

# Exosome-Reversed Chemoresistance to Cisplatin in Non-Small Lung Cancer Through Transferring miR-613

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**Introduction:** Non-small lung cancer (NSCLC) is one of the most common malignant tumors in the world. Chemoresistance is the main reason of adverse effects leading to the death of patients; thus, it is important to discover the potential target of chemotherapeutic resistance.

**Methods:** The expression of differentially expressed miRNA was detected in BEAS-2B, A549 and A549/cisplatin (DDP) by qRT-PCR. Transmission electron microscopy (TEM) and exosome biomarkers were used to validate the extracted exosome. Cells incubated with miR-613 enriched exosomes were used to detect the function of exo-miR-613 in vitro. Then, exo-miR-613 was injected to mice treated with DDP to investigate the function role of exo-miR-613 in vivo.

**Results:** Comparing to BEAS-2B, the expression of miR-613 in A549 was significantly reduced, which was more obvious in A549/DDP. After incubated with exo-miR-613 and corresponding exo-negative control (NC), we found overexpression of miR-613 remarkably increased the inhibition of cell proliferation induced by cisplatin. Exo-miR-613 fused into cells to significantly enhance the inhibited effect of DDP on the proliferation, migration and showed a promotion on cell apoptosis and DNA damage. The in vivo study showed that exo-miR-613 significantly inhibited the tumor growth, and promote the sensitivity to DDP, probably by down-regulating the expressions of GJA1, TBP and EIF-4E in tumor cells and tissues.

**Conclusion:** Exo-miR-613 reversed chemoresistance to DDP in NSCLC cell to involve in the process of tumor progression, and might be a potential therapeutic strategy for NSCLC.

**Keywords:** exosome, miRNA, non-small cell lung cancer, cisplatin, chemoresistance, apoptosis

## Introduction

Non-small lung cancer (NSCLC) constitutes 80–85% of total lung cancer, with a characteristic of slow cell division and relatively late metastasis.<sup>1,2</sup> Currently, surgical resection, adjuvant radio chemotherapy, molecular targeted therapy and immunotherapy have made great development these years, the 5-year overall survival rate of patients with lung cancer is only about 10–20%.<sup>3–5</sup> Cisplatin (DDP) is a first-line chemotherapy of NSCLC and chemoresistance of DDP seriously leads to poor prognosis of patients. Thus, to understand the pathogenesis and chemoresistance of NSCLC should be of great importance to improve NSCLC prognosis. In addition, more and more researches have proven that miRNAs and exosome involved in the development of chemoresistance.<sup>6–8</sup>

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miRNA is one type of endogenous small non-coding RNA molecule (containing about 19–22 nucleotides).<sup>9,10</sup> In NSCLC, miRNAs were illustrated to function in regulating apoptosis, proliferation, and chemotherapeutic sensitivity of cancer cells. miRNA-153-3p was demonstrated to promote the sensitivity of Gefitinib in NSCLC by down-regulating the expression of ATG5 and regulating autophagy.<sup>11</sup> miR-613 was confirmed to inhibit the development of various cancers, including laryngeal squamous cell cancer,<sup>12</sup> breast cancer,<sup>13</sup> stomach carcinoma<sup>14</sup> and hepatocellular carcinoma.<sup>15</sup> In addition, miR-613 also served as an important biomarker in NSCLC,<sup>16</sup> and inhibited the development and progression of NSCLC by regulating its target genes.<sup>17</sup>

Exosomes are one kind of 30 nm-100 nm uniformed nanoscale vesicles, which play a critical role in tumorigenesis, growth, invasion, metastasis and chemoresistance in various cancers,<sup>18</sup> including intestinal cancer,<sup>19</sup> melanoma,<sup>20</sup> and gastric cancer.<sup>21</sup> Moreover, it has been reported that exosomes involved in the process of chemoresistance by transferring protein, DNA and RNAs to the targeted cells.<sup>22</sup> In colorectal cancer, exosome-derived miR-128-3p enhanced the chemosensitivity of colorectal cancer cells by negatively regulating Bmi1 and MRP5.<sup>19</sup> miRNAs have been shown to be closely associated with the development of NSCLC. However, there are still few studies focusing on the role of exosome-derived miRNAs in the chemoresistance of NSCLC.

In this study, we aimed to relieve the chemoresistance by use of exosome-derived miR-613 in NSCLC. Next, the role of exosomal-miR-613 was explored to discover whether exo-miR-613 could reverse drug resistance to DDP in both cell lines and mice model by measuring the cell proliferation, apoptosis *in vitro* and tumor growth *in vivo*. Our study provided a new sight for gene treatment of NSCLC, and suggested a potential alternative for NSCLC patients with DDP-resistance.

## Methods

### Animal Study

5–6 weeks old BALB/c-nu mice (male) were obtained from Mode Animal Research Centre of Shanxi. The study (No.201614) was approved by the Ethics Committee of Shanxi Cancer Hospital. The study was approved by the Ethics Committee of the Animal Care and Use committee of the Shanxi Cancer Hospital. The research followed the National Institutes of Health (NIH) Animal Welfare Guidelines for the use and care of

animals. All *in vivo* experiments were performed in Shanxi Cancer Hospital. The prepared A549/DDP cell suspension ( $1 \times 10^7$  cells) was subcutaneously injected to axillary of nude mice. 16 days later, the nude mice were undergoing tail intravenous injection with 20  $\mu$ g exosome (suspended in 40  $\mu$ L PBS) or PBS every two days, and were intraperitoneally injected with 5 mg/kg DDP every four days. After exosome injection, the tumor size was measured every 4 days. After 32 days of injection, the animals were sacrificed. The tumor tissues were weighed and collected for further qRT-PCR and Western blot analysis.

