OncoTargets and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Transmission of Exosomal TPX2 Promotes Metastasis and Resistance of NSCLC Cells to Docetaxel

Jiaru Hu[1], Qing He[1], Tian Tian[2], Na Chang[3], Liting Qian[1]

[1]Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, 230001, People's Republic of China; [2]Department of Respiratory Oncology, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Anhui Provincial Cancer Hospital, Hefei, 230001, People's Republic of China; [3]Department of Radiation Oncology, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Anhui Provincial Cancer Hospital, Hefei, 230001, People's Republic of China

Correspondence: Liting Qian, Email money2014@ustc.edu.cn

Background: Lung cancer, most of which is non-small cell lung cancer (NSCLC), is the most common tumor in the world, and drug resistance, as a major problem in clinical treatment, has attracted extensive attention. However, the role and mechanism of Targeting protein for Xenopus kinesin-like protein 2 (TPX2), which is highly expressed in NSCLC, is still unclear.

Methods: Bioinformatics analysis was used to analyze the relationship between TPX2 and the clinicopathological features of NSCLC. Stable TPX2 overexpression cell lines with were constructed by lentivirus infection, and the effect of TPX2 on proliferation, migration, invasion and chemoresistance to docetaxel was characterized by the CCK8, wound healing, transwell, colony formation assay and FACS. An in vivo lung homing mouse model was used to further confirmed the role of TPX2 on metastasis. Exosomes were extracted by differential centrifugation from the culture supernatant, and their functions were investigated by co-culture with tumor cells. Gene expression was detected via Western blot and real time PCR (RT-qPCR).

Results: Overexpression of TPX2 was related to the poor prognosis of NSCLC. Promoted migration, invasion and metastasis, and reduced the sensitivity of NSCLC cells to docetaxel. The abundance of TPX2 can be packaged in vesicles and transported to other cells. In addition, overexpression of TPX2 induced the accumulation of β-catenin and C-myc.

Conclusion: Our findings indicated that intercellular transfer of exosomal TPX2 triggered metastasis and resistance against to docetaxel in lung cancer cells, through activating downstream WNT/β-catenin signaling pathway.

Keywords: metastasis, docetaxel, TPX2, exosome, NSCLC

Introduction

Lung cancer is the leading global cause of mortality worldwide,[1,2] it is mainly divided into small cell lung cancer and Non-small cell lung cancer, which constitutes 85% of lung cancer, and the common treatments are surgical resection, chemotherapy and targeted therapy.[2,3] Docetaxel, which has a unique mechanism of inhibiting mitosis, has been widely used as a chemotherapeutic drug to treat lung cancer.[4,5] However, there are still a large number of treatment failure because of drug resistance.[6,7] Therefore, understanding the molecular determinants of the regulation of chemotherapy resistance in NSCLC will provide the basis for molecular targeted therapy.

TPX2 is a microtubule-related protein that is a key aspect in mobile mitosis and spindle assembly.[8–10] In many cancers, TPX2 has been identified as a diagnostic and prognostic marker.[11–14] A large amount of evidence have shown that increased TPX2 can lead to spindle dysfunction, chromosome instability or DNA damage, resulting in a poor prognosis.[8,9] It has been suggested that TPX2 could be used as a marker for poor prognosis in lung adenocarcinoma (LUAD) by data analysis.[15–17] TPX2 overexpression also caused resistance to EGFR TKI and radiation in NSCLC.[18] So, understanding the mechanism of TPX2 has become an important issue.

Received: 14 December 2022
Accepted: 16 March 2023
Published: 27 March 2023

Exosomes are nanosized vesicles (between 30 and 150 nm) that can be ingested. They exist in all biological fluids, mediate cell-to-cell communication, and can also be used as intercellular carriers for various biomolecules, including proteins, mRNA, microRNA and DNA.[19,20] Studies have shown that tumor cells release exosomes at a significantly higher rate, and exosomes can carry active proteins or RNA, transmit and activate downstream pathways between cells or affect the expression of target cell proteins and play a key function in events related to tumor growth and metastasis.[21–23]

In this study, we determined the expression of TXP2 and its relationship with the TMN stage and survival of patients in NSCLC. We also investigated its effect on drug resistance, and interestingly, we found that exosomes play a transmission role during this process. Meanwhile, we explored the potential molecular mechanism. Our results may shed light on the role of TPX2 in the progression of NSCLC and help develop potential strategies for clinical treatment.

Materials and Methods

Cell Lines and Cell Culture

The lung cancer cell lines CALU-3, pc-9, H1650, H1299, H1975 and A549 were provided by the China Cell Resource Center. All cell lines were negative for mycoplasma. A549, H1650, PC-9 and H1299 cells were cultured under normal conditions (37 °C, 5% CO2) with RPMI medium modified (cat. #SH30809.01, Cytiva) +1% (v/v) penicillin–streptomycin (cat. #C0222, Beyotime) and 10% (VBV) heat inactivated fetal bovine serum (Gibco). Calu-3 and H1975 cells were cultured under normal conditions in DMEM (cat. #SH30022.01, cytosol) +1% (v/v) penicillin–streptomycin and 10% (v/v) heat-inactivated fetal bovine serum supplemented with high glucose and glutamine.

Data Collection and Bioinformatics Analysis

The level 3 HTSeq FPKM format RNA-sequencing data of non–small cell lung cancer patients, including 108 normal and 1037 tumor samples, was downloaded from the TCGA data portal (https://portal.gdc.cancer.gov/), then was transformed into TPM and log2-transformed for subsequent analyses. The non-small cell lung cancer dataset from TCGA was used by combing TCGA LUAD and TCGA LUSC datasets. The clinical data of these patients were also obtained from the TCGA. The receiver operating curve (ROC) was used to determine the diagnostic accuracy of TPX2 expression in differentiating normal tissues from NSCLC tissues. The ROC curve was created, and the area under the curve (AUC) value was calculated using the R package "pROC" (v.1.17.0.1). An AUC value greater than 0.9 indicates excellent predictive power. The RNA sequencing data (TPM values) in TCGA-TARGET-GTEx cohort were obtained from UCSC XENA (https://xenabrowser.net/datapages/) and used to run pan-cancer analysis of TPX2 expression between normal and tumor tissues.

Gene Set Enrichment Analysis (GSEA) was performed to elucidate differential pathways between high- and low-expression groups according to the median TPX2 expression level. Firstly, differential gene expression analysis between these two groups was performed using DESeq2 (v.1.18.1) to generate a gene list. Secondly, the sorted (by log fold change of expression level) gene list was applied for GSEA by "clusterProfiler" (v.3.14.3) package using the gene-set h.all.v7.2.symbols.gmt. The analysis was based on 1000-times permutations. The pathways were considered to be significantly enriched when adjusted p-value < 0.05 and FDR q-value < 0.25.

All analytics and plots in the bioinformatic analysis were generated using R (v.3.6.3).2.3 Gene Overexpression Experiment

The lentivirus packaged with TPX2 expression vector or negative control vector (vector as NC) was purchased from Hanheng organism. To infect the target cell line, the cell culture medium diluted to 0.5 mL was spread in a 24-well plate, and the culture medium was removed after adhering to the wall. Then, 0.25 mL of medium diluted with 10 μL lentivirus and polybrene (the final concentration was 8 μg/mL) was replaced for 4 hours and then added to 0.5 mL. Once the cells reached 70% confluence, the selection began: the cells were added to culture medium containing 0.5 μg/mL puromycin (Gibco) and replaced every 2 to 3 days. Once the cells recovered and expanded, the expression of TPX2 was verified by WB and RT–qPCR.

Western Blot

NSCLC cell lines' proteins were extracted with lytic buffer (cat.# P0015A, Beyotime). The protein was separated by SDS–PAGE and transferred to PVDF membranes (0.22 μm microwell). The membrane was blocked in 5% skim milk for 1 hour, incubated overnight with specific antibodies at 4 °C, and then incubated with a horseradish peroxidase-conjugated secondary antibody (Proteintech). The signal band was detected with an ECL kit (Thermo). All the antibodies used in this article are shown in Table 1. The pixel density of the protein bands was analyzed by ImageJ software. The ratio of protein strength to GAPDH protein strength or whole protein strength was calculated and expressed as a percentage.

