European Review for Medical and Pharmacological Sciences 2021; 25: 127-138

Exosomes derived from Taxol-resistant nasopharyngeal carcinoma (NPC) cells transferred DDX53 to NPC cells and promoted cancer resistance to Taxol

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Abstract. – OBJECTIVE: Nasopharyngeal carcinoma (NPC) is a common cancer with high incidence in Southern China. Taxol is one of the first-line chemotherapeutic drugs for treating NPC; however, Taxol resistance has become the main difficulty for clinical treatment and the mechanisms remain not fully understood. In this study, we mainly focus on exploring whether exosomes from Taxol-resistant NPC cells played some roles in the resistance and progression of NPC.

MATERIALS AND METHODS: Taxol was used to treat NPC cell line CNE1 and Taxol-resistant NPC cell line CNE1-TR cells to measure cell viability and IC50 by CCK-8 assay. Exosomes from these two cells were extracted and identified by transmission electron microscopy (TEM), and special protein markers were determined by Western blot (WB) assay. Real-time PCR was performed to detect levels of mRNAs in exosomes, CNE1 and CNE1-TR cells. WB was performed to detect protein levels. The p-DDX53 and si-DDX53 were constructed and cloned into cells, resulted with DDX53 overexpression and inhibition, then resistant associated protein levels and IC50 were measured. Finally, GW4869, an inhibitor to block exosome secretion, was used to verify that the exosomes derived from CNE1-TR cells transferred DDX53 to CNE1 cells and contributed to promote NPC resistance.

RESULTS: We found that the IC50 to Taxolin CNE1-TR was much higher than that in CNE1 cells and DDX53 was highly expressed in Taxol-resistant CNE1-TR cells. Furthermore, exosomes were successfully extracted and determined, showing high levels of DDX53 and MDR1. Thus, they could promote cell resistance for CNE1 after adding CNE1-TR exosomes into CNE1 cells. Moreover, DDX53 overexpression increased the IC50 and upregulated MDR1 in CNE1 cells, while DDX53 inhibition showed the opposite results. In addition, the DDX53 inhibition decreased the IC50 and repressed MDR1 in CNE1-TR cells. Besides, blocking exosome released from CNE1-TR by using GW4869 treatment significantly repressed the levels of DDX53 and MDR1, and the IC50 of CNE1 cells was reversed. Finally, the increased levels of MDR1 were significantly reversed following with adding DDX53 si-DDX53-CNE1-TR exosomes, and the increased IC50 to Taxol was obviously reversed.

CONCLUSIONS: This study firstly discovered that DDX53 was highly expressed in Taxol-resistant NPC cells, which could be transferred into normal NPC cells via exosome secretion. The transferred DDX53 could upregulate the expression of MDR1 in NPC cells to promote the resistant capacity to Taxol, which provided a novel insight for understanding NPC and might be a potential therapeutic target for NPC.

Key Words:
Exosomes, Taxol-resistance, Nasopharyngeal carcinoma (NPC), DDX53, Multidrug resistance 1 (MDR1).

Introduction

Nasopharyngeal carcinoma (NPC) is a common head and neck cancer with high incidence in Southern China[1,2]. NPC patients at early-stage can be cured by multiple improved therapies[3,4]. However, most NPC patients are diagnosed at middle-late stage or advanced stage because there are no specific symptoms at early-stage[3,4]. Chemotherapy has been proved to be an effective treatment for NPC combined with radiotherapy[4-6]. However, drug resistance is the most common problem that leads to unsatisfactory consequences and poor survival rates for NPC patients[4-7].

Taxol is one of the first-line chemotherapeutic drugs for ovarian cancer[8], breast cancer[9,10] and NPC[11,12]. Taxol is a mitotic inhibitor that can in-

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hibit cell division by stabilizing the formation of microtubule, leading to cell death and inhibiting cancer progression[10-13]. Taxol resistance has been the main difficulty for clinical treatment, which imposes some restrictions for long-term anticancer consequences[10-13]. The mechanisms of Taxol resistance in NPC are not fully understood; therefore, it is necessary to make better understandings and deeper investigations on Taxol resistance to improve the antitumor effects of Taxol.

Multidrug resistance (MDR) is a resistant process to various chemotherapies in cancers and it has been proved to be correlated with drug efflux pumps, which have been called ATP-binding cassette (ABC) transporters[14-17]. These ABC transporters include MDR1 (ABCB1), MDR2 (ABCB2), MRP1 (ABCC1), MRP2 (ABCC2), BCRP (ABCG2), etc.[16,18]. These membrane transporters can transfer the anticancer drugs out of cancer cells[16,18]. MDR1 is one of the most famous ABC transporters that pumps anticancer drugs out of cells, and it has been demonstrated to be upregulated in couple of cancers, promoting resistance and declining the effects of chemotherapies[19-21].

DDX53 has been found to be upregulated in various cancers, which lead to drug resistance, such as Taxol, through upregulating MDR1[22-25]. For example, it was reported that DDX53 was highly expressed in Taxol-resistant cervix cancer cells compared to the parental HeLa cells, which could upregulate the expression of MDR1, thereby promoting the anti-cancer drug resistance in cervix cancer[22]. Although evidences suggested that DDX53 was a potential target for developing anti-cancer drug for some tumors[22-25], no reports have reported the functions of DDX53 in NPC. In this study, we found that DDX53 was increased in Taxol-resistant NPC cells compared with normal NPC cells. Therefore, we aimed at exploring the roles of DDX53 in NPC.

