Article

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Exosome-Mediated Transfer of ALDH2 in Nasopharyngeal Carcinoma Cells Confers Increased Resistance to Paclitaxel Treatment

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Background: Nasopharyngeal carcinoma (NPC) is an aggressive and highly metastatic malignant tumor. Despite recent therapeutic advances, resistance to Taxol (the generic name of paclitaxel) therapy remains a major challenge in clinical management. Therefore, it is imperative to explore the potential mechanisms of paclitaxel resistance in NPC. This study aimed to investigate the expression of aldehyde dehydrogenase 2 (ALDH2) in NPC cells and its critical role in paclitaxel resistance.
Methods: Paclitaxel-resistant cell line CNE1/Taxol (CNE1-TR), a drug-resistant cell line, was established by exposing the CNE1 nasopharyngeal carcinoma cell line to progressively increasing concentrations of paclitaxel. Furthermore, we investigated the role of ALDH2 in paclitaxel resistance and the function of exosomes using cell culture, Western blotting, reverse transcription-polymerase chain reaction (RT-PCR), Cell Counting Kit-8 (CCK-8), and nanoparticle tracking analysis.
Results: The results showed that in the presence of paclitaxel, the CNE1-TR cells manifested higher survival rate and half-maximal inhibitory concentration (IC50) value compared to the parental cell line, indicating strong resistance to paclitaxel. CNE1-TR cells had significantly upregulated mRNA and protein levels of ALDH2. In addition, exosome analysis showed that CNE1-TR cells were able to deliver ALDH2 via exosomes, increasing paclitaxel resistance in the recipient cells. We observed that the *ALDH2* expression levels and paclitaxel resistance in CNE1-TR cells were effectively reduced by blocking the release of exosomes.
Conclusion: ALDH2 is not only a key molecular marker indicative of therapeutic efficacy, but also a potential therapeutic target for developing novel anticancer strategies. By blocking the exosomal transport of ALDH2 or directly inhibiting its activity, it may be possible to overcome paclitaxel resistance, thus improving the success rate of clinical treatment.

Keywords: nasopharyngeal carcinoma; paclitaxel resistance; *ALDH2*; exosomes; chemotherapy

Introduction

Nasopharyngeal carcinoma (NPC) affects the epithelial cells in the head and neck region and is known for its distinct geographic prevalence [1]. According to cancer reports of 2020, over three-quarters of NPC cases occur in Southeast Asia and regions of Southern China [2]. Nasopharyngeal cancer has always been a serious health challenge due to its aggressive nature and high likelihood of metastasizing [3,4]. This cancer is associated with a high mortality rate due to the challenging detection at an early stage and the high potential of local and distant metastasis.

The existing treatment regimens do not always lead to satisfactory outcomes for NPC, despite the advances in radiotherapies such as intensity-modulated radiation therapy, which can precisely target the tumor while protecting adjacent critical structures like optic nerve and brainstem [5]. It has been reported that patients with locally advanced NPC who receive a single radiotherapy treatment have a five-year survival rate of between 67% and 77%, highlighting the aggressiveness of the disease and the difficulty in achieving long-term disease control [6]. In addition, treatment failure is commonplace among patients experiencing distant metastasis or local recurrence of NPC, even if a combination of chemotherapy and radiotherapy—the standard of care for advanced stages—is used [7,8]. Recent shifts in therapeutic strategies, especially the combination of immunotherapy and targeted therapy, have shown some achievements in treating recurrent or metastatic NPC [9,10], but there is a need for further exploring the precise mechanism of cancer and developing more effective therapeutic strategies.

Exosomes play a key part in the progression and treatment resistance of NPC. These extracellular vesicles can facilitate communication between tumor cells and the surrounding microenvironment, affecting multiple aspects of tumor behavior, including immune escape, angiogenesis, and drug resistance [11]. An important aspect of exo-

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some research in NPC is concerning its interaction with the Epstein–Barr virus (EBV) [12]. It has been found that exosomes in NPC contain EBV components such as latent membrane protein 1 (LMP1) and latent membrane protein 2A (LMP2A), which contribute to the immunosuppressive tumor microenvironment [13,14]. For example, LMP1 has been shown to inhibit T cell activation and facilitate the evasion of tumor cells from immune surveillance. It also promotes oncogenic signaling pathways such as nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1), enhancing tumor cell survival and proliferation [15]. In addition, the presence of tumor-derived exosomes in NPC not only supports tumor growth and metastatic potential but also interferes with effective immune responses against the tumor. It has been shown that exosomes can carry NPC-specific markers that can be used for early diagnosis and monitoring of disease progression through non-invasive liquid biopsy [16]. These findings highlight the potential of targeting the exosome pathway as a therapeutic strategy for NPC by disrupting signals that contribute to tumor growth and treatment resistance. Thus, deciphering the molecular content of exosomes and their specific role in NPC may pave the way for discovering new diagnostic and therapeutic approaches.

Taxol (generic name of paclitaxel) remains the key medication in the treatment of NPC, especially for patients in advanced stages of the disease. Contemporary research surrounding Taxol is focused on optimizing its use and overcoming the resistance that often occurs during treatment. A previous study has attempted to examine the combined use of nanoparticle albumin-bound paclitaxel (nab-paclitaxel) and cisplatin, a promising drug for metastatic nasopharyngeal cancer treatment. This combined therapy was designed to determine the maximum tolerated dose and improve outcomes in patients who have previously shown resistance to standard therapy [17]. In addition, the combination of paclitaxel with other agents such as carboplatin and cetuximab has been explored as first-line treatment for patients with recurrent and/or metastatic NPC, with the aim to improve remission rates and effectively manage drug resistance [18]. However, paclitaxel resistance remains a major challenge, with approximately 30% of patients developing resistance due to a wide-ranging spectrum of genetic and epigenetic changes.

Aside from that, efforts are underway to better understand these resistance mechanisms and to find new targets to overcome this huge challenge. For example, it has been suggested that targeting specific signaling pathways (the IRAK1-S100A9 axis) may re-sensitize tumors to paclitaxel [19]. Aldehyde dehydrogenase 2 *(ALDH2)* has been found to be a paclitaxel resistance-associated gene, with upregulated *ALDH2* expression highly correlated with paclitaxel resistance [20]. However, the mechanism underlying ALDH2-mediated paclitaxel resistance in NPC remains unclear. Thus, this research was designed to explore the mechanism underlying the resistance to paclitaxel in NPC through exosome-mediated transfer of ALDH2. The major findings of this study suggest that the strategy of targeting ALDH2 may provide a potential therapeutic avenue to overcome paclitaxel resistance in NPC.

Materials and Methods

Cell Culture and Transfection

The human NPC cell line CNE1 was obtained from Shanghai Zhongqiao Xinzhou Biotechnology Co., Ltd. (cat no. ZQ0479, Shanghai, China). According to a previous study [21], paclitaxel-resistant cell line CNE1/Taxol (CNE1-TR) was established by exposing parental CNE1 cells to progressively increasing the concentration of paclitaxel. Roswell Park Memorial Institute (RPMI) 1640 medium (Cat. SH30809.01B, HyClone, South Logan, UT, USA) supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (FBS) (Cat. A3161001C, Gibco, Carlsbad, CA, USA) was used for culturing the cells in a 5% CO_2 incubator at 37 °C. To deplete exosomes, the cells were centrifuged at 200,000 $\times g$ for 18 hours (h). Transwell inserts (Cat. 3422, Costar, New York, NY, USA) were employed to culture the CNE1 and CNE1-TR cells. Specifically, CNE1 cells were seeded at a density of 1×10^5 cells/mL in the lower chamber, and CNE1-TR cells were seeded at a density of 1×10^5 cells/mL in the upper chamber. The upper chamber with a pore size of 0.4 µm allows exosomes to interact with CNE1 cells but prevents cells from invading the membrane. Cells were subjected to STR identification and mycoplasma detection, which showed negative results. Cell transfection of negative control (vector) and small interfering RNA (siRNA) was performed using Lipofectamine 2000 (Cat. 11668500, Invitrogen, Carlsbad, CA, USA). The sequences of the siRNA used in this experiment are shown in Table 1.