RT–qPCR

Total RNA was extracted by TRIzol reagent (cat.# R401-01, Vazyme). A HiScrip ®II First Strand gene synthesis kit (cat.# R211-01, Vazyme) was used to synthesize cDNA. In line with the manufacturer's instructions, SYBR Green (cat.# C0008, TargetMol) was used to quantify RT–qPCR gene expression. GAPDH was used as the endogenous control. Table 2 lists the primer pairs for each target gene. Finally, the relative expression was calculated using $2-\Delta\Delta CT$.[24]

Wound Healing Assay

The cells (1.5×105) used for wound healing analysis were inoculated in a 24-well plate. When the cell confluence reached approximately 80%, the cell layer on each culture plate was scratched with the tip of a 200 μL pipette to form scratches. After injury, the cells were washed with PBS, and the debris was removed. The wound culture was cultured in medium without FBS. An Olympus IX73 inverted microscope was used to image wound healing every 24 hours for 48 hours. Wound healing ability was determined by measuring the average migration distance using Prism 8 statistics. The experiment was carried out three times.

Transwell Migration and Invasion Assays

Cell migration analysis was carried out in a 24-well tissue culture plate using a transwell (cat. #353097, BD Falcon, USA) membrane filter plug-in. The NSCLC cells to be detected were trypsinized and suspended in serum-free medium. Then, the cells were inoculated in the upper transwell chamber, and medium containing 10% FBS was added to the lower chamber. After incubating at 37 °C for 24 hours, the cells were stained with crystal violet. The unmigrated cells within

Table 1 Antibodies Used in This Study

Atibody	Supplier	Working Dilutions
Anti-GAPDH	Proteintech (cat.# 60004-1-Ig)	1:5000
Anti-TPX2	Proteintech (cat.# 11741-1-AP)	1:2000
Anti-C-myc	Cell Signaling (cat.# 18583)	1:1000
Anti-β-catenin	Abmart (cat.# M24002)	1:1000
Anti-CD63	Cell Signaling (cat.# 52090)	1:1000
Anti-tsg101	Cell Signaling (cat.# 72312)	1:1000
IgGs, rabbit and mouse	Proteintech	1:5000

Table 2 Primer Sequences Used for RT–qPCR Experiments from Generalbiol

Gene	Forward Primer 5′-3′	Reverse Primer 3′-5′
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
TPX2	ATGGAACTGGAGGGCTTTTTC	TGTTGTCAACTGGTTTCAAAGGT
WNT6	GGCAGCCCCTTGGTTATGG	CTCAGCCTGGCACAACTCG
WNT5B	GCTTCTGACAGACGCCAACT	CACCGATGATAAACATCTCGGG
C-myc	GGCTCCTGGCAAAAGGTCA	CTGCGTAGTTGTGCTGATGT

the upper cavity were eliminated, and the migrated cells were imaged via an Olympus IX73 microscope. ImageJ software was used to analyze and count and Prism 8 statistical data. For intrusive analysis, BioCoatMatrigel (cat.#356234, CorningBioCoat) cross-hole inserts are used, and the process is similar to the migration evaluation and conforms to the manufacturer's instructions.

In vivo Metastasis Model

Female BALB/c nude mice (4-week-old) was purchased from Hangzhou Ziyuan Laboratory Animal Technology Co., Ltd. To construct an in vivo lung transfer model, each nude mouse is injected with 100 μL vector or TPX2 overexpressed A549 cells (1×10^6 cells per mouse, n=5 per group) respectively through the tail vein. Four weeks after injection, mice are killed and metastatic lung tumors are analyzed. All animal research procedures are carried out with the approval of the Ethics Committee of the Animal Laboratory Center of the First Affiliated Hospital of University of Science and Technology of China.

Hematoxylin and Eosin (H&E) Staining

First, put the tissue sections into a drying oven and bake the pieces at 66 °C for 20–30min. It is then treated three times sequentially with xylene and ethanol. Slowly rinse and slice under running water until it is clean and transparent, then soak with hematoxylin. Bluing of saturated lithium carbonate solution and redness of eosin dyeing solution. Dehydrated with 95% ethanol several times, and then transparent sections with phenol-xylene, xylene I and II. Finally, a neutral gum seal is used and the results are observed under a microscope.2.10 Proliferation and Docetaxel Sensitivity Assays.

The CCK-8 assay (cat. #C0005, TargetMol, Shanghai, China) and the colony formation assay were both used to monitor cell viability and sensitivity recovery. To quantify the cell growth rate, 2000 cells were inoculated in 96-well plates. At 24, 48, 72 and 96 hours after cell attachment, CCK-8 solution was added to the cells and then cultured for 2 hours. The absorbance value (OD) at 450 nm was measured using a universal microplate spectrophotometer. For the drug sensitivity test, docetaxel sensitivity was evaluated by docetaxel treatment in 96-well plates for 72 hours before adding CCK-8 solution.

For the colony formation test, the cells of different treatments were resuspended and inoculated into each hole of the six-well plate with the same number of cells. The cells were treated with docetaxel for 72 hours and then cultured in new medium without docetaxel. After being cultured for the same time, the clones were fixed with methanol and then stained with crystal violet.

Apoptosis Analysis

To study apoptosis, an Annexin V-Alexa Fluor660/PI (cat.# BB-41037-50T MagneBestbio) kit was used. After washing the tumor cells with PBS twice, 5×10^5 cells were suspended in 100 μL 1× binding buffer and incubated in the dark with 5 μL Annexin V-AF660 for 10 min, and then 10 μL PI was added for 3 min. Then, 400 UL PBS was added prior to detection and immediately measured by flow cytometry (LSRFortessa, BD Biosciences, USA), and 10,000 signals were collected from each sample. FlowJo software was used for further analysis.

Isolation of Exosomes by Differential Centrifugation

The cells were cultured in conventional medium until 80% confluence, and then the medium was replaced with RPMI-1640 without FBS. The cell supernatant was collected directly after 48 h. The samples were centrifuged at 3000×g for 30 min at 4 °C to remove cells and cell debris. The supernatant was transferred into ultracentrifuge tubes and centrifuged at 16,000×g for 1 h in an L-100xp ultracentrifuge (Beckman, USA) equipped with a Type 70 rotor. The supernatant was centrifuged again at a rate of 100,000×g for 1 h. The supernatant was discarded, resuspended in PBS, and filtered into a 1.5 mL ultra-detachment tube with a 0.2 μm membrane. Then, the samples were centrifuged at 100,000×g for 1 h in an Optima MAX-XP ultracentrifuge (Beckman, USA) equipped with a TLA55 rotor, the supernatant was discarded, and exosome samples were obtained by resuspension with 20 μL PBS.[25] Exosomes samples (20 μL) were obtained from the initial 20 mL supernatant, and the exosome protein concentration was determined with a two-inch nickel acid (BCA) detection kit (Beyotime). Exosome preparations can be stored at −80 °C for 30 days. The morphological characteristics of

exosomes were observed by transmission electron microscopy (Tecnai G2 F20). The size distribution of the Exosomes body was measured with a nanoparticle tracking analyzer (PMX220, Particle Metrix, Germany). The expression levels of tsg101 and CD63, representative exosome-associated positive markers, were detected by Western blotting.

Statistical Analysis

Student's *t*-test and two-tailed χ^2 test methods were used to determine statistical significance. Data with $p < 0.05$ were considered statistically significant.

Results

TPX2 is Upregulated in NSCLC and is Associated with Poor Prognosis

The pan-cancer analysis showed that compared with the normal tissues, the expression level of TPX2 was significantly higher in most types of cancers, including NSCLC (Figure 1A and C). The results were furthered validated in paired cancer- corresponding normal tissues (Figure 1B and D). In addition, the ROC curve indicated that TPX2 expression had a good predictive power with an area under the curve (AUC) of 0.981 (95% confidence interval [CI] = 0.973–0.988) to discriminate NSCLC tissues from normal tissues (Figure 1E). Meanwhile, we found that high expression of TPX2 was significantly associated with advanced pathologic stage, T stage, N stage, smoking, shorter overall survival (OS), and disease-specific survival (DSS) events (Figure 1F–K). These results indicated that TPX2 acting as an oncogene, might be participated in the occurrence and development of NSCLC.