### Cell Culture

BEAS-2B, DDP-resistant cell line A549/DDP and A549 and were purchased from ATCC. The cells were cultured in PRMI-1640 medium which contains 10% FBS and penicillin-streptomycin (100  $\mu$ g/mL streptomycin and 100 IU penicillin) at 37°C with 5% CO<sub>2</sub>. The cells were digested with trypsin and passaged every 2 to 3 days according to the growth condition, cells at logarithmic growth phase were collected for further experiment.

### Collection of Exosomes

The supernatant of HEK-293T cells with logarithmic growth was collected for exosome extraction when the cells grew to a number of  $10^7, 10^8$ . The supernatant was centrifuged at 300 $\times$ g, 4°C for 10 min in a precooling centrifuge, to exclude the cell contamination. The supernatant was centrifuged at 2000 $\times$ g, 4°C for 20 min to remove the cell debris. Then the supernatant was aspirated again and centrifuged at 10,000 $\times$ g, 4°C for 70 min, and the pellet was re-suspended with PBS buffer and filtrated with a 0.22  $\mu$ m filter. The exosomes were preserved at –80°C for further use.

### Transmission Electron Microscope

The aliquot of exosome in cell culture was obtained from a –80°C refrigerator, thawed and mixed. 10  $\mu$ L exosome suspensions were dropped to a 2 mm gilder grid and placed the grid at room temperature for 1 min. The excess water was sucked with filter paper. 10  $\mu$ L phosphotungstic acid (20 g/L) was added for negative staining for 2 min and was sucked by filter water, and then the grid was baked under incandescent light. The grid was placed in the Hitachi-7500 Transmission Electron Microscope tube, after focus and magnification adjustment, the exosome was observed and photographed.

## Immunofluorescence Technique

A549 and A549/DDP cells were planted in 12-well plates. After culturing for 24 h, the cells were fixed with 4% paraformaldehyde, and after permeabilized with 0.2% Triton X-100 for 10 min, the cells were blocked for 1 h. Afterwards, specific antibodies, such as CD63 antibody (1:100, Abcam),  $\gamma$ H2AX antibody (1:100, Abcam) were incubated with cells at 4°C overnight. After incubation with Goat Anti-Rabbit IgG antibody Alexa Fluor 488 (Invitrogen) for 1 h, the cells were photographed with Olympus fluorescence microscope.

## Protein Extraction and Western Blot

The cells were washed twice with PBS before collection. 100  $\mu$ L RIPA and 2  $\mu$ L protease inhibitor were used for protein extraction. After dissolved on ice for 45 min, the cells were scraped. The scraped cell was centrifuged at 4°C, 12,000 rpm/min for 20 min, and the supernatant was the total protein. Exosomes were dissolved with PBS. The protein samples were mixed with 5 $\times$ SDS loading buffer at a ratio of 4:1 at room temperature. 30  $\mu$ g protein was used for the Western blot. The specific experimental process follows that has been described before.<sup>23</sup> The antibodies used in this study include: GJA1 (1:1000, Abcam), TBP (1:1000, Abcam), EIF-4E (1:1000, CST), ALIX (1:1000, CST), CD63 (1:100, Abcam), TSG-101 (1:1000, CST). Cell protein samples were normalized to GAPDH (1:1000, Proteintech).

## RNA Extraction and qRT-PCR

TRIzol agent (Invitrogen, Thermo Fisher Scientific, Inc.) was applied to extract exosome RNA and total RNA. The experiment was carried out in accordance with the instructions. RNA was transcribed to cDNA by reverse transcriptase (PrimeScript™ RT Reagent Kit (TaKaRa, Japan; Code No. RR037A (miRNAs)/RR036A (mRNAs))) according to the following procedure: 42°C for 15 min, 85°C for 5s and 4°C for 5 min. The reaction condition of qRT-PCR includes: pre-denaturation at 95°C for 10 min, denaturation at 95°C for 10 second, annealing at 60°C for 20 second, extension at 72°C for 10 second. The practice was performed on two-step PCR amplification standard operation machine. The Ct value was obtained after amplification, and 2 duplicate wells were set for each sample. The mi-RNA613 expression level was measured by the comparative  $2^{-\Delta\Delta CT}$  method.  $\Delta\Delta CT = \text{target CT} - \text{ref CT} - \text{control CT}$  (target CT - ref CT) - control  $\Delta CT$  (target CT - ref CT). The specific primer sequence of miR-631 was:

GGCTGCGCACAGTGGGGTCT. mRNA expression levels were normalized to U6.

## Cell Transfection

A549/DDP and A549 were transfected with miR-NC and miR613 mimics, respectively. After 4–6 h of transfection, the medium was changed. A549/DDP and A549 cells with miR-613 over-expression were obtained after 48 h of transfection. The cells were co-transfected with lipofectamine 3000 liposomes. All the experiments were performed according to the manufacturer's instructions.

## Cell Counting Kit-8 (CCK-8) Assay

The cell viability was tested by CCK-8. A549 and A549-DDP cells were planted in a 96-well plate. After transfection or co-cultured with exosome-miR-613, DDP (MCE, China) at different concentrations was added for 24 h. 200  $\mu$ L of the CCK-8 solution was added to each well and incubated for 2 h. The optical density (OD) at 450 nm was detected by a microplate reader (Thermo). The corresponding OD values showed cell proliferation ability.

## Flow Cytometry

A549 and A549/DDP cells were planted in a 24-well plate, with 4 parallel wells for each group. The cells were co-incubated with exo-miR-613 and DDP (3  $\mu$ g/mL) for 24 h. After digested with 0.25% trypsin, the cells were washed with PBS for three times. After that, the cells were incubated with PI and Annexin V-FITC at dark condition and room temperature for 25 min. Flow cytometry was carried out to detect cell apoptosis. The experiment was repeated for three times.

