 80% the promoter-luciferase reporter [CPT1C or Snail (6 ng)] and pCMV6 plasmids (1 ng) were co-transfected. After 48 h cells were lysed and luciferase activity was measured following the manufacturer's instructions. Each experiment was repeated three times and the average value ± SD was used.

Transfection and small interfering RNA (siRNA)

H1299 and A549 cells were seeded in 6-well plates (10^5/well). When the density of the cells reached 80%, the cells were transfected using a PolyJet DNA Transfection Reagent (SignaGen Laboratories, Gaithersburg, MD, USA) according to the manufacturer's instructions. A549 and H1299 cells were inoculated into 6-well plates for siRNA. When the density of the cells reached 60%, the cells were transfected with 10 nM siRNA using 1 μL of GenMute siRNA Transfection Reagent (SignaGen Laboratories). The siRNA sequences are shown in **Table 1**.

Immunoprecipitation (IP)

Samples were collected in a lysis buffer (P2175S-1; Beyotime) containing 1x protease inhibitor cocktail (P2175S-3; Beyotime) at a 100:1 ratio. Each sample was incubated overnight with anti-CPT1C antibody at 4°C and normal rabbit IgG (P2175S-6; Beyotime) was used as a control group. Then, BeyoMag™ protein A magnetic beads (P2175S-4; Beyotime) were used in a 2-h incubation. The immune complex was centrifuged and washed with lysis buffer containing protease inhibitors to remove unbound proteins. SDS-PAGE sample loading buffer (1X) (P2175S-9; Beyotime) was added to each sample and protein expression was determined by Western blot analysis.

Table 1 siRNA sequence

Gene	Genebank accession number	Target sequence (5′–3′)
siCPT1C	XM_05431977	GAAAUCCGCUGAUGGUGAA
siNEDD4L	NM_001144967.3	GGUCCUCAGCUGUUUACAA
siEZH2	NM_001203248.1	GACUCUGAAUGCAGUUGCUTT

Cloning and DNA construction

The CPT1C promoter luciferase reporter was constructed by inserting the amplified promoter fragments into the luciferase reporter vector, PGL3-basic (Promega, Madison, WI, USA) at the Kpn I and Hind III restriction sites. The primer sequences are shown below:

CPT1C forward: 5′-CCGCTCGAGCTCCATCACGCTCCATCTCTCAATT-3′; and CPT1C reverse: 5′-CCCAAGCTTATTGGACATATGCAAGCGGGAGATT-3′.

Statistical analysis

The data are presented as the mean ± SD from independent experiments that were performed in triplicate. ANOVA was performed using SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA) for continuous data following a normal distribution and a significant difference was considered when the *P*-value was < 0.05.

Results

CPT1C expression was associated with DDP resistance in NSCLC cells

The relationship between lipid metabolism and lung cancer prognosis was not clear based on the retrospective analysis from clinical reviews. However, inhibition of lipid metabolism suppresses the growth of NSCLC in many animal models[12]. Therefore, the relationship between some key genes related to lipid metabolism and lung cancer prognosis was determined by analyzing publicly available data. Acetyl-coenzyme A carboxylase, fatty acid synthase (FASN), and CPT1 (a and c types) are often overexpressed in human cancers[13,14]. Surprisingly, in addition to an increase in lung tumors (TCGA-LUAD), it was found that the level of CPT1C expression was also correlated with poor prognosis [GEPIA (http://gepia.cancer-pku.cn)] (**Figure 1A**). Therefore, the current study focused on exploiting the correlation between CPT1C and lung cancer prognosis because there has been little relevant research to date. A549 cells were shown to exhibit the highest level of CPT1C expression based on a comparison of various NSCLC cell lines (**Figure 1B**). Currently, systemic chemotherapy remains the primary treatment for NSCLC mediated by KRAS/TP53. The A549 cell line, which has an activating mutation in KRAS and