TPX2 Promotes the Invasion and Migration Ability of NSCLC Cells

To investigate potential function of TPX2 in the progression of NSCLC, firstly the expression of TPX2 in NSCLC cell lines was determined by Western blot. A549 and H1299, two TPX2 lowly expressing cell lines were selected for further experiments (Figure 2A). Then TPX2 was overexpressed in H1299 and A549 by lentivirus transfection, RT–qPCR and Western blotting analysis showed that the mRNA and protein expression level of TPX2 was significantly increased (Figure 2B and C). Subsequently, we carried out wound healing experiments and transwell assays to determine the effect of TPX2 upregulation on cell invasion and migration. The results showed that compared with the control group, the migration and invasion abilities of A549 OE-TPX2 cells were significantly enhanced (Figure 2D–F), and similar results was confirmed in H1299 cells (Figure 2G–I), which was consistent with previously report.[26] To further examine the role of TPX2 in metastasis in vivo, we assessed the metastatic nodules in the lungs in nude mice, which were injected with A549 cells into tail vein. Ectopic overexpression of TXP2 markedly increased the lung homing potential of A549 cells (Figure 2J and K). However, overexpression of TPX2 did not affect the proliferation of both two NSCLC cell lines (Figure 2L and M). All these results demonstrated that upregulation of TXP2 could promote migration, invasion and metastasis in NSCLC.

TPX2 Promotes the Resistance of NSCLC Cells to Docetaxel

Docetaxel is a microtubule depolymerization inhibitor which arrests the cell cycle at G2/M and leads to cell apoptosis, thus killing cancer cells. It has been widely used a second-line chemotherapeutic for treatment of patients with locally advanced and metastatic non-small cell lung cancer.[4] TPX2, a microtubule-associated protein that mediates spindle assembly during mitosis, is speculated to affect the response of cells to docetaxel. To test this hypothesis, the sensitivity to docetaxel of NSCLC cells after TXP2 overexpression was evaluated by the CCK-8 assay. The results showed that the IC50 value of TPX2-overexpressing cells was significantly higher than that of negative control cells (Figure 3A and B), suggesting that TPX2 desensitized NSCLC cells to the cell death triggered by docetaxel. Besides, colony formation experiments showed that the colony formation ratio was significantly higher in TPX2 overexpressing A549 and H1299 cells after docetaxel treatment than in control cells (Figure 3C and D). Both suggested the promoting role of TPX2 in NSCLC cells against to docetaxel.

In addition, to examine the effect of TXP2 on apoptosis, cells were treated with docetaxel or not for 72 h, then detected by flow cytometry. We found that TPX2 did not inhibit the apoptosis individually but only in combination with

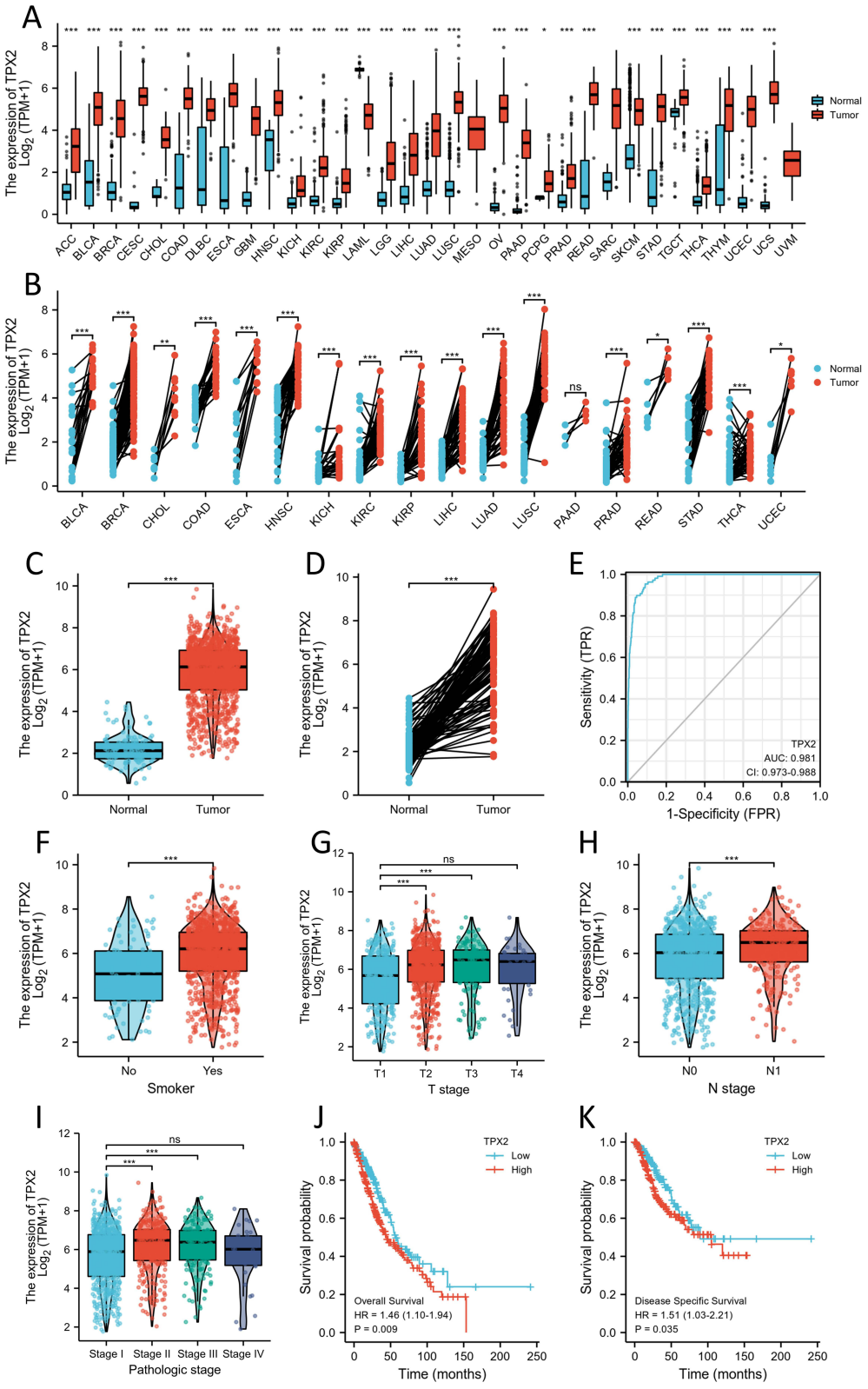

Figure 1 TPX2 expression level in NSCLC and its relationship with clinicopathological features. (**A**) Expression of TPX2 in different tumor types compared to nonmatching normal tissues in TCGA and GTEx databases. (**B**) TPX2 expression in different tumor types compared to matched normal tissues in TCGA and GTEx databases. (**C**) TPX2 expression in NSCLC and nonmatching normal tissues in TCGA databases. (**D**) TPX2 expression in NSCLC in TCGA database with matching normal tissues. (**E**) ROC curves for classifying NSCLC versus normal breast tissues in the TCGA database. (**F**–**K**) Data are shown for (**F**) smoker; (**G**) T stage; (**H**) N stage; (**I**) Pathologic stage; (**J**) Overall Survival; and (**K**) Disease Specific Survival. *p<0.05, **p<0.01 and ***p<0.001.

Abbreviations: TCGA, The Cancer Genome Atlas; GTEx, Genotype Tissue Expression Project; ROC, receiver operating characteristic; OS, overall survival; DSS, disease-specific survival.

