Targeting transient receptor potential canonical 1 reduces non-small cell lung cancer chemoresistance and stemness via inhibition of PI3K/AKT signaling

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Abstract. TRPC1 enhances cell proliferation and migration in non-small cell lung cancer (NSCLC); however, its effect on NSCLC chemoresistance and stemness remains to be determined. The aim of the current study was to investigate the effect of TRPC1 on NSCLC chemoresistance and stemness and to determine the underlying mechanism of action. Cisplatin-resistant A549 (A549/CDDP) and H460 (H460/CDDP) cells were first established and were then transfected with negative control small interfering (si)RNA (si-NC) or TRPC1 siRNA (si-TRPC1). Cells were then treated with 740 Y-P, a PI3K/Akt agonist. Subsequently, the sensitivity of A549/CDDP and H460/CDDP cells to CDDP was evaluated. Furthermore, the expression levels of CD133 and CD44, and sphere formation ability were also determined. The results showed that the half-maximal inhibitory concentration (IC_{50}) of CDDP was significantly higher in A549/CDDP cells compared with A549 cells and in H460/CDDP cells compared with H460 cells. TRPC1 silencing decreased the IC_{50} value of CDDP compared with the si-NC group in A549/CDDP (11.78 vs. 21.58 μM; P<0.01) and H460/CDDP (23.76 vs. 43.11 μM; P<0.05) cells. Additionally, TRPC1 knockdown in both cell lines decreased the number of spheres formed compared with the si-NC group. Furthermore, compared with the si-NC group, A549/CDDP cells transfected with si-TRPC1 exhibited decreased levels of both CD133 (P<0.01) and CD44 (P<0.05). However, only CD133 (P<0.05) was downregulated in TRPC1-depleted H460/CDDP cells compared with the si-NC group. In addition, TRPC1 knockdown repressed PI3K/AKT signaling compared with the si-NC group in both A549/CDDP and H460/CDDP cells (all P<0.05). Finally, cell treatment with 740 Y-P reversed the effect of TRPC1 knockdown on PI3K/AKT signaling, chemoresistance, and cancer stemness in A549/CDDP and H460/CDDP cells (all P<0.05). In conclusion, the results of the current study suggested that targeting TRPC1 could attenuate cancer stemness and chemoresistance via suppression of PI3K/AKT signaling in NSCLC.

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Introduction

Lung cancer is one of the most fatal and common types of cancer globally, accounting for ~11.4% of new cancer cases and 18.0% of cancer-related deaths in 2020. Notably, non-small cell lung cancer (NSCLC) accounts for the majority of lung cancer cases (1-3). To date, chemotherapy remains the most common therapeutic approach for patients with NSCLC. However, a proportion of patients with NSCLC develop chemoresistance during treatment, resulting in a less favorable survival profile (4-7). Cancer stemness is considered the most crucial factor contributing to chemoresistance (8). Therefore, exploring the mechanism underlying chemoresistance and stemness in NSCLC is of great importance.

Transient receptor potential canonical 1 (TRPC1) mediates the influx of extracellular Ca^{2+} and plays a critical role in cell proliferation, differentiation, apoptosis, and migration (9,10). The regulatory role of TRPC1 in chemoresistance in solid carcinomas has been well established (11-13). A previous study showed that TRPC1 could promote hypoxia-associated epithelial-mesenchymal transition (EMT), subsequently contributing to chemoresistance in endometrial carcinoma (11,13). Another study demonstrated that TRPC1 attenuated the sensitivity of breast cancer cells to chemotherapy (12). However, the role of TRPC1 in NSCLC chemoresistance to cisplatin (CDDP, one of the most widely used and effective compounds in cancer treatment, which functions by binding to the DNA bases in the nucleus and inhibits DNA replication and transcription to exhibit its anti-tumor efficacy) and stemness remains unclear. Therefore, the current study aimed to evaluate the regulatory

effect of TRPC1 on NSCLC chemoresistance and stemness, as well as its underlying mechanism of action.