Table 1. Primer sequences of siRNAs.

Gene	Primer sequence (5′–3′)
si-*ALDH2*_1	AACCAGATTTTCATAAACAATGA
si-*ALDH2*_2	CTCAAATGTCTCCGGTATTATGC

ALDH2, aldehyde dehydrogenase 2.

Cell Counting Kit-8 (CCK-8) Assay

Cultured cells were seeded into 96-well plates (4×10^3 cells/mL). Then, the plates were placed in an incubator under 37 °C for 48 h after. Briefly, 10 µL of CCK-8 reagent (Cat. DV652, Dojindo Molecular Technologies, Kumamoto, Japan) was added to each well, followed by a 2-hour incubation at 37 °C in the dark. CCK-8 assay kit was employed for measuring cell viability in accordance with the manufacturer's instructions. The absorbance was measured at 450 nm by using a microplate reader (Cat. 1410101, Thermo Fisher Scientific, Waltham, MA, USA).

Table 2. Primer sequences used in RT-PCR experiment.

Gene names	Forward primer (5′–3′)	Reverse primer (5′–3′)
ALDH2	TTGCCTCCCATGAGGATGTGGA	GGTCACTCTCTTGAGGTTGCTG
ABCB1	GCCTGGCAGCTGGAAGACAAAT	CAGACAGCAGCTGACAGTCC
ABCB2	CCAATAGTATGTCAAGCCTGT	ATACGACATCACGGCCTCCA
ABCG2	GTTCTCAGCAGCTCTTCGGCTT	TCCTCCAGACACACCACGGATA
GAPDH	GGAGTCCACTGGTGTCTTCA	GGGAACTGAGCAATTGGTGG

ABCB1, multidrug resistance 1 (MDR1); *ABCB2*, multidrug resistance 2 (MDR2); *ABCG2*, breast cancer resistance protein (BCRP); *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; *RT-PCR*, reverse transcription-polymerase chain reaction.

Finally, the half-maximal inhibitory concentration (IC50) was calculated. The experiments were conducted in triplicate.

Extraction and Validation of Exosomes

To remove exosomes, RPMI 1640 supplemented with 10% FBS was used for culturing the cells. The cells were centrifuged at 200,000 $\times g$ for 18 h to obtain CNE1-TR cell culture medium, which was further centrifuged at 700 $\times g$ for 10 min to collect the supernatant as a conditioned medium. Following the protocol, the medium was supplemented with exosome precipitation solution (Cat. EXOQ5A-1, System Bioscience, Mountain View, CA, USA) and frozen at 4 °C overnight and then further centrifuged at 180 $\times g$ for 30 min at 4 °C and at 700 $\times g$ for 5 min. Next, after resuspending the exosome microspheres in phosphate-buffered saline (PBS) at –80 °C, the exosomes were further resuspended in the cell culture medium (500 µL) for a 48-hour incubation with recipient cells. The exosome-free medium was obtained by centrifugation at 20,000 $\times g$ for 90 min. The concentration of isolated exosomes was measured by using a bicinchoninic acid (BCA) kit (Cat. 23227, Thermo Fisher Scientific, Waltham, MA, USA).

Transmission electron microscopy (TEM) (JEM 1230, JEOL, Tokyo, Japan) was used to characterize the morphology of the isolated exosomes. Briefly, isolated exosomes were added to 200 mesh copper electron microscope grids coated with formvar carbon prior to a 5-minute incubation at room temperature. Next, standard uranyl acetate staining was performed after washing the cells with PBS three times. Transmission electron microscopy (JEM 1230, JEOL, Tokyo, Japan) was used to observe exosomes and measure their diameter. Nanoparticle tracking analysis was conducted to determine the size of isolated exosomes using a ZetaView PMX 110 (Particle Metrix, Meerbusch, Germany), which had been calibrated using 100 nm polystyrene particles.

A specific phosphatidylcholinesterase inhibitor, GW4869, acquired from Sigma-Aldrich (Cat. 6823-69-4, St. Louis, MO, USA) and stored at –20 °C in the dark, was used to assess the effectiveness of the inhibitors in reversing paclitaxel resistance in CNE1 cells induced by exosome-mediated delivery of *ALDH2*. Exosome release was blocked by treating the CNE1-TR cells with a medium containing 10 µM GW4869 within 24 hours after inoculation for 48 hours. Finally, we verified the effect of GW4869 in inhibiting exosome release by comparing the levels of ALDH2 protein and mRNA in exosomes before and after treatment.

Table 3. Antibodies used in this study.

Proteins	Host	kDa	Catalog	Dilution
CD63	Rabbit	32	25682-1-AP	1:1000
TSG101	Rabbit	44	28283-1-AP	1:1000
β-tubulin	Rabbit	54	10094-1-AP	1:10000
ALDH2	Rabbit	56	15310-1-AP	1:5000
MDR1	Rabbit	141	22336-1-AP	1:1000
MDR2	Rabbit	87	27726-1-AP	1:1000
GAPDH	Rabbit	36	81640-5-RR	1:10000

TSG101, Tumor susceptibility gene 101.

RNA Extraction and RT-PCR

TRIzol (Cat. 15596018CN, Invitrogen, Carlsbad, CA, USA) was employed to isolate total RNA, from which cDNA was synthesized using the PrimeScript™ RT reagent kit (Cat. RR047A, TaKaRa, Otsu, Japan) in accordance with the protocol. Relative mRNA expression was quantified using standard reverse transcription-polymerase chain reaction (RT-PCR) using SYBR Premix Ex Taq II (Cat. 640022, Clontech, Otsu, Japan). RT-PCR primers, as presented in Table 2, were obtained from Gene Pharma (Shanghai, China). The mRNA expression was normalized to that of glyceraldehyde 3-phosphate dehydrogenase (*GAPDH)*, and the $2^{-\Delta\Delta CT}$ method was used for calculating the relative expressions for selected genes.

Protein Extraction and Western Blotting

RIPA protein extraction buffer (Cat. P0013B, Beyotime Institute of Biotechnology, Nanjing, China) with protease inhibitors (Cat. P8340, Roche Diagnostics, Indianapolis, IN, USA) was used to extract total proteins from the cultured cells. BCA kit (Cat. 23227, Thermo Fisher