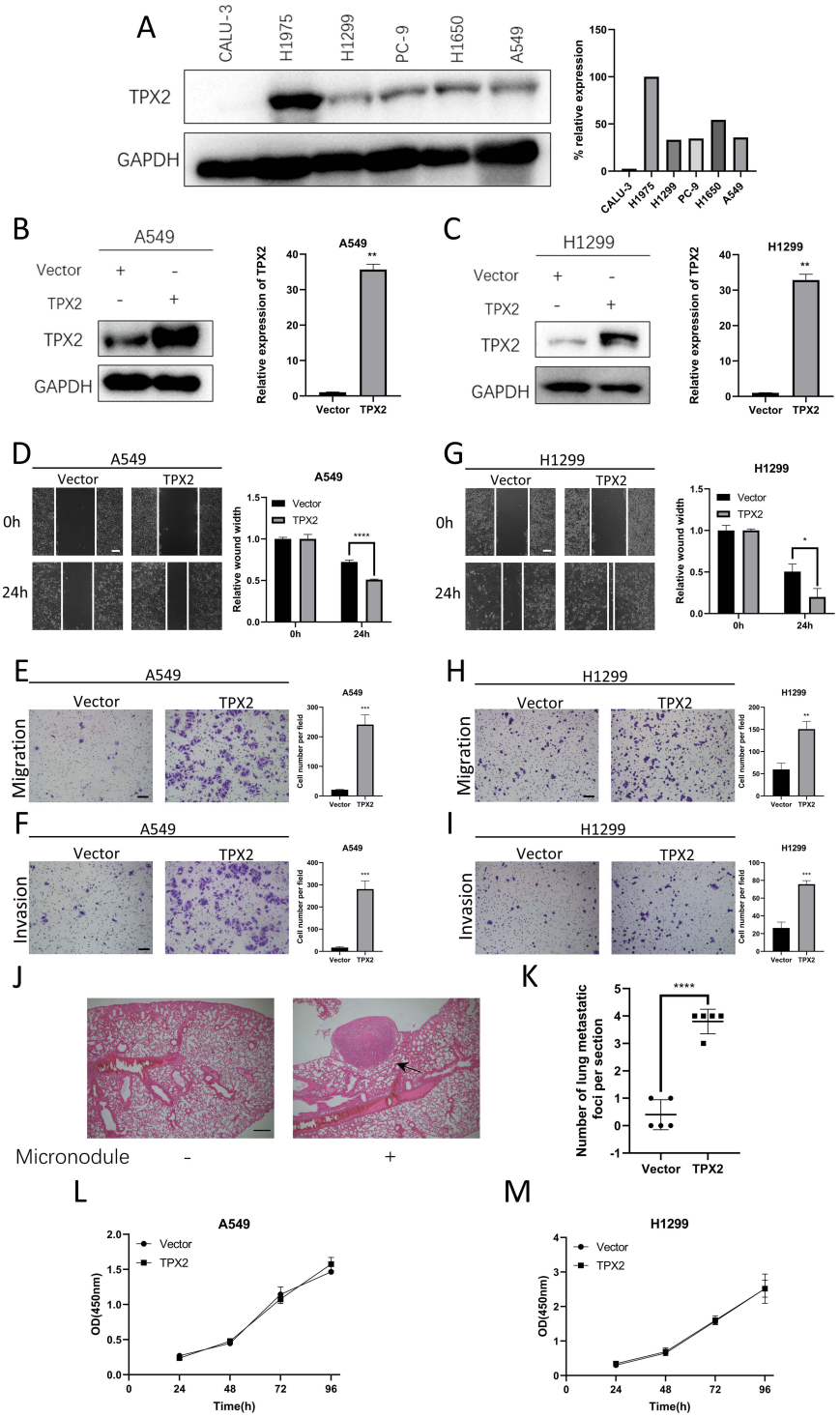

Figure 2 TPX2 overexpression promoted the migration and invasion abilities of NSCLC cells. (**A**) Western blotting analysis of TPX2 expression in six NSCLC cell lines. (**B** and **C**) The overexpression efficiency of TPX2 in A549 (**B**) and H1299 (**C**) was identified at the protein and RNA levels using Western blotting (left) and RT–qPCR (right). (**D**–**F**) Effects of TPX2 overexpression on wound healing (**D**), transwell migration (**E**) and invasion (**F**) Abilities of A549 cells. Scale bar: 100 μm. (**G**–**I**) Effects of TPX2 overexpression on wound healing (**G**), transwell migration (**H**) and invasion (**I**) abilities of H1299 cells. Scale bar: 100 μm. (**J**) H&E staining showing representative photos of positive and negative metastatic nodules in the lung. Scale bar: 200 μm. (**K**) The scatterplot shows the number of lung metastatic foci per mouse. (**L** and **M**) Growth curve of A549 (**L**) and H1299 (**M**) cell lines (Vector represents negative control of overexpression, TPX2 represents overexpression of the TXP2 gene). Each bar displays the mean±SD of 3 independent experiments as analyzed by paired two-tailed students, *t*-test. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.

https://doi.org/10.2147/OTT.S401454 DovePress

docetaxel treatment (Figure 3E and F). In summary, these results strongly pointed out that TPX2 triggered drug resistance in NSCLC cells.

TPX2 Can Affect the Expression of TPX2 in Surrounding Cells Through Exosomes

At present, there is a great deal of evidence showed that DNA, mRNA and protein can be transmitted between cells through exosomes, thus affecting the function of tumor cells. It is also commonly used in the diagnosis and treatment of NSCLC.[27,28] The exosomes in the conditional medium were isolated by differential centrifugation and characterized by transmission electron microscopy (TEM) (Figure 4A and B), nanotracker (NTA) (Figure 4C and D) and Western blot analysis (positive marker: CD63, TSG101) (Figure 4E). At the same time, the RT–qPCR results showed that the level of TPX2 mRNA in exosomes from cells overexpressing TPX2 was significantly higher than that in exosomes from control cells (Figure 4F). This indicates that abundant TPX2 mRNA can be packaged into vesicles and transported outside of the cells.

Next, we studied whether TPX2 in the exosomes can be untaken by the surrounding cells. A549 cells were co-incubated with exosomes derived from different sources, 24 hours later, cells were collected for TXP2 expression examination by Western blot. We found that TXP2 protein level was significantly increased in A549 cells treated with TXP2-overexpressing exosomes than control exosomes (Figure 4G). Consistent results were obtained in H1299 cells (Figure 4H). Taken together, the abundant TXP2 in NSCLC cells could be transferred intracellular via exosomes.

TPX2 Derived from Exosomes Can Affect the Motility and Drug Sensitivity of NSCLC Cells

Next, we wondered whether TXP2 derived from exosomes could induced similar phenotypes as overexpressed by lentivirus transfection. Exosomes isolated from A549-NC and A549 TPX2-overexpression cells were incubate with A549 and H1299 cells. Then the migration, invasion, drug sensitivity to docetaxel and apoptosis of NSCLC cells were