Exosomes are membranous vesicles (EVs), which are released in the endosomal compartment of cells and range from 30 to 100 nanometers (nm)[26-28]. It has been reported that exosomes transfer some DNA, miRNAs, mRNAs and proteins to target cells, which may help to promote progression and facilitate drug resistance of cancers[29-34], such as breast cancer[31,32], colorectal cancer[30,33], ovarian cancer[29], etc. In fact, it was reported that Tumor-associated macrophages (TAMs) secreted exosomes, which transferred some miRNAs to colorectal cancer (CRC) cells and induced M2 polarization of macrophages by regulating PTEN and PI3K/Akt signaling pathway, thereby enhancing the metastatic capacity of CRC[33]. However, whether exosomes played some roles in the progression or in the resistance of NPC remained unknown.

In this study, we mainly focus on exploring whether exosomes from Taxol-resistant NPC cells played some roles in the resistance of NPC. And we found that DDX53 was obviously increased in exosomes derived from Taxol-resistant NPC cells. Thus, we investigated whether DDX53 participated in the process of NPC resistance to Taxol and explored the potential mechanism.

Materials and Methods

Cell Culture

Human NPC cell line CNE1 was purchased from the Cancer Research Institute of Central South University (Changsha, China). The Taxol-resistant cell line CNE1/Taxol (CNE1-TR) was obtained by exposing the parental CNE1 cells to gradually increasing concentrations of Taxol according to previous studies[35,36]. Cells were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA) with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA) and 1% penicillin/streptomycin in an incubator with 5% CO_2 at 37°C. Cells were centrifuged with 200,000 g for 18 h to deplete exosomes. Transwell inserts (Costar, NY, USA) were used to co-culture CNE1 and CNE1-TR cells. The CNE1 cells were seeded on the lower chambers, and CNE1-TR cells were seeded on the upper chambers. The pore diameter of the upper chambers was 0.4 μm, which could prevent CNE1-TR cells from crossing the membranes but allow exosomes to pass through to interact with CNE1 cells.

Construction of Plasmid, siRNA and Cell Transfection

The full length of human DDX53 cDNA was synthesized and cloned into the vector of pCDNA3.1 (Invitrogen, Carlsbad, CA, USA), which resulted in a stable cell line with DDX53 overexpression (p-DDX53). In addition, small interfering RNA (siRNA) for DDX53 (si-DDX53) and the negative control siRNA (si-NC) were provided by Invitrogen (Invitrogen, Carlsbad, CA, USA). siRNA sequences were as follows: si-DDX53 sense5'-GAAGAUUCCAGGCAUGUU-3' and antisense 5'-UAACAUGCCUGGAAUCUU-3'; si-NCsense5'-GTCAATGAATCGCAGTGT-3'

and antisense 5'-GCATAGTAAGTCTCTTCA-3'. Cells were prepared until about 40% confluence on six-well plates and transfected with plasmids or siRNAs with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the protocol and cells with p-DDX53 and si-DDX53 were prepared and obtained for further analysis.

Exosome Isolation

Cells were cultured in RPMI-1640 with 10% FBS, which was centrifuged at 200,000×g for 18 h to deplete exosomes. The media from CNE1-TR cells was collected and centrifuged at 700 g for 10 mins. As a result, the supernatant was collected as conditioned media. Exosomes from CNE1 and CNE1-TR cells were isolated by using the Exosome Precipitation Solution (Exo-Quick; System Bioscience, Mountain View, CA, USA) in accordance with the manufacturer's protocol. The solution was added into the medium and refrigerated at 4°C overnight, and then centrifuged at 180 g for 30 mins at 4°C and 700 g for 5 mins. Exosome pellets were resuspended with PBS and stored at -80°C. Exosomes were resuspended in 500 μl of cell medium and incubated for 48 h with recipient cells, and exosome-depleted medium was obtained after centrifuging at 20,000 g for 90 mins. The concentrations of isolated exosomes were measured by using a bicinchoninic acid (BCA) kit (Thermo Fisher Scientific, Waltham, MA, USA) according to its protocol.

Exosomes Identification

The morphologies of isolated exosomes were identified by transmission electron microscopy (TEM, JEOL JEM 1230, Peabody, MA, USA). Briefly, the isolated exosomes were added on formvar carbon-coated 200 mesh copper electron microscopy grids, and then incubated for 5 mins at room temperature. After that, they were subjected to standard uranyl acetate staining and washed with PBS for three times before the observation by TEM (JEOL JEM 1230, Peabody, MA, USA). The diameters of exosomes were determined by Micrographs. The sizes of isolated exosomes were measured by nanoparticle tracking analysis (NTA) with ZetaView PMX 110 (Particle Metrix, Meerbusch, Germany), which was calibrated by using 100 nm polystyrene particles.

CCK-8 Assay

Cell viability was measured by using a Cell Counting Kit-8 (CCK-8) assay (Dojindo Molecular Technologies, Kumamoto, Japan). Cells were seeded on 96-well plates (4×10^3 cells/ml) and cultured in an incubator with appropriate conditions, which were treated with the presupposed treatments for 48 h. Briefly, 10 μl CCK-8 agent was treated into the medium, which was cultured at the darkness for 2 h at 37°C, and then the cell viability was measured by CCK8 assay according to the protocol. Besides, the absorbance (OD) was measured at 450 nm by using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). Finally, the half inhibition concentration (IC50) was calculated. Each experiment was repeated three times.

Exosomes and GW4869 Treatment

CNE1 cells were prepared on 6-well plates (1×10^6 cells/well) and 20 μg exosomes isolated from CNE1 cells or CNE1-TR cells were added into the medium. The supernatants were harvested after 48 h for further study. GW4869, an inhibitor for exosome secretion, was used to block exosome secretion[37-39]. Briefly, GW4869 (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO) (Thermo Fisher Scientific, Waltham, MA, USA), 20 μM GW4869 was then added into the prepared media with the presupposed treatments for 48 h, and finally, supernatants were collected for further study.