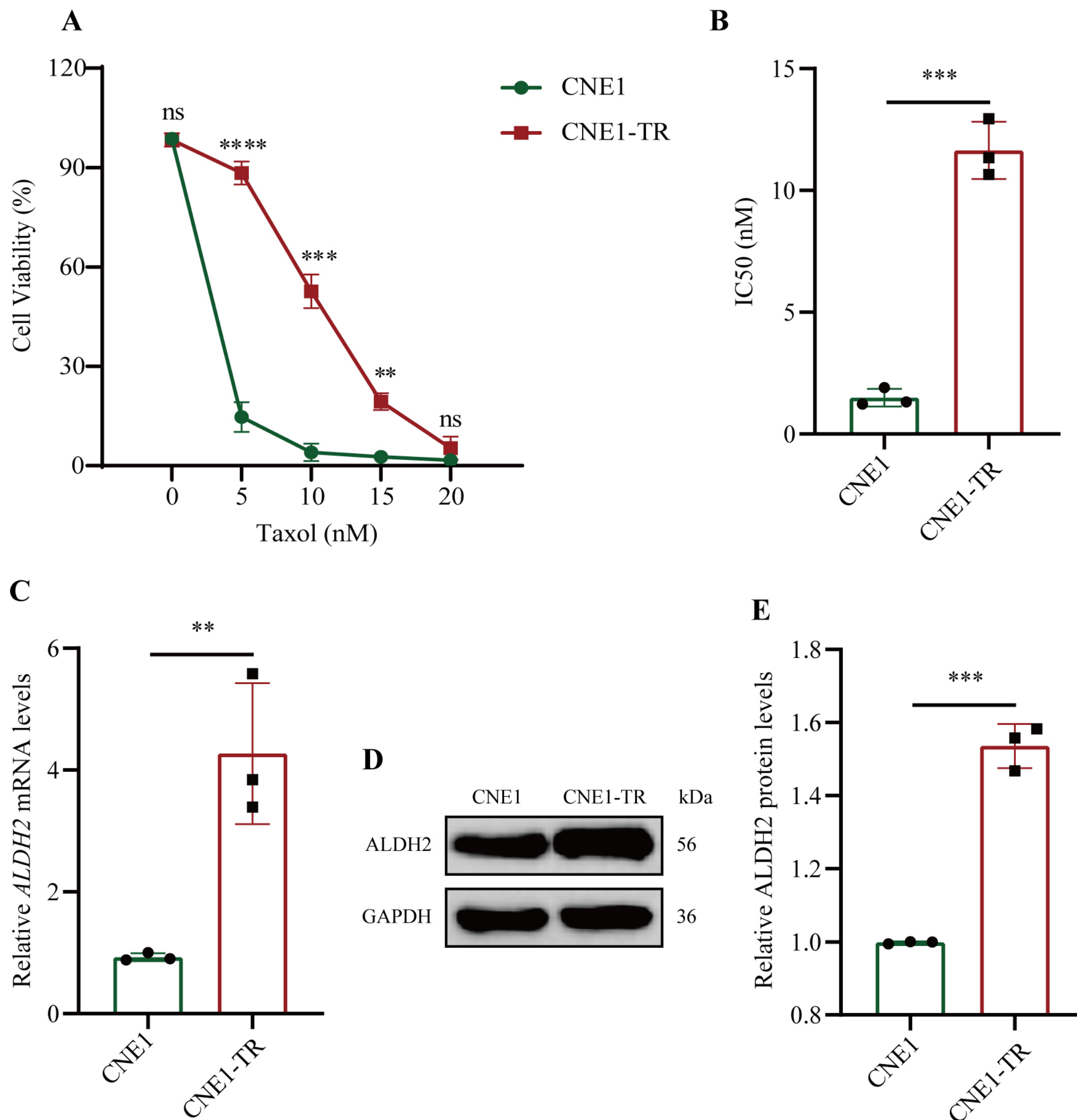

Fig. 1. *ALDH2* is highly expressed in paclitaxel-resistant NPC cells. (A) Viability of CNE1 and CNE1-TR cells under 1, 2, 3, and 4 nM paclitaxel treatment. (B) Half-maximal inhibitory concentration (IC50) of CNE1 and CNE1-TR cells. (C) Expression level of *ALDH2* in CNE1 and CNE1-TR cells. (D,E) Western blotting detection of ALDH2 protein expression levels in CNE1 and CNE1-TR cells. ns $p > 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Scientific, Waltham, MA, USA) was utilized, in conformance with the manufacturer's instructions, to measure protein concentration. Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE; 10%, Cat. P0014B, Beyotime Institute of Biotechnology, Nanjing, China) was used to separate protein (40 µg). The separated proteins on the gel were then transferred onto a polyvinylidene difluoride (PVDF) membrane (Cat. IPVH00010, Millipore, Billerica, MA, USA). The membrane was blocked by 5% nonfat milk at 25 °C for 1 h and further incubated together with the primary antibody overnight at 4 °C. Primary antibodies were obtained from Abcam (Abcam, Cambridge, MA, USA; see Table 3 for all the antibodies used in this study). Next, after washing, these membranes were incubated with the corresponding secondary antibody (1:50,000, sc-2357, Santa Cruz Biotechnology, Dallas, Texas, USA) for 1 h. Finally, Pierce™ ECL Western blotting substrate (Cat. 32109, Thermo Fisher Scientific, Waltham, MA, USA) was used to detect immunoreactive proteins with the ECL detection system, and protein quantification was carried out employing Image J software (Version 1.8.0.112, NIH, Bethesda, MD, USA).

Statistical Analysis

SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0 software (GraphPad software, La Jolla, CA, USA) were employed for data analysis. Analysis of variance (ANOVA) was performed to analyze dif-

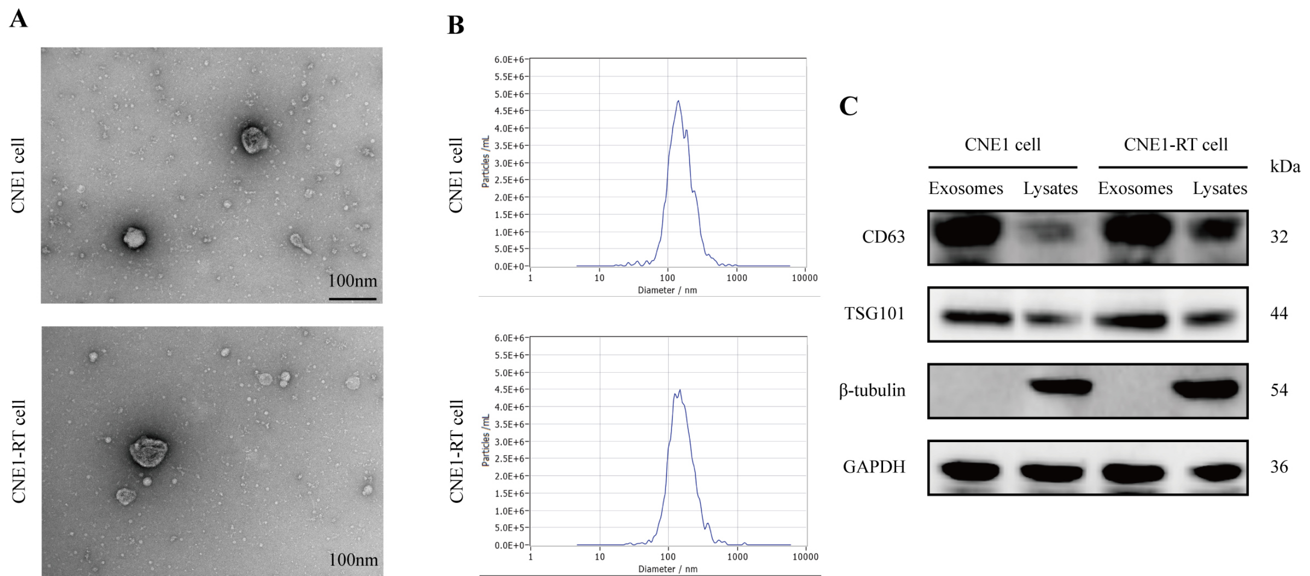

Fig. 2. Exosomes from CNE1 and CNE1-TR cells. (A) Exosomes extracted from CNE1 and CNE1-TR cells were observed by transmission electron microscopy. (B) Size distribution of exosomes in CNE1 and CNE1-TR cells measured by nanoparticle tracking analysis. (C) Protein expression levels of CD63, TSG101 and β-tubulin.

ferences between groups, whereas two-group differences were compared using the Student–Newman–Keuls (SNK) method. A p-value < 0.05 was considered statistically significant.