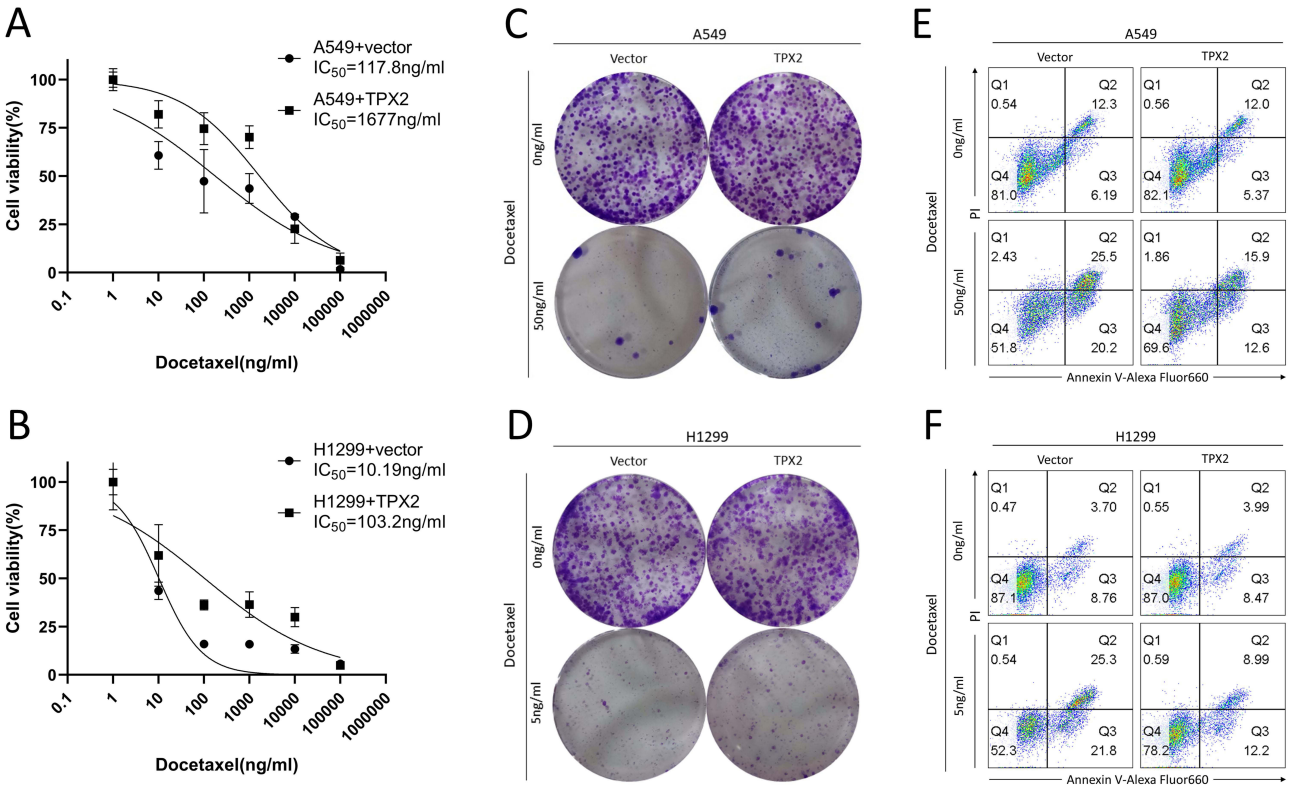

Figure 3 TPX2 overexpression reduced sensitivity to docetaxel. (**A** and **B**) Dose–response docetaxel viability curves in A549 (**A**) and H1299 (**B**) cell lines. Cell viability was estimated by CCK-8 reagent after 72 h of drug exposure. (**C** and **D**) A colony formation assay was used to analyze the clone formation ability of A549 (**C**) and H1299 (**D**) cell lines treated with 50 ng/mL docetaxel or not. (**E** and **F**) Flow cytometry was used to detect apoptosis in A549 (**E**) and H1299 (**F**) cell lines after treated with docetaxel for 72 h.

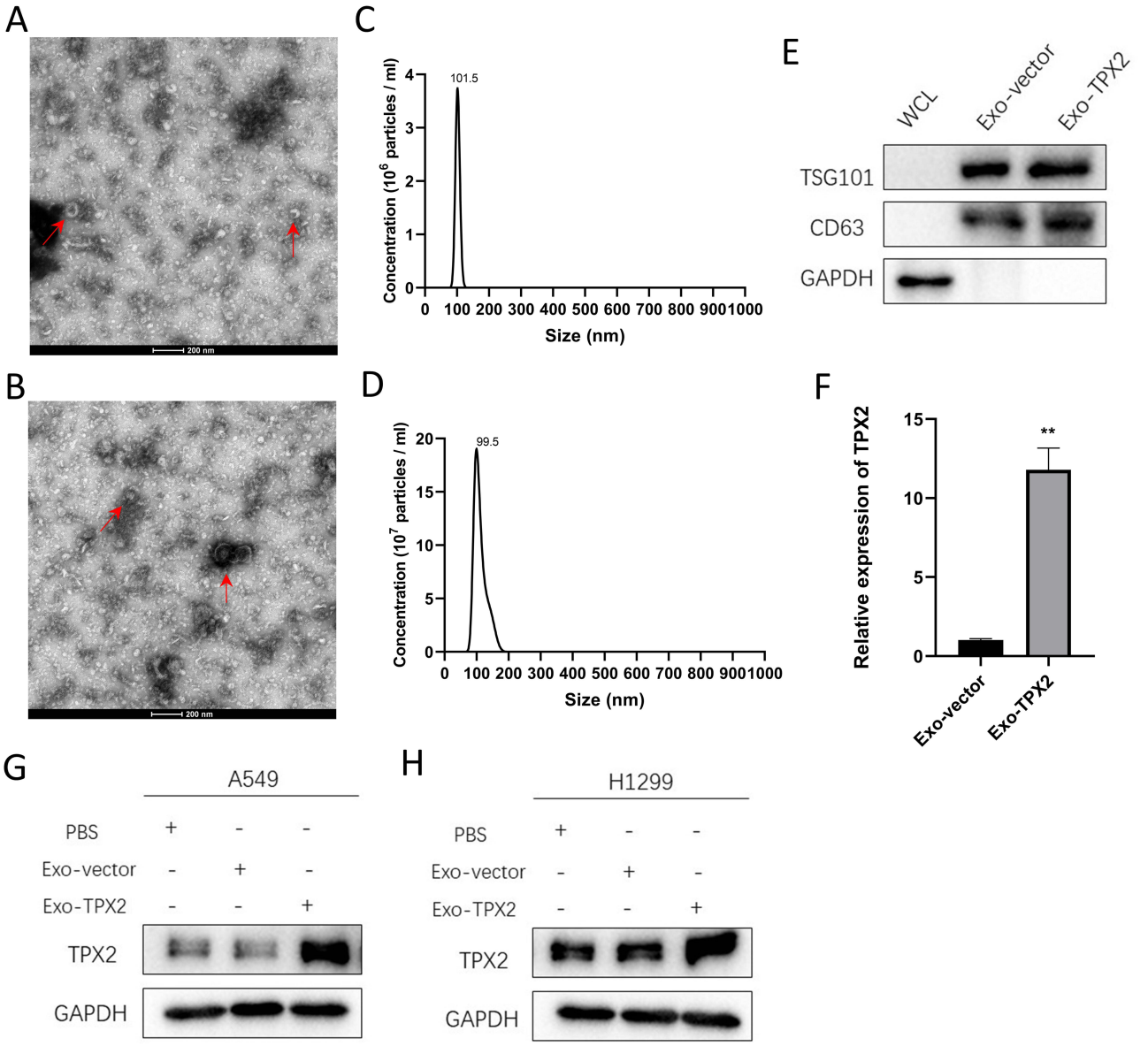

Figure 4 TPX2 can be transferred intercellularly through exosomes. (**A** and **B**) Transmission electron microscopy (TEM) images of exosomes isolated from A549 cells (Vector: A, TPX2: (**B**). Red arrows indicate exosomes. Scale bar: 200 μm. (**C** and **D**) The size distribution of exosomes determined from A549 cells (Vector: A, TPX2: (**B**) by nanoparticle tracking analysis (NTA). (**E**) Western blot analysis of exosome markers (tsg101 and CD63) in equivalent amounts of protein from A549-derived exosomes and A549 whole cell lysates (WCL) (as a control). (**F**) The amount of TPX2 mRNA in exosomes isolated from cell supernatant was detected by RT–qPCR. (**G** and **H**) Western blot analysis of TPX2 expression in A549 (**G**) and H1299 (**H**) cells after incubation with exosomes. Data were statistically analyzed with paired two-tailed students, *t*-test. **p<0.01.

determined by wound healing (Figure 5A and B), transwell (Figure 5C–F), CCK-8 assay (Figure 6A and B), colony formation experiments (Figure 6C and D) and flow cytometry (Figure 6E and F). Results showed that exosomal TPX2 could promote the migration, invasion and drug resistance to docetaxel, but inhibited apoptosis in recipient A549 and H1299 cells. Altogether, TXP2 in the NSCLC cells could be secreted into exosomes and ingested by the surrounding cells, and induced higher migratory and invasive capacity, triggered docetaxel resistance, and suppressed apoptosis in NSCLC cells.