## Transwell Assay

We divided cells co-incubated with exosomes for 24 h into three groups: exo-mock group, exo-NC group, and exo-miR613 mimics group. The cells were planted in a 24-well plate at a density of  $10^5$ /mL. 200  $\mu$ L cell supernatant was added to the upper chamber, and 600  $\mu$ L medium containing 10% FBS was added to the lower chamber. The plate was incubated at 37°C for 36 h. For measuring, the cells were washed with PBS for 3 times, fixed with 37% formaldehyde for 10 min and stained with crystal violet for 5 min. The average cell numbers per field under the microscope were calculated.

## Statistical Analysis

Data are showed as mean  $\pm$  standard deviation. The data and images were analyzed with Graphpad Prism 5 and Image G. ANOVA and *t*-test was conducted. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

## Results

### Expression of miR-613 in Different Cells and Its Possible Target Genes

Firstly, we analysis the NSCLC microarray GSE292482 and found differentially expressed hsa-miR-96, hsa-miR-183, hsa-miR-134, hsa-miR-330-3p, hsa-miR-34b, hsa-miR-34c-5p, hsa-miR-433, hsa-miR-22, hsa-miR-31, hsa-miR-613. Then their expression was detected in BEAS-2B, and A549/DDP and A549 cells. Among this, miR-613 was significantly decreased in A549/DDP and A549 cells, especially in A549/DDP cells (Figure 1A and B). miRNAs inhibit mRNA translation and degrades target RNA via combining to 3'UTR of mRNA, thus inhibit gene expression. The bioinformatics software TargetsScan, PicTar, miRanda, microT and miRmap were applied. Several target genes of miRNAs were obtained after screening (Figure 1C). Furthermore, we explored the regulation of miR-613 on the potential target genes by overexpressing and inhibiting miR-613. The results showed the expression levels of GJA1, TBP and EIF-4E were remarkably down-regulated by miR-613 mimics and increased by miR-613 inhibitor in both A549/DDP and A549 cells (Figure 1D-G). To analyze the biological functions of miR-613, we transfected miR-613 mimics, miR-613 inhibitor and the corresponding NC, respectively. DPP at different concentrations were added for stimulation. It was showed that proliferation of these two cell lines was significantly inhibited by DDP dose dependently. Meanwhile, the inhibition rate in the two cell lines was significantly increased by miR-613 over-expression and slightly decreased by miR-613 inhibitor (Figure 1H). The above results suggest miR-613 greatly enhanced sensitivity to DDP in A549 cells and reversed chemoresistance in A549/DDP cells.

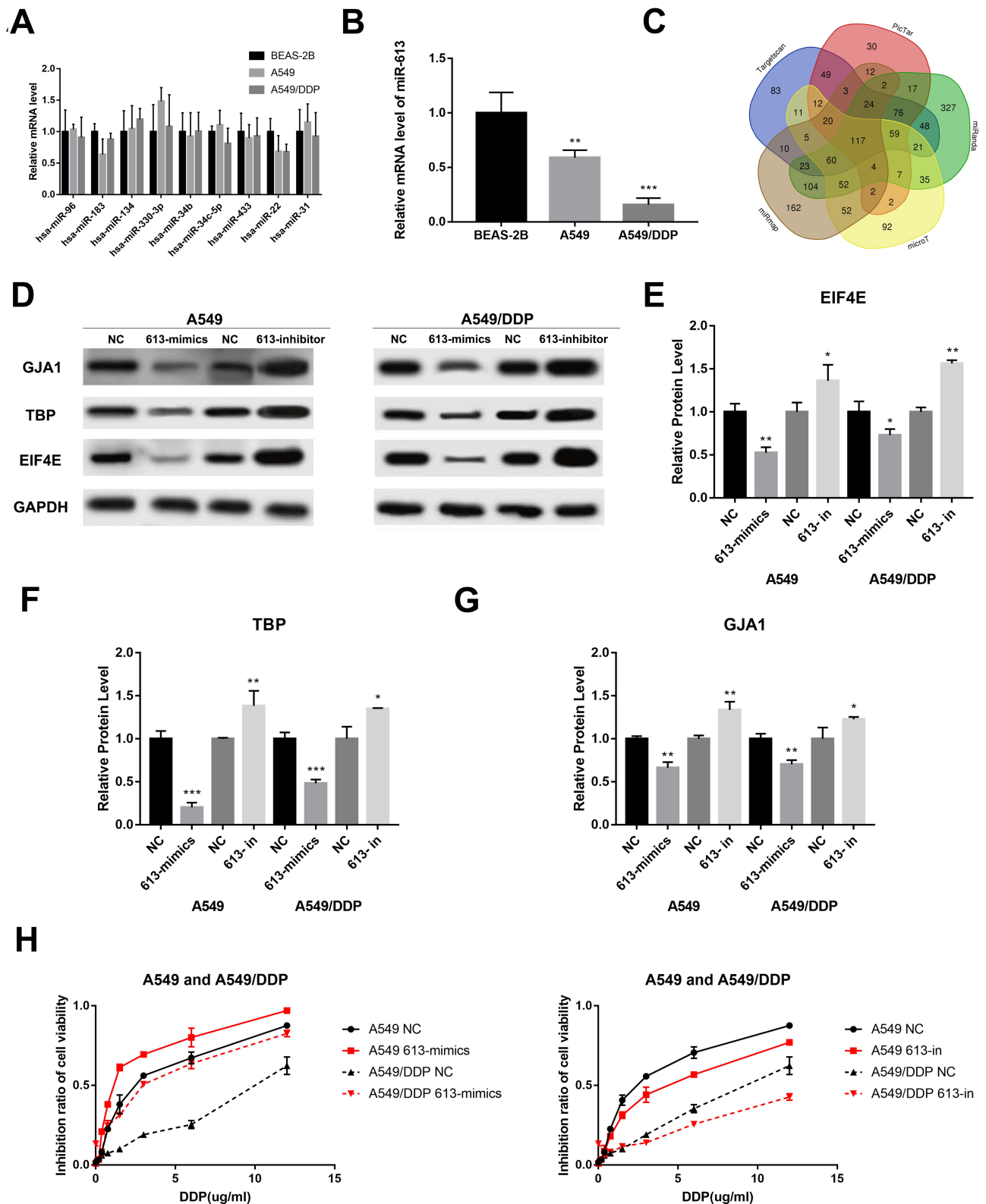
### Exo-miR-613 Fuses with Cells to Modulate Possible Target Genes