Results

High Expression of ALDH2 in Paclitaxel-Resistant NPC Cells

First, CNE1 cells were treated under different concentrations of paclitaxel, and we obtained a paclitaxel-resistant CNE1 cell line (CNE1-TR). The cell viability of CNE1 was remarkably reduced under different concentrations of paclitaxel, and the cell viability of CNE1-TR was almost unaffected by paclitaxel treatment (Fig. 1A). The IC50 of CNE1-TR was noticeably higher than that of CNE1 cells, indicating the paclitaxel resistance of CNE1-TR (Fig. 1B). Additionally, the expression level of *ALDH2* was elevated in CNE1-TR cells (Fig. 1C). Compared to CNE1, CNE1-TR cells had much higher protein expression of ALDH2 (Fig. 1D,E). Overall, CNE1-TR cells manifested resistance to paclitaxel and elevated *ALDH2* expression levels. Thus, we postulated that *ALDH2* expression may increase paclitaxel resistance in NPC.

Exosomes Extracted from CNE1 and CNE1-TR Cells

Exosomes play a critical part in the occurrence and progression of certain types of tumors and drug resistance. Therefore, we explored whether paclitaxel-resistant NPC cells release exosomes and assessed whether these exosomes affect the biological behavior of NPC. We used exosome precipitation solution to isolate exosomes from CNE1 and CNE1-TR cells. The morphology of exosomes was analyzed by means of transmission electron microscopy, and their diameter was found to be approximately 100 nm (Fig. 2A). This result was confirmed by the nanoparticle tracking analysis, which found that the initial exosomes isolated from CNE1 and CNE1-TR cells were each 100 nm in diameter (Fig. 2B). We also employed Western blotting to detect specific protein markers of exosomes, such as CD63 and TSG101, derived from CNE1 and CNE1-TR cells. We found that CD63 and TSG101 were overexpressed but β-tubulin was barely detected (Fig. 2C). These results confirmed our success in isolating exosomes from the two types of cells.

Exosome-Mediated Transfer of ALDH2 from CNE1-TR Cells to CNE1 Cells and Promotion of Paclitaxel Resistance

This current study investigated whether exosomes in the CNE1-TR cells could be transferred to CNE1 cells, thereby causing paclitaxel resistance in the CNE1 cells. Exosomes were separated from the two types of cells. The expression of breast cancer resistance protein (BCRP) (ABCG2), Multidrug Resistance 1 (MDR1) (ABCB1), and Multidrug Resistance 2 (MDR2) (ABCB2), which play critical roles in the chemoresistance of NPC, in CNE1 and CNE1-TR cells was detected. We found that CNE1-TR had elevated mRNA and protein levels of ALDH2 and MDR1, with the former manifesting a more profound surge in expression (Fig. 3A–C). To demonstrate that ALDH2 can be transferred through exosomes of CNE1-TR and affects the function of normal NPC cells, we treated CNE1 cells with CNE1-TR exosomes or control for 48 h. The

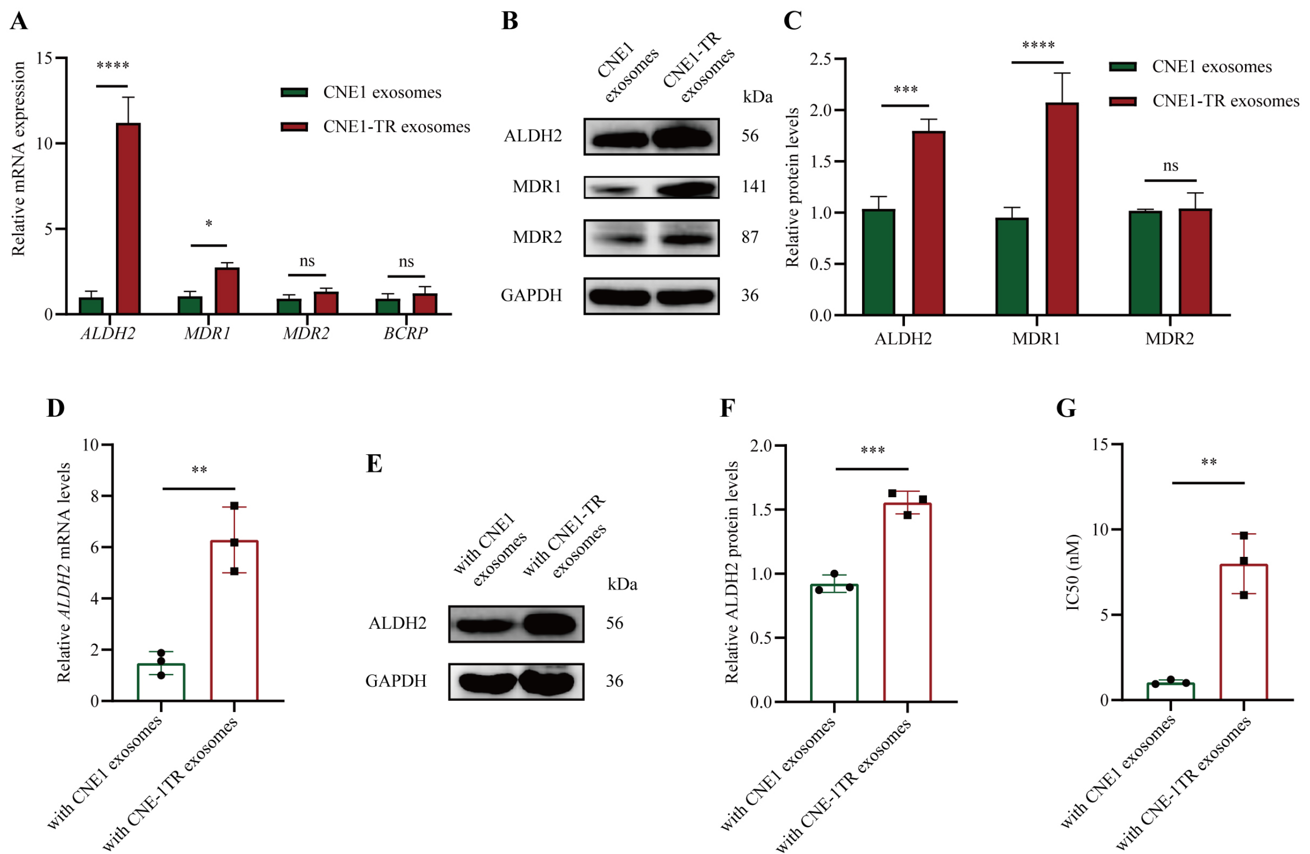

Fig. 3. Exosome-mediated transfer of ALDH2 from CNE1-TR cells to CNE1 cells and promotion of paclitaxel resistance. (A–C) mRNA (A) and protein expression. (B,C) Levels of ALDH2, MDR1, MDR2, and BCPR in CNE1 and CNE1-TR cells. (D) mRNA expression levels of *ALDH2* in CNE1 cells after the addition of CNE1-TR exosomes and control. (E,F) Expression levels of ALDH2 protein in CNE1 cells after the addition of CNE1-TR exosomes and control. (G) IC50 of paclitaxel in CNE1 cells after the addition of CNE1-TR exosomes and control. ns $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant.

mRNA and protein levels of ALDH2 were significantly elevated in CNE1 cells after CNE1-TR exosomes were added (Fig. 3D–F). Importantly, we found that the IC50 value of paclitaxel in CNE1 cells was significantly increased after the addition of CNE1-TR exosomes (Fig. 3G). These findings indicate that exosomes from CNE1-TR cells can efficiently deliver ALDH2, which enhances resistance to paclitaxel in CNE1 cells.