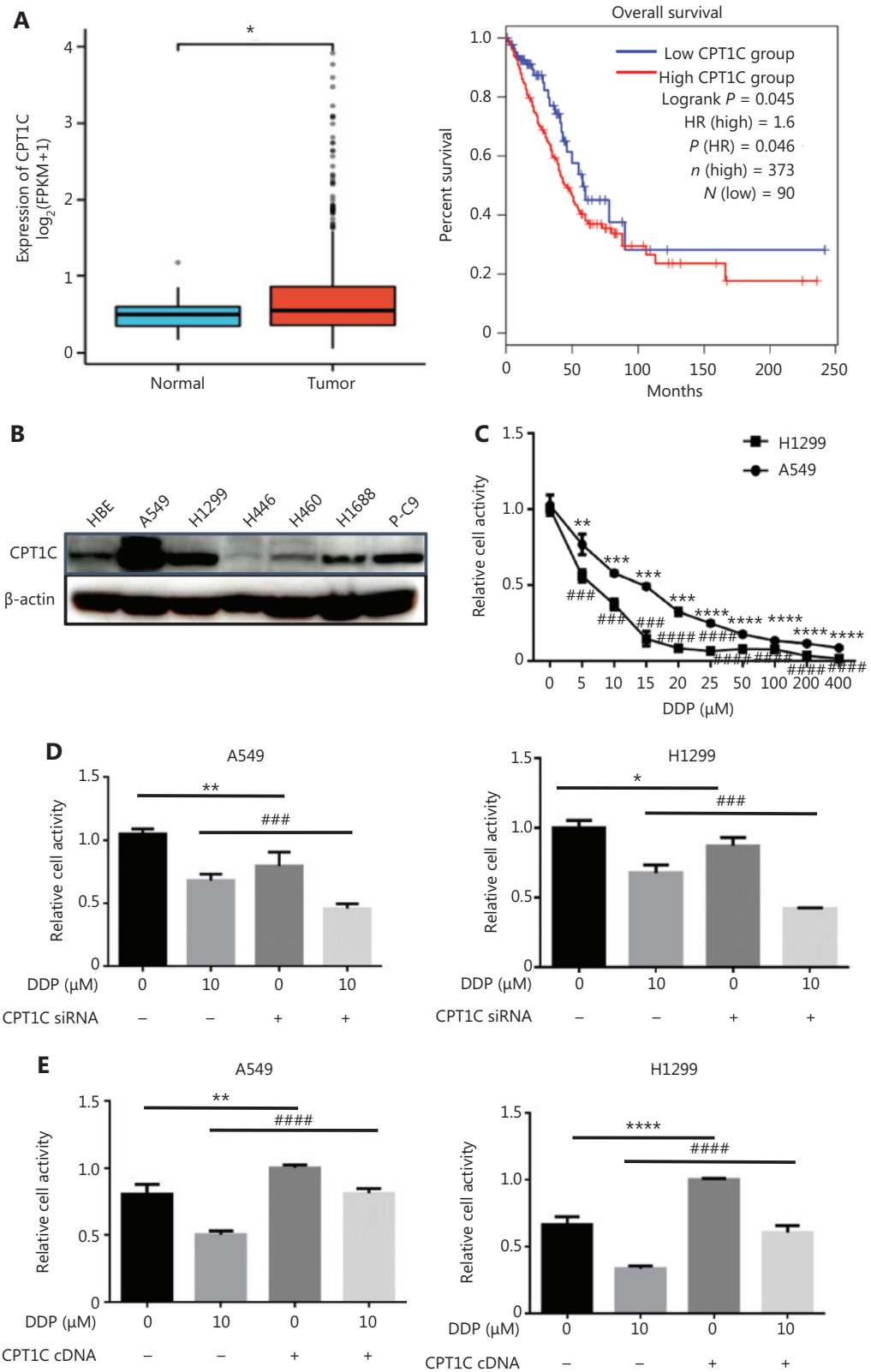


Figure 1 Continued

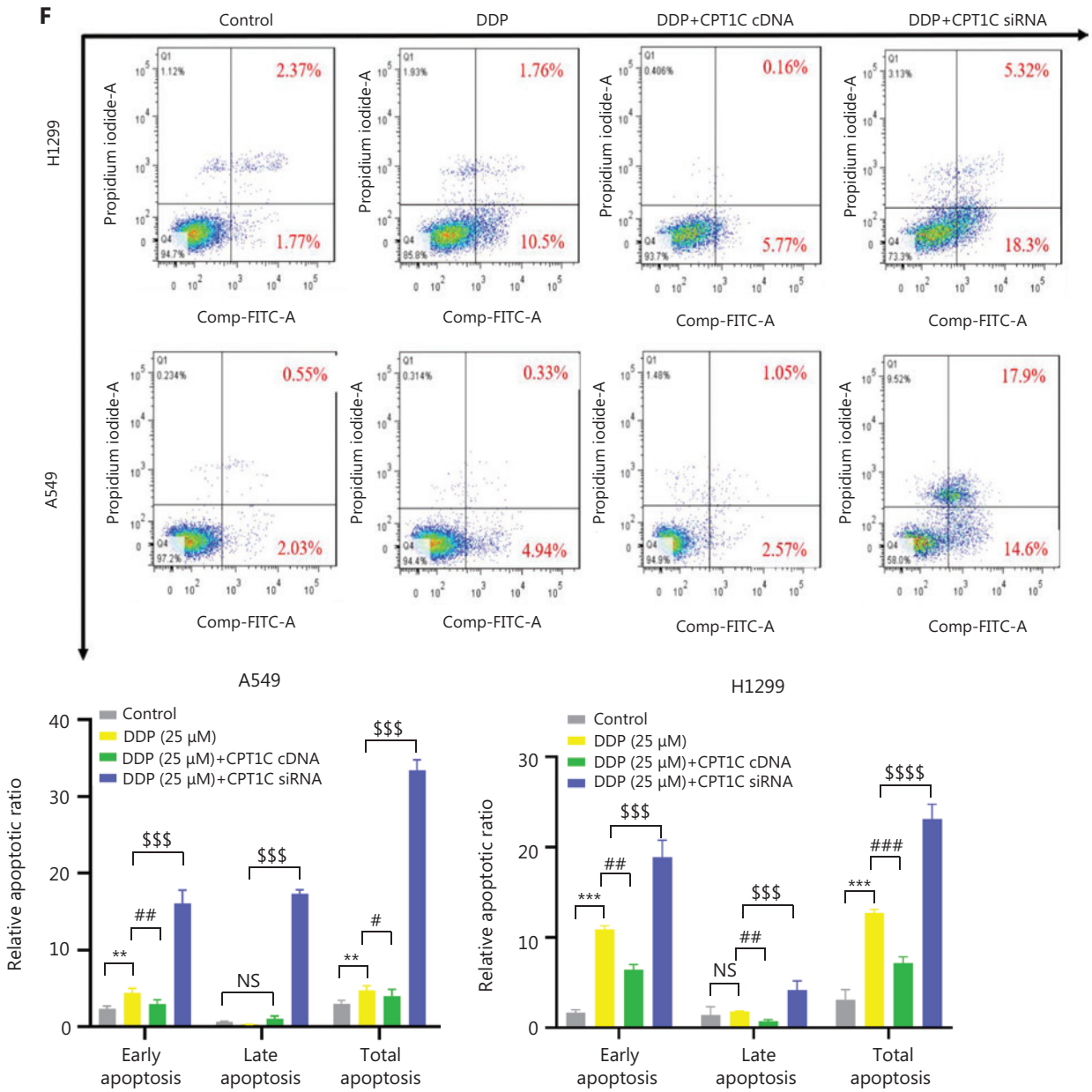


Figure 1 CPT1C expression is associated with cisplatin resistance in NSCLC cells. (A) The differential analysis of CPT1C expression between normal cells and non-small cell lung cancer in the LUCD cohort of the TCGA database showed that CPT1C is highly expressed in lung adenocarcinoma, with a median difference of 0.149 (0.003–0.298) between the two groups, which was statistically significant (*P < 0.05 indicates that there was a significant difference compared to normal tissues). Based on the GEPIA2 website, the impact of CPT1C on the overall survival rate of lung adenocarcinoma patients was analyzed and the differences were statistically significant (P < 0.05 indicates that there was significant difference between groups with high and low CPT1C expression). (B) Western blot analysis was performed in seven cell lines (HBE, A549, H1299, H446, H460, H1688, and P-C9) to detect the level of CPT1C protein expression in the above cell lines. (C) Two cell lines (H1299 and A549) were treated with cisplatin (0, 5, 10, 15, 20, 25, 50, 100, 200, and 400 μM) for 48 h in preparation for MTT analysis. The bars represent the mean ± S.D. of triplicates (**P < 0.01, ***P < 0.001, ****P < 0.0001 for differences from DDP-untreated A549 cells and $^{\#\#\#}P$ < 0.001 and $^{\#\#\#\#}P$ < 0.0001 for the differences from DDP-untreated H1299 cells by ANOVA with Dunnett's correction for multiple comparisons). (D) The cells were transfected with control siRNA or CPT1C siRNA, then treated with 10 μM cisplatin for 48 h in preparation for MTT analysis. The bars represent the mean ± S.D. of triplicates (*P < 0.05 and **P < 0.01 for the difference from the control group, and $^{\#\#\#}P$ < 0.001 for the difference from the DDP-treated group by ANOVA with Dunnett's correction for multiple comparisons). (E) After transfection of CPT1C cDNA, cell lines (H1299 and A549) were treated with 10 μM cisplatin for 48 h for MTT analysis. The bars represent the mean ± S.D. of triplicates (**P < 0.01

and ****P < 0.0001 for the difference from the control group, and $^{\#\#\#\#}P$ < 0.0001 for the difference from the DDP-treated group by ANOVA with Dunnett's correction for multiple comparisons). (F) After transfection of CPT1C cDNA or CPT1C siRNA, cell lines (H1299 and A549) were treated with vehicle or the indicated doses of cisplatin (25 μM) for 6 h in preparation for flow cytometry assays. The bars represent the mean ± S.D. of triplicates (**P < 0.01 and ***P < 0.001 for the difference from the control group; $^{\#\#}P$ < 0.01 and $^{\#\#\#}P$ < 0.001 for the difference from the DDP-treated group; and $^{\$\$}P$ < 0.01 and $^{\$\$\$}P$ < 0.001 for the difference from the DDP-treated group by ANOVA with Dunnett's correction for multiple comparisons, NS indicates no statistical difference).

p53-deficient H1299 cells, was specifically selected to represent our *in vitro* model for NSCLC chemotherapy resistance research. A549 cells exhibit a higher survival rate than H1299 cells following treatment with varying concentrations of DDP (**Figure 1C**), suggesting a positive correlation between cell viability and CPT1C expression. To verify the role of CPT1C in DDP resistance, siRNA technology was used to knock down CPT1C in A549 and H1299 cells. A549 and H1299 cells increased the sensitivity to DDP (**Figure 1D**). In addition, overexpression of CPT1C increased resistance to DDP (**Figure 1E**). Similar results were obtained using the apoptosis assay (**Figure 1F**).

CPT1C-induced EMT enhances DDP resistance

Because CPT1C is a key enzyme in lipid metabolism of cancer cells[10,11], the molecular mechanism underlying CPT1C in chemoresistance was explored. Several reports indicate that EMT has an important role in chemoresistance[4,5]. Bioinformatic analysis showed that CPT1C was positively correlated with the key EMT transcription factor, Snail, and the mesenchymal cell markers, fibronectin and N-cadherin, whereas CPT1C was negatively correlated with the epithelial cell marker, E-cadherin (**Figure 2A**). Interestingly, Western blot analysis results showed that overexpression of CPT1C induced EMT, causing Snail, fibronectin, and N-cadherin to be upregulated, while E-cadherin was downregulated (**Figure 2B**). Conversely, when CPT1C was depleted by siRNA, the levels of Snail and fibronectin were inhibited and the E-cadherin protein level increased (**Figure 2C**). In agreement with this finding, the wound scratch assay also confirmed that CPT1C promoted EMT (**Figure 2D, 2E**). The contribution of CPT1C-induced EMT in DDP resistance was assessed. A luciferase reporter harboring Snail promoter was first generated. Strikingly, the promoter activity of Snail was induced in a dose-dependent fashion by DDP treatment in H1299 and A549 cells (**Figure 2F**). In addition, Western blot analysis showed a decrease in E-cadherin protein expression with an increase in the fibronectin level and an induction of Snail and CPT1C protein expression in response to DDP treatment (**Figure 2G**). To directly test whether CPT1C was involved in DDP-induced EMT, the impact of CPT1C level manipulation on Snail promoter activity and EMT markers was measured. As shown in **Figure 2H**, the DDP-induced Snail promoter activity was significantly repressed by CPT1C siRNA (**Figure 2H**), which was accompanied by repression of the EMT (**Figure 2I**). Consistent results were obtained in cells overexpressing CPT1C (**Figure 2J, 2K**). Furthermore, the cell viability assay showed that Snail reversed the increase in DDP sensitivity triggered by CPT1C knockdown (**Figure 2L**). This finding suggested that DDP enhanced resistance *via* the CPT1C-induced EMT pathway.

CPT1C-induced ROS was involved in promoting EMT and chemoresistance

The next step was to investigate the molecular mechanism underlying CPT1C-induced EMT. CPT1C functions in transporting FAs into the mitochondria to generate acetyl-CoA, which enters the tricarboxylic acid cycle and promotes oxidative phosphorylation to produce ATP. The induction of invasion and EMT has been linked to activation of the PI3K/AKT pathway. Phosphorylation of GSK3β at Ser9 by AKT causes GSK3β activity to be reduced. GSK3β binds to and phosphorylates the Snail transcription repressor, causing Snail to be degraded[15]. Our prior work demonstrated that ROS produced by oxidative phosphorylation promote Snail-dependent EMT *via* the AKT pathway[16]. Indeed, CPT1C overexpression increased intracellular ROS, while knockdown of CPT1C reduced intracellular ROS (**Figure 3A**). The anti-oxidant, NAC, reversed the CPT1C-induced phosphorylation of AKT as well as Snail-dependent EMT (**Figure 3B**). NAC also reversed CPT1C-activated cell migration in scratch assays