Materials and methods

Cell culture. A549 and H460 cells were purchased from BeNa Culture Collection. A549 and H460 cells resistant to cis-Diamminedichloroplatinum (cisplatin/CDDP, MilliporeSigma; A549/CDPP and H460/CDDP respectively) were established by gradually exposing parental cells to increasing concentrations of CDDP as described previously (14). All cells were maintained in RPMI-1640 medium (Lonza Pharma & Biotech) supplemented with 10% FBS (Lonza Pharma & Biotech) and 1% penicillin/streptomycin solution (Beijing Solarbio Science & Technology Co., Ltd.) at 37˚C in a humidified incubator supplied with 5% CO_2. To maintain resistance, A549/CDDP and H460/CDDP cells were cultured in the presence of 2 and 4 μM CDDP, respectively. The mRNA and protein expression levels of TRPC1 in A549, A549/CDDP, H460, and H460/CDDP cells were detected by reverse transcription-quantitative PCR (RT-qPCR) and western blot analysis, respectively.

CDDP sensitivity assay. The sensitivity of A549, A549/CDDP, H460, and H460/CDDP cells to CDDP was assessed using Cell Counting Kit-8 assays (CCK-8; MedChemExpress). Briefly, cells were seeded into 96-well plates at a density of $2x10^3$ cells/well. Subsequently, A549 and H460 cells were treated with 0, 0.25, 0.5, 1, 2, 4 or 8 μM CDDP for 48 h. Additionally, A549/CDDP and H460/CDDP cells were treated with 0, 2, 4, 8, 16, 32, or 64 μM CDDP for 48 h. Following treatment, cells were incubated for 2 h in the presence of CCK-8 reagent. The absorbance values were subsequently detected at a wavelength of 450 nm using a plate reader (Molecular Devices, LLC). The half-maximal inhibitory concentration (IC_{50}) of CDDP was evaluated using a sigmoidal dose-response curve, as previously described (15).

Cell transfection. The small interfering RNA (siRNA) constructs targeting TRPC1 (si-TRPC1-1, si-TRPC1-2, and si-TRPC1-3) and the corresponding negative control (si-NC) were obtained from Shanghai GenePharma Co., Ltd. A549/CDDP and H460/CDDP cells were plated into 6-well plates at a density of $2x10^6$ cells/well and then transfected with the above siRNAs using Lipofectamine™ 3000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. The knockdown efficiency of siRNAs was detected by RT-qPCR and western blot analysis. Since si-TRPC1-2 exhibited the most potent knockdown effect on TRPC1 expression, this siRNA clone was used for the subsequent interference experiments. Further assays, including CDDP sensitivity, sphere formation assay, and western blot analysis were performed after transfection. The sequences of the siRNAs used were as follows: For siRNA-1 sense, CGA UCAUCAAGACCAACUAUA and antisense, UAGUUGGUC UUGAUGAUCGUU; siRNA-2 sense, GGAAGUCUCUUU AAUGCAAUG and antisense, UUGCAUUAAAGAGAC UUCCUA); siRNA-3 sense, GCUUUCAGUUGAUAGCAA AUC and antisense, UUUGCUAUCAACUGAAAGCUU); and si-NC sense, UUCUCCGAACGUGUCACGUTT and antisense, ACGUGACACGUUCGGAGAATT.

Cell treatment with 740 Y-P. A549/CDDP and H460/CDDP cells were seeded into 6-well plates and transfected as described above. Subsequently, to evaluate the regulatory effect of TRPC1 on PI3K/AKT signaling, cells were treated with 25 μg/ml 740 Y-P (a PI3K/AKT activator; MedChemExpress) as described previously (16). Following cell treatment with 740 Y-P for 48 h, western blot analysis was performed. Additionally, CDDP sensitivity and sphere formation assays were also performed in cells treated with 740 Y-P for 48 h.

RNA isolation and RT-qPCR. Total RNA was isolated from A549, A549/CDDP, H460, and H460/CDDP cells using Beyozol (Beyotime Institute of Biotechnology) and subsequently reverse transcribed into cDNA using the QuantiNova RT Kit according to the manufacturer's protocol (Qiagen GmbH). qPCR was performed using the SYBR® Premix DimmerEraser™ kit (Takara Bio, Inc.). The thermocycling conditions were: 95˚C for 30 sec; followed by 40 cycles of 95˚C for 5 sec and 61˚C for 30 sec. The expression of TRPC1 was assessed using the $2^{-\Delta\Delta Cq}$ method and GAPDH was used as the internal control (17). The sequences of the primers were: TRPC1 forward, 5'-ACCTTCCATTCGTTCATTGG-3' and reverse, 5'-TGGTGAGGGAATGATGTTGA-3'; and GAPDH forward, 5'-GAGTCCACTGGCGTCTTCAC-3' and reverse, 5'-ATCTTGAGGCTGTTGTCATACTTCT-3'.