Effect of ALDH2 on Paclitaxel Resistance and MDR1 Expression in CNE1 Cells

The mechanism of exosome-mediated promotion of paclitaxel resistance by ALDH2 was investigated. We examined the mRNA and protein expression levels of ALDH2, MDR1, and MDR2 in CNE1 cells transfected with plasmids overexpressing or inhibiting *ALDH2*. *ALDH2* expression was significantly downregulated in the si-ALDH2 group, while it was significantly upregulated in the p-ALDH2 group. *MDR1* expression was also significantly upregulated in the p-ALDH2 group, while the expression of *MDR2* presented a meager change (Fig. 4A). The results of Western blotting showed a downregulated expression of ALDH2 protein in the si-ALDH2 group and an upregulated expression in the p-ALDH2 group. The expression of MDR1 protein was decreased in the si-ALDH2 group and increased in the p-ALDH2 group (Fig. 4B,C). The p-ALDH2 group also featured a significant increase in IC50 of paclitaxel, which indicates increased cellular resistance to paclitaxel. In contrast, the IC50 decreased in the si-ALDH2 group, indicating that the cells were more sensitive to paclitaxel (Fig. 4D). These results suggest that exosome-mediated ALDH2 transfer promotes resistance to paclitaxel by regulating MDR1 in CNE1 cells.

Suppression of Paclitaxel Resistance in CNE1-TR Cells through ALDH2 Inhibition

To investigate whether ALDH2 regulates MDR1 to enhance the tolerance of CNE1-TR cells to paclitaxel, we performed transfection experiments with si-NC (control) and si-ALDH2 on CNE1-TR cells. Through RT-PCR and Western blotting analysis, we observed that inhibition of *ALDH2* expression resulted in a simultaneous downregulation of the protein and mRNA levels of MDR1; however, the reduction of MDR1 expression level was not as signif-

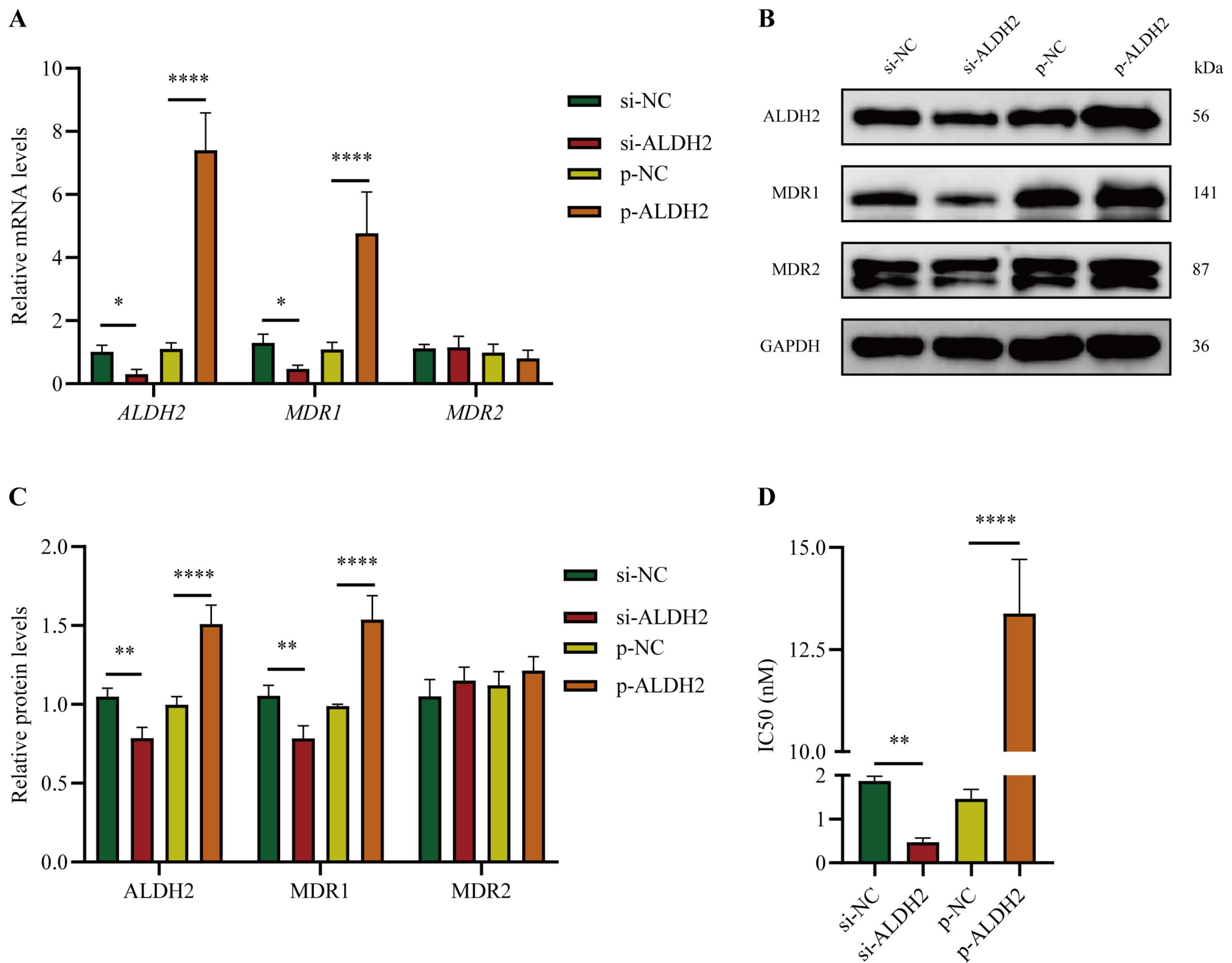

Fig. 4. Effect of *ALDH2* on paclitaxel resistance and MDR1 expression in CNE1 cells. (A–C) Effects of inhibition and overexpression of *ALDH2* on mRNA (A) and protein expression levels (B,C) of ALDH2, MDR1, and MDR2 in CNE1 cells. (D) IC50 of paclitaxel treatment in CNE1 transfected with si-ALDH2, si-NC, p-ALDH2, and p-NC. * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$.

icant as that of ALDH2 (Fig. 5A–C). In addition, the decrease in ALDH2 levels was followed by a significant decrease in the IC50 value of the cells to Taxol, which is a clear indication of elevated drug sensitivity (Fig. 5D). This series of experimental results revealed that ALDH2 effectively reduced paclitaxel resistance in CNE1-TR cells probably by downregulating MDR1 expression.

Reversal of Drug Resistance in CNE1 Cells through Abrogation of CNE1-TR Exosomes Release

This study explored whether paclitaxel resistance in CNE1 cells could be enhanced by exosomes secreted by CNE1-TR cells and tested whether blocking exosome secretion by GW4869 could reverse this resistance. GW4869 acts as an inhibitor of sphingomyelinase and effectively blocks exosome release. Our experimental results showed that the expression of ALDH2 and MDR1 was significantly decreased in CNE1-TR cells after GW4869 treatment, and this change was reflected at the mRNA and protein levels (Fig. 6A–C). In addition, blocking the release of exosomes remarkably reduced the IC50 of paclitaxel, indicating that drug resistance was reversed in CNE1 cells (Fig. 6D). We further co-cultured CNE1 cells with exosomes isolated from CNE1-TR and *ALDH2*-silenced CNE1-TR cells. Upon addition of ALDH2-depleted exosomes derived from CNE1-TR cells, a significant decrease in mRNA and protein levels of MDR1 was observed (Fig. 6E–G), with a corresponding decrease in IC50 of paclitaxel (Fig. 6H). These data suggest that CNE1-TR cells upregulate MDR1 expression by releasing ALDH2-rich exosomes, thereby promoting paclitaxel resistance in CNE1 cells.