RNA Extraction and Quantitative RT-PCR

Total RNAs from cells were extracted by using TRIzol (Invitrogen, Carlsbad, CA, USA), which were purified by using GeneJET RNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) in accordance with their protocols. Total RNAs were reverse transcribed into cDNA by using a PrimeScript™ RT reagent Kit (TaKaRa, Otsu, Shiga, Japan) according to the protocol. The qPCR amplification was performed by using a TaKaRa system. The relative mRNA expressions were quantified by standard Real-time PCR protocol with SYBR Premix Ex Taq II (TaKaRa, Otsu, Shiga, Japan). Primers for RT-PCR were obtained from Gene Pharma (Shanghai Gene Pharma, Shanghai, China), which were listed in Table I. The mRNA expressions were normalized to GAPDH and $2^{-\Delta\Delta CT}$ method was used to calculate the relative gene expressions.

Protein Extraction and Western Blot Analysis

Total protein was extracted by using a RIPA protein extraction buffer (Beyotime Biotechnology Co., Shanghai, China) containing a protease inhibitor (Roche Diagnostics, Indianapolis, IN,

Table I. Primers for RT-PCR.

Gene names	Forward primer (5′-3′)	Reverse primer
Age, yrs		
DDX53	TGGCCAGATACTGTACGTCAA	CTTGGGTGAGAGCTCGTTTTT
ABCB1	GCCTGGCAGCTGGAAGACAAAT	CAGACAGCAGCTGACAGTCC
ABCB2	CCAATAGTATGTCAAGCCTGT	ATACGACATCACGGCCTCCA
ABCC1	ATCGCCACACCACTAGTTGCTA	AAGCGCTCCTGTTCACCAAA
ABCC2	CGAGTTCCCATGGTGTTCTT	TGACAAACAGTGTCCCCAAA
ABCG2	GGTGCCATTTACTTTGGGC	ACAAAGAGTTCCACGGCTGA
β-actin	ACCCACACAGTACCCATCT	CGGCGGTGCCCATCT
GAPDH	GGAGTCCACTGGTGTCTTCA	GGGAACTGAGCAATTGGTGG

USA). Protein concentrations were measured by using a bicinchoninic acid (BCA) kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the protocol. 40 μg proteins were used to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for separation, and then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Membranes were blocked in 5% non-fatty milk at 25°C for 1 h, which were incubated with primary antibodies overnight at 4°C. Primary antibodies were obtained from Abcam (Abcam, Cambridge, MA, USA), which were listed in Table II. After this step, these membranes were washed and incubated with matched secondary antibodies (1:5000) for 1 hour. Finally, immunoreactive proteins were detected via Pierce ECL Western blot substrate (Thermo Fisher Scientific, Waltham, MA, USA) with ECL detection system, which were quantified by using Image J software (NIH, Bethesda, MD, USA).

Statistical Analysis

The data was analyzed by SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA, USA). The difference between groups was analyzed by one-way Analysis of Variance (ANOVA) and SNK method was used after ANOVA analysis to analyze differences in two groups. p-value <0.05 was considered to be statistically significant.

Results

DDX53 Was Highly Expressed in Taxol-Resistant Nasopharyngeal Carcinoma Cell

To explore the mechanisms of Taxol resistance in NPC, various concentrations of Taxol were treated for CNE1 and Taxol-resistant NPC cells CNE1-TR. After that, the cell viabilities and IC50 were measured by CCK8 assay. Results showed that Taxol induced concentration-dependent cell death and repressed cell viability in CNE1 cells, while no significant difference was found in CNE1-TR cells (Figure 1A). Furthermore, the IC50 to Taxolin CNE1-TR was much higher than that in CNE1 cells (Figure 1B), suggesting that CNE1-TR was resistant to Taxol. Surprisingly,

Table II. Antibodies for Western blot.

Proteins	Host	KDa	Catalog	Antibody dilution
DDX53	Rabbit	71 kDa	ab103545	1:1000
ABCB1	Rabbit	141 kDa	ab170904	1:1000
ABCB2	Rabbit	87 kDa	ab83817	1:1000
ABCC1	Rabbit	172 kDa	ab233383	1:1000
ABCC2	Rabbit	174 kDa	ab172630	1:2000
ABCG2	Rabbit	72 kDa	ab207732	1:1000
CD81	Rabbit	26 kDa	ab79559	1:2000
CD9	Rabbit	25 kDa	ab92726	1:2000
GAPDH	Rabbit	36 kDa	ab9485	1:5000
β-actin	Rabbit	42 kDa	ab179467	1:5000