Western blot analysis. Total protein was extracted from cells using RIPA lysis reagent supplemented with 1% PMSF (both from Wuhan Servicebio Technology Co., Ltd.). The protein concentration was measured using a BCA kit according to the manufacturer's protocol (Beyotime Institute of Biotechnology). Subsequently, 40 μg total protein was loaded per a lane on a BeyoGel™ Plus Precast PAGE Gel (Beyotime Institute of Biotechnology), resolved using SDS-PAGE, and then transferred to a nitrocellulose membrane (Wuhan Servicebio Technology Co., Ltd.). Following blocking with 5% nonfat milk (Beyotime Institute of Biotechnology), the membranes were incubated with primary and secondary antibodies for 1 h at 37˚C, successively. Signals were visualized using the ECL-PLUS reagent (Beyotime Institute of Biotechnology). The following antibodies were used: Anti-TRPC1 (1:500; cat. no. DF12783; Affinity Biosciences), anti-AKT (dilution, 1:500; cat. no. AF6261; Affbiotech), anti-PI3K (1:500; cat. no. AF6241, Affbiotech), anti-CD133 (1:1,000; cat. no. 51917; Cell Signaling Technology, Inc.), anti-CD44 (1:1,000; cat. no. 37259; Cell Signaling Technology, Inc.), anti-phospho (p)-PI3K (1:2,000; cat. no. ab182651; Abcam), anti-p-AKT (1:1,000; cat. no. ab38449; Abcam), anti-GAPDH (1:5,000; cat. no. GB15004; Wuhan Servicebio Technology Co., Ltd.), and goat anti-rabbit secondary antibody (1:10,000; cat. no. GB23303; Wuhan Servicebio Technology Co., Ltd.).

Sphere formation assay. Sphere formation assays in A549/CDDP and H460/CDDP cells were performed 48 h after transfection. Briefly, cells at a density of $1x10^3$ cells/well were seeded into 6-well ultra-low attachment plates (Corning, Inc.) and were then cultured in spheroid medium, composed of DMEM/Nutrient Mixture F-12 (DMEM-F12; Gibco; Thermo Fisher Scientific, Inc.) containing B-27™ Supplement (Thermo Fisher Scientific, Inc.), 20 ng/ml epidermal growth factor

