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EDN1 facilitates cisplatin resistance of non-small cell lung cancer cells by regulating the TNF signaling pathway

Jie Zhen[1], Long Sun[2], Li Ji[3], Shaochong Zhou[1], Yijin Cui[4] and Zhenwei Li[5*]

Abstract

Background Cisplatin (DDP) is a commonly utilized chemotherapeutic agent. Nevertheless, the development of resistance to DDP significantly diminishes the effectiveness of DDP-based chemotherapy in patients with non-small cell lung cancer (NSCLC). In this study, we investigated the impact of endothelin 1 (EDN1) on the resistance to DDP in NSCLC.

Methods The proliferation, invasion, and migration of NSCLC cells were detected by cell counting kit-8 and Transwell migration and invasion assays. ELISA was performed to analyze the inflammatory cytokines concentrations. The related protein levels of tumor necrosis factor (TNF) signaling pathway were analyzed by Western blot. Besides, a xenograft tumor mice model was established to explore the role of EDN1 in vivo.

Results The results showed that DDP-resistance upregulated EDN1 expression, cell viability, invasion, migration, and inflammation in NSCLC cells, while the results were reversed after EDN1 inhibition. EDN1 affected DDP-resistance of NSCLC by regulating TNF signaling pathway. Overexpression of TNF receptor-1 (TNFR1) reversed the decreased cell viability, invasion, migration, and inflammation induced by silencing EDN1 in A549/DDP cells. Moreover, silencing EDN1 inhibited tumor growth and the protein levels of EDN1 and TNFR1.

Conclusion EDN1 promoted DDP resistance in NSCLC cells through the modulation of the TNF signaling pathway, suggesting a potential therapeutic intervention strategy for NSCLC.

Keywords Non-small cell lung cancer, Cisplatin resistance, EDN1, TNF signaling pathway

*Correspondence:
Zhenwei Li
zhenweiii84@163.com
[1]Department of Thoracic Surgery, Qidong People's Hospital, Qidong Liver Cancer Institute, Affiliated Qidong Hospital of Nantong University, Qidong, China
[2]Department of Pathology, Qidong People's Hospital, Qidong Liver Cancer Institute, Affiliated Qidong Hospital of Nantong University, Qidong, China
[3]Department of Blood Transfusion, Qidong People's Hospital, Qidong Liver Cancer Institute, Affiliated Qidong Hospital of Nantong University, Qidong, China
[4]Department of Neurology, Qidong People's Hospital, Qidong Liver Cancer Institute, Affiliated Qidong Hospital of Nantong University, Qidong, China
[5]Department of Operating Room, Qidong People's Hospital, Qidong Liver Cancer Institute, Affiliated Qidong Hospital of Nantong University, No.753, Jianghai Middle Road, Qidong, Jiangsu 226200, China

Introduction

Non-small cell lung cancer (NSCLC) is a term that encompasses a group of lung cancers that behave similarly and are characterized by the presence of large cells. It accounts for approximately 85% of all lung cancer diagnoses, making it the most prevalent type of lung cancer [1]. Long-term smoking, heredity, chronic obstructive pulmonary diseases, occupational causes (exposure to silica, asbestos, and inorganic arsenic), and obesity are common inductive factors for NSCLC [2, 3]. Cisplatin (DDP), also known as cis-diamminedichloroplatinum(II), is a platinum-based metal complex that plays a significant role in cancer treatment due to its unique mechanism of action. Upon administration, cisplatin enters the cells and undergoes hydrolysis, leading to the formation of reactive platinum-DNA adducts, which primarily induce cross-linking between DNA strands, disrupting the normal structure and function of the DNA helix [4]. Unfortunately, the emergence of DDP-resistance poses an obstacle to the therapeutic efficacy and reduces the survival rate of NSCLC patients [5]. Research has identified potential biomarkers for DDP resistance in NSCLC, including ubiquitin-specific protease 14 (USP14) and the long non-coding RNA small nucleolar RNA host gene 7 (SNHG7) [6, 7]. Additionally, several innovative treatment strategies for DDP-resistant NSCLC are under investigation, such as the combination of cordycepin with DDP, aptamer-guided nano-zinc carriers loaded with USP14 siRNA, and immune checkpoint inhibitors [7–9].

Yanagisawa and his co-workers first identified endothelin 1 (EDN1) in porcine aortic endothelial cells in 1988 [10]. Endothelin-1 (EDN1) is a powerful vasoconstrictor and mitogen, consisting of a chain of 21 amino acids. This peptide is essential in numerous physiological and pathological processes occurring in the body, such as blood supply, tissue metabolism, inflammation, homeostasis, and antioxidant stress, making it a significant focus of research in cardiovascular health and disease [11]. Besides, the role of EDN1 in the pathogenesis of different diseases has been illustrated, including obesity, infective diseases, periodontal diseases, and cancers [12–15]. In NSCLC, a previous study has found that EDN1 promotes drug resistance in NSCLC by promoting vasoconstriction and restricting blood and drug delivery [16]. In addition, another study suggests that EDN1 level in exhaled breath condensate in NSCLC patients is of diagnostic value for NSCLC [17]. While previous studies have highlighted the significance of EDN1 in NSCLC, its specific contributions to the mechanisms underlying drug resistance in NSCLC are still largely unknown.

Tumor necrosis factor (TNF), a pro-inflammatory cytokine, significantly influences tumor progression [18]. It influences a wide range of cellular functions and functions through complex signaling pathways, serving an essential role in the management of numerous diseases [19]. TNF receptor-1 (TNFR1) is a critical transducer of inflammatory pathways. TNF/TNFR1 pathway playing pleiotropic roles in the development of many cancers, including NSCLC [20, 21]. The impact of the TNF/TNFR1 signaling pathway on DDP-resistant NSCLC remains unclear.

In light of this context, the objective of this study was to investigate the role of EDN1 in DDP resistance in NSCLC and to elucidate the underlying mechanisms. The results could provide a promising therapeutic approach for managing NSCLC.