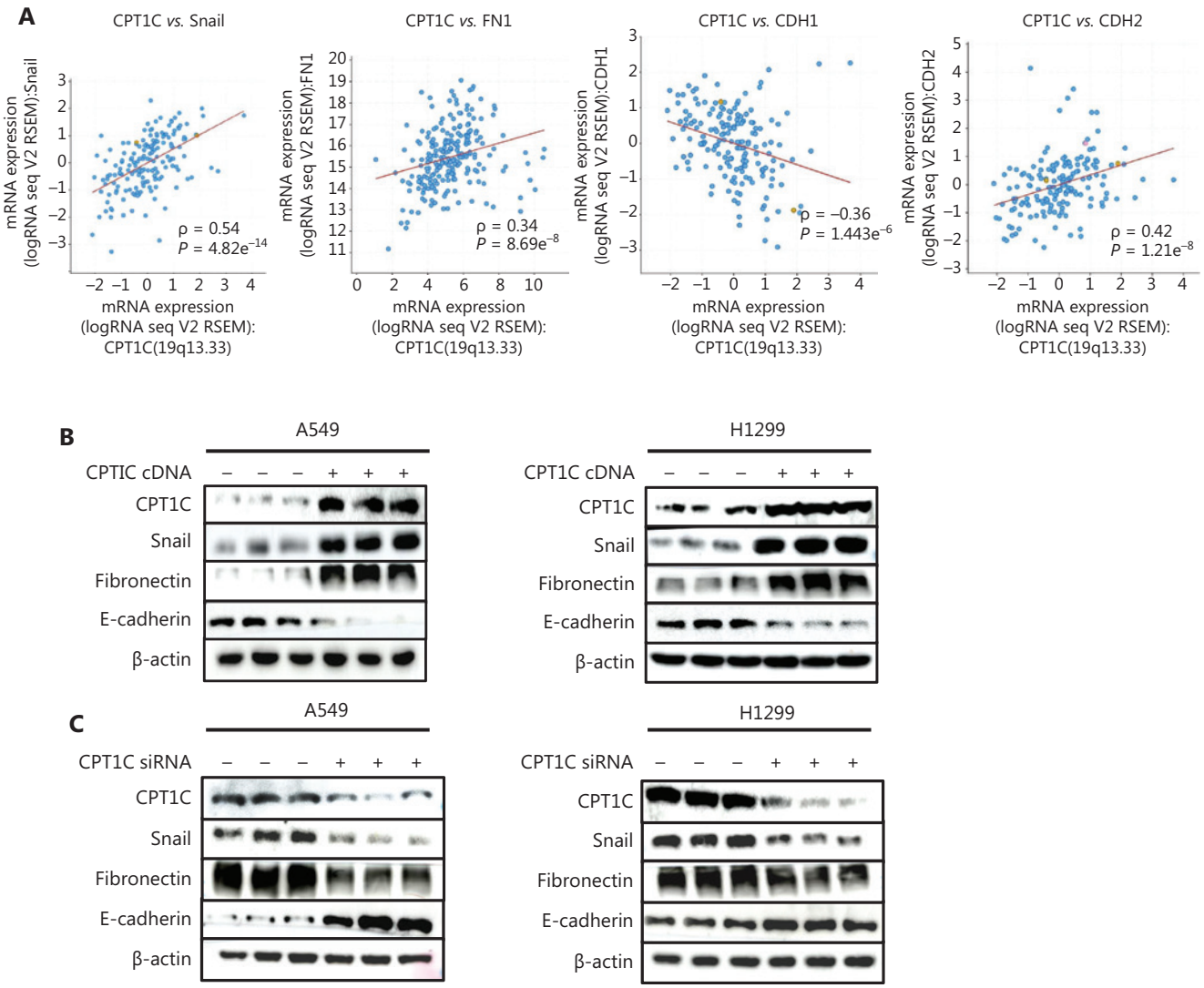


Figure 2 Continued

(**Figure 3C**). To demonstrate the involvement of AKT signaling in CPT1C-induced EMT, cells were treated with the AKT inhibitor, LY294002, and LY294002 reversed CPT1-induced EMT (**Figure 3D**). Surprisingly, DDP-induced EMT was also reversed by LY294002 and NAC (**Figure 3E, 3F**). To further confirm that ROS induced by CPT1C are derived from FA oxidation, a pharmacologic inhibitor (etomoxir) was used. Etomoxir is a widely used small molecule inhibitor of FAO which has irreversible inhibitory effects on CPT1 to inhibit β-oxidation. As shown in **Figure 3G**, etomoxir treatment of H1299 cells overexpressing CPT1C dramatically reduced ROS production. Notably, etomoxir treatment reduced CPT1C-induced AKT phosphorylation and Snail expression (**Figure 3H**). Of greater significance, etomoxir reversed DDP sensitivity of CPT1C-overexpressed cells (**Figure 3I**). These results demonstrated the essential role of ROS produced by CPT1C-induced lipid oxidation in promoting EMT and chemoresistance.

Suppression of an E3 ubiquitin ligase, NEDD4L, by DDP-induced EZH2 increased CPT1C expression

In the next step, how DDP regulates CPT1C expression was determined (**Figure 4A**). We first quantified CPT1C mRNA expression in DDP-treated H1299 and A549 cells