TPX2 Functions Through WNT/β-Catenin/C-Myc Signaling Pathway

We next addressed the molecular mechanism through which TPX2 regulates lung cancer progression. GSEA enrichment analysis of lung cancer data in TCGA showed WNT, MYC, unfolded protein response and spermatogenesis were

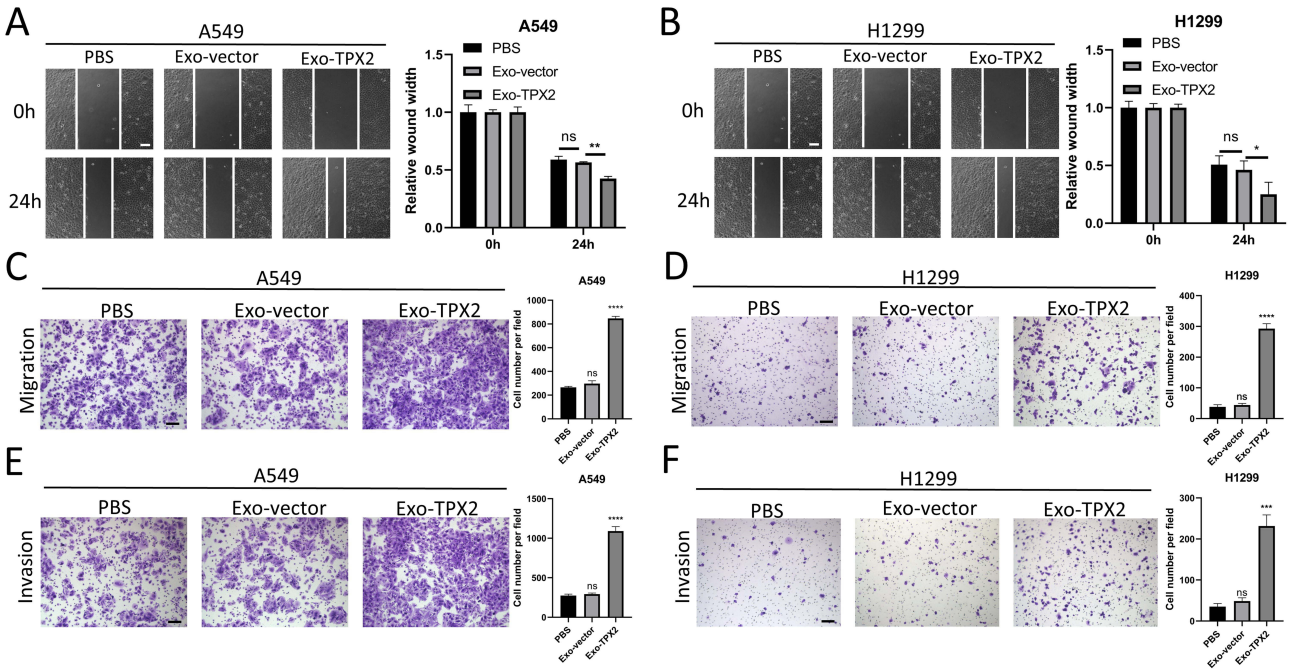

Figure 5 Exosomes from cells with high TPX2 expression promote cell invasion and migration. (**A** and **B**) The migration of A549 (**A**) and H1299 (**B**) cells after treatment with exosomes from different sources were determined by wound healing. Scale bar: 100 μm. (**C** and **D**) The migration capacity of A549 (**C**) and H1299 (**D**) cells after exosomes treatment is determined by transwell assay. Scale bar: 100 μm. (**E** and **F**) The invasive capacity of A549(**E**) and H1299(**F**) cells after treatment with exosomes is determined by transwell assay. Scale bar: 100 μm. Each bar shows the mean±SD of 3 independent experiments. Data were statistically analyzed with paired two-tailed students, *t*-test. *p<0.05; **p<0.01; ***p<0.001 and ****p<0.0001.

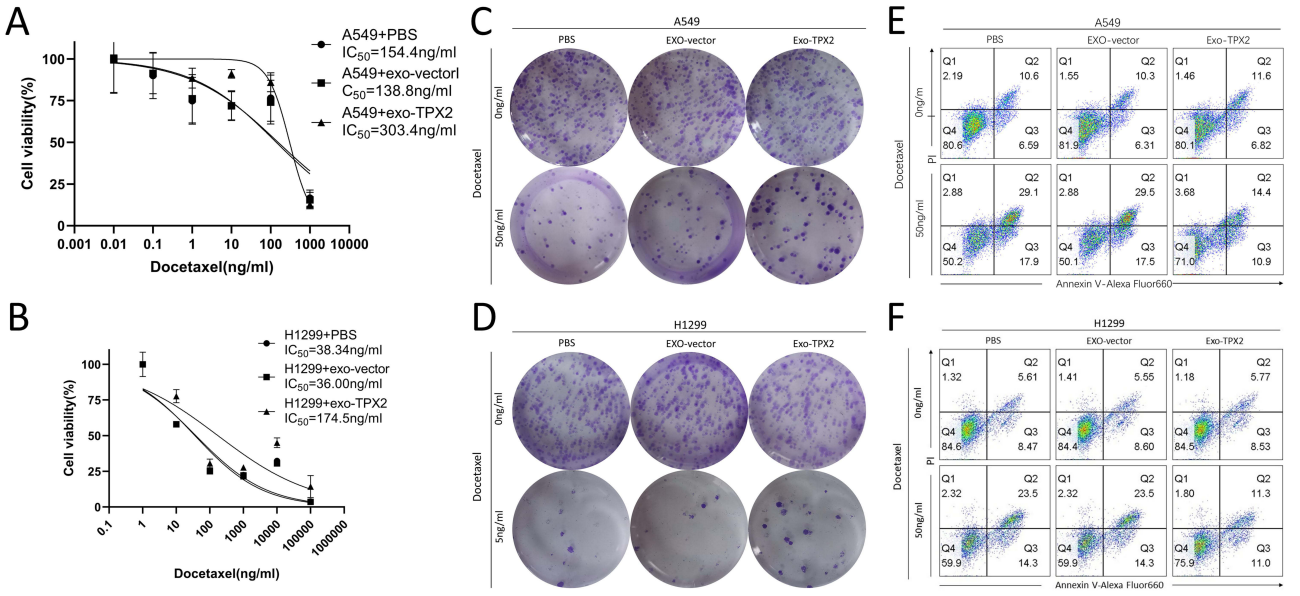

Figure 6 Exosomes from TPX2 high-expressing cells reduce the sensitivity of NSCLC cells to docetaxel. (**A** and **B**) Dose-responsive docetaxel activity curves in A549 (**A**) and H1299 (**B**) cells treated with exosomes. Cell viability was estimated with CCK-8 reagent after 72 h of drug exposure. (**C** and **D**) Effect of exosomes on the clonogenic capacity of A549 (**C**) and H1299 (**D**) cells treated with docetaxel or not. (**E** and **F**) Flow cytometry was used to detect apoptosis in A549 (**E**) and H1299 (**F**) cells treated with docetaxel or not after exosome co-incubation.

positively correlated with TPX2, while coagulation was negatively correlated with TPX2 (Figure 7A and B). Studies have shown that TPX2 and MYC work together in tumors, and c-myc is one of the downstream of WNT pathway.[29] Then, we analyzed the relationship between WNT/β-catenin pathway-related genes and TPX2 expression, and the results