Methods and materials

Clinical study

Samples were harvested from 80 NSCLC patients undergoing DDP therapy from the Qidong People's Hospital, Qidong Liver Cancer Institute, Affiliated Qidong Hospital of Nantong University. NSCLC patient tumors and adjacent paracancerous tissues (>3 cm from malignant tissues) were harvested during surgical biopsy and surgical procedures, including 80 DDP-sensitive tissue samples and 80 DDP-resistant tissue samples, which were immediately stored at -80℃ for further analysis. All patients who participated in this study signed an informed consent form, and this study was approved by the Ethics Committee of Qidong People's Hospital, Qidong Liver Cancer Institute, Affiliated Qidong Hospital of Nantong University. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Bioinformatics analysis

The datasets GSE108214 and GSE135720 were obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). Data analysis was performed using the R programming language, with differentially expressed genes (DEGs) identified based on the criteria of $p < 0.05$ and|log2(fold change)|>1. KEGG pathway enrichment analysis was carried out utilizing the DAVID database (https://david.ncifcrf.gov/) and visualized through the clusterProfiler package in R software.

Antibodies

For Western blot: GAPDH (1/2500, ab9485, Abcam), TNFR1 (1/1000, ab223352, Abcam, Cambridge, MA, USA); TNFAIP3 ((1/1000, ab92324, Abcam); MAP2K6 (1/2000, K108065P, Thermo Fisher Scientific, Waltham, MA, USA), goat anti-rabbit IgG (1/2000, ab6721, Abcam).

For immunohistochemistry (IHC): EDN1 (5 μg/mL, ab113697, Abcam); Ki67 (1 μg/mL, ab15580, Abcam); and TNFR1 (1/100, ab223352, Abcam).

Table 1 Primer sequences used in qPCR

Name	Forward (5′-3′)	Reverse (5′-3′)
EDN1	AGAGTGTGTCTACTTCTGCCA	CTTCCAAGTCCATACGGAACAA
TNFR1	CAGGGAGAAGAGAGATAGTG	TGTACAAGTAGGTTCCTTTG
GAPDH	GACTCATGACCACAGTCCATGC	AGAGGCAGGGATGATGTTCTG

EDN1, endothelin 1; **TNFR1**, tumor necrosis factor receptor-1; **GAPDH**, glyceraldehyde-3-phosphate dehydrogenase

Table 2 Correlation analysis of EDN1 expression level and clinical features

Subjects	Low ($n=40$)	High ($n=40$)	X^2	*p*-value
Age (years)			0.205	0.651
<50	16	18		
≥50	24	22		
Sex			0.202	0.653
Male	21	23		
Female	19	17		
Tumor size			6.084	0.014*
<50 mm	27	16		
≥50 mm	13	24		
Differentiation			7.366	0.007**
I/II	29	17		
III/IV	11	23		
Tumor number			3.208	0.073
1	23	15		
≥2	17	25		
Distant Metastasis			5.013	0.025*
YES	16	26		
NO	24	14		

Cell culture

A549 cells and their DDP resistant cells (A549/DDP) were sourced from Procell Life Technology Co., LTD, (Wuhan, China). A549 cells were maintained in Ham's F-12 K medium (Procell) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin mixture. A549/DDP cells were grown in Ham's F-12 K medium containing 10% FBS, 1 μg/mL DDP (Yuanye Biotechnology Co., LTD, Shanghai, China) and 1% penicillin/streptomycin mixture.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from cells using the commercial RNA Easy Fast Extraction kit (Tiangen Biochemical Technology Co., LTD, Beijing, China). HiScript III 1st Strand cDNA Synthesis Kit (Vazyme Biotechnology Co., LTD, Nanjing, China) was used to reverse transcribe RNA into cDNA and ChamQ Universal SYBR qPCR Master Mix (Vazyme) was used for qPCR amplification experiment. Gene primes were obtained from Genescript Biotechnology Technology Co., LTD, (Nanjing, China) and listed in Table 1 and 2. This experiment consisted of three biological replicates.

Cell transfection

EDN1 short hairpin RNA (sh-EDN1) and its negative control (sh-NC) as well as overexpression vector (pcDNA3.1-TNFR1) and the empty vector (pcDNA3.1-NC) were sourced from Genescript. Transfection was conducted utilizing Lipofectamine 3000 (Vazyme) in accordance with the supplied guidelines when the cell confluence approached approximately 80%, for a duration of 48 h.

Cell counting kit-8 (CCK-8) assay

Cell viability was detected by a commercial CCK-8 kit (Beyotime). A549 and A549/DDP cells were seeded into 96-well plates (Beyotime) at the density of 1×10^3 cells/well and treated with DDP at the dose of 1, 2, 4, 8, 16, 32, or 64 μmol/L for 48 h at 37˚C, respectively. Then, 90 μL of Ham's F-12 K medium and 10 μL of CCK-8 solution were added into each well of the plates to incubate for 2 h in the incubator after the medium with DDP were removed. The 50% inhibiting concentration (IC50) value was calculated via GraphPad Prism (v8.0.1). Additionally, A549 and A549/DDP cells were maintained in the incubator for 24 h at 37˚C. After that, 10 μL of CCK-8 solution was added to incubate with cells for 2 h at 37˚C. The absorbance was assessed at 450 nm using a microplate reader (Thermo Fisher). This experiment consisted of three biological replicates.

Lactic dehydrogenase (LDH) release

A549 and A549/DDP cells (1×10^5 cells/mL) were seeded on 96-well plates, followed by centrifugation to collect the supernatants. LDH assay kit (ab65393, Abcam) was utilized to assess the release of LDH in the supernatant of the cell culture medium, following the guidelines provided by the manufacturer. Finally, the colorimetric change of cells at the absorbance of 492 nm was determined using a microplate reader.

Transwell migration and invasion assays

A549 and A549/DDP cells were resuspended in a serum-free medium. For the migration assay, a cell suspension of 5 × 10^4 cells in 100 μL was placed in the chamber without Matrigel. In the case of the invasion assay, the same number of cells was seeded in a chamber containing 100 μL of Matrigel. The lower chamber was filled with Ham's F-12 K medium enriched with 10% FBS to serve as a chemoattractant. Following a 24-h incubation period, the cells on the outer surface were rinsed with PBS, fixed using 4% paraformaldehyde (Beyotime) for 20 min, and subsequently stained with a dye solution of 0.1% crystal

violet (Beyotime) for visualization purposes. This experiment consisted of three biological replicates.