Exosomes contain non-coding RNAs, which can be transferred by exosomes to affect the development of cancers.<sup>22</sup> Exosomes were collected from the supernatant of HEK-293T and A549 cells and the morphology of exosome was determined by transmission electron microscopy (Figure 2A). We conducted the Nanoparticle tracking analysis (NTA) to value

the particle size of our extracellular vesicle and the results showed that the mean diameter of them was 79.22 nm (Figure 2B) which was consistent with the diameter of exosome (40–160nm).<sup>24</sup> Furthermore, the expressions of several marker proteins, ALIX, CD63 and TSG-101 were much higher in exosomes than that in cells (Figure 2C). Exosomes from HEK-293T cells were labeled with CD63 to incubate with A549 and A549/DDP cells for 24 h. We found that CD63 were distributed around the nucleus as small dots, confirming the fusion of exosomes into the cells (Figure 2D). To explore whether the exo-miR-613 has a similar effect as miR-613 on the drug-resistance of cancer cells, the HEK-293T cells were firstly transfected with miR-613 and corresponding NC for 48 h. After over-expressing miR-613 successfully (Figure 2E), exosomes in the cell supernatant were extracted and co-cultured with A549 and A549/DDP cells treated with DDP. Meanwhile, the regulation role of exo-miR-613 on its target genes was also examined. Similar to miR-613, exo-miR-613 could remarkably down-regulate the expressions of GJA1, TBP and EIF-4E as well (Figure 2F and G), suggesting exo-miR-613 plays a vital role in the development of NSCLC and the drug resistance process of cancer cells, possibly by regulating the expressions of its target genes.

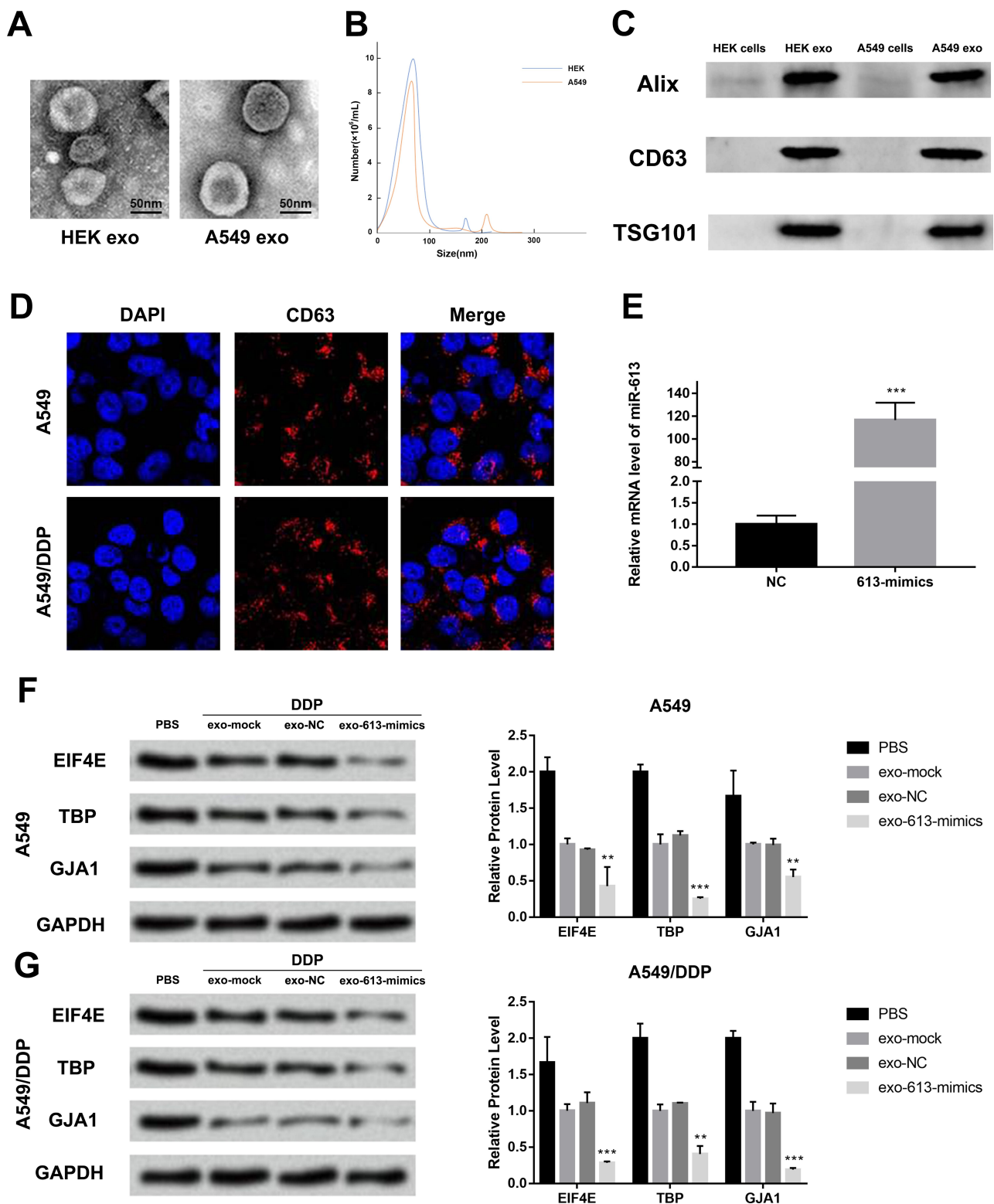
### Exo-miR-613 Enhances the Chemotherapy Sensitivity to DDP in NSCLC Cells