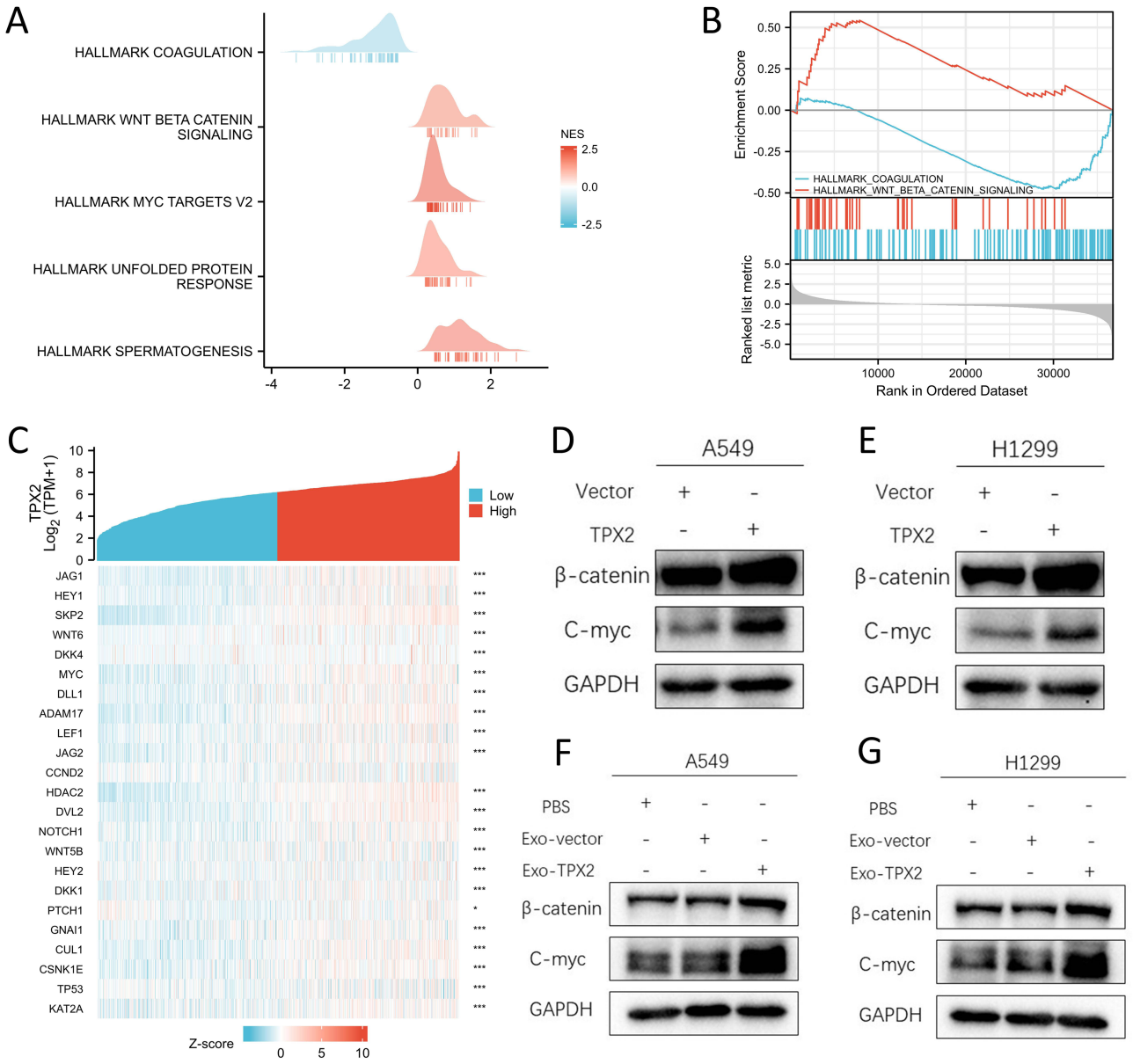

Figure 7 TPX2 activates the downstream WNT/β-catenin signaling pathway. (**A** and **B**) GSEA of the hallmark gene sets deposited in MSigDB. (**C**) Heatmap of the correlation between TPX2 expression and WNT pathway-associated proteins. (**D** and **E**) The amount of β-catenin and C-myc proteins in A549 (**D**) and H1299 (**E**) cells with different levels of TPX2 expression was compared by Western blot. (**F** and **G**) The amount of β-catenin and C-myc proteins in A549 (**F**) and H1299 (**G**) cells treated with different exosomes was compared by Western blot.

showed that TPX2 activated the WNT pathway (Figure 7C). To verify this conjecture, we tested it at the protein level. The Western blot results showed that the levels of β-catenin and C-myc were also higher in the cell lines overexpressing TPX2 (Figure 7D and E), or treated with exosomal TPX2 (Figure 7F and G). These results suggest that TPX2 may play a role through the WNT/β-catenin/C-myc axis.

Discussion

TPX2 is a microtubule-associated protein whose expression is precisely managed in the cell; it appears in the G1/S phase, moves to the mitotic spindle poles during mitosis and disappears after the completion of cytokinesis.[9,30] A large number of studies have shown that the overexpression of TPX2 is related to the stage, grade and poor prognosis of some malignant tumors,[31–33] and it has been identified as a driving oncogene. Previous study also pointed out TXP2 could be

used as a poor prognostic marker for NSCLC.[16,34,35] To verify this, TCGA database analysis showed that the advanced clinical stage and poor prognosis of NSCLC were associated with high TPX2 expression.

Many studies have elucidated the relationship between TPX2 and proliferation, metastasis, and apoptosis in liver cancer, stomach cancer and other cancers,[13,36,37] including NSCLC.[26] In this study, both in vitro and in vivo experiments confirmed that high TPX2 expression can promote NSCLC metastasis. TPX2 has been reported to play an important role as a microtubule-associated protein in the mitosis process and generally promotes cell proliferation.[13,38,39] A previous bioinformatics analysis predicted that TPX2 is related to the proliferation of LUAD.[40] However, here we found that although high TPX2 positivity correlated with T stage of NSCLC, which represented tumor size, but it did not affect NSCLC cells proliferation in vitro. This contradiction was probably due to that TPX2 overexpression was the concomitant phenomenon during NSCLC rapid proliferation but not the cause. Or TPX2 promoted larger tumor size not through fast proliferation but other reasons, eg tumor immunity. Indeed, the overexpression of TPX2 was discovered to be associated with reduced immune infiltration in LUAD.[15] Docetaxel is a commonly used chemotherapeutics for NSCLC, which promotes microtubule polymerization, resulting in cell-cycle arrest and apoptosis. We speculated that the microtubule-associated protein, TXP2, could affect the response to docetaxel. Here we demonstrated that overexpression of TPX2 induced resistance against docetaxel in NSCLC cells. Interestingly, we also found that TPX2 could be secreted outside of the cells via exosome which further affect the expression of TPX2 in the surrounding cells and then change their invasion and migration ability and sensitivity to docetaxel. This is a completely new discovery. Bioinformatics data analysis showed that TPX2 may affect the expression of the proto-oncogene MYC through the WNT/β-catenin signaling pathway, and we further verified this conjecture by protein detection.

Conclusions

This study demonstrates that TPX2 promotes the metastasis of NSCLC cells and induces docetaxel resistance. TPX2 may play a role in NSCLC by further promoting the transcription of C-myc through the accumulation of β-catenin, and it can be intercellularly transferred by exosomal TPX2. In summary, TPX2 may be a marker of NSCLC metastasis and is a key gene involved in the induction of docetaxel resistance, and it may become an important molecular target for the clinical treatment of NSCLC.

Data Sharing Statement

The analyzed datasets for this study can be found in the TCGA (https://portal.gdc.cancer.gov).

Acknowledgments

We thank Yan Hua, Qiyi Yi and Lei Lv on guiding experimental techniques and the staff who participated in this study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This research was funded by the Youth Fund of Anhui Cancer Hospital (grant number 2020YJQN005), the Hefei Science and Technology Bureau Fund (grant number J2020Y01) and the Fundamental Research Funds for the Central Universities (grant number WK9110000188).