Western blot

Total protein was extracted by a commercial kit (Solarbio, Beijing China) and the BCA method (Vazyme) was used for protein concentration detection. Then, 50 μg of protein was subjected to separation via 10% SDS-PAGE (Beyotime) and subsequently transferred to a PVDF membrane (Vazyme). Following this, the membrane was blocked with 5% skim milk for one hour. The membrane was then incubated overnight at 4 °C with primary antibodies obtained from a commercial source. After three washes with TBST (Thermo Fisher), the membrane was treated with the secondary antibody for one hour at room temperature. Finally, protein signal detection was carried out using an enhanced chemiluminescence solution (Vazyme). This experiment consisted of three biological replicates.

Enzyme-linked immunosorbent assay (ELISA)

Commercial ELISA kits (Beyotime) were used for TNF-α, IL-6, and IL-1β concentration detection in accordance with the manufacturer's guidelines. Absorbance readings were obtained utilizing a microplate reader. This experiment consisted of three biological replicates.

Xenograft mouse model establishment

Forty male BALB/c nude mice, aged 6 to 8 weeks and weighing approximately 25 g, were acquired from Charles River (Beijing, China). Following a one-week acclimatization period, the mice were randomly assigned to four groups ($n = 10$). Subcutaneous injections were administered in the left flank, using A549 or A549/DDP cells that had been stably transfected with either sh-NC or sh-EDN1 plasmids, at a concentration of 1 × 10^6 cells in 100 μL. After a duration of 28 days, the mice were euthanized with pentobarbital sodium at a dosage of 40 mg/kg (Sigma), and the tumors were subsequently excised, weighed, and preserved at -80℃.

Hematoxylin-eosin (H&E) staining

The tumor tissues in each group ($n = 10$) were preserved in 4% paraformaldehyde for a duration of 24 h, followed by dehydration using ethanol (Beyotime) and embedding in paraffin. Subsequently, the paraffin-embedded tissues were sectioned into slices of 4 μm thickness. The sections were then stained with hematoxylin (Beyotime) for 5 min and eosin (Beyotime) for 3 min. In conclusion, the sections were dehydrated and sealed, and images were captured using a biopathology microscope (Olympus, Japan).

IHC

Paraffin-embedded tumor tissues in each group ($n = 10$) were sectioned, dewaxed, and rehydrated before being treated with 3% hydrogen peroxide. Subsequently, the sections were subjected to treatment with 10 mM citrate buffer (pH 6.0, Beyotime), heated to 95 °C, and blocked at room temperature. Following this, the sections were incubated with primary antibodies overnight at 4 °C, and then were treated with goat anti-rabbit IgG at 37 °C for 1 h. The sections were then counterstained with hematoxylin (Beyotime) and blued using 1% ammonia water after the addition of DAB solution (Vazyme). Finally, the sections were dehydrated, sealed, and examined under a light microscope (Leica, Wetzlar, Germany).

Statistical analysis

The analysis of data was conducted using SPSS version 21.0. The results are presented as mean ± standard deviation (SD). For comparisons between two groups, Student's t-test was employed, while ANOVA was utilized for comparisons among three groups. Statistical evaluations were carried out using GraphPad Prism (version 8.0.1). A p-value of less than 0.05 was considered indicative of a statistically significant difference.

Results

EDN1 was an upregulated gene in DDP-resistant NSCLC

The GSE108214 and GSE135720 datasets in GEO database were used to obtain DEGs in DDP-resistant NSCLC. The findings indicated that EDN1 was upregulated in DDP-resistant NSCLC (Fig. 1A and B). No previous studies have been conducted on EDN1 in NSCLC; consequently, this research seeks to explore the role of EDN1 in NSCLC that is resistant to DDP. The RT-qPCR analysis revealed that resistant NSCLC patients exhibited markedly higher expression level of EDN1 in NSCLC tissues compared to their sensitive counterparts (Fig. 1C). Furthermore, as detailed in Table 1, a cohort of 80 NSCLC patients was stratified into two groups, High ($n = 40$) and Low ($n = 40$), based on the expression levels of EDN1. Notably, there were no significant differences between these groups concerning age, sex, and the number of tumors. However, a greater proportion of patients in the High group presented with tumor sizes of ≥ 50 mm, advanced III/IV tumor stages, and evidence of distant metastasis when compared to those in the Low group.

A549/DDP cell lines showed upregulated EDN1 expression, cell viability, invasion, migration, and inflammation

To assess the DDP resistance of the acquired A549/DDP cell lines, CCK-8 assay was conducted to evaluate cell viability. The findings revealed that A549/DDP cell viability was significantly lower compared to that of A549 cells. Additionally, the IC50 values for A549/DDP