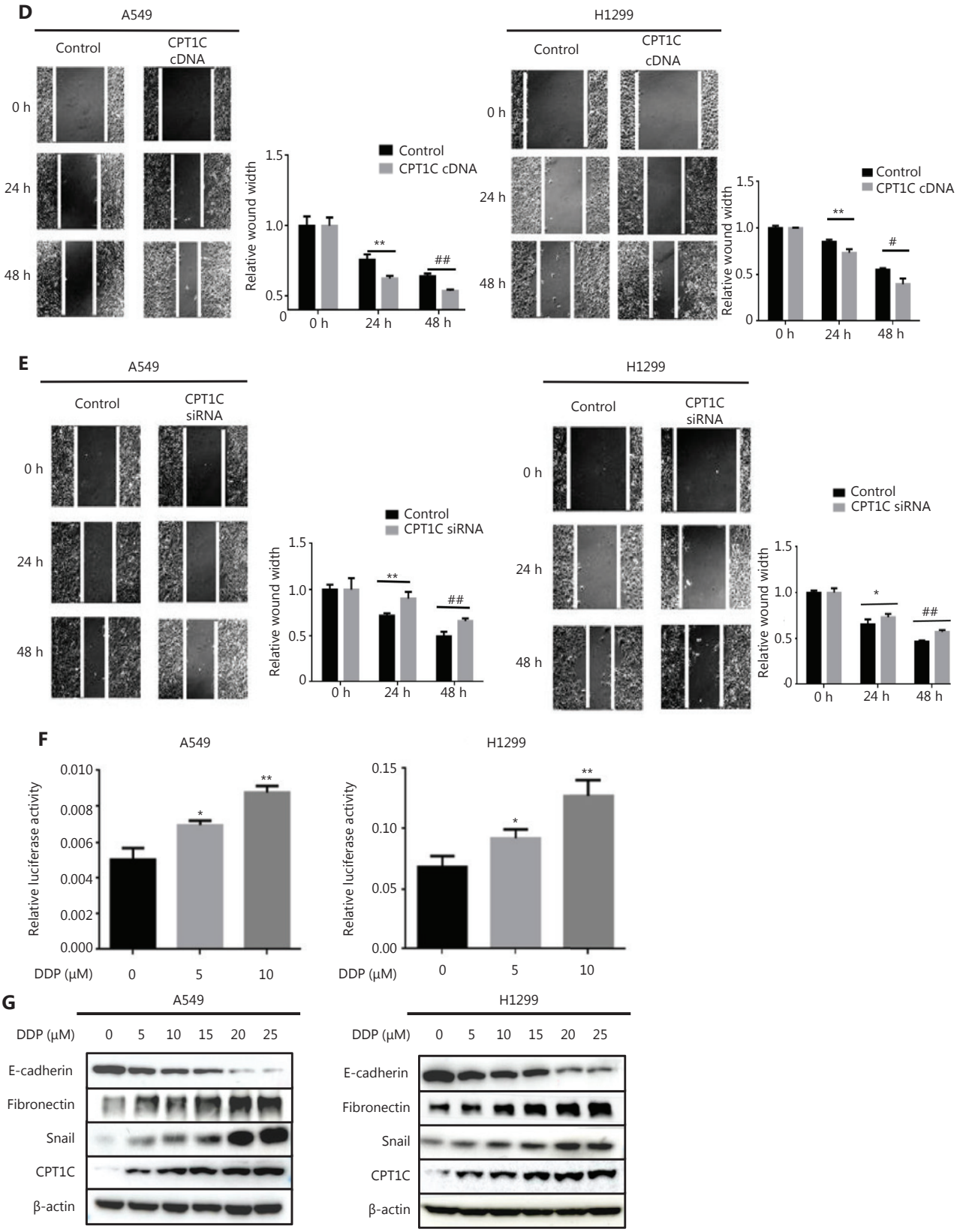


Figure 2 Continued

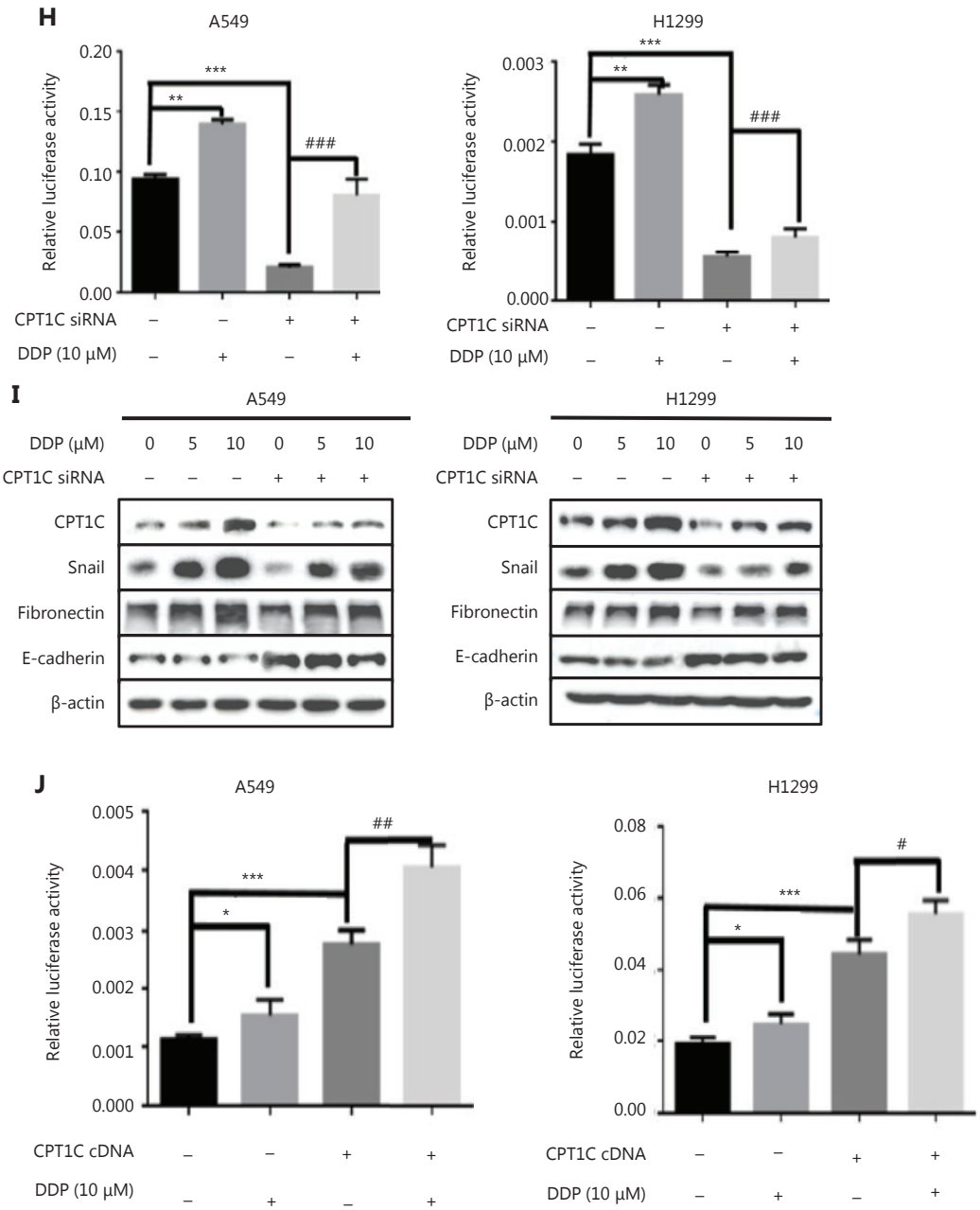


Figure 2 Continued

and showed that CPT1C mRNA levels were not affected. Therefore, we proposed the possibility that DDP might promote the stability of CPT1C protein. A protein synthesis inhibitor [cycloheximide (CHX)] chase assay was used to detect the effect of DDP on the half-life of the CPT1C protein. Indeed, the CPT1C protein half-life was significantly increased in H1299 and A549 cells treated with DDP (**Figure 4B**), suggesting that DDP regulates CPT1C through post-translational mechanisms.

Ubiquitination is a protein modification system that regulates a broad range of protein degradation and stability. To determine if DDP-induced CPT1C stability was mediated by the ubiquitination pathway, IP was used in DDP-treated H1299 and A549 cells to detect ubiquitin levels. The IP results showed

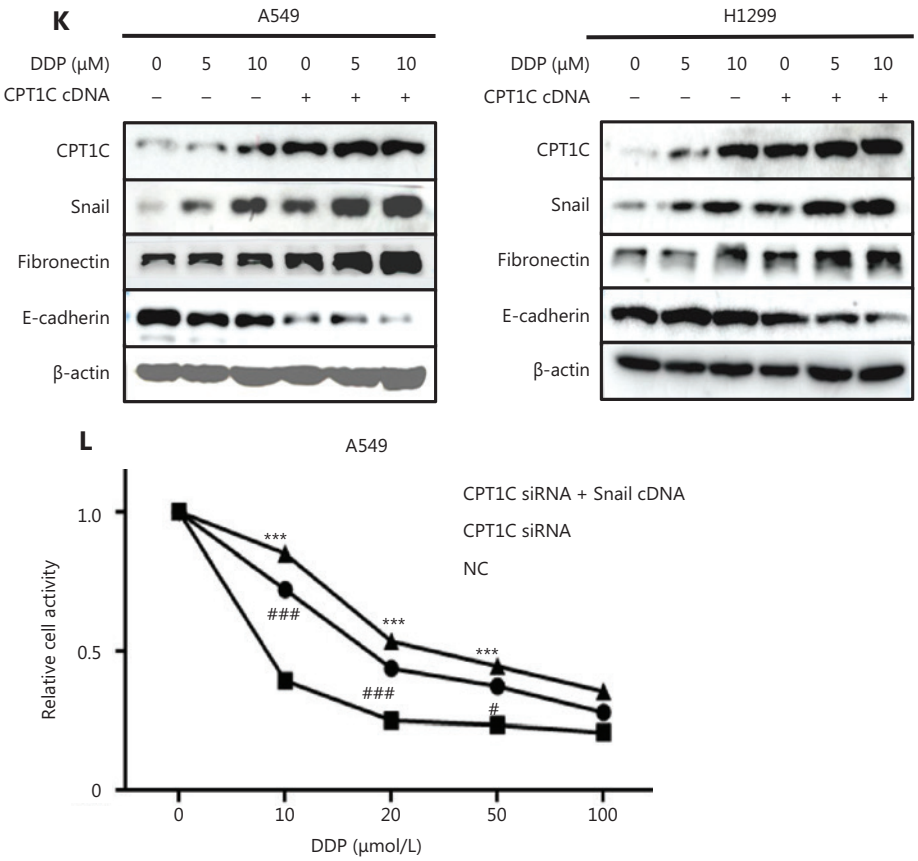


Figure 2 CPT1C-induced EMT enhances cisplatin resistance. (A) Using the lung adenocarcinoma database (TCGA, Nature 2014, sample: 230) in the website (http://www.cbioportal.org/), the correlations between the expression of the *CPT1C* gene and the expression of *SNAI1*, *CDH1*, *CDH2*, and *FN1* were analyzed. (B) After transfection of A549 and H1299 cells with *CPT1C* cDNA for 24 h, Western blot analysis was performed to detect CPT1C, Snail, fibronectin, and E-cadherin protein expression. (C) The cells were transfected with CPT1C siRNA for Western blot analysis of CPT1C, Snail, fibronectin, and E-cadherin expression. (D) After transfection of A549 and H1299 cells with CPT1C cDNA, wound healing assays were performed at various times (0, 24, 48 h) to determine the migration ability. The bars represent the mean ± S.D. of triplicates (***P* < 0.01 for the difference from untreated control at 24 h; $^{\#}P$ < 0.05 and $^{\#\#}P$ < 0.01 for the difference from untreated control at 48 h by ANOVA with Dunnett's correction for multiple comparisons). (E) After transfection of A549 and H1299 cells with CPT1C siRNA, wound healing assays were performed. The bars represent the mean ± S.D. of triplicates (**P* < 0.05 and ***P* < 0.01 for the difference from untreated control at 24 h; and $^{\#\#}P$ < 0.01 at 48 h by ANOVA with Dunnett's correction for multiple comparisons). (F) A549 and H1299 cells were co-transfected with Snail promoter and control Renilla luciferase reporter gene plasmid and treated with the indicated cisplatin concentrations (0, 5, and 10 μM) after 48 h. Luciferase activity was determined and normalized using the dual luciferase reporter system. The bars represent the mean ± S.D. of triplicates (**P* < 0.05 and ***P* < 0.01 for the difference from DDP-untreated A549 and H1299 cells by ANOVA with Dunnett's correction for multiple comparisons). (G) The cells were untreated or treated with the indicated doses of cisplatin (0, 5, 10, 15, 20, and 25 μM) for 6 h. Then, Western blot analysis was performed to determine CPT1C, Snail, fibronectin, and E-cadherin. (H) After transfection of A549 and H1299 cells with CPT1C siRNA for 24 h, the cell lines were co-transfected with Snail promoter and control Renilla luciferase reporter gene plasmid and treated with the indicated cisplatin concentration (10 μM) for 48 h. Luciferase activity was determined and normalized using the dual luciferase reporter system. The bars represent the mean ± S.D. of triplicates (***P* < 0.01 and ****P* < 0.001 for the difference from untreated cells; and $^{\#\#\#}P$ < 0.001 for the difference from transfection with CPT1C siRNA cells by ANOVA with Dunnett's correction for multiple comparisons). (I) After transfection of A549 and H1299 cells with CPT1C siRNA for 24 h, Western blot analysis was performed to determine CPT1C, Snail, fibronectin, and epithelial marker (E-cadherin) expression in A549 and H1299 cells treated with cisplatin (0, 5, and 10 μM) for 6 h. (J) After transfection of A549 and H1299 with CPT1C cDNA for 24 h, the cell lines were co-transfected with Snail promoter and control Renilla luciferase reporter gene plasmid and treated with 10 μM cisplatin for 48 h. Luciferase activity was determined and normalized using the dual luciferase reporter system. The bars represent the mean ± S.D. of triplicates (**P* < 0.05 and ****P* < 0.001 for the difference from untreated cells and $^{\#}P$ < 0.05 and $^{\#\#}P$ < 0.01 for difference from transfection with CPT1C cDNA cells by ANOVA with Dunnett's correction for multiple comparisons).

(K) After transfection of A549 and H1299 cells with CPT1C cDNA for 24 h, Western blot analysis of CPT1C, Snail, fibronectin, and E-cadherin in A549 and H1299 cells treated with cisplatin (0, 5, and 10 μM) for 6 h. (L) Transfection of CPT1C siRNA alone or CPT1C siRNA combined with Snail cDNA, the A549 cells were treated with different concentrations of cisplatin for 48 h in preparation for MTT analysis. The bars represent the mean ± S.D. of triplicates (***P < 0.001 for the difference of CPT1C siRNA + Snail cDNA from the control group; and $^{###}P$ < 0.001 for the difference in CPT1C siRNA from the control group by ANOVA with Dunnett's correction for multiple comparisons).

that the addition of DDP significantly reduced ubiquitination of CPT1C (**Figure 4C**). We attempted to uncover the regulatory mechanism underlying CPT1C using the UniProt approach of proteomics (https://www.uniprot.org). CPT1C protein contains the P700-D701-Y702 sequence, which is similar to the conserved PxY binding motif of the E3 ubiquitin ligase, NEDD4L, located on the membrane. Intriguingly, bioinformatics analysis revealed that CPT1C expression was negatively correlated with the NEDD4L level (GEPIA http://gepia.cancer-pku.cn) (**Figure 4D**). When the levels of CPT1C and NEDD4L expression in various cell lines were determined, A549 was shown to have the lowest NEDD4L expression in contrast to the highest CPT1C levels. The levels of both genes exhibited a negative correlation in H1299, P-C9, and H1688 cells (**Figure 4E**). To determine whether NEDD4L was involved in CPT1C ubiquitination, cells were transfected with NEDD4L cDNA. Indeed, CPT1C levels were significantly suppressed in both H1299 and A549 cells upon overexpression of NEDD4L, which was accompanied by the suppression of Snail protein (**Figure 4F**). Conversely, when NEDD4L siRNA was introduced ectopically into H1299 and A549 cells the levels of CPT1C and Snail protein increased significantly (**Figure 4G**). Similar results were obtained in the DDP-treated cells (**Figure 4H, 4I**). Furthermore, the CHX chase assay demonstrated that NEDD4L considerably reduced the half-life of the CPT1C protein (**Figure 4J**).