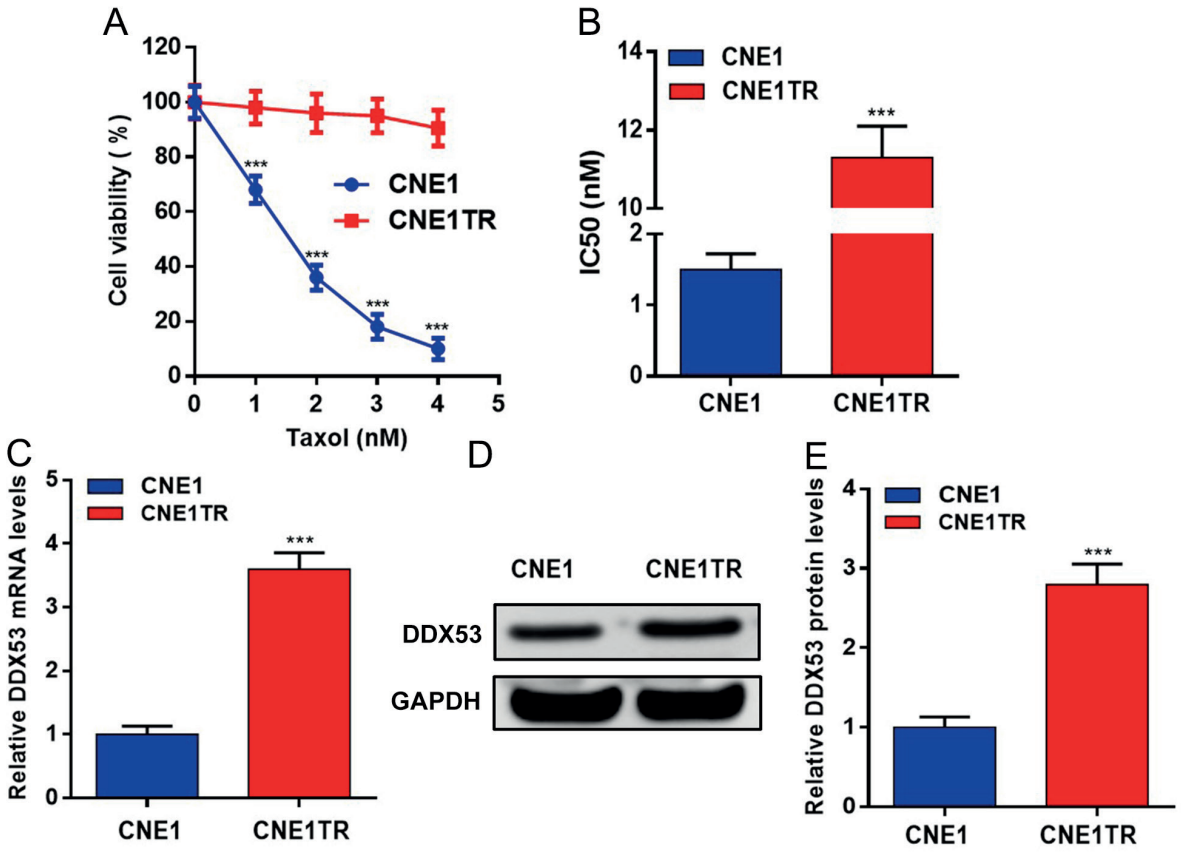

Figure 1. DDX53 was highly expressed in Taxol-resistant nasopharyngeal carcinoma cell. **A-B,** Various concentrations of Taxol were used to treat human NPC cells CNE1 and CNE1-TR cells; the cell viabilities and IC50 were measured by CCK8 assay. **C,** RT-PCR was performed to detect the mRNA expressions of DDX53 in two cells. D, E, Western blot (WB) assay was performed to detect the protein levels of DDX53. ***p<0.001.

we found that the mRNA and protein expressions of DDX53 in CNE1-TR cells were much higher than these in CNE1 cells (Figure 1C-E), which was reported to be an oncogene associated with cell resistance and cancer progression in several cancers[22-25] such as cervix cancer[22], melanomas[23], breast cancer[24], lung cancer[25], etc. Above results revealed that DDX53 was highly expressed in Taxol-resistant NPC cell.

Exosomes Were Extracted From CNE1 and CNE1-TR Cells

It has been reported that exosomes can deliver some molecules to affect cancer progression and resistant capacities in some cancers[29-34] such as breast cancer[31, 32], colorectal cancer[30,33], ovarian cancer[29], etc. As a result, we investigated whether Taxol-resistant NPC cell could release exosomes and whether that could contribute to affect the biological functions of NPC. Exosome Precipitation Solution was used to isolate the exosomes from CNE1 and CNE1-TR cells. We successfully extracted the exosomes which were determined by transmission electron microscopic (TEM) morphological analysis. Exosomes were about 100 nm in diameter (Figure 2A). WB assay was performed to detect some protein markers of exosomes. CD81 and CD9 were two markers for exosomes, β-actin was a marker for lysates. WB assay showed that CD81 and CD9 were over-expressed while β-actin was little expressed in exosomes from CNE1 and CNE1-TR cells (Figure 2B). Collectively, these results demonstrated that the exosomes were successfully isolated from CNE1 and CNE1-TR cells. However, whether the exosomes played some roles in NPC remained unknown.

Exosomes-Mediated DDX53 From CNE1-TR Cells was Transferred Into CNE1 Cells and Promoted Resistance in CNE1 Cells

To further confirm whether the exosomes from Taxol-resistant NPC cells could be transferred

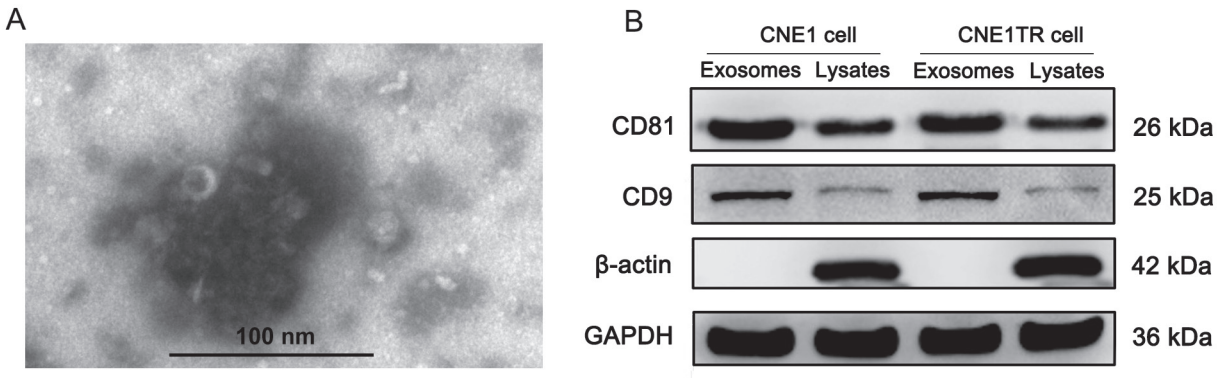

Figure 2. Exosomes were extracted from CNE1 and CNE1-TR cells. **A,** Exosomes were determined by transmission electron microscopic morphological analysis. Scale bar=100 nm. **B,** The markers (CD81 and CD9) for exosomes and the marker (β-actin) for lysates were detected by WB assay.