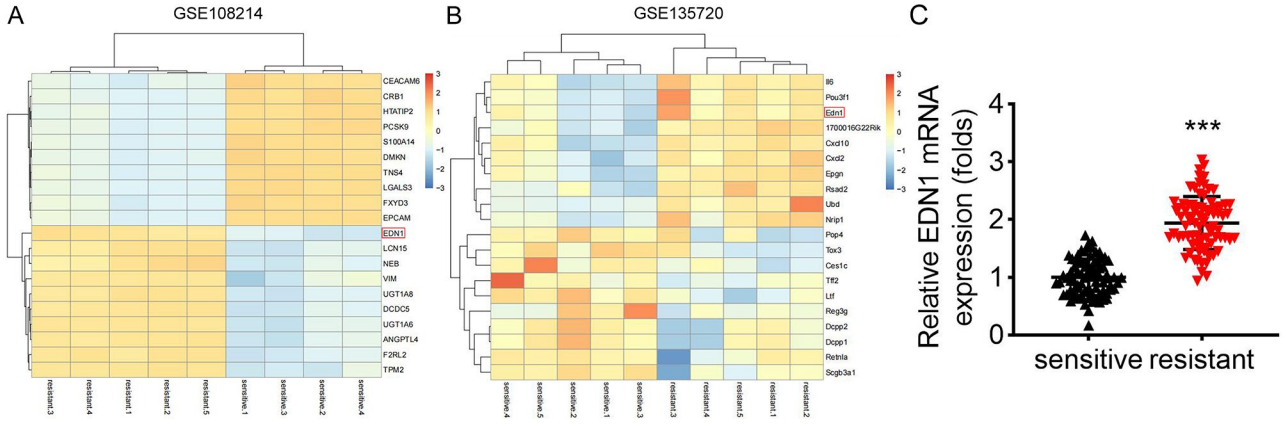

Fig. 1 EDN1 was an upregulated gene in DDP-resistant NSCLC. The **A**, GSE108214 and **B**, GSE135720 datasets in GEO database were used to obtain differentially expressed genes in DDP-resistant NSCLC; **C**, RT-qPCR was performed to detect the mRNA level of EDN1 in the sensitive ($n=80$) and resistant ($n=80$) groups. **GEO**, Gene Expression Omnibus; **DDP**, cisplatin; **NSCLC**, non-small cell lung cancer; **RT-qPCR**, reverse transcription-quantitative polymerase chain reaction; **EDN1**, endothelin 1

cells exceeded those of A549 cells (Fig. 2A), indicating that the A549/DDP cell lines exhibit resistance to DDP. Besides, A549/DDP cells showed an upregulated EDN1 mRNA level compared with A549 cells (Fig. 2B). Transwell migration and invasion assays results indicated that DDP-resistance A549 cells exhibited increases in invasion and migration in comparison with A549 cells (Fig. 2C-E). Furthermore, A549/DDP cells exhibited elevated levels of TNF-α, IL-6, and IL-1β in comparison to A549 cells (Fig. 2F-H), indicating that DDP resistance heightened the inflammatory response in A549 cells.

Silencing EDN1 decreased the cell viability, invasion, migration, and inflammation and increased cytotoxicity in A549/DDP cells

To investigate the impact of EDN1 on the development of DDP resistance, we introduced sh-EDN1 into A549/DDP cells, resulting in the suppression of EDN1 expression (Fig. 3A). Furthermore, the suppression of EDN1 resulted in a reduction of cell viability and an increased of LDH release when compared to the sh-NC group in A549/DDP cells, suggesting that loss of EDN1 increased cytotoxicity in A549/DDP cells. Besides, EDN1 inhibition downregulated the cell invasion, migration, and levels of inflammatory cytokines (TNF-α, IL-6, and IL-1β) in A549/DDP cells compared with the sh-NC group (Fig. 3B-G).

EDN1 affected DDP-resistant NSCLC by regulating TNF signaling pathway

Through the construction of the Venn diagram, we obtain the differentially expressed genes that were co-expressed in the two datasets (GSE108214 and GSE 135720) (Fig. 4A). KEGG enrichment analysis was conducted to assess gene functional enrichment. The findings revealed that the 15 differentially expressed genes were predominantly associated with TNF signaling pathways (Fig. 4B). Thus, we analyzed the related protein levels of TNF signaling pathways using Western blot, and the results found EDN1 inhibition decreased the protein levels of TNFR1, TNFAIP3, and MAP2K6 (Fig. 4C), illustrating that EDN1 affected DDP-resistant NSCLC by regulating TNF signaling pathway.

Overexpressing of TNFR1 increased cell viability, invasion, migration, and inflammation in A549/DDP cells

To investigate the role of TNFR1 in the progression of DDP-resistant NSCLC, we introduced pcDNA-3.1-TNFR1 into A549/DDP cells, resulting in an elevated expression of TNFR1 (Fig. 5A). Furthermore, the overexpression of TNFR1 counteracted the reduction in cell viability, invasion, migration, and levels of inflammatory cytokines (TNF-α, IL-6, and IL-1β) that were caused by the silencing of EDN1 in A549/DDP cells (Fig. 5B-G).

Silencing EDN1 inhibited tumor growth of mice

Finally, the in vivo findings revealed that the silencing of EDN1 resulted in a reduction of both tumor size and weight in A549 and A549/DDP cells (Fig. 6A and B). H&E results indicated that EDN1 inhibition alleviated the outcomes of cytoplasmic reduction, cytoboundary ambiguity, and cell necrosis. In addition, IHC assay showed that silencing EDN1 downregulated the protein levels of EDN1, Ki67, and TNFR1 (Fig. 6C).

Discussion

The emergence of DDP resistance in patients with NSCLC represents a significant challenge in the treatment of this disease. As cancer cells develop resistance to DDP, their sensitivity to the drug diminishes, leading to a situation where higher doses may be required to achieve the same therapeutic effect. This reduced sensitivity can