The above results raised an important question. Why can DDP affect the ubiquitination of CPT1C by NEDD4L? Previous reports showed that NEDD4L can be inhibited by DDP-induced EZH2[17,18]. Western blot results showed that DDP can upregulate EZH2 and downregulate NEDD4L expression (**Figure 4K**). Overexpression of EZH2 repressed NEDD4L expression and promoted CPT1C and Snail expression (**Figure 4L**). Conversely, NEDD4L expression increased in tandem with a decrease in CPT1C and Snail expression upon EZH2 depletion by siRNA (**Figure 4M**). Importantly, overexpression of EZH2 greatly decreased the ubiquitination of CPT1C (**Figure 4N**). Overall, the results indicated that suppression of NEDD4L by DDP-induced EZH2 is functionally relevant to CPT1C regulation.

Discussion

Many lines of evidence suggest that changes in FA metabolism are a common characteristic of several solid tumors. The results are consistent with multiple reports, emphasizing the increasing importance of FA metabolism in tumor development. Recent studies have shown that the growth and survival of cancer cells relies on the activity of CPT1C[10,11,19]. Sustained expression of CPT1C increases FAO and ATP production, while depletion of CPT1C reduces ATP production. Under hypoxic or limited glucose conditions, experiments have shown that knocking out CPT1C in human cancer cell lines reduces cell growth *in vitro* and reduces the formation of new tumors *in vivo*, which is consistent with the results demonstrated that CPT1C has an impact on cell proliferation. Notably, we were the first to show the necessary role of CPT1C in chemotherapy resistance of lung cancer cells. Intriguingly, in DDP-resistant cells CPT1C activity has been linked to induction of Snail-dependent EMT program. Overexpression of CPT1C induced Snail expression and EMT, and Snail reversed the increase in DDP sensitivity triggered by CPT1C knockdown. Moreover, we demonstrated the essential role of CPT1C-induced ROS in promoting EMT and chemoresistance. Taken together, the results suggested that CPT1C is involved in regulation of bioenergetics that mediates resistance to chemotherapy.

The results of the current study have expanded our understanding of the function of this unconventional member of the CPT family. CPT1C is primarily expressed in the neurons of normal mammals[20,21]. It has been proposed that CPT1C plays an important role in maintaining the overall energy homeostasis of mice. Our findings are consistent with several studies showed that CPT1C has functional roles at both the systemic and cellular levels in non-neuronal tumors[10,11]. It is not clear why this particular *CPT1* gene is overexpressed in tumors. In the current study we identified the E3-ubiquitin ligase, NEDD4L, as a major regulator of CPT1C stability. NEDD4L, also known as NEDD4-2, is an HECT E3 ubiquitin ligase comprised of a phospholipid-binding (C2) domain, four WW domains that interact with the PY motif (L/PPXY) of