into CNE1 TR cells, the exosomes from CNE1 cells and CNE1 TR cells were isolated. MDR1 (ABCB1), MDR2 (ABCB2), MRP1 (ABCC1), MRP2 (ABCC2) and BCRP (ABCG2) are critical for cancer chemoresistance in NPC[14-18]. Then, we detected the expressions of DDX53, MDR1, MDR2, MRP1, MRP2 and BCRP. Results showed that mRNA and protein levels of DDX53 and MDR1 were increased, especially DDX53 was significantly increased in cells with CNE1-TR exosomes (Figure 3A-C) (p<0.05). Furthermore, to prove that the DDX53 was transferred by exosomes from CNE1-TR and affected the functions of normal NPC cells, we added the CNE1-TR exosomes or control into CNE1 cells for 48 h. Results revealed that the mRNA and protein levels of DDX53 were significantly increased after adding with CNE1-TR exosomes (Figure 3D-F) (p<0.001). Besides, the IC50 to Taxol was detected by CCK-8 assay and results showed that

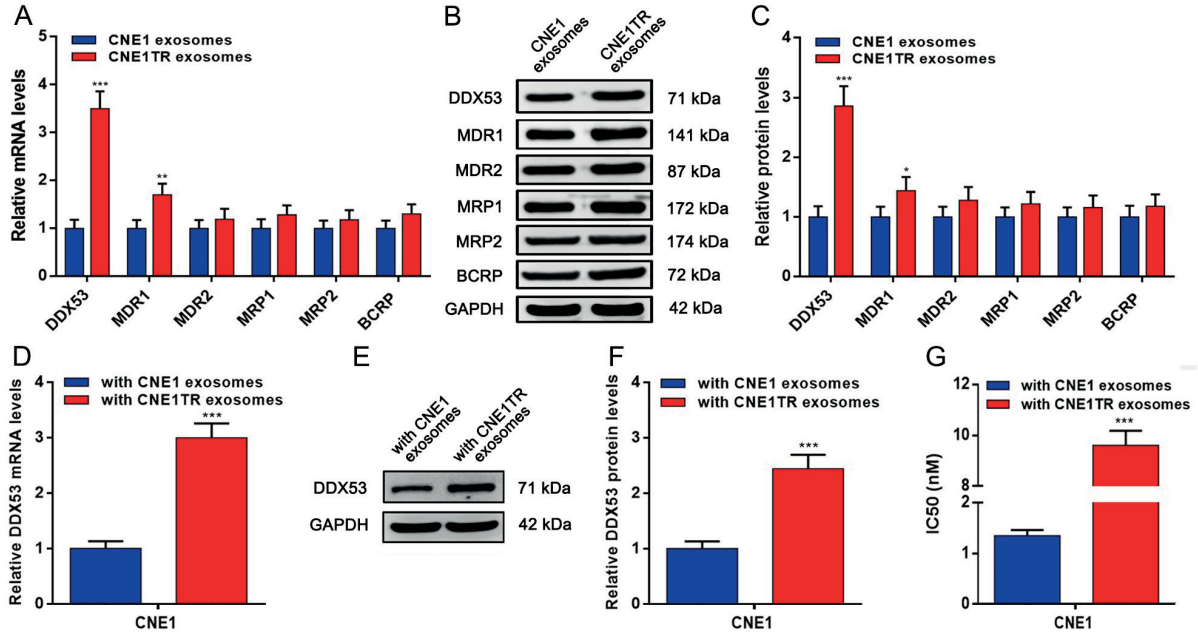

Figure 3. Exosomes-mediated DDX53 from CNE1-TR cells was transferred into CNE1 cells and promoted resistance in CNE1 cells. Exosomes were isolated from CNE1 and CNE1-TR cells. **A-C,** The mRNA and protein levels of DDX53, MDR1, MDR2, MRP1, MRP2 and BCRP were detected by RT-PCR and WB assay in exosomes. **D-E,** CNE1-TR exosomes or the control were added into CNE1 cells for 48 h, the mRNA and protein levels of DDX53 were detected by RT-PCR and WB assay. **F,** The IC50 to Taxol in two groups was measured by CCK8 assay. **p<0.05, **p<0.01, ***p<0.001.