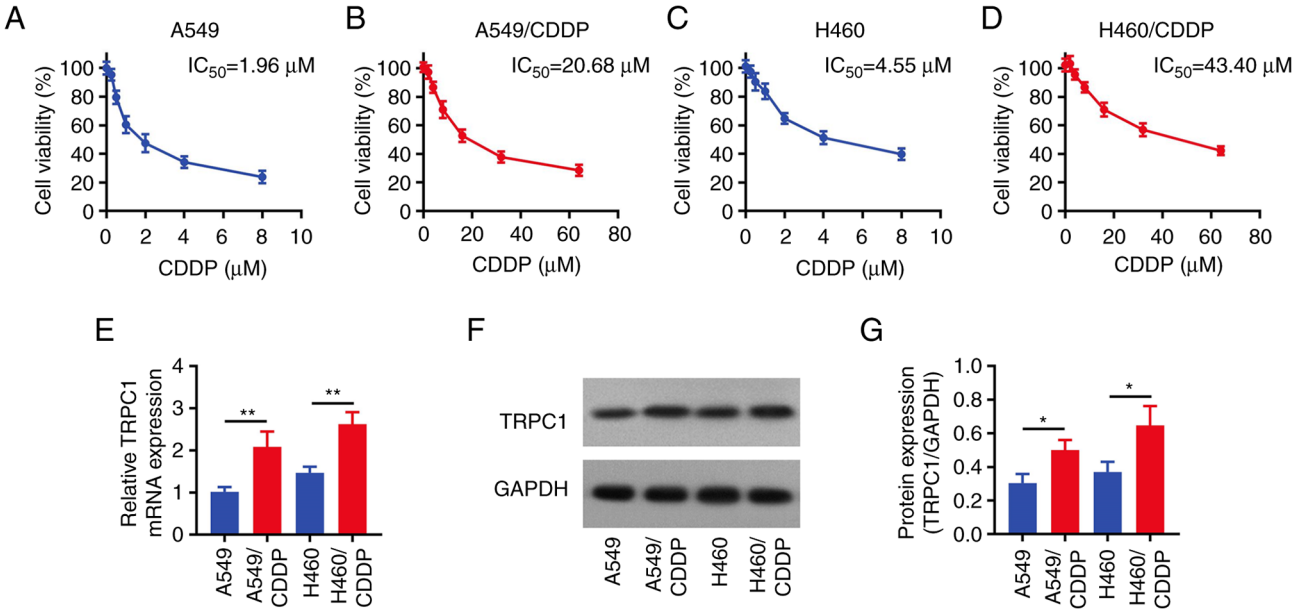


Figure 1. TRPC1 expression in normal and CDDP-resistant NSCLC cells. The dose-response curve of CDDP in (A) A549 cells, (B) A549/CDDP cells, (C) H460 cells, and (D) H460/CDDP cells. (E) TRPC1 mRNA expression. (F) Representative western blots of TRPC1 expression. (G) Densitometry analysis of TRPC1 protein expression. *P<0.05, **P<0.01. TRPC1, transient receptor potential canonical 1; CDDP, cis-Diamminedichloroplatinum.

(EGF, MedChemExpress), 20 ng/ml basic fibroblast growth factor (MedChemExpress), and 1% penicillin/streptomycin solution (Beijing Solarbio Science & Technology Co., Ltd.). Following 10 days of culture, the number of formed spheres (diameter, >50 μm) was counted, and images were captured under a light microscope with a magnification of x200.

Statistical analysis. All data were compared using an unpaired Student's t-test or a one-way ANOVA followed by a Tukey's post hoc test in GraphPad Prism 7 (GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

TRPC1 is upregulated in chemoresistant NSCLC cells. The IC_{50} values of CDDP were 1.96 (Fig. 1A), 20.68 (Fig. 1B), 4.55 (Fig. 1C), and 43.40 μM (Fig. 1D) in parental A549 cells, A549/CDDP cells, parental H460 cells, and H460/CDDP cells, respectively, thus confirming that CDDP-resistant NSCLC cells were successfully established. Subsequently, TRPC1 expression was detected in the above cells and the results showed that the mRNA and protein expression levels of TRPC1 were notably increased in A549/CDDP cells compared with the parental A549 cells. Consistently, the same trend was observed in H460/CDDP cells compared with the parental H460 cells (all P<0.05; Fig. 1E-G).

TRPC1 knockdown attenuates the chemoresistance of NSCLC cells to CDDP. To further investigate the effect of TRPC1 knockdown on NSCLC chemoresistance, A549/CDDP and H460/CDDP cells were transfected with one of three siRNAs targeting TRPC1. RT-qPCR (Fig. 2A and B) and western blot analysis (Fig. 2C-E) revealed that the si-TRPC1-2 construct exhibited the highest TRPC1 knockdown activity in both A549/CDDP and H460/CDDP cells. Therefore, si-TRPC1-2 was chosen for the subsequent experiments. The IC_{50} value of CDDP was significantly reduced in TRPC1-knockdown A549/CDDP cells compared with the si-NC group (11.78 vs. 21.58 μM; P<0.01); this trend was also observed in H460/CDDP cells (23.76 vs. 43.11 μM; P<0.05; Fig. 3A-D).

TRPC1 knockdown reduces NSCLC stemness. The number of spheres formed was markedly decreased in both the TRPC1-knockdown A549/CDDP (P<0.01; Fig. 4A and B) and H460/CDDP (P<0.05; Fig. 4A and C) cells compared with the respective si-NC group. Furthermore, in A549/CDDP cells, the expression levels of CD133 (P<0.01) and CD44 (P<0.05) were reduced in the si-TRPC1 group compared with the si-NC group (Fig. 4D and E). However, only CD133 expression was significantly downregulated in TRPC1-depleted H460/CDDP cells (the transfection efficiency of si-TRPC1 transfected H460/CDDP cells was not sufficient) (P<0.05; Fig. 4D and F).

TRPC1 knockdown reduces PI3K/AKT signaling. TRPC1 knockdown reduced the p-PI3K/PI3K and p-AKT/AKT ratios in both A549/CDDP (both P<0.05; Fig. 5A and B) and H460/CDDP cells (both P<0.01; Fig. 5A and C) compared with the respective si-NC group.