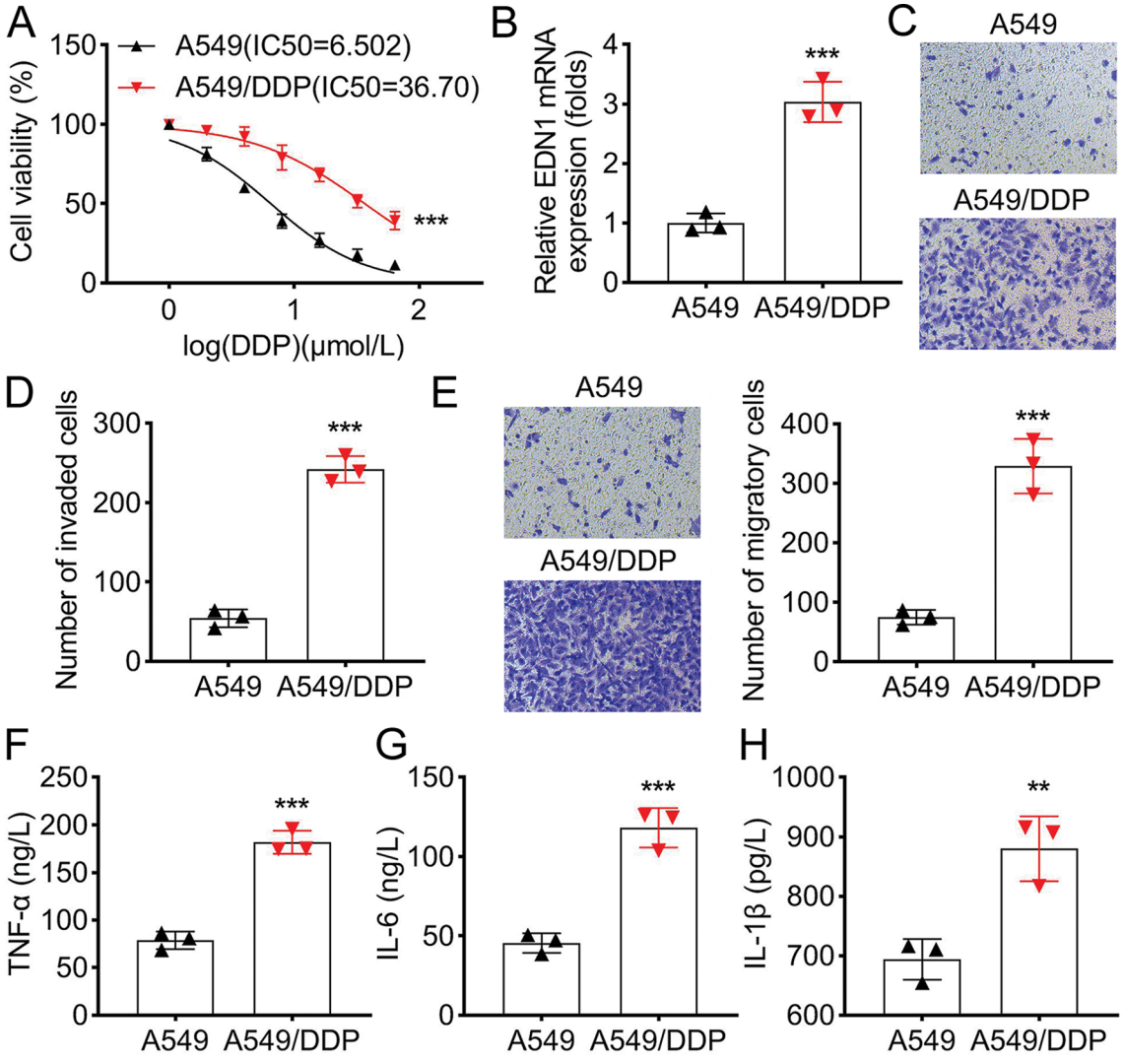

Fig. 2 A549/DDP cell lines showed upregulated EDN1 expression, cell viability, invasion, migration, and inflammation. **A**, CCK-8 assay was performed to assess the cell viability of A549 and A549/DDP cells; **B**, Quantification analysis of EDN1 in A549 and A549/DDP cells; **C**, Transwell invasion assay was performed to detect the cell invasion in A549 and A549/DDP cells; **D**, The number of invaded cells in A549 and A549/DDP cells; **E**, Transwell migration assay was performed to detect the cell migration in A549 and A549/DDP cells; ELISA was used to analyze the concentrations of **F**, TNF-α, **G**, IL-6, and **H**, IL-1β in A549 and A549/DDP cells. ($n=3$; $^{**}p<0.01$; $^{***}p<0.001$).**DDP**, cisplatin; **CCK-8**, cell counting kit-8; **TNF-α**, tumor necrosis factor α; **IL**, interleukin; **ELISA**, enzyme-linked immunosorbent assay; **EDN1**, endothelin 1

occur through various mechanisms, including alterations in drug uptake and efflux, changes in DNA repair pathways, and modifications in cellular signaling that promote survival despite the presence of the drug [22, 23].

Research indicates that the upregulation of EDN1 could contribute to various cellular processes that promote tumor survival and proliferation despite the presence of chemotherapeutic agents. EDN1 is recognized for its role in pathways that govern cell proliferation, angiogenesis, and apoptosis, all of which are essential elements in the advancement of cancer and the response to treatment [24, 25]. In our study, GEO databases showed that EDN1 was an upregulated gene in DDP-resistant NSCLC. Similarly, Pulido et al. [16] indicate that secretion of EDN1 mediates drug resistance in epidermal growth factor receptor mutant NSCLC by promoting vasoconstriction, limiting blood and drug delivery. Besides, we found that DDP-resistance upregulated EDN1 expression, cell viability, invasion, migration, and inflammation in A549 cells, suggesting that DDP-resistance promoted the malignant progression of NSCLC. Similarly, other DDP-resistant tumor cells also show greater migration and invasion abilities and increased inflammation levels [26, 27]. EDN1 is a crucial peptide in cancer progression and is upregulated in different cancers [13, 28]. In the current investigation, the inhibition of EDN1 resulted in a reduction of cell viability, invasion, migration, and inflammation in A549/DDP cells, aligning with findings from