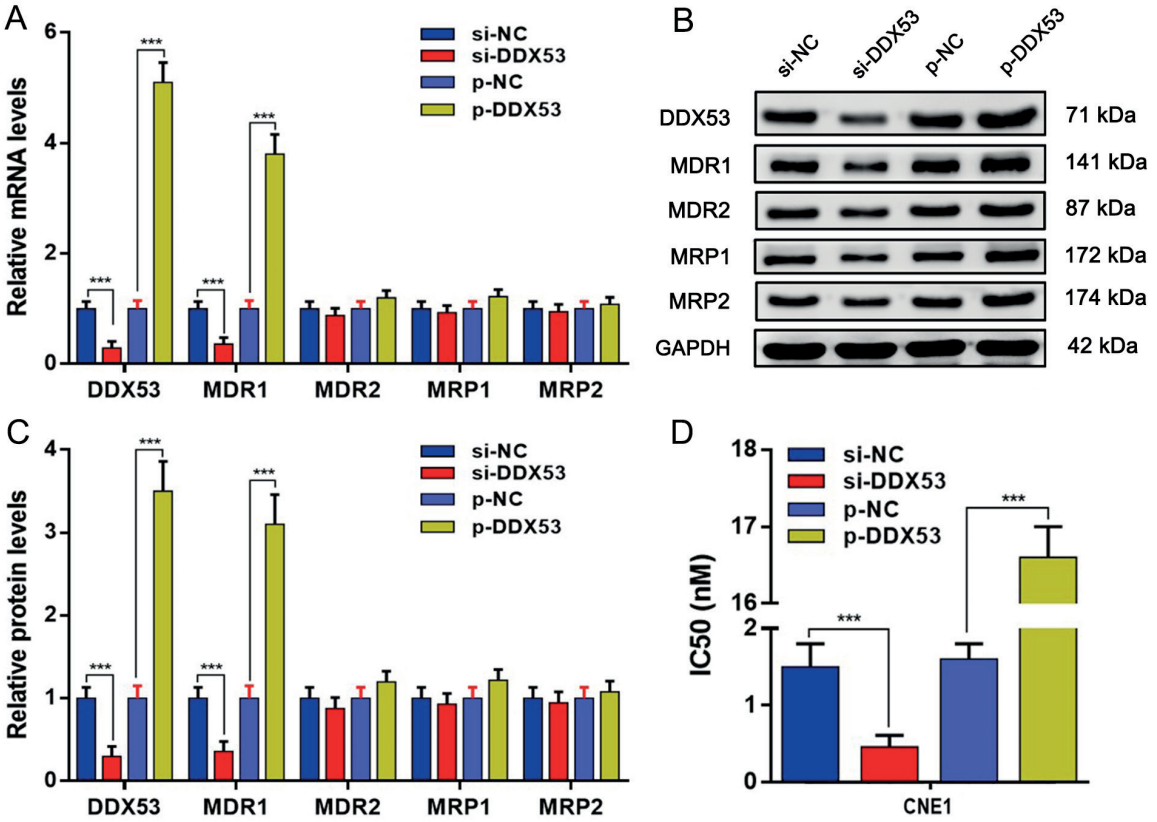

Figure 4. DDX53 promoted resistance through upregulating MDR1 in CNE1 cells. Si-DDX53 or p-DDX53 was respectively constructed and transfected into CNE1 cells. **A-C,** The mRNA and protein levels of DDX53, MDR1, MDR2, MRP1, MRP2 and BCRP were detected by RT-PCR and WB. D, The IC50 to Taxol in these four groups was measured by CCK8 assay. ***p<0.001.

the IC50 to Taxol was increased in cells with CNE1-TR exosomes compared with the control (Figure 3G**)** (p<0.001). These data indicated that exosomes-mediated DDX53 from CNE1-TR cells was transferred into CNE1 cells and promoted resistance to Taxolin CNE1 cells. However, how exosomes-mediated DDX53 promoted resistance to Taxol remained unknown.

DDX53 Promoted Resistance Through Upregulating MDR1 in CNE1 Cells

To further understand the mechanism that exosomes-mediated DDX53 promoted resistance to Taxol, plasmids of DDX53 overexpression or inhibition were respectively constructed and transfected into CNE1 cells. After that, the expressions of DDX53, MDR1, MDR2, MRP1, MRP2 and BCRP were detected. Results showed that the mRNA and protein levels of MDR1 were significantly repressed followed with DDX53 inhibition, while these were significantly increased followed with DDX53 overexpression (Figure 4A-C**)** (p<0.001). However, no significant difference was found in MDR2, MRP1, MRP2 and BCRP. MDR1 had been revealed as an important transporter that could pump the drugs out of the cells through special membrane transporter in cancers[17, 20, 21, 40], and DDX53 could regulate the expression of MDR1[22, 24]. Furthermore, the IC50 to Taxol was measured by CCK-8 assay and results showed that the IC50 to Taxol was obviously decreased followed with DDX53 inhibition and it was significantly increased followed with DDX53 overexpression (Figure 4A-C) (p<0.001). These results might indicate that the exosomes-mediated DDX53 promoted resistance to Taxol through regulating with MDR1 in CNE1 cells.

DDX53 Inhibition Reduced the Resistant Capacity to Taxol in CNE1-TR Cells

To verify that DDX53 promoted resistance to Taxol through regulating with MDR1 in CNE1-