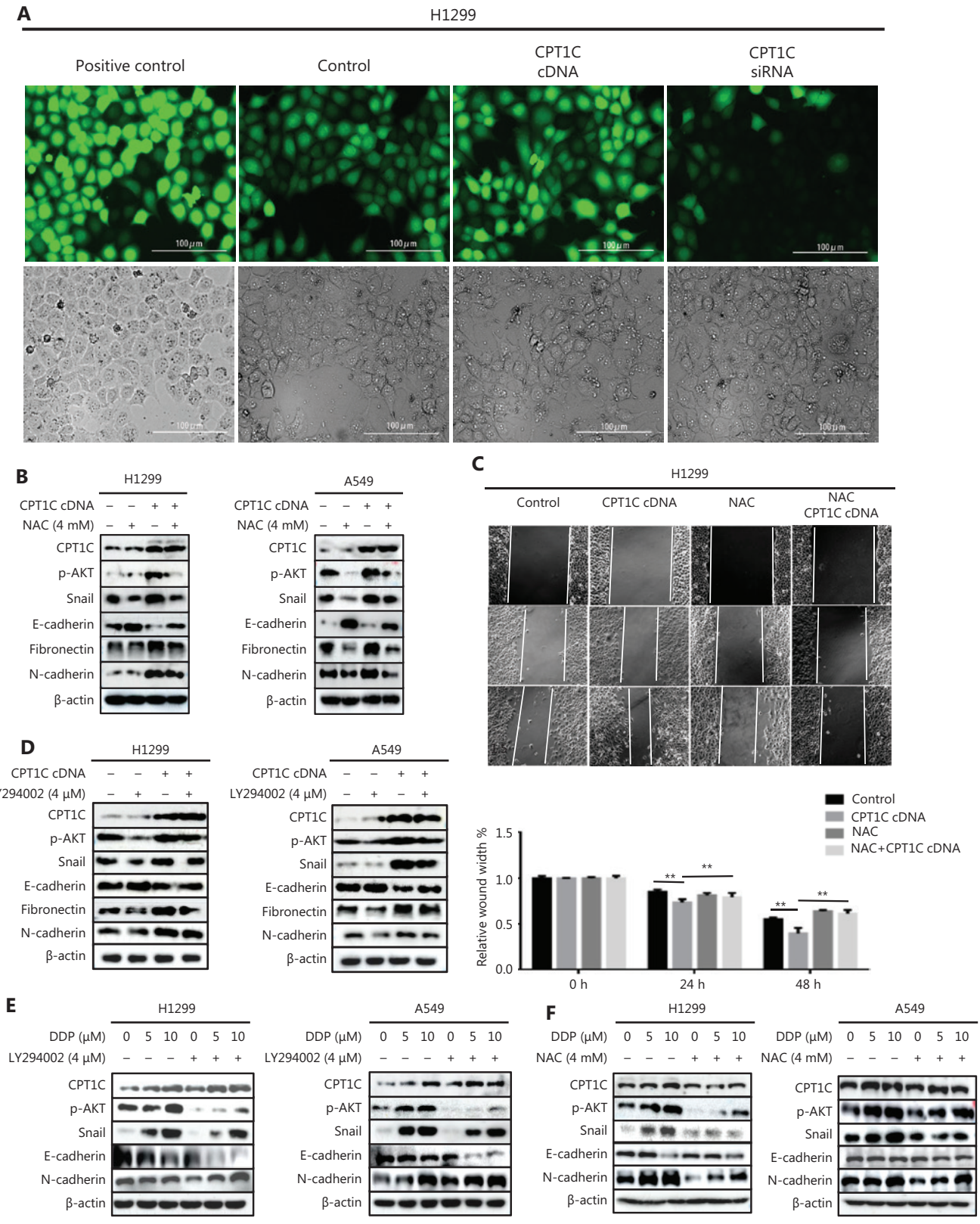


Figure 3 Continued

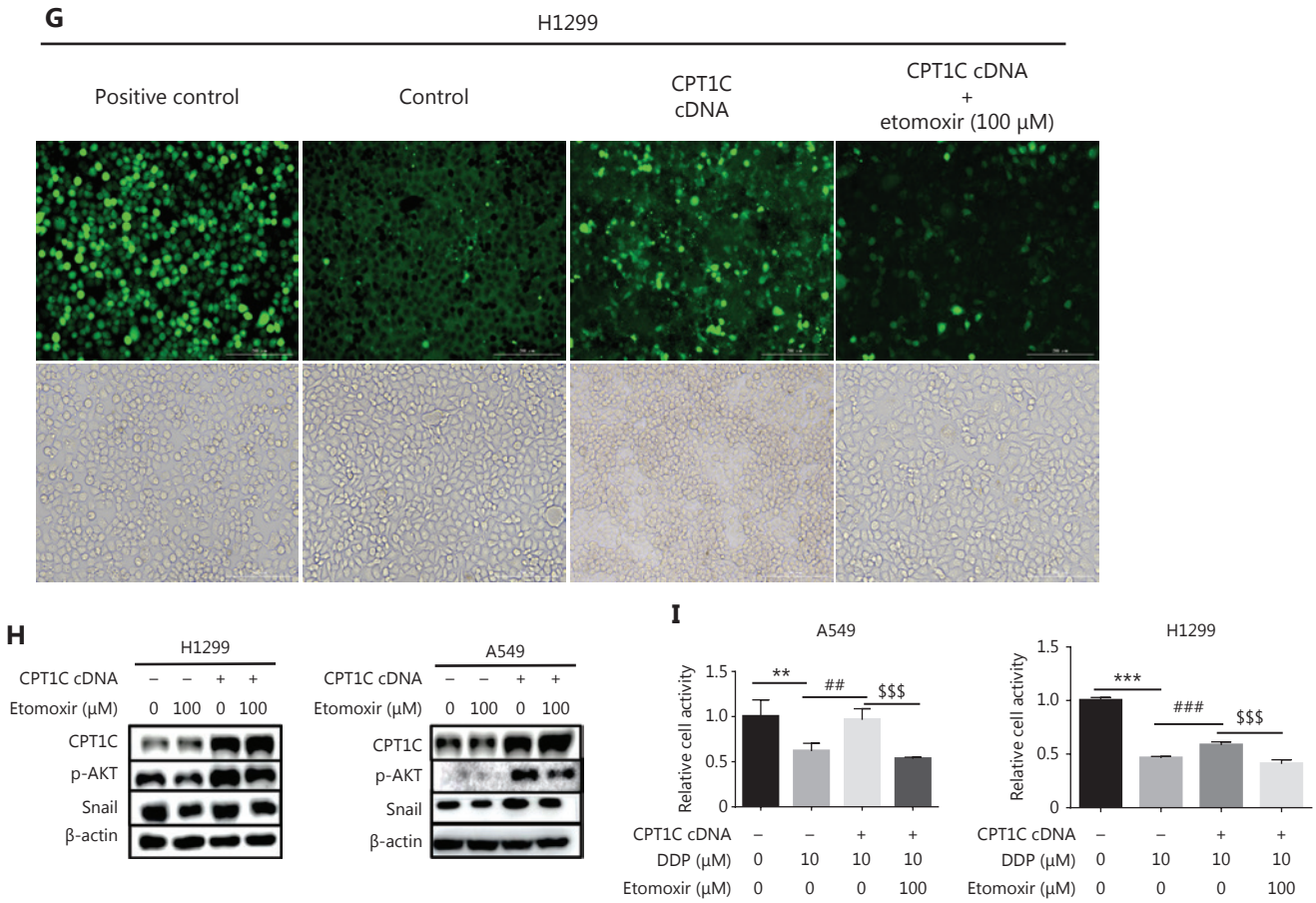


Figure 3 CPT1C-induced ROS is involved in promoting EMT and chemoresistance. (A) H1299 cells were transfected with CPT1C cDNA and CPT1C siRNA and the ROS levels were detected using fluorescent probe DCFH-DA (start magnification × 100 scale, 100 μM). (B) A549 and H1299 cells were treated with 4 mM N-acetyl-cysteine (NAC) after CPT1C cDNA transfection for 3 h. Western blot analysis was performed to measure CPT1C, p-AKT, Snail, E-cadherin, fibronectin, and N-cadherin protein levels. (C) After transfection of H1299 cells with CPT1C cDNA for 24 h, then the cell line was treated with vehicle or the indicated doses of 4 mM N-acetyl-cysteine (NAC). Wound healing assays were performed at various times (0, 24, and 48 h) for migration ability. A quantitative analysis is shown below. The bars represent the average ± S.D. (**P < 0.01 for the difference from the CPT1C cDNA-treated group by ANOVA with Dunnett's correction for multiple comparisons). (D) 48 h after transfection of CPT1C cDNA, the cell lines (A549 and H1299) were treated with AKT inhibitors (4 μM LY294002) for 3 h in preparation for Western blot analysis. (E) A549 and H1299 cells were treated with vehicle or the indicated doses of cisplatin (0, 5, and 10 μM) for 3 h, then the cells were treated with vehicle or the indicated doses of AKT inhibitors (4 μM LY294002) for 6 h before Western blot analysis. (F) Representative Western blot analysis of CPT1C, p-AKT, Snail, E-cadherin and N-cadherin after the cells (A549 and H1299) were treated with the indicated doses of cisplatin (0, 5, and 10 μM) for 3 h, followed by the indicated doses of 4 mM N-acetyl-cysteine (NAC) for 3 h. (G) 24 h after H1299 cells were transfected with CPT1C cDNA, cells were treated with the CPT1 inhibitor, etomoxir (100 μM), for 1 h. The ROS levels were detected using the fluorescent probe, DCFH-DA (start magnification × 100 scale, 100 μM). (H) 24 h after H1299 cells were transfected with CPT1C cDNA, cells were treated with the CPT1 inhibitor, etomoxir (100 μM), for 1 h. Western blot analysis was performed for analysis of CPT1C, p-AKT, and Snail expression. (I) 24 h after transfection of CPT1C cDNA, the cell lines (H1299 and A549) were treated with 10 μM of cisplatin or cisplatin with etomoxir (100 μM) for additional 24 h for MTT analysis. The bars represent the mean ± S.D. of triplicates (**P < 0.01 and ***P < 0.001 for the difference from the control group; $^{\#\#}P$ < 0.01 and $^{\#\#\#}P$ < 0.001 for the difference from the DDP-treated group; and $^{\$\$\$}P$ < 0.001 for the difference from the CPT1C-transfected combined with the DDP-treated group by ANOVA with Dunnett's correction for multiple comparisons).

target proteins, and a carboxy-terminal ubiquitin ligase HECT domain[22,23]. The most studied target proteins of NEDD4L are membrane proteins, including epithelial sodium channels (ENaC), cystic fibrosis transmembrane conductance regulator (CFTR), and GPX4[23,24]. We demonstrated for the first time that NEDD4L is the E3 ubiquitin ligase of CPT1C and

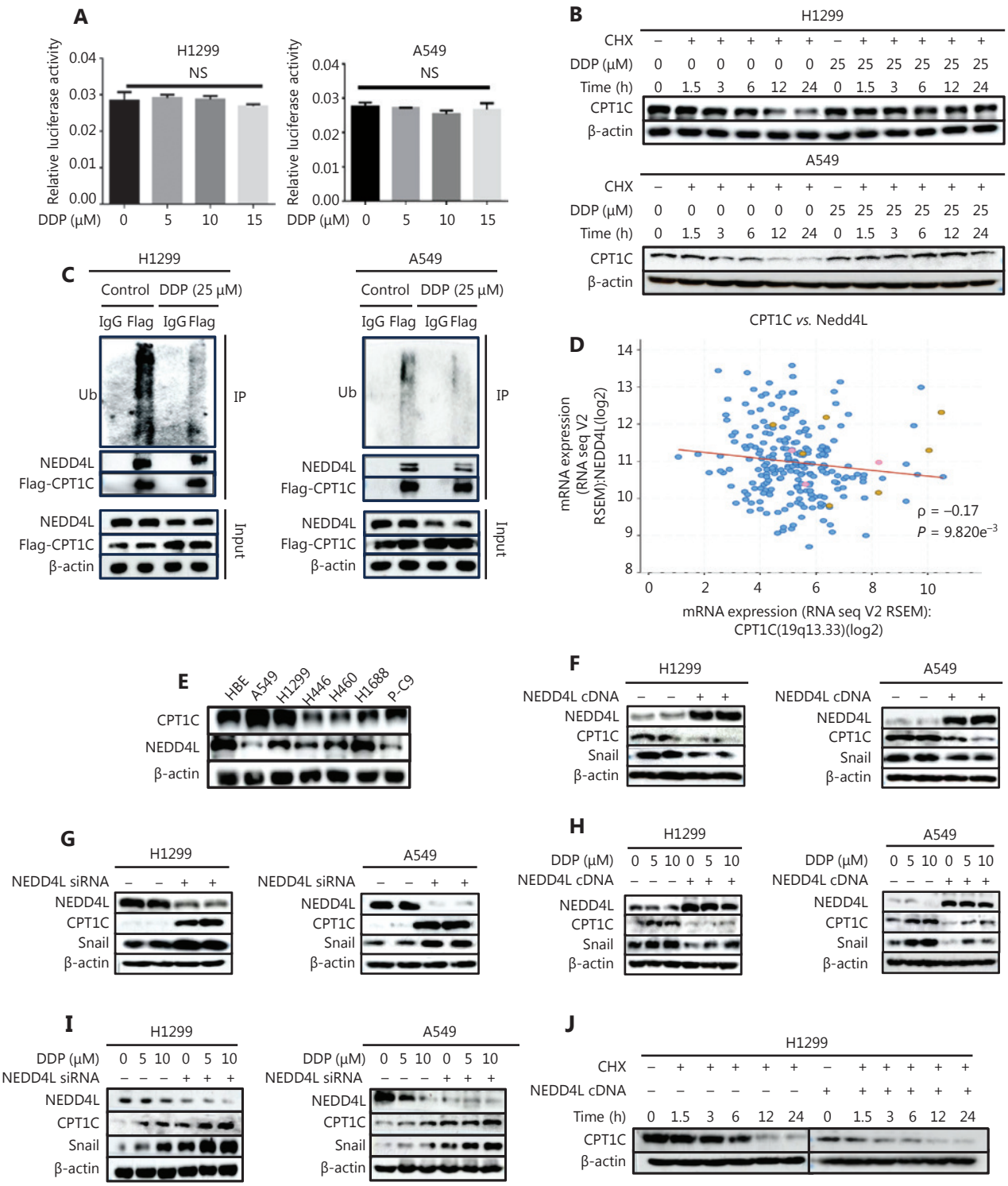


Figure 4 Continued

specifically mediates regulation of CPT1C by chemotherapy. Remarkably, our evidence showed that NEDD4L inactivation is mediated by the DDP-induced EZH2 pathway.