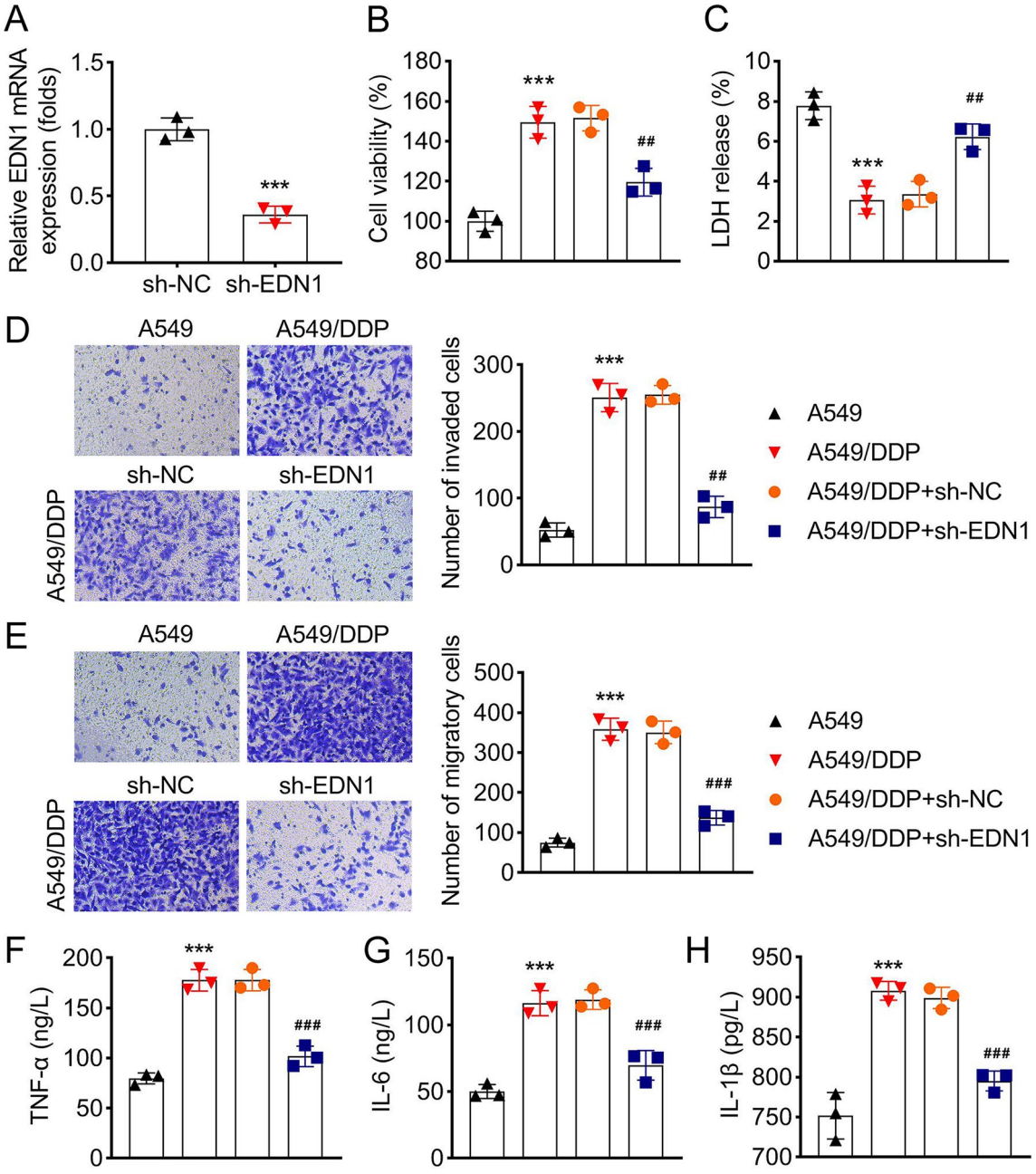

Fig. 3 Silencing EDN1 decreased the cell viability, invasion, migration, and inflammation and increased cytotoxicity in A549/DDP cells. **A**, RT-qPCR was used to detect the expression of EDN1 in A549/DDP cells after EDN1 silenced; **B**, The cell viability was assessed by CCK-8 assay; **C**, LDH release in each group was analyzed by a commercial kit; Detection and quantification of cell **D**, invasion and **E**, migration; The levels of **F**, TNF-α, **G**, IL-6, and **H**, IL-1β in A549 and A549/DDP cells were analyzed by ELISA. ($n = 3$; $^{***}p < 0.001$ vs. sh-NC group or A549 group; $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ vs. A549/DDP + sh-NC group).**DDP**, cisplatin; **RT-qPCR**, reverse transcription-quantitative polymerase chain reaction; **TNF-α**, tumor necrosis factor α; **IL**, interleukin; **ELISA**, enzyme-linked immunosorbent assay; **CCK-8**, cell counting kit-8; **LDH**, lactate dehydrogenase; **EDN1**, endothelin 1; **shRNA**, short hairpin RNA

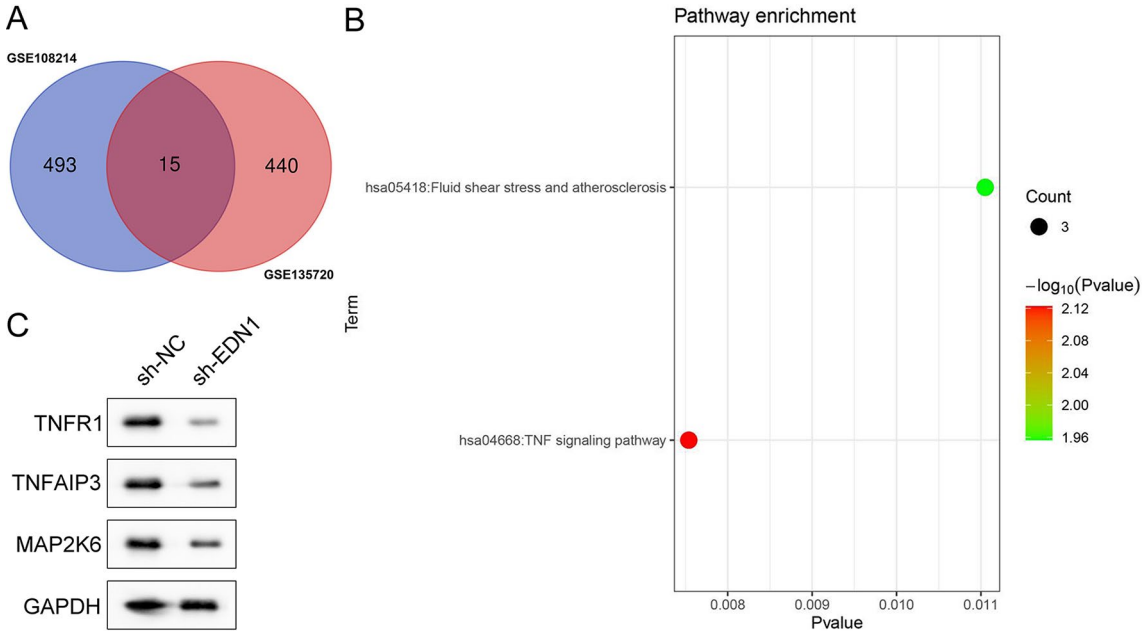

Fig. 4 EDN1 affected DDP-resistant NSCLC by regulating TNF signaling pathway. **A**, The Venn diagram was used to screen genes that were upregulated in both GSE108214 and GSE135720 datasets; **B**, The KEGG pathway enrichment analysis. The color indicates the p-value (from the lowest in green to the highest in red), and the bubble size indicates the number of genes; **C**, The related protein levels of TNF signaling pathway were assayed by Western blot ($n = 3$). **KEGG**, Kyoto Encyclopedia of Genes and Genomes; **TNF**, tumor necrosis factor; **EDN1**, endothelin 1

earlier research [29]. In addition, among other lung diseases, EDN1 has been shown to be associated with pulmonary airway dysfunction [30], idiopathic pulmonary fibrosis [31], and pulmonary inflammation [32].