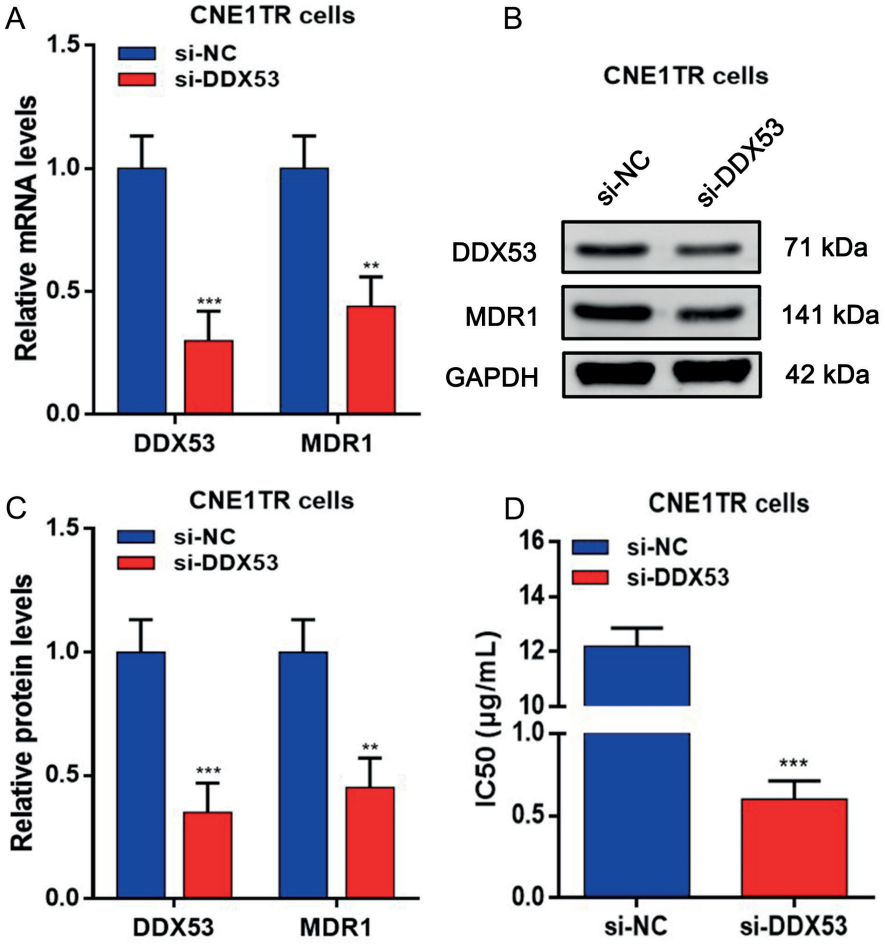

Figure 5. DDX53 inhibition reduced the resistant capacity to Taxol in CNE1-TR cells. Si-NC or si-DDX53 was respectively transfected into CNE1-TR cells. **A-C,** The mRNA and protein levels of DDX53 and MDR1 were detected by RT-PCR and WB. **D,** The IC50 to Taxol in these two groups was measured by CCK8 assay. **p<0.01, ***p<0.001.

TR cells, the si-NC or si-DDX53 was respectively transfected into CNE1-TR cells. Then, the expressions of DDX53 and MDR1 were detected by RT-PCR and WB, which showed the mRNA and protein levels of MDR1 were inhibited followed with DDX53 inhibition in CNE1-TR cells (Figure 5A-C) (p<0.01). In addition, the IC50 to Taxol was obviously decreased followed with DDX53 inhibition (Figure 5D) (p<0.001). These results showed that DDX53 inhibition reduced the resistant capacity to Taxol by regulating with MDR1 in CNE1-TR cells.

Blocking Exosomes from CNE1-TR Reversed Resistant Capacity in CNE1 Cells

Previous results showed that CNE1-TR could promote the resistant capacity to Taxol in CNE1 cells through exosomes, then we assessed whether blocking exosome secretion from CNE1-TR could prevent the resistance in CNE1 cells. GW4869, a non-competitive inhibitor of sphingomyelinase, was revealed to block exosome secretion in cells[37-39]. Then, we used GW4869 to confirm whether GW4869-treated CNE1-TR could reverse resistance to Taxolin CNE1 cells. Results showed that blocking exosome release by CNE1-TR using GW4869 treatment significantly repressed the expressions of DDX53 and MDR1 (Figure 6A-C) (p<0.01). Furthermore, the IC50 to Taxol was reversed following with blocking exosome (Figure 6D) (p<0.001). Finally, we isolated the exosomes from CNE1-TR cells and si-DDX53-CNE1-TR cells, which were co-cultured with CNE1 cells. Results showed that the increased mRNA and protein levels of MDR1 were significantly reversed following with adding DDX53si-DDX53-CNE1-

TR exosomes (Figure 6E-G) (p<0.001). The increased IC50 to Taxol was reversed following with adding DDX53 si-DDX53-CNE1-TR exosomes (Figure 6H**)** (p<0.001).

Above all, these data suggested that CNE1-TR-derived exosomes were the critical regulators of resistance to Taxol through transferring DDX53 and upregulating MDR1 in NPC cells; therefore, blocking the exosome secretion by CNE1-TR might prevent cancer resistance to Taxol.

Discussion

It is known that NPC is a malignant cancer with high incidence in Southern China[1,2]. Patients at middle-late stage or advanced stage are mainly treated with chemotherapy and radiotherapy[4-6]. Taxol treatment has been widely proved to be an effective auxiliary treatment in tumors, such as ovarian cancer[8], breast cancer[9,10], NPC[11,12], etc. However, the overall efficacy is still unsatisfactory because of Taxol resistance, which is a difficult problem for clinical treatment and long-term anticancer consequences[10-13]. The mechanisms of Taxol resistance in NPC are not fully understood, thus, in this study, we explored the mechanisms for Taxol resistance. We found that DDX53 was highly expressed in Taxol-resistant NPC cells compared to NPC cells. DDX53 was an oncogene that was associated with cancer resistance and progression in some cancers[22-25], such as cervix cancer[22], melanomas[23], breast cancer[24], lung cancer[25], etc. However, no reports found the roles of DDX53 in NPC, then we aimed at investigating the function of DDX53 and its potential mechanism in NPC.

Exosomes have been revealed to participate in facilitating drug resistance for cancers[29-34], such as breast cancer[31, 32], colorectal cancer[30, 33], ovarian cancer[29], etc. However, whether exosomes played some roles in Taxol resistance of NPC remained unknown. Then, we isolated the exosomes from CNE1 and CNE1-TR cells, which were proved using TEM assay and WB assay. And we found that the DDX53 and MDR1 were highly expressed in exosomes from CNE1-TR cells compared to those of CNE1 cells. Moreover, the isolated exosomes were added into CNE1 for 48 h, which showed that the level of DDX53 was significantly increased after adding with CNE1-TR exosomes compared to that of CNE1 cells. Besides, the IC50 to Taxol was significantly increased after treating with CNE1-TR exosomes