Regarding that exo-miR-613 significantly down-regulated the expressions of GJA1, TBP and EIF-4E, which are illustrated to be closely related to rapid progression, poor prognosis and metastasis of multiple cancers.<sup>25–27</sup> We explored the effects of exo-miR-613 on cell proliferation and apoptosis in A549 and A549/DDP cells treated with DDP. The CCK-8 results demonstrated that the cell proliferation of exo-miR-613 group was distinctively inhibited in A549 cell line, compared to control group (Figure 3A). In addition, similar evidence was also observed in A549/DDP cell lines (Figure 3B). Besides, compared to A549 cell line, the inhibitory effect of exo-miR-613 on proliferation was more obvious in A549/DDP cell line. Simultaneously, the flow cytometry was applied to detect the regulation of exo-miR-613 to DDP-induced cell apoptosis. As a whole, apoptosis of A549 and A549/DDP cells were both enhanced by exo-miR-613 mimics (Figure 3C and D). These results indicated that exo-miR-613 could increase the chemotherapy sensitivity to DDP in NSCLC cells by inhibiting cell proliferation and promoting apoptosis.

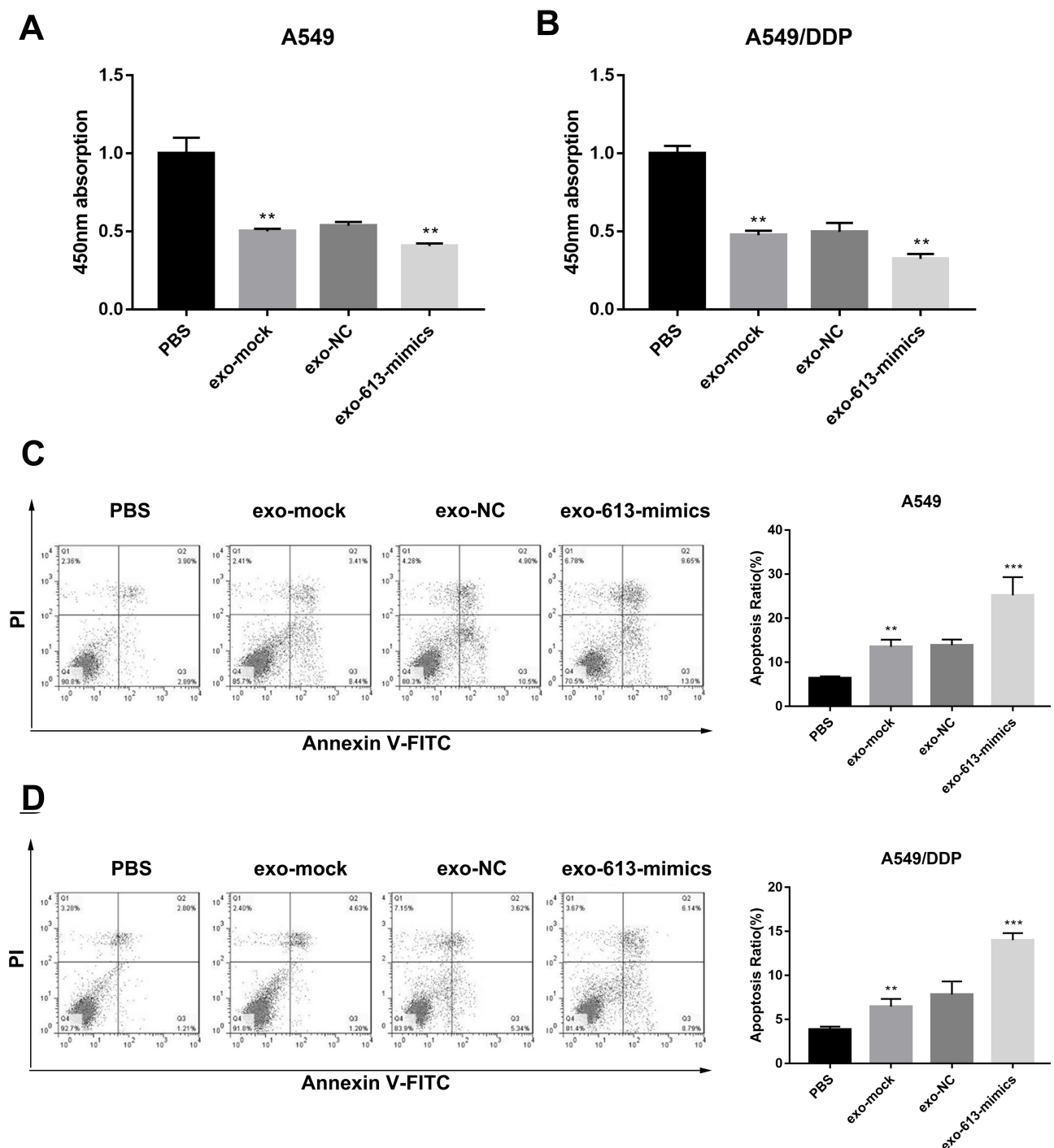


**Figure 1** miR-613 expression and possible targets of it. **(A-B)** Differentially expressed miRNAs of NSCLC microarray GSE292482 and miR-613 expressions in BEAS-2B, A549 and A549/DDP were detected via qRT-PCR. **(C)** The possible targets of miR-613 were verified by TargetsScan, PicTar, miRanda, microT and miRmap. **(D)** Western blot was carried out to confirm the potential targets in A549/DDP and A549 cell lines. **(E-G)** The gray analysis results of **(C)**. **(H)** Cell vitality at various concentrations of drug in A549 and A549/DDP was examined via CCK-8 to calculate the inhibition rate of cells transfected with miR-613 mimics (left) and miR-613 inhibitor (right). \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . Each error bar represents SE.