To further explore the mechanisms of EDN1, we used the KEGG database for enrichment function analysis and found that differentially expressed genes were mainly involved in TNF signaling pathways. The role of TNF signaling pathways in pathophysiology in different cancers has been intensively investigated [33]. We found that related protein levels of TNF signaling pathways were decreased after EDN1 silenced and overexpression of TNFR1 reversed the decreased cell viability, invasion, migration, and inflammation induced by silencing EDN1 in A549/DDP cells, implying that EDN1 facilitated DDP resistance of NSCLC by regulating the TNF signaling pathway. TNF-α can bind to two different receptors, TNFR1 and TNFR2 [34]. TNFR1, a member of TNFR superfamily, is a 455 amino acid polypeptide that contains a death domain in its C-terminal region [35]. Similar with our results, a recent review has indicated that TNFR1 overexpression promotes cell dedifferentiation and metastasis in lung squamous cell carcinoma [35]. In addition, Yang et al. demonstrates that upregulation of squalene synthase promotes lung cancer metastasis by increasing TNFR1 expression [36]. Interestingly, inflammation has been described as a favorable environment for tumor development in breast cancer, and TNF-α alone or in combination with chemotherapy and radiotherapy can be used as adjuvant treatment for breast cancer [37], implying that TNF signaling pathway plays different roles in different tumors. Moreover, the results of in vivo study demonstrated that silencing EDN1 inhibited tumor growth of mice and the protein levels of EDN1 and TNFR1, which was consistent with our in vitro study.

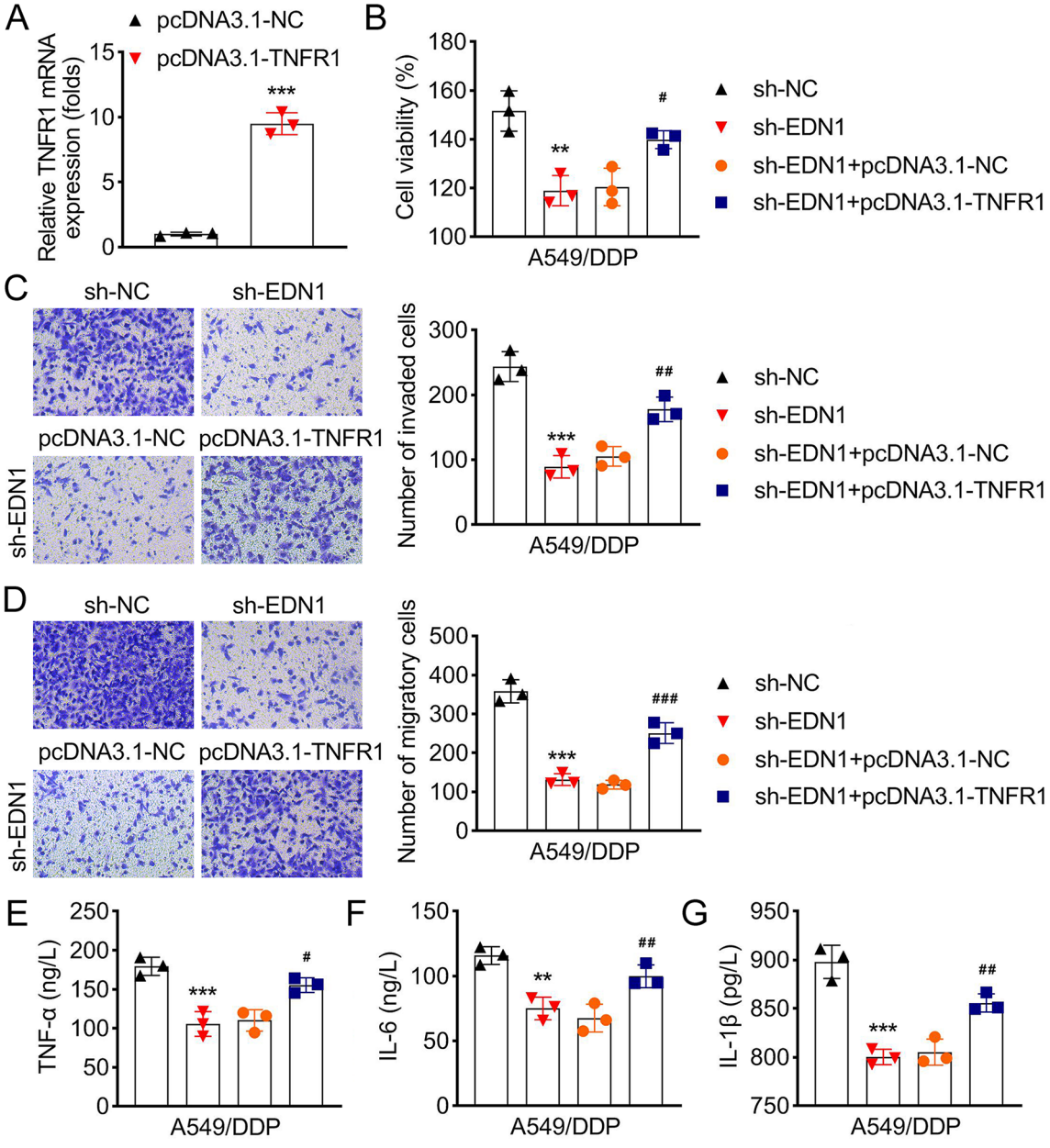

Fig. 5 Overexpressing of TNFR1 reversed the decreased cell viability, invasion, migration, and inflammation induced by silencing EDN1 in A549/DDP cells. **A**, RT-qPCR was performed to detect the mRNA level of TNFR1 in A549/DDP cells after TNFR1 overexpression; **B**, The cell viability in each group was assessed by CCK-8 assay; Detection and quantification of cell **C**, invasion and **D**, migration in each group; The levels of **E**, TNF-α, **F**, IL-6, and **G**, IL-1β in A549 and A549/DDP cells were assessed using ELISA. ($n = 3$; $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. pcDNA3.1-NC group or sh-NC group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ vs. sh-EDN1 + pcDNA3.1-NC group). **RT-qPCR**, reverse transcription-quantitative polymerase chain reaction; **TNF-α**, tumor necrosis factor α; **IL**, interleukin; **ELISA**, enzyme-linked immunosorbent assay; **CCK-8**, cell counting kit-8; **EDN1**, endothelin 1; **DDP**, cisplatin; **shRNA**, short hairpin RNA; **TNFR1**, tumor necrosis factor receptor-1