The adverse prognosis of advanced lung cancer can be partially explained by the strong resistance to chemotherapy. Despite the recent development of targeted therapy and

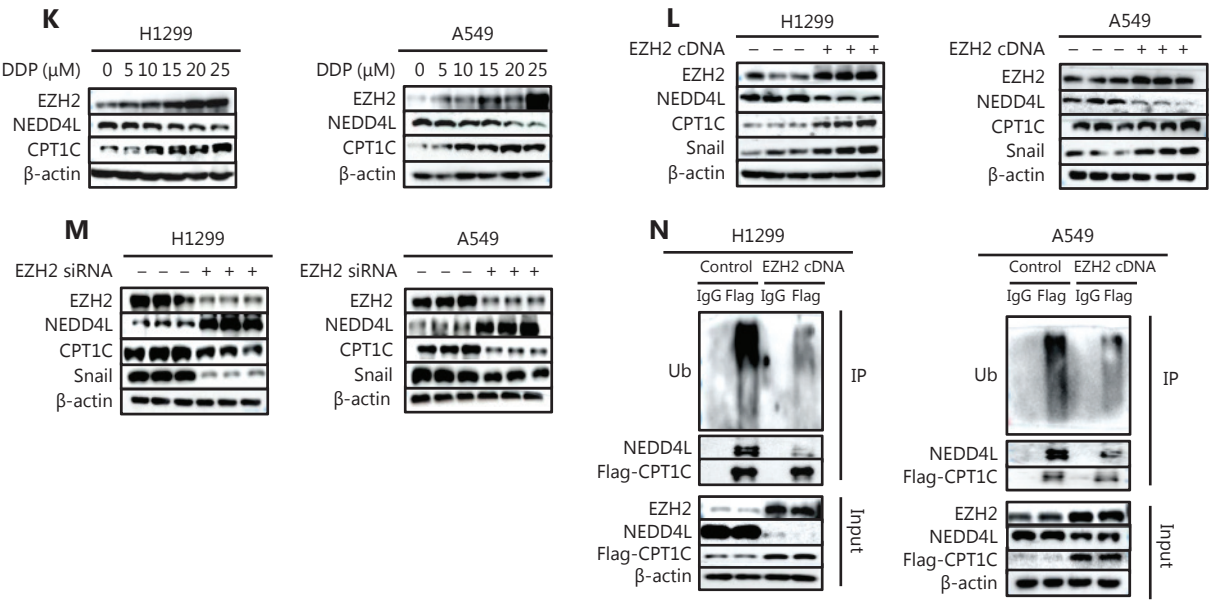


Figure 4 Suppression of the E3 ubiquitin ligase, NEDD4L, by cisplatin-induced EZH2 increases CPT1C expression. (A) A549 and H1299 cells were co-transfected with CPT1C promoter and control Renilla luciferase reporter gene plasmid for 24 h. After transfection, cells were treated with cisplatin (0, 5, 10, and 15 μM) for 6 h. Luciferase activity was determined and normalized using the dual luciferase reporter system. The bars represent the mean ± S.D. of triplicates (NS indicates no statistical difference). (B) The cells were first treated with 25 μM cisplatin for 6 h, followed by 178 μM cycloheximide (CHX) treatment for the indicated time (0, 1.5, 3, 6, 12, and 24 h). Then, Western blot analysis of CPT1C in A549 and H1299 cells. (C) The cells were transfected with flag-tagged CPT1C cDNA constructs for 24 h, then treated with cisplatin (25 μM) for an additional 6 h. The cells were lysed using lysis buffer and the supernatant was removed after centrifugation. Then, magnetic beads bound to primary antibody (Flag) were added to the supernatant and incubated overnight for 4°C before Western blot analysis was performed for analysis of Ub, NEDD4L, and CPT1C proteins. (D) Bioinformatics analysis (GEPIA http://gepia.cancer-pku.cn) revealed that the *CPT1C* gene expression was inversely correlated with NEDD4L expression. (E) Western blot analysis was performed in seven cell lines (HBE, A549, H1299, H446, H460, H1688, and P-C9) to detect the level of CPT1C and NEDD4L protein expression. (F) H1299 and A549 cells were transfected with NEDD4L cDNA for 24 h, then Western blot analysis was performed to detect CPT1C and Snail protein expression. (G) Cell lines (H1299 and A549) were transfected with NEDD4L siRNA for 24 h in preparation for Western blot analysis. (H) After transfection of NEDD4L cDNA, H1299 and A549 cells were treated with the indicated concentrations of cisplatin (0, 5, and 10 μM) for 24 h in preparation for Western blot analysis. (I) After transfection of NEDD4L siRNA, H1299 and A549 cells were treated for 24 h with the indicated concentrations of cisplatin (0, 5, and 10 μM) for 24 h in preparation for Western blot analysis. (J) Western blot analysis of CPT1C in the H1299 cell line. The cells were treated with 178 μM cycloheximide (CHX) at various times (0, 1.5, 3, 6, 12, and 24 h) 48 h after NEDD4L cDNA transfection. (K) H1299 and A549 cells were treated with the indicated doses of cisplatin (0, 5, 10, 15, 20, and 25 μM) for 6 h in preparation for Western blot analysis. (L) H1299 and A549 cells were transfected with EZH2 cDNA for 24 h and Western blot analysis was performed to detect the levels of NEDD4L, CPT1C, and Snail protein expression. (M) H1299 and A549 cells were transfected with EZH2 siRNA for 48 h and Western blot analysis was performed to detect the levels of NEDD4L and Snail protein expression. (N) After transfection of EZH2 cDNA and flag-tagged CPT1C cDNA construct for 24 h, cells were lysed using lysis buffer for immunoprecipitation. The magnetic beads bound to primary antibody (Flag) were used for immunoprecipitation and Western blot analysis of Ub, NEDD4L, and CPT1C proteins.

immunotherapy, platinum-based chemotherapy remains the primary treatment method for patients with NSCLC. Multiple mechanisms are responsible for this resistance but upregulation of ABC transporters has received considerable attention. These transporters are known to mediate drug efflux and therefore confer multidrug resistance to cancer cells[2]. Ample evidence indicates that the emergence of therapeutic drugs resistance in tumor cells have undergone the EMT process[5,25,26]. In agreement with this finding, we have previously shown that Snail, a major EMT-inducing transcriptional factor, forms complexes with TAZ and AP-1 at the ABC transporter, MRP1 promoter, to activate MRP1 expression and is critical for drug resistance in NSCLC cells. Herein we report, for the first time, that the increased expression of CPT1C induces EMT and mediates

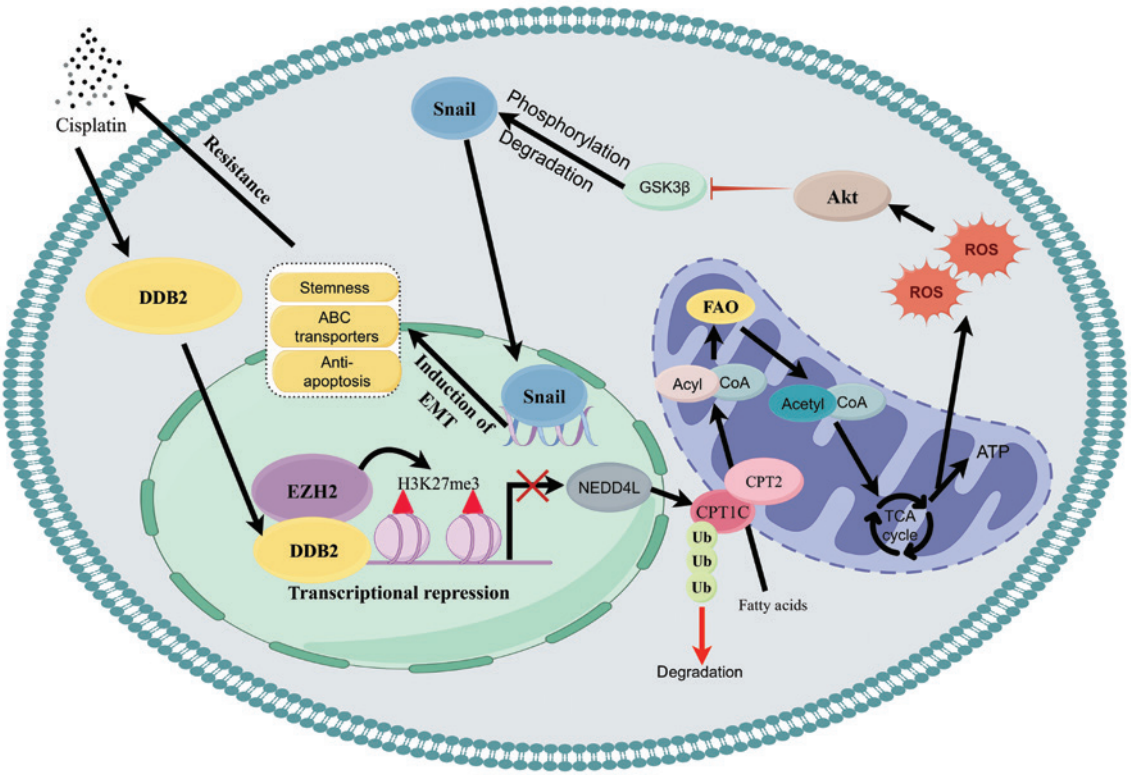


Figure 5 A schematic illustration of our major findings. Cisplatin treatment induces expression of damaged DNA binding protein complex subunit 2 (DDB2), DDB2 can bind to the promoter region of NEDD4L and recruit EZH2 to repress NEDD4L transcription by facilitating histone H3 lysine 27 trimethylation (H3K27me3) at the NEDD4L promoter. Consequently, reduced NEDD4L decreases ubiquitination and degradation of CPT1C. Enhanced CPT1C expression increased FAO, ATP generation, and ROS production. ROS induces phosphorylation and activity of AKT, which phosphorylates GSK3β to reduce GSK3β activity. The inability of GSK3β to phosphorylate the Snail transcription factor results in Snail stability and translocation into nucleus. Eventually, Snail targets several related genes to increase cisplatin resistance by promoting activation of the Snail-dependent EMT program and expression of ABC transporters (drawn by Figdraw, ID: OWRPR94604). EZH2, enhancer of zeste homolog 2 histone methyltransferase; NEDD4L, a HECT E3 ubiquitin ligase; CPT1C, carnitine palmitoyltransferase 1C; FAO, fatty acid oxidation; ROS, activated oxygen species.

DDP resistance in NSCLC cells. We also showed that CPT1-induced ROS regulates activation of the AKT pathway and EMT program. Taken together, these results have potential implications for future cancer therapy by targeting the EMT.

Our results are consistent with numerous studies that have emphasized the emerging importance of lipid metabolism[27]. There is mounting evidence suggesting a link between obesity and different types of cancer risk, such as breast, colorectal, and pancreatic cancer[28]. Intriguingly, the data on lung cancer is not consistent, with some reports indicating an inverse correlation with body mass index (BMI) and lung cancer risk[29]. However, clinical studies have shown that abdominal visceral obesity is associated with an increased risk of NSCLC[30]. It has been shown that obese NSCLC patients have a lower response rate to chemotherapy and radiotherapy, as well as a higher risk of recurrence and death[31-33]. Nevertheless, the present findings suggest that lipid metabolism is closely related to the development of lung cancer. Taken together, these results may be very important for the development of potential new treatment strategies for combining CPT1C inhibition with other chemotherapeutic agents.