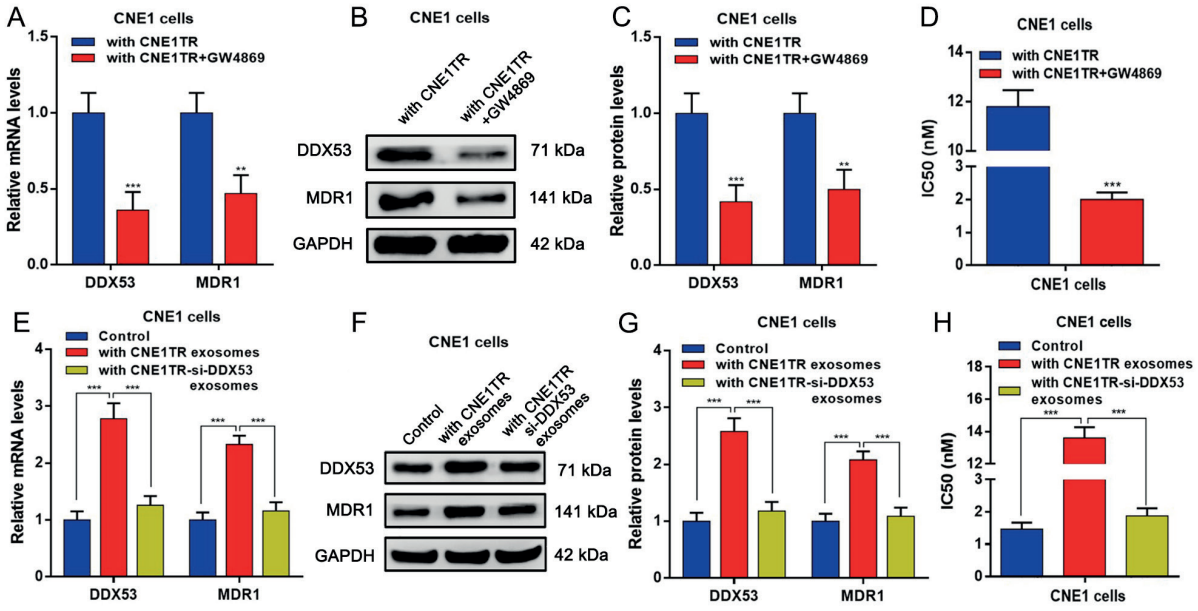

Figure 6. Blocking exosomes from CNE1-TR reversed resistant capacity in CNE1 cells. GW4869 was used to block exosome secretion of CNE1-TR cells. **A-C,** The mRNA and protein levels of DDX53 and MDR1 were detected by RT-PCR and WB. **D,** The IC50 to Taxol was measured by CCK8 assay. **E-G,** The mRNA and protein levels of DDX53 and MDR1 were detected after adding with CNE1-TR exosomes and si-DDX53-CNE1-TR exosomes by RT-PCR and WB. **H,** The IC50 to Taxol in these three groups was measured by CCK8 assay. **p<0.01, ***p<0.001.

compared with that of CNE1 cells. These results might indicate that exosomes-mediated DDX53 from CNE1-TR cells was transferred into CNE1 cells and might contribute to promote resistance to Taxol for NPC. However, the mechanism that exosomes-mediated DDX53 promoted resistance to Taxol remained unknown.

DDX53, a cancer antigen, was reported to be abnormally expressed in some tumors and cancer cells, which could upregulate the expression of MDR1 to increase resistance against Taxol and cisplatin in cervix cancer[22-25]. To further investigate the mechanism that exosomes-mediated DDX53 promoted resistance to Taxol, p-DDX53 and si-DDX53 were respectively transfected into CNE1 cells. As a result, MDR1 was significantly repressed followed with DDX53 inhibition, while it was increased followed with DDX53 over expression. Furthermore, the IC50 to Taxol was markedly decreased followed with DDX53 inhibition, while it was increased followed with DDX53 overexpression, which might indicate that the exosomes-mediated DDX53 promoted resistance to Taxol through upregulating with MDR1 in CNE1 cells.

To verify that exosomes-mediated DDX53 promoted Taxol resistance through upregulating MDR1 in CNE1-TR cells, the si-NC or si-DDX53 was respectively transfected into CNE1-TR cells, which showed the mRNA and protein levels of MDR1 were inhibited followed with DDX53 inhibition in CNE1-TR cells. Moreover, the IC50 to Taxol was obviously decreased followed with DDX53 inhibition in CNE1-TR cells. These results demonstrated that DDX53 inhibition reduced the resistant capacity to Taxol through repressing MDR1 in CNE1-TR cells.

Furthermore, we used GW4869, an exosome inhibitor, to block exosome secretion of CNE1-TR to confirm whether blocking exosome secretion of CNE1-TR could prevent the resistant capacity. Results showed that blocking exosome release by CNE1-TR repressed the expressions of DDX53 and MDR1 in CNE1 cells. Besides, the IC50 to Taxol was obviously reversed following with adding GW4869. Finally, we isolated the exosomes from CNE1-TR cells and si-DDX53-CNE1-TR cells, which were treated into CNE1 cells. And results showed that the increased MDR1 was significantly reversed and the increased IC50 to Taxol was obviously reversed following with adding DDX53 si-DDX53-CNE1-TR exosomes.

Above all, for the first time, we found that CNE1-TR-derived exosomes were the critical regulators of resistance to Taxol through transferring DDX53 and upregulating MDR1 in NPC cells. Therefore, blocking the exosome secretion by CNE1-TR might prevent cancer resistance to Taxol, which might provide a novel target for NPC.

Conclusions

This study firstly discovered that DDX53 was highly expressed in Taxol-resistant human NPC cells, which was surprisingly transferred into normal NPC cells through exosome secretion. The exosome-mediated DDX53 could upregulate the expression of MDR1 in normal NPC cells to promote the Taxol-resistant capacity, thereby declining the effects of Taxol. This finding provided a novel insight for understanding NPC and might be a potential therapeutic target for treating NPC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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