Discussion

The current study offers an interesting front that high expression of ALDH2 in paclitaxel-resistant NPC cells plays a potential role in the mechanism of drug resistance. Paclitaxel-resistant CNE1-TR cell lines showed a high de-

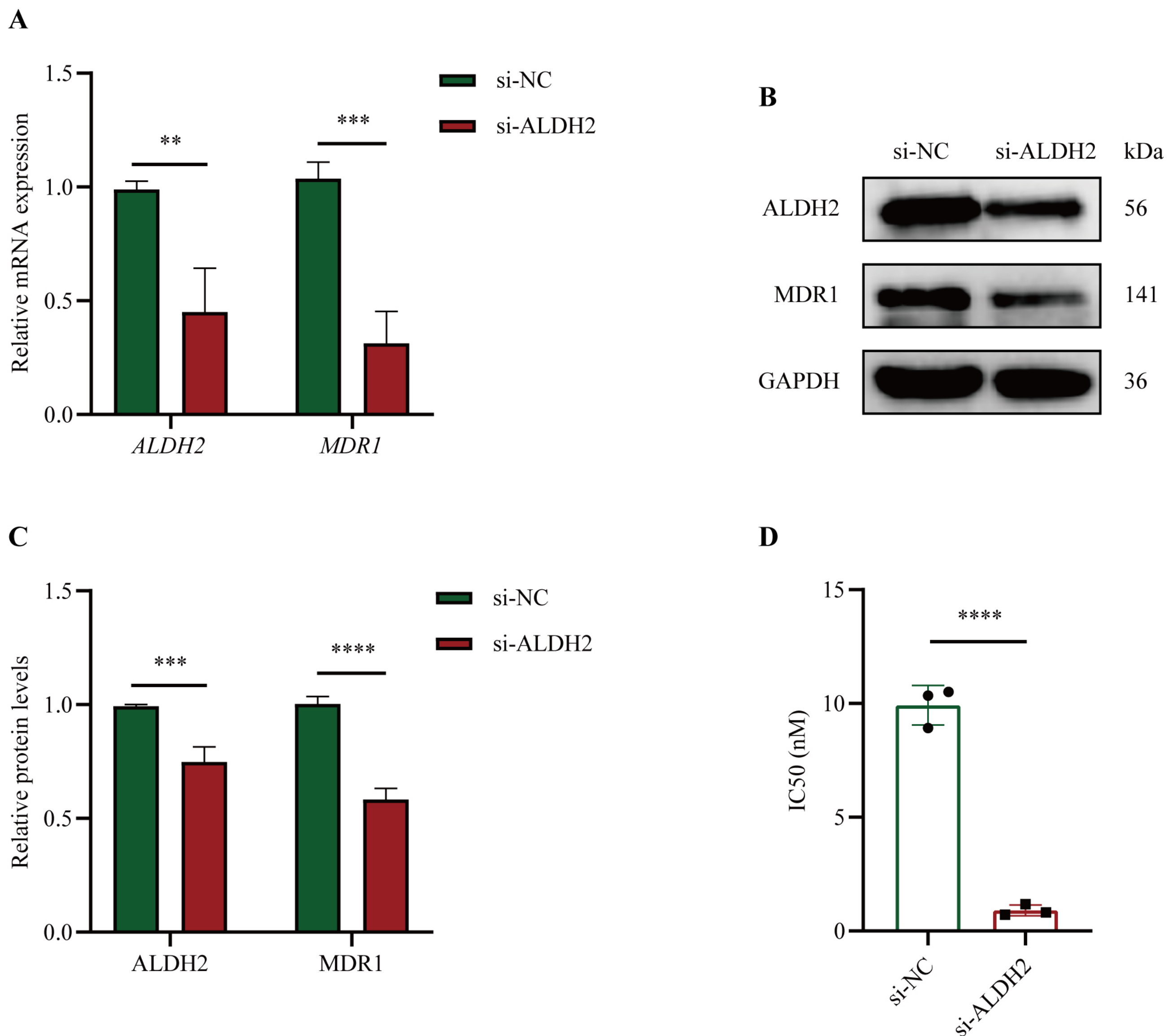

Fig. 5. Inhibition of *ALDH2* expression suppresses paclitaxel resistance in CNE1-TR cells. (A–C) Effect of *ALDH2* inhibition on mRNA (A) and protein expression levels (B,C) of ALDH2 and MDR1 in CNE1-TR cells. (D) IC50 of CNE1-TR transfected with si-NC or si-ALDH2 after paclitaxel treatment. ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

gree of resistance to paclitaxel, as evidenced by their relatively high IC50 value. *ALDH2* showed an upregulation in expression level not only at the mRNA level of CNE1-TR cells but also at the protein level, strengthening the hypothesis that ALDH2 may play a role in paclitaxel resistance. *ALDH2* was found to be a paclitaxel resistance-associated gene, and upregulation of *ALDH2* expression was highly correlated with paclitaxel resistance. It has been reported that under *in vitro* conditions, *ALDH2*-overexpressing NSCLC cells were significantly less sensitive to paclitaxel and displayed enhanced malignant properties such as proliferation and invasion; whereas under *in vivo* conditions, these cells promoted tumor growth and metastasis [20]. Wang *et al.* [22] indicated that the main function of ALDH2 in paclitaxel resistance is to maintain cancer stem cell (CSC) properties and ALDH activity in drug-resistant cancer cells, as well as promote drug resistance. DSF/Cu reversed drug resistance by inhibiting *ALDH2* expression, and the addition of Cu enhanced DSF activity. ALDH2 is an important regulator in ethanol metabolism, and a study has found that *ALDH2*-deficient breast cancer patients treated with paclitaxel, a solvent containing alcohol, experience extreme side effects from paclitaxel [23]. In ovarian, lung and prostate cancers, enrichment of ALDH2 protein was associated with enhanced paclitaxel resistance [24]. This is consistent with our findings that ALDH2 is highly expressed in paclitaxel-resistant NPC cells, a phenomenon that contributes to paclitaxel treatment resistance in NPC.