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72(1):7–33. doi:10.3322/caac.21708
2. Thai AA, Solomon BJ, Sequist LV, Gainor JF, Heist RS. Lung cancer. *Lancet*. 2021;398(10299):535–554. doi:10.1016/S0140-6736(21)00312-3
3. Hirsch FR, Scagliotti GV, Mulshine JL, et al. Lung cancer: current therapies and new targeted treatments. *Lancet*. 2017;389:299–311. doi:10.1016/S0140-6736(16)30958-8
4. Cortes JE, Pazdur R. Docetaxel. *J Clin Oncol*. 1995;13(10):2643–2655. doi:10.1200/JCO.1995.13.10.2643
5. Zhang E, Xing R, Liu S, Li P. Current advances in development of new docetaxel formulations. *Expert Opin Drug Deliv*. 2019;16(3):301–312. doi:10.1080/17425247.2019.1583644
6. Passaro A, Brahmer J, Antonia S, Mok T, Peters S. Managing resistance to immune checkpoint inhibitors in lung cancer: treatment and novel strategies. *J Clin Oncol*. 2022;40(6):598–610. doi:10.1200/JCO.21.01845
7. Chen Z, Shi T, Zhang L, et al. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family in multidrug resistance: a review of the past decade. *Cancer Lett*. 2016;370(1):153–164. doi:10.1016/j.canlet.2015.10.010
8. Wadsworth P. TPX2. *Curr Biol*. 2015;25(24):R1156–R1158. doi:10.1016/j.cub.2015.10.003
9. Neumayer G, Belzil C, Gruss OJ, Nguyen MD. TPX2: of spindle assembly, DNA damage response, and cancer. *Cell Mol Life Sci*. 2014;71(16):3027–3047. doi:10.1007/s00018-014-1582-7
10. King MR, Petry S. Phase separation of TPX2 enhances and spatially coordinates microtubule nucleation. *Nat Commun*. 2020;11(1):270. doi:10.1038/s41467-019-14087-0
11. Zou Z, Zheng B, Li J, et al. TPX2 level correlates with cholangiocarcinoma cell proliferation, apoptosis, and EMT. *Biomed Pharmacother*. 2018;107:1286–1293. doi:10.1016/j.biopha.2018.08.011
12. Gomes-Filho SM, Dos Santos EO, Bertoldi ERM, et al. Aurora A kinase and its activator TPX2 are potential therapeutic targets in KRAS-induced pancreatic cancer. *Cell Oncol*. 2020;43:445–460. doi:10.1007/s13402-020-00498-5
13. Wang F, Zhao W, Gao Y, et al. CDK5-mediated phosphorylation and stabilization of TPX2 promotes hepatocellular tumorigenesis. *J Exp Clin Cancer Res*. 2019;38(1):286. doi:10.1186/s13046-019-1297-6
14. Wang X, Wang J, Shen H, Luo Z, Lu X. Downregulation of TPX2 impairs the antitumor activity of CD8+ T cells in hepatocellular carcinoma. *Cell Death Dis*. 2022;13(3):223. doi:10.1038/s41419-022-04645-8
15. Zeng H, Ji J, Song X, et al. Stemness related genes revealed by network analysis associated with tumor immune microenvironment and the clinical outcome in lung adenocarcinoma. *Front Genet*. 2020;11:549213. doi:10.3389/fgene.2020.549213
16. Wang H, Wang X, Xu L, Cao H, Zhang J. Nonnegative matrix factorization-based bioinformatics analysis reveals that TPX2 and SELENBP1 are two predictors of the inner sub-consensuses of lung adenocarcinoma. *Cancer Med*. 2021;10(24):9058–9077. doi:10.1002/cam4.4386
17. Huo C, Zhang M-Y, Li R, et al. Comprehensive analysis of TPX2-related ceRNA network as prognostic biomarkers in lung adenocarcinoma. *Int J Med Sci*. 2020;17(16):2427–2439. doi:10.7150/ijms.49053
18. Shah KN, Bhatt R, Rotow J, et al. Aurora kinase A drives the evolution of resistance to third-generation EGFR inhibitors in lung cancer. *Nat Med*. 2019;25(1):111–118. doi:10.1038/s41591-018-0264-7
19. Pegtel DM, Gould SJ. Exosomes. *Annu Rev Biochem*. 2019;88(1):487–514. doi:10.1146/annurev-biochem-013118-111902
20. Dai J, Su Y, Zhong S, et al. Exosomes: key players in cancer and potential therapeutic strategy. *Signal Transduct Target Ther*. 2020;5(1):145. doi:10.1038/s41392-020-00261-0
21. Zhang L, Yu D. Exosomes in cancer development, metastasis, and immunity. *Biochim Biophys Acta Rev Cancer*. 2019;1871:455–468. doi:10.1016/j.bbcan.2019.04.004
22. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science*. 2020;367(6478):eaau6977. doi:10.1126/science.aau6977
23. Zhou Y, Zhang Y, Gong H, Luo S, Cui Y. The role of exosomes and their applications in cancer. *Int J Mol Sci*. 2021;22(22):12204. doi:10.3390/ijms222212204
24. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res*. 2001;29:e45. doi:10.1093/nar/29.9.e45
25. Lobb RJ, Becker M, Wen SW, et al. Optimized exosome isolation protocol for cell culture supernatant and human plasma. *J Extracell Vesicles*. 2015;4:27031. doi:10.3402/jev.v4.27031
26. Zhou F, Wang M, Aibaidula M, et al. TPX2 promotes metastasis and serves as a marker of poor prognosis in non-small cell lung cancer. *Med Sci Monit*. 2020;26:e925147-1–e925147-15. doi:10.12659/MSM.925147
27. Xu K, Zhang C, Du T, et al. Progress of exosomes in the diagnosis and treatment of lung cancer. *Biomed Pharmacother*. 2021;134:111111. doi:10.1016/j.biopha.2020.111111
28. Jiang C, Zhang N, Hu X, Wang H. Tumor-associated exosomes promote lung cancer metastasis through multiple mechanisms. *Mol Cancer*. 2021;20:117. doi:10.1186/s12943-021-01411-w
29. Rohrberg J, Van de Mark D, Amouzgar M, et al. MYC dysregulates mitosis, revealing cancer vulnerabilities. *Cell Rep*. 2020;30:3368–3382.e7. doi:10.1016/j.celrep.2020.02.041
30. Pérez de Castro I, Malumbres M. Mitotic stress and chromosomal instability in cancer: the case for TPX2. *Genes Cancer*. 2012;3:721–730. doi:10.1177/1947601912473306
31. Liu Q, Tu K, Zhang H, Zheng X, Yao Y, Liu Q. TPX2 as a novel prognostic biomarker for hepatocellular carcinoma. *Hepatol Res*. 2015;45:906–918. doi:10.1111/hepr.12428
32. Wei P, Zhang N, Xu Y, et al. TPX2 is a novel prognostic marker for the growth and metastasis of colon cancer. *J Transl Med*. 2013;11(1):313. doi:10.1186/1479-5876-11-313
33. Huang Y, Guo W, Kan H. TPX2 is a prognostic marker and contributes to growth and metastasis of human hepatocellular carcinoma. *Int J Mol Sci*. 2014;15(10):18148–18161. doi:10.3390/ijms151018148
34. Schneider MA, Christopoulos P, Muley T, et al. AURKA, DLGAP5, TPX2, KIF11 and CKAP5: five specific mitosis-associated genes correlate with poor prognosis for non-small cell lung cancer patients. *Int J Oncol*. 2017;50(2):9058–9077. doi:10.1002/cam4.4386
35. Shen Y, Tang X, Zhou X, et al. Screening of key prognosis genes of lung adenocarcinoma based on expression analysis on TCGA database. *J Oncol*. 2022;2022:4435092. doi:10.1155/2022/4435092

36. Liang B, Zheng W, Fang L, et al. Overexpressed targeting protein for Xklp2 (TPX2) serves as a promising prognostic marker and therapeutic target for gastric cancer. *Cancer Biol Ther*. 2016;17:824–832. doi:10.1080/15384047.2016.1195046
37. Huang D-H, Jian J, Li S, Zhang Y, Liu L-Z. TPX2 silencing exerts anti-tumor effects on hepatocellular carcinoma by regulating the PI3K/AKT signaling pathway. *Int J Mol Med*. 2019;44:2113–2122. doi:10.3892/ijmm.2019.4371
38. Sawada J-I, Matsuno K, Ogo N, Asai A. Various effects of two types of kinesin-5 inhibitors on mitosis and cell proliferation. *Biochem Pharmacol*. 2021;193:114789. doi:10.1016/j.bcp.2021.114789
39. Hsu P-K, Chen H-Y, Yeh Y-C, et al. TPX2 expression is associated with cell proliferation and patient outcome in esophageal squamous cell carcinoma. *J Gastroenterol*. 2014;49(8):1231–1240. doi:10.1007/s00535-013-0870-6
40. Wang K, Zhang M, Wang J, et al. Identifies key regulators involved in cell proliferation and potential drugs for the treatment of human lung adenocarcinoma. *Front Oncol*. 2021;11:737152. doi:10.3389/fonc.2021.737152