Conclusions

CPT1C, which is highly expressed in lung cancer, was shown to be linked to DDP resistance in NSCLC cells. EMT induced by CPT1C was shown to contribute to chemoresistance. Snail ectopic expression reversed the CPT1C knockdown-induced

increase in DDP sensitivity. Moreover, CPT1C was post-translationally regulated and an E3-ubiquitin ligase, NEDD4L, was a key regulator of CPT1C stability and activity (**Figure 5**). Taken together, these results may be very important for the development of potential new treatment strategies for combining CPT1C inhibition with other chemotherapeutic agents.

Acknowledgments

We thank Professor Yuelong Jin from the School of Public Health at Wannan Medical College for his valuable and professional statistical support.

Grant support

This work was supported by the National Natural Science Foundation of China (Grant No. 81872371), the Open Project Program of State Key Laboratory of Molecular Oncology (Grant No. SKL-KF-2019-11), the Program for Excellent Sci-tech Innovation Teams of Universities in Anhui Province (Grant No. 2023AH010073), the Provincial-level Quality Project in Higher Education Institutions of Anhui Province (Grant No. 2022jyxm1710), the College Students Innovation and Entrepreneurship Training Program (Grant No. S202310368028 and S202410368035), and the Health Science Research Project of Anhui Province (Grant No. AHWJ2022a028). Renjie Chen, Jiahui Wang, Xuefeng Hu, Shuoyu Huang, Xinran He are team members of Precision Medicine Beidou Research Group.

Conflict of interest statement

No potential conflicts of interests are disclosed.

Author contributions

Conceived and designed the analysis: Zhihao Wu, Yushu Huang.
Collected the data: Xuefeng Hu, Xinran He, Tiange Zhang, Yunhan Hu.
Contributed data or analysis tools: Huijun Wei, Sihui Nian.
Performed the analysis: Renjie Chen, Jiahui Wang, Shuoyu Huang, Xuefeng Hu.
Wrote the paper: Zhihao Wu.

Data availability statement

The data generated in this study are available upon request from the corresponding authors.

References

1. Shea M, Costa DB, Rangachari D. Management of advanced non-small cell lung cancers with known mutations or rearrangements: latest evidence and treatment approaches. Ther Adv Respir Dis. 2016; 10: 113-29.
2. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. Nat Rev Cancer. 2013; 13: 714-26.
3. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nat Rev Cancer. 2016; 16: 275-87.
4. Brabletz T, Kalluri R, Nieto MA, Weinberg RA. EMT in cancer. Nat Rev Cancer. 2018; 18: 128-34.
5. Shibue T, Weinberg RA. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. Nat Rev Clin Oncol. 2017; 14: 611-29.
6. Li X, Zhang Z, Zhang Y, Cao Y, Wei H, Wu Z. Upregulation of lactate-inducible snail protein suppresses oncogene-mediated senescence through $p16^{INK4a}$ inactivation. J Exp Clin Cancer Res. 2018; 37: 39.
7. Dong Q, Zhou C, Ren H, Zhang Z, Cheng F, Xiong Z, et al. Lactate-induced MRP1 expression contributes to metabolism-based etoposide resistance in non-small cell lung cancer cells. Cell Commun Signal. 2020; 18: 167.
8. Hirsch HA, Iliopoulos D, Joshi A, Zhang Y, Jaeger SA, Bulyk M, et al. A transcriptional signature and common gene networks link cancer with lipid metabolism and diverse human diseases. Cancer Cell. 2010; 17: 348-61.
9. Casals N, Zammit V, Herrero L, Fado R, Rodriguez-Rodriguez R, Serra D. Carnitine palmitoyltransferase 1C: from cognition to cancer. Prog Lipid Res. 2016; 61: 134-48.
10. Zaugg K, Yao Y, Reilly PT, Kannan K, Kiarash R, Mason J, et al. Carnitine palmitoyltransferase 1C promotes cell survival and tumor growth under conditions of metabolic stress. Genes Dev. 2011; 25: 1041-51.
11. Chen T, Wu G, Hu H, Wu C. Enhanced fatty acid oxidation mediated by CPT1C promotes gastric cancer progression. J Gastrointest Oncol. 2020; 11: 695-707.
12. Chen H, Yang Z, Sun Y, Yin S, Tang M, Zhang F. Targeting the key enzymes of abnormal fatty acid β-oxidation as a potential strategy for tumor therapy. Front Biosci (Landmark Ed). 2022; 27: 95.
13. Reilly PT, Mak TW. Molecular pathways: tumor cells Co-opt the brain-specific metabolism gene CPT1C to promote survival. Clin Cancer Res. 2012; 18: 5850-5.

14. Kuhajda FP. Fatty acid synthase and cancer: new application of an old pathway. Cancer Res. 2006; 66: 5977-80.
15. Zhou BP, Deng J, Xia W, Xu J, Li YM, Gunduz M, et al. Dual regulation of snail by GSK-3beta-mediated phosphorylation in control of epithelial-mesenchymal transition. Nat Cell Biol. 2004; 6: 931-40.
16. Huang T, Zhou X, Mao X, Yu C, Zhang Z, Yang J, et al. Lactate-fueled oxidative metabolism drives DNA methyltransferase 1-mediated transcriptional co-activator with PDZ binding domain protein activation. Cancer Sci. 2020; 111: 186-99.
17. Wang X, Duan J, Fu W, Yin Z, Sheng J, Lei Z, et al. Decreased expression of NEDD4L contributes to NSCLC progression and metastasis. Biochem Biophys Res Commun. 2019; 513: 398-404.
18. Zhao R, Cui T, Han C, Zhang X, He J, Srivastava AK, et al. DDB2 modulates TGF-β signal transduction in human ovarian cancer cells by downregulating NEDD4L. Nucleic Acids Res. 2015; 43: 7838-49.
19. Zhuang W, Lian G, Huang B, Du A, Gong J, Xiao G, et al. CPT1 regulates the proliferation of pulmonary artery smooth muscle cells through the AMPK-p53-p21 pathway in pulmonary arterial hypertension. Mol Cell Biochem. 2019; 455: 169-83.
20. Wolfgang MJ, Kurama T, Dai Y, Suwa A, Asaumi M, Matsumoto S, et al. The brain-specific carnitine palmitoyltransferase-1c regulates energy homeostasis. Proc Natl Acad Sci U S A. 2006; 103: 7282-7.
21. Price N, van der Leij F, Jackson V, Corstorphine C, Thomson R, Sorensen A, et al. A novel brain-expressed protein related to carnitine palmitoyltransferase I. Genomics. 2002; 80: 433-42.
22. Escobedo A, Gomes T, Aragon E, Martin-Malpartida P, Ruiz L, Macias MJ. Structural basis of the activation and degradation mechanisms of the E3 ubiquitin ligase Nedd4L. Structure. 2014; 22: 1446-57.
23. Goel P, Manning JA, Kumar S. NEDD4-2 (NEDD4L): the ubiquitin ligase for multiple membrane proteins. Gene. 2015; 557: 1-10.
24. Cheng F, Dou J, Yang Y, Sun S, Chen R, Zhang Z, et al. Drug-induced lactate confers ferroptosis resistance via p38-SGK1-NEDD4L-dependent upregulation of GPX4 in NSCLC cells. Cell Death Discov. 2023; 9: 165.
25. Debaugnies M, Rodriguez-Acebes S, Blondeau J, Parent MA, Zocco M, Song Y, et al. RHOJ controls EMT-associated resistance to chemotherapy. Nature. 2023; 616: 168-75.
26. Fischer KR, Durrans A, Lee S, Sheng J, Li F, Wong ST, et al. Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. Nature. 2015; 527: 472-6.
27. Rohrig F, Schulze A. The multifaceted roles of fatty acid synthesis in cancer. Nat Rev Cancer. 2016; 16: 732-49.
28. Steele CB, Thomas CC, Henley SJ, Massetti GM, Galuska DA, Agurs-Collins T, et al. Vital signs: trends in incidence of cancers associated with overweight and obesity – United States, 2005-2014. MMWR Morb Mortal Wkly Rep. 2017; 66: 1052-8.
29. Lam VK, Bentzen SM, Mohindra P, Nichols EM, Bhooshan N, Vyfhuis M, et al. Obesity is associated with long-term improved survival in definitively treated locally advanced non-small cell lung cancer (NSCLC). Lung Cancer. 2017; 104: 52-7.
30. Barbi J, Patnaik SK, Pabla S, Zollo R, Smith RJ Jr, Sass SN, et al. Visceral obesity promotes lung cancer progression-toward resolution of the obesity paradox in lung cancer. J Thorac Oncol. 2021; 16: 1333-48.
31. Ten Haaf K, Jeon J, Tammemagi MC, Han SS, Kong CY, Plevritis SK, et al. Risk prediction models for selection of lung cancer screening candidates: a retrospective validation study. PLoS Med. 2017; 14: e1002277.
32. Dhillon SS, Groman A, Meagher A, Demmy T, Warren GW, Yendamuri S. Metformin and not diabetes influences the survival of resected early stage NSCLC patients. J Cancer Sci Ther. 2014; 6: 217-22.
33. Zhang C, Rexrode KM, van Dam RM, Li TY, Hu FB. Abdominal obesity and the risk of all-cause, cardiovascular, and cancer mortality: sixteen years of follow-up in US women. Circulation. 2008; 117: 1658-67.

Cite this article as: Chen R, Wang J, Huang S, Hu X, He X, Zhang T, et al. Carnitine palmitoyltransferase 1C promotes EMT-associated cisplatin resistance in non-small cell lung cancer cells. Cancer Biol Med. 2025; 22: 48-66. doi: 10.20892/j.issn.2095-3941.2024.0459