**Figure 2** Exo-miR-613 fuses with cells to modulate possible target genes. **(A)** Exosome image of HEK293T and A549 cell in the culture medium via a TEM. **(B)** The NTA analysis of exosomes from HEK293T and A549 cells. **(C)** The exosome marker proteins were detected through Western blot and analyzed with gray analysis. **(D)** CD63-stained exosome fused with NSCLC cells. **(E)** The miR-613 expression in HEK exosome was tested by conducting qRT-PCR. **(F-G)** The possible target proteins expression in A549 **(E)** and A549/DDP **(F)** Western blot was applied in cells. \*\*\*p < 0.001, \*\*p < 0.01. Each error bar represents SE.

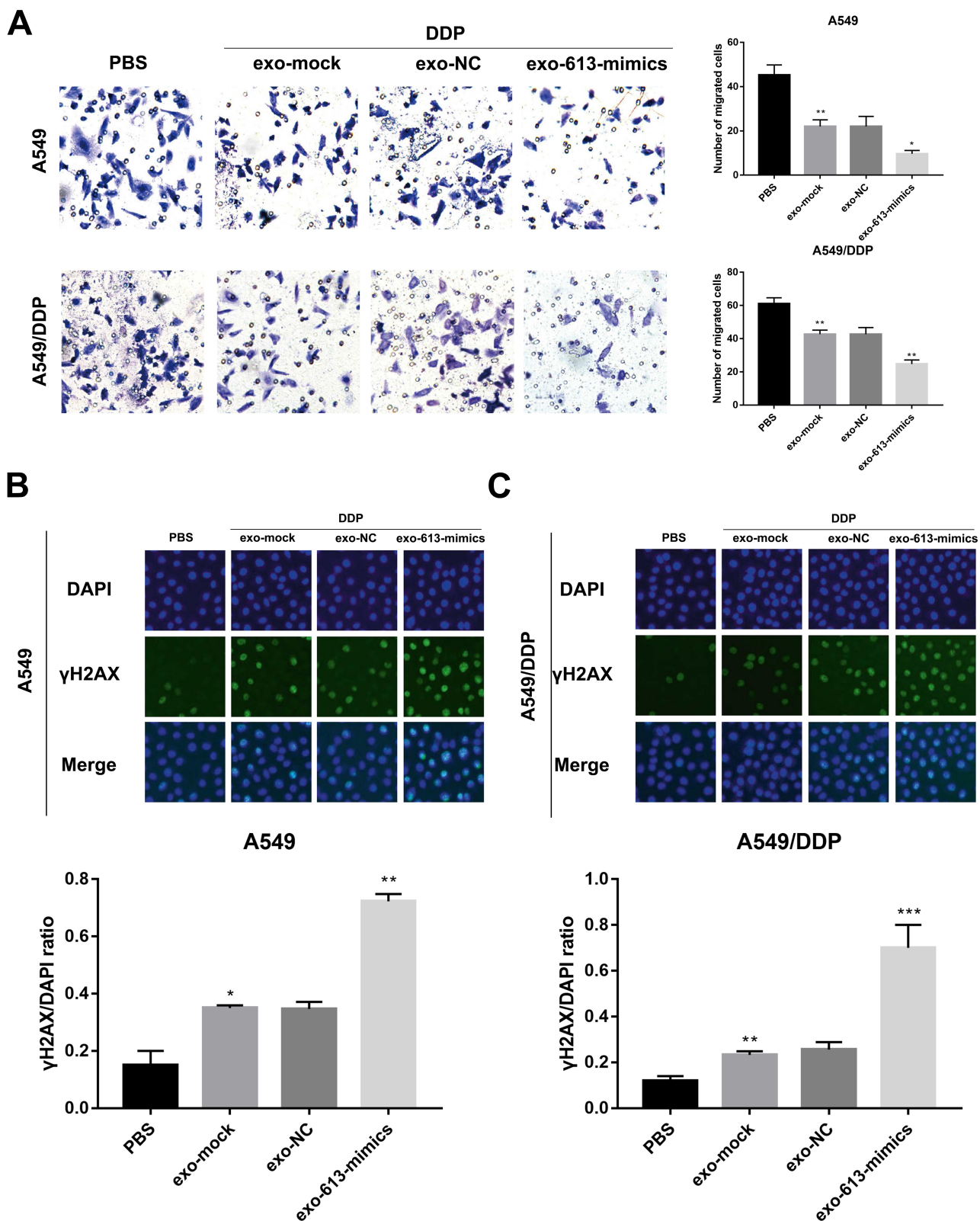


**Figure 3** Reverse of exo-miR-613 on DDP-resistant NSCLC. (A) Cell vitality in A549 was detected by CCK-8 assay. (B) The cell vitality of A549/DDP cell line. (C-D) Flow cytometry was applied to test cell apoptosis in A549 (C) and A549/DDP (D) cells. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ . Each error bar represents SE.

## Exo-miR-613 Enhance the Cytotoxicity of DDP in NSCLC Cells

In addition to drug resistance, tumor metastasis is another major cause leading to cancer associated death. To explore the effect of exo-miR-613 on cell migration, Transwell was used to examine the migration of these two cell lines.