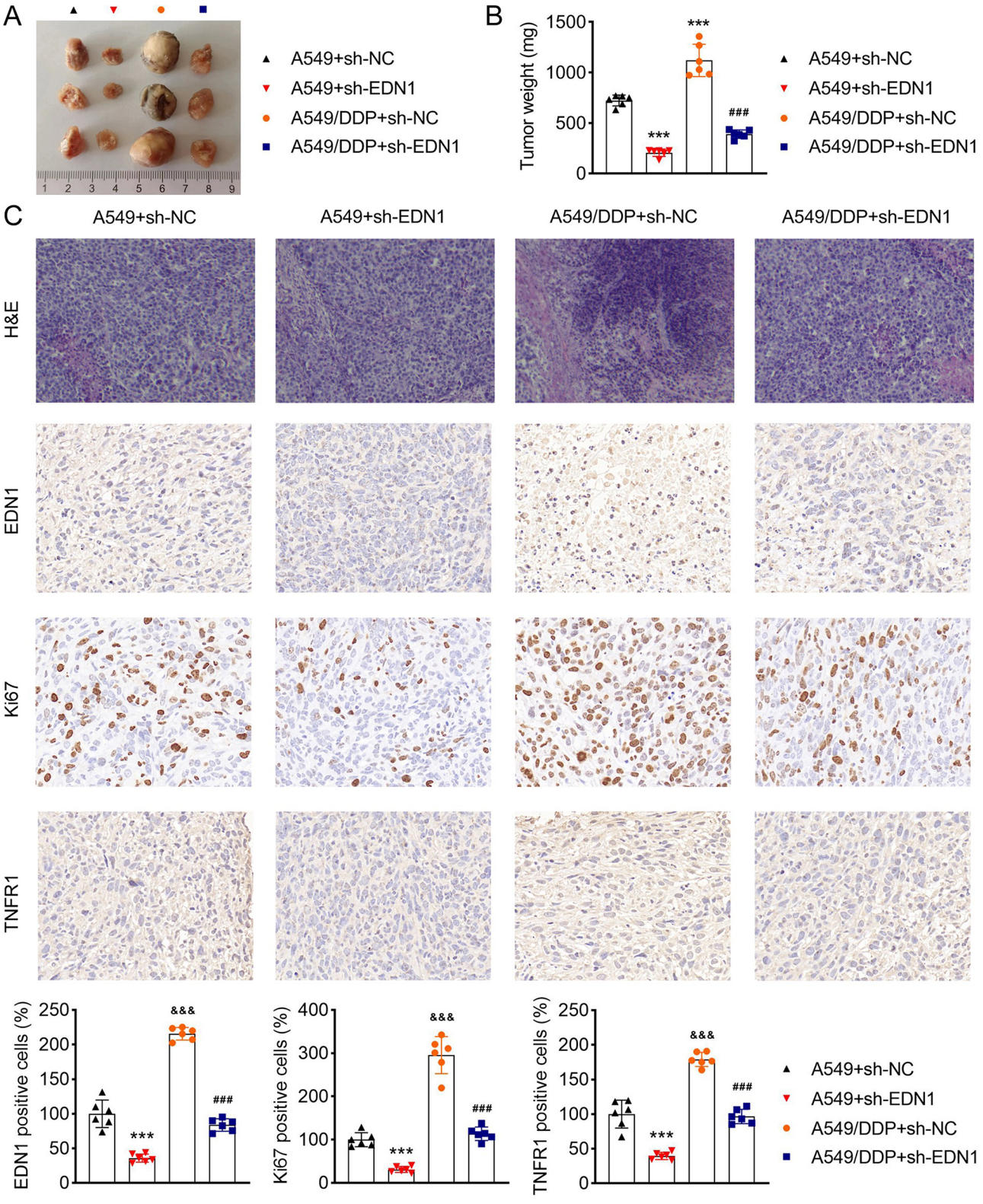

Fig. 6 Silencing EDN1 inhibited tumor growth of mice. **A**, The tumor size in each group; **B**, Tumor weight in each group; **C**, H&E and IHC assays were performed to assess the pathological status of tumor tissue and the protein levels of EDN1, Ki67, and TNFR1 in each group. ($n = 10$; $^{***}p < 0.001$ vs. A549 + sh-NC group; $^{\#\#\#}p < 0.001$ vs. A549/DDP + sh-NC group). **H&E**, hematoxylin-eosin; **sh-RNA**, short hairpin RNA; **IHC**, immunohistochemistry; **TNFR1**, tumor necrosis factor receptor-1; **DDP**, cisplatin

In summary, this research indicated that EDN1 enhances DDP resistance in NSCLC through the modulation of the TNF signaling pathway, potentially offering a novel reference for the treatment of NSCLC. Nevertheless, it is necessary to note that the findings of this study were validated solely through cellular and animal experiments, lacking clinical verification. This limitation will be addressed in future comprehensive investigations.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12957-025-03692-7.

Supplementary Material 1

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Not applicable.

Author contributions
All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript. J Z drafted the work and revised it critically for important intellectual content; L S, L J, S Z and Y C were responsible for the acquisition, analysis, or interpretation of data for the work; Z L made substantial contributions to the conception or design of the work. All authors read and approved the final manuscript.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study protocol was approved by the Ethics Committee of Qidong People's Hospital, Qidong Liver Cancer Institute, Affiliated Qidong Hospital of Nantong University. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All animal experiments should comply with the ARRIVE guidelines. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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