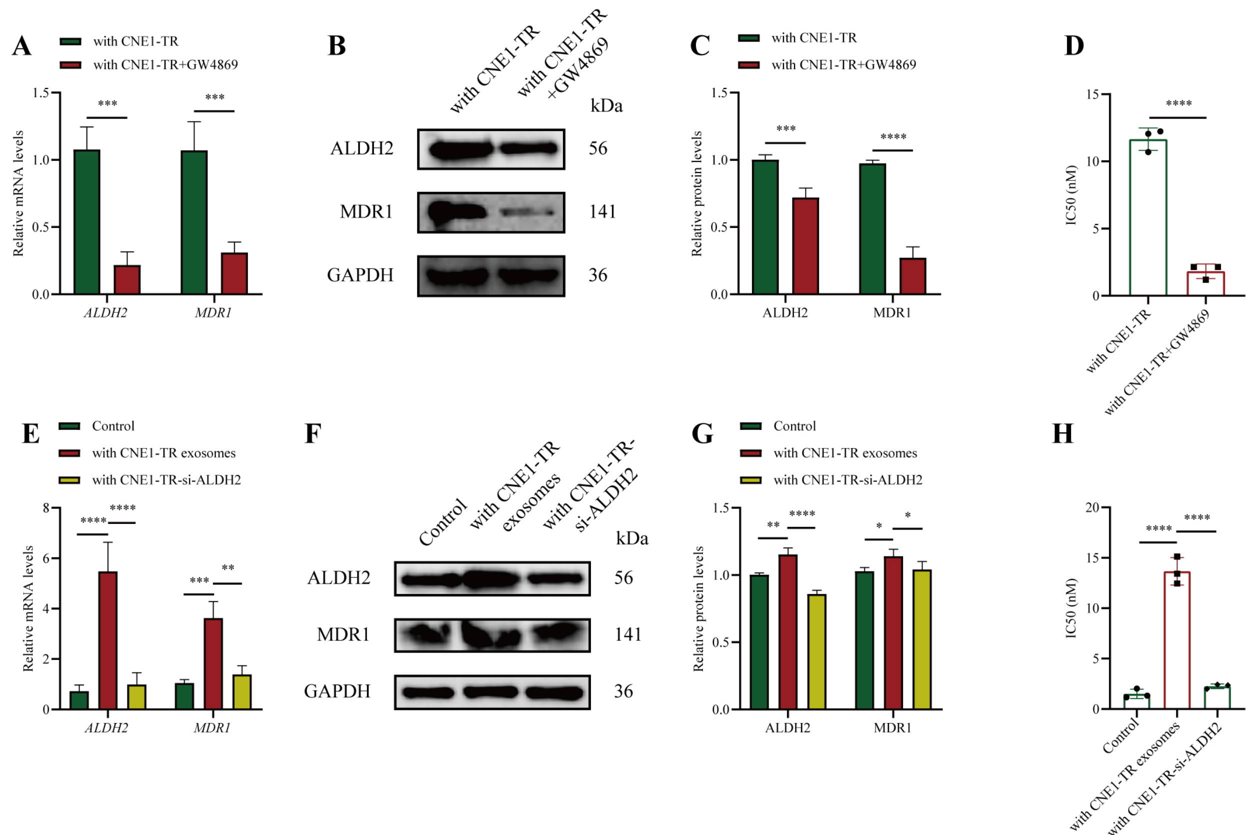

Fig. 6. Blockade of CNE1-TR exosomes release reverses drug resistance in CNE1 cells. (A) Comparison of the mRNA expression levels of *ALDH2* and *MDR1* between GW4869-treated and GW4869-untreated CNE1-TR cells. (B) Western blotting detection of ALDH2 and MDR1 protein expression in GW4869-treated CNE1TR cells as compared to untreated cells. (C) Relative protein levels of ALDH2 and MDR1 in GW4869-treated CNE1-TR cells as compared to untreated cells. (D) IC50 of paclitaxel in GW4869-treated CNE1-TR cells in comparison to untreated cells. (E) *ALDH2* and *MDR1* mRNA expression levels of *ALDH2* and *MDR1* in control cells, cells treated with CNE1-TR exosomes, and cells treated with exosomes from *ALDH2*-silenced CNE1-TR cells. (F) Western blotting detection of ALDH2 and MDR1 protein levels in control cells, cells treated with CNE1-TR exosomes, and cells treated with exosomes from *ALDH2*-silenced CNE1-TR cells. (G) Relative protein expression levels of ALDH2 and MDR1 in cells treated with CNE1-TR exosomes and exosomes from *ALDH2*-silenced CNE1-TR cells. (H) Comparison of paclitaxel IC50 value in control cells, cells treated with CNE1-TR exosomes, and cells treated with exosomes from *ALDH2*-silenced CNE1-TR cells. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Aside from providing a means of intercellular communication, exosomes play a multifaceted role in the dynamics of cancer therapy and drug resistance [25]. These small extracellular vesicles released by most cells contain various biologically active substances, including nucleic acids such as RNA and DNA, proteins, and lipids, which are involved in intercellular communication [26]. Exosomes can contribute to cancer progression by carrying and transferring molecules that promote tumorigenesis, metastasis, and resistance to therapy. Therapeutic resistance, in particular, is a complex process, which is highly influenced by exosomes. They can mediate the confer of drug resistance by delivering functional proteins, RNA, or even exporting active drugs from cells or acting as decoys for therapeutic antibodies [27]. For example, exosomes have been shown to display receptors such as CD20 and HER2 on their surfaces that sequester therapeutic antibodies and protect cancer cells from immune-mediated destruction. They can also influence cell cycle regulation and DNA repair, which are key to building up resistance to chemotherapy and radiation [28]. Exosomes have also been implicated in the maintenance and expansion of CSCs, a subset of cancer cells associated with treatment resistance and tumor recurrence, by providing factors that promote stemness and epithelial mesenchymal transition, which allows non-CSCs to acquire CSC-like properties and contributes to the formation of a more resistant cellular phenotype [29]. The current research demonstrated for the first time that paclitaxel resistance can be conferred from CNE1-TR cells to CNE1 cells via exosomal transfer of key molecules. In particular, exosomes from CNE1-TR cells, after being delivered to CNE1 cells, increased the latter's resistance to paclitaxel, suggesting that ALDH2 in exosomes may play a role in mediating paclitaxel resistance.

Furthermore, by inhibiting *ALDH2* expression, we observed that CNE1-TR cells had downregulated mRNA and protein levels of MDR1, underscoring the importance of ALDH2 in regulating MDR1 expression and influencing paclitaxel resistance. This suggests that strategies targeting ALDH2 may offer a potential therapeutic avenue for overcoming paclitaxel resistance. It has been demonstrated that paclitaxel can be pumped out of cancer cells via MDR1, in a process called the "pump effect"—a mechanism that reduces drug concentration and effectiveness in tumor cells [30]. Therefore, overexpression of MDR1 can directly lead to the excretion of the drug from the cells, reducing drug accumulation and intracellular concentration, thereby resulting in paclitaxel resistance. Further, a study utilizing other cancer cell types, such as ovarian and breast cancer, has confirmed that MDR1 overexpression is associated with increased paclitaxel resistance. For example, in ovarian cancer, resistance mechanisms centered on MDR1 may be directly related to poorer clinical outcomes after paclitaxel treatment [31]. In addition, the regulation of MDR1 expression may be influenced by other cellular components, such as glucose ceramide synthase, which has been shown to upregulate *MDR1* gene expression. This interaction suggests that targeting pathways that control *MDR1* expression may serve as a potential strategy for surmounting drug resistance [32]. According to the current set of results, blocking the release of exosomes using the GW4869 could downregulate the expression levels of *ALDH2* and *MDR1* in CNE1-TR cells, thereby increasing the sensitivity of CNE1 cells to paclitaxel and reversing their resistance to the drug. This further supports the role of exosomes in the build-up of cellular drug resistance, especially those carrying ALDH2.

Nevertheless, this study is not without any shortcomings. First, the current research did not attempt to construct drug-resistant mouse models to explore the mechanism of action of ALDH2 and to test the hypothesis under *in vivo* conditions. Second, more in-depth mechanistic investigations using inhibitors or gene editing methods to explore how ALDH2 exerts drug resistance through MDR1 are lacking.

Conclusion

Overall, this study reveals a possible mechanism underlying paclitaxel resistance in NPC cells involving the regulation of ALDH2 and MDR1 expression. Our findings suggest that CNE1-TR cells promote paclitaxel resistance in CNE1 cells by exosomal delivery of ALDH2, considerably adding to the present knowledge of the complex mechanisms of paclitaxel resistance. Furthermore, this study unveils new potential therapeutic targets for overcoming paclitaxel resistance.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

All authors contributed to this present work: JYY & LRW designed the study, MMZ acquired the data, FFP made substantial contributions to analysis and interpretation of data. FFP improved the figure quality. JYY & FFP drafted the manuscript, JYY & MMZ revised the manuscript. All authors contributed to the important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors participated sufficiently in the work and agreed to be accountable for all aspects of the work. We confirmed that all authors follow the 4 criteria in ICMJE guidelines.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Wong KCW, Hui EP, Lo KW, Lam WKJ, Johnson D, Li L, *et al*. Nasopharyngeal carcinoma: an evolving paradigm. Nature Reviews. Clinical Oncology. 2021; 18: 679–695.
[2] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians. 2021; 71: 209–249.
[3] YUE H, WANG C, ZHU H, DU Q, LI J, OU X, *et al*. SIRT2 interacts with DDX24 to promote nasopharyngeal carcinoma growth. BIOCELL. 2023; 47: 2445–2452.
[4] Jain S, Kaushal M, Bhardwaj M. A case of neck swelling with an unusual presentation. Cytojournal. 2022; 19: 2.
[5] Tang LL, Guo R, Zhang N, Deng B, Chen L, Cheng ZB, *et al*. Effect of Radiotherapy Alone vs Radiotherapy With Concurrent Chemoradiotherapy on Survival Without Disease Relapse in Patients With Low-risk Nasopharyngeal Carcinoma: A Randomized Clinical Trial. JAMA. 2022; 328: 728–736.
[6] Liu Z, Chen Y, Su Y, Hu X, Peng X. Nasopharyngeal Carci-

DM DISCOVERY MEDICINE 2001

noma: Clinical Achievements and Considerations Among Treatment Options. Frontiers in Oncology. 2021; 11: 635737.