Generally, cell migration was almost totally inhibited by DDP in A549 cells incubated with untreated exosomes (exo-mock), exo-miR-613 or exo-NC. In A549/DDP cells treated with DDP, cell migration still existed, and could be reduced by exo-miR-613 mimics (Figure 4A). By directly binding DNA, cisplatin destroys the DNA structure and causes



**Figure 4** Exo-miR-613 increases DDP cytotoxicity in NSCLC. **(A)** Cell migration was tested with transwell assay in the two cell types and the quantitative analysis of cell migration. **(B-C)** DNA damage of A549 (left) and A549/DDP (right) cell lines was measured by immunofluorescence of  $\gamma$ H2AX staining and the quantitative analysis of DNA damage. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . Each error bar represents SE.



damage to cells, thereby exerting an anti-tumor effect. Therefore, we explored whether exo-miR-613 involved in DDP-induced DNA damage. By staining with  $\gamma$ H2AX, the DNA damage marker protein, we showed that DDP induced DNA damage of A549 cells. However, exo-miR-613 mimics showed little effect on DNA damage in A549 cell line (Figure 4B), whereas in drug-resistance cell lines, exo-miR-613 significantly increased the level of DNA damage induced by DDP (Figure 4C), furtherly suggesting exo-miR-613 plays an important role in drug-resistance of lung cancer cells by enhance the cytotoxicity of DDP.

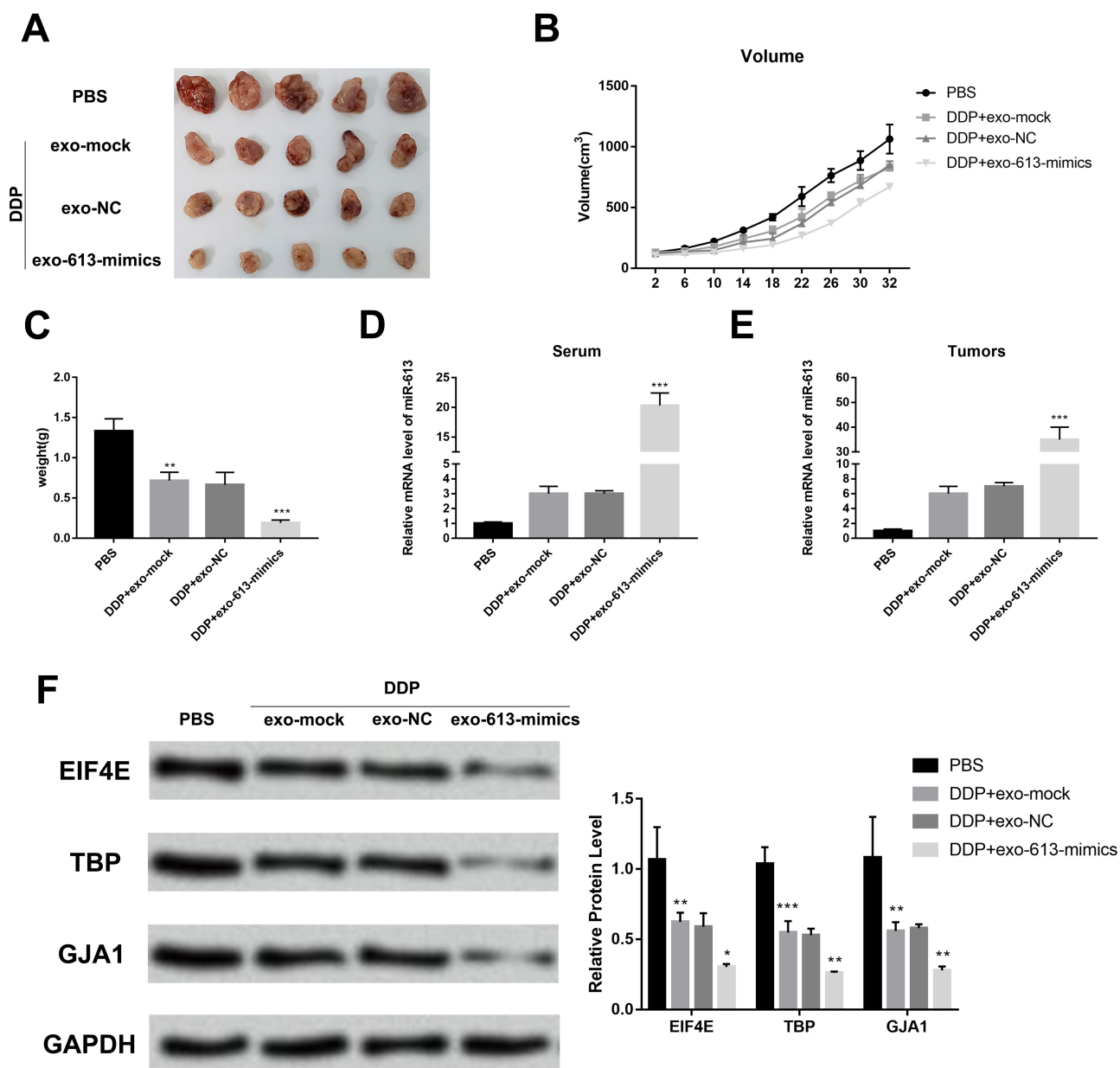
### Injection of Exo-miR-613 in Mice Significantly Enhance Sensitivity of NSCLC to DDP