Cell treatment with 740 Y-P alleviates the effect of TRPC1 knockdown on NSCLC cell chemoresistance and stemness. A549/CDDP (both P<0.01; Fig. 6A and B) and H460/CDDP (both P<0.001; Fig. 6A and C) cell treatment with 740 Y-P increased the p-PI3K/PI3K and p-AKT/AKT ratios, which were previously decreased by TRPC1 knockdown. Additionally, treatment with 740 Y-P increased the IC_{50} values of CDDP in si-TRPC1-transfected A549/CDDP cells (23.18 vs.

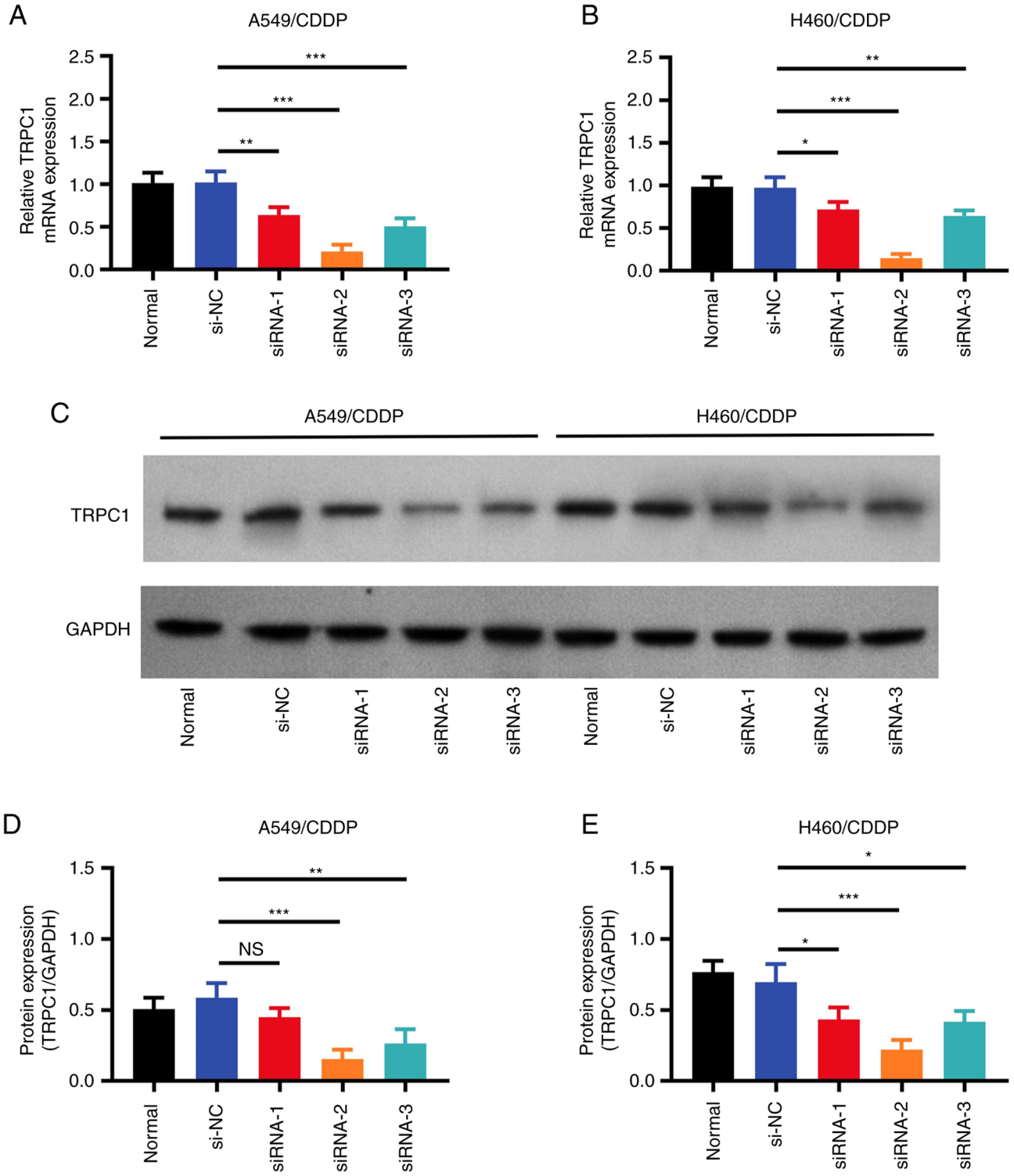