[7] Sun X, Su S, Chen C, Han F, Zhao C, Xiao W, *et al*. Long-term outcomes of intensity-modulated radiotherapy for 868 patients with nasopharyngeal carcinoma: an analysis of survival and treatment toxicities. Radiotherapy and Oncology: Journal of the European Society for Therapeutic Radiology and Oncology. 2014; 110: 398–403.

[8] Tsang J, Lee VHF, Kwong DLW. Novel therapy for nasopharyngeal carcinoma–where are we. Oral Oncology. 2014; 50: 798–801.

[9] Perri F, Della Vittoria Scarpati G, Caponigro F, Ionna F, Longo F, Buonopane S, *et al*. Management of recurrent nasopharyngeal carcinoma: current perspectives. OncoTargets and Therapy. 2019; 12: 1583–1591.

[10] Chen YP, Chan ATC, Le QT, Blanchard P, Sun Y, Ma J. Nasopharyngeal carcinoma. Lancet (London, England). 2019; 394: 64–80.

[11] Liu Y, Wen J, Huang W. Exosomes in nasopharyngeal carcinoma. Clinica Chimica Acta; International Journal of Clinical Chemistry. 2021; 523: 355–364.

[12] Miskovic R, Miljanovic D, Cumic MD, Lazarevic I, Banko A. Epstein–Barr virus positive gastric adenocarcinoma with systemic EBV reactivation in a patient with persistently active systemic lupus erythematosus. Oncologie. 2023; 25: 93–97.

[13] Canitano A, Venturi G, Borghi M, Ammendolia MG, Fais S. Exosomes released in vitro from Epstein-Barr virus (EBV)-infected cells contain EBV-encoded latent phase mRNAs. Cancer Letters. 2013; 337: 193–199.

[14] Aga M, Bentz GL, Raffa S, Torrisi MR, Kondo S, Wakisaka N, *et al*. Exosomal HIF1α supports invasive potential of nasopharyngeal carcinoma-associated LMP1-positive exosomes. Oncogene. 2014; 33: 4613–4622.

[15] Deng Y, Liu X, Huang Y, Ye J, He Q, Luo Y, *et al*. STIM1-regulated exosomal EBV-LMP1 empowers endothelial cells with an aggressive phenotype by activating the Akt/ERK pathway in nasopharyngeal carcinoma. Cellular Oncology (Dordrecht). 2023; 46: 987–1000.

[16] Liao C, Liu H, Luo X. The emerging roles of exosomal miRNAs in nasopharyngeal carcinoma. American Journal of Cancer Research. 2021; 11: 2508–2520.

[17] Mai HQ, Chen QY, Chen D, Hu C, Yang K, Wen J, *et al*. Toripalimab Plus Chemotherapy for Recurrent or Metastatic Nasopharyngeal Carcinoma: The JUPITER-02 Randomized Clinical Trial. JAMA. 2023; 330: 1961–1970.

[18] Ueda Y, Enokida T, Okano S, Fujisawa T, Ito K, Tahara M. Combination Treatment With Paclitaxel, Carboplatin, and Cetuximab (PCE) as First-Line Treatment in Patients With Recurrent and/or Metastatic Nasopharyngeal Carcinoma. Frontiers in Oncology. 2020; 10: 571304.

[19] Liu L, Liu S, Deng P, Liang Y, Xiao R, Tang LQ, *et al*. Targeting the IRAK1-S100A9 Axis Overcomes Resistance to Paclitaxel in Nasopharyngeal Carcinoma. Cancer Research. 2021; 81: 1413–1425.

[20] Wang W, Wang J, Liu S, Ren Y, Wang J, Liu S, *et al*. An EHMT2/NFYA-ALDH2 signaling axis modulates the RAF pathway to regulate paclitaxel resistance in lung cancer. Molecular Cancer. 2022; 21: 106.

[21] Peng X, Cao P, Li J, He D, Han S, Zhou J, *et al*. MiR-1204 sensitizes nasopharyngeal carcinoma cells to paclitaxel both *in vitro* and *in vivo*. Cancer Biology & Therapy. 2015; 16: 261–267.

[22] Wang NN, Wang LH, Li Y, Fu SY, Xue X, Jia LN, *et al*. Targeting ALDH2 with disulfiram/copper reverses the resistance of cancer cells to microtubule inhibitors. Experimental Cell Research. 2018; 362: 72–82.

[23] Yagi T, Fujiishi K, Hasegawa A, Otsuka T, Yoshinami T, Nishio M, *et al*. Aldehyde dehydrogenase 2 genotype in tolerability of alcohol contained in paclitaxel in Japanese breast cancer patients. Breast Cancer (Tokyo, Japan). 2019; 26: 229–234.

[24] Kletzmayr A, Clement Frey F, Zimmermann M, Eberli D, Millan C. An Automatable Hydrogel Culture Platform for Evaluating Efficacy of Antibody-Based Therapeutics in Overcoming Chemoresistance. Biotechnology Journal. 2020; 15: e1900439.

[25] Paskeh MDA, Entezari M, Mirzaei S, Zabolian A, Saleki H, Naghdi MJ, *et al*. Emerging role of exosomes in cancer progression and tumor microenvironment remodeling. Journal of Hematology & Oncology. 2022; 15: 83.

[26] Wandrey M, Jablonska J, Stauber RH, Gül D. Exosomes in Cancer Progression and Therapy Resistance: Molecular Insights and Therapeutic Opportunities. Life (Basel, Switzerland). 2023; 13: 2033.

[27] Steinbichler TB, Dudás J, Skvortsov S, Ganswindt U, Riechelmann H, Skvortsova II. Therapy resistance mediated by exosomes. Molecular Cancer. 2019; 18: 58.

[28] Mostafazadeh M, Samadi N, Kahroba H, Baradaran B, Haiaty S, Nouri M. Potential roles and prognostic significance of exosomes in cancer drug resistance. Cell & Bioscience. 2021; 11: 1.

[29] Lin Z, Wu Y, Xu Y, Li G, Li Z, Liu T. Mesenchymal stem cell-derived exosomes in cancer therapy resistance: recent advances and therapeutic potential. Molecular Cancer. 2022; 21: 179.

[30] Maráz A, Furák J, Pálföldi R, Eller J, Szántó E, Kahán Z, *et al*. Roles of BCL-2 and MDR1 expression in the efficacy of paclitaxel-based lung cancer chemoradiation. Anticancer Research. 2011; 31: 1431–1436.

[31] Mozzetti S, Ferlini C, Concolino P, Filippetti F, Raspaglio G, Prislei S, *et al*. Class III beta-tubulin overexpression is a prominent mechanism of paclitaxel resistance in ovarian cancer patients. Clinical Cancer Research: an Official Journal of the American Association for Cancer Research. 2005; 11: 298–305.

[32] Liu YY, Gupta V, Patwardhan GA, Bhinge K, Zhao Y, Bao J, *et al*. Glucosylceramide synthase upregulates MDR1 expression in the regulation of cancer drug resistance through cSrc and beta-catenin signaling. Molecular Cancer. 2010; 9: 145.