To explore whether exo-miR-613 possess the identical functions in vivo, we established a NSCLC mice model by subcutaneously injecting A549/DDP cells into nude mice. Exo-miR-613 overexpressed mice model was further constructed by caudal vein injection of exo-miR-613. The exo-miR-613 injection was performed every four days and DDP injection was executed every two days. Tumor size was recorded every four days. After 32 days of injection, the tumors of mice were obtained and photographed (Figure 5A). The results showed that exo-miR-613 led to a smaller size of tumor comparing with the control group. In addition, tumor size was comparable in mice injected with exo-NC and exo-miR-613 at the first 14 days. However, after 14 days of exo-miR-613 injection, tumor size was stable in the treatment group, while tumor size of the control group significantly increased (Figure 5B). Mirrored with tumor size, tumor weight of exo-miR-613 group was significantly decreased than that of control group (Figure 5C). The mRNA expression level of miR-613 was significantly increased in serum and tumor tissues of DDP+exo-613-mimics group compared with the control group, indicating the efficiency of intravenous injection (Figure 5D and E). Regarding the regulation of exo-miR-613 on its target genes in NSCLC cells, expression level of GJA1, TBP, and EIF-4E in tumor tissues was extracted, and the expression of three proteins consistent with in vitro results was down-regulated by systemic injection of exo-miR-613 (Figure 5F), which provided possible mechanism for the enhanced sensitivity of NSCLC to DDP in vivo.

### Discussion

The drug-resistance of chemotherapeutics is an important factor for the high morbidity and mortality of NSCLC. Therefore, it is important to explore the possible target of drug-resistance to prevent the occurrence of drug-resistance.<sup>28,29</sup> In recent years, more and more studies show that miRNAs play a key role in regulating drug-resistance of cancer cells.<sup>30-33</sup> Previous studies have showed that miR-613 inhibited the development and progression of NSCLC by targeting CDK4.<sup>17</sup> In the present study, we found miR-613 were significantly down-regulated in both A549 and A549/DDP cell lines. Meanwhile, miR-613 over-expression reversed the inhibition effect of DDP on cell proliferation, suggesting that miR-613 may be involved in the development of NSCLC and the drug-resistance of tumor cells to DDP. In addition, GJA1, TBP and EIF-4E were selected as target genes of miR-613. In glioblastoma (GBM), overexpression of GJA1 was observed in temozolomide-resistant GBM cells.<sup>34</sup> In ovarian cancer, TATA-box binding protein (TBP) contributes to regulation of dedifferentiation states that promote metastasis and chemoresistance.<sup>35</sup> In breast cancer, miR-141 mediated docetaxel resistance through direct interactions with EIF-4E.<sup>36</sup>

As a secreted miRNA, the exo-miRNA appeared to be more appropriate as the biomarkers of cancers for its non-invasive feature. Several serum exosomal microRNAs served as predictive markers for chemoresistance in advanced colorectal cancer.<sup>37</sup> Tumor-derived exosomes contain informative microRNAs involved in carcinogenesis, cell migration, invasion, eventually contributing to metastasis of cancers.<sup>38</sup> Gemcitabine-resistant cell-derived exosome could transfer malignant phenotype by delivering miRNA-222-3p.<sup>39</sup> Exosomes were reported to promote resistance to Gefitinib in the gastric cancer cells by transferring miR-21.<sup>40</sup> However, only few researches identified the role of exo-miRNAs in NSCLC. It has been identified that several exo-miRNAs could regulate the sensitivity of NSCLC cells to DDP. Exo-miR-21 from hypoxia treated NSCLC enhanced the resistance of normal oxygen treated cells to DDP.<sup>31</sup> The therapeutic effect of DDP in NSCLC could be predicted by expression level of exo-miR-146a-5p.<sup>41</sup> After transfected with miR-613 in HEK-293T cells, the exo-miR-613 was extracted. Then, the roles of exo-miR-613, which was verified by TEM, NTA and the markers of exosomes, were investigated in NSCLC. The results showed that exo-miR-613 significantly enhanced the inhibition effect of DDP on proliferation, migration



**Figure 5** Exo-miR-613 was injected systemically to sensitize the reaction to DDP in vivo. **(A)** Tumor Images of mice (n = 5). **(B)** Tumor size alterations. **(C)** Tumors weight detection. **(D and E)** qRT-PCR was applied to measure the miR-613 expression of serum exosome **(D)** and mice tumor **(E)**. **(F)** Western blot was applied to analyze the miR-613 expression in tumors and potential target proteins. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . Each error bar represents SE.

in A549 and A549/DDP and promoted cell apoptosis induced by DDP, especially in A549/DDP cells.  $\gamma$ H2AX, the first and most prominent protein for DNA damage foci formation, which has been utilized as markers of DNA damage in recent studies, was also examined in our research. By fluorescence staining, exo-miR-613 also aggravated the DNA damage induced by DDP in A549/DDP cells, evidenced by elevated expression of  $\gamma$ H2AX. However, the detailed mechanisms for DNA damage by exo-miR-613 needed to be further studied. The above

results indicated that exo-miR-613 involved in the DDP-resistance mechanism of NSCLC cells. Furthermore, exosomes isolated from cell media were intravenously injected into mice to maintain the up-regulation of exo-miR-613 in serum and tumors, therefore verifying the role of exo-miR-613 in NSCLC tumor growth and DDP-resistance.

Besides, the present study also screened the possible target genes of exo-miR-613, and validated the regulation of exo-miR-613 on those genes both in vivo and in vitro,

providing possible mechanisms for the enhanced sensitivity of NSCLC to DDP. In summary, we investigated the role of exo-miR-613 in drug-resistance of NSCLC, and showed that exo-miR-613 could reverse the resistance to DDP in NSCLC, by accelerating cell apoptosis and suppressing proliferation and migration in vitro and inhibiting tumor growth in vivo. Our results may provide new sights for the diagnosis and treatment of NSCLC and lay a foundation for the clinical application of exosome-derived miRNAs.

## Disclosure

The authors report no funding and no conflicts of interest for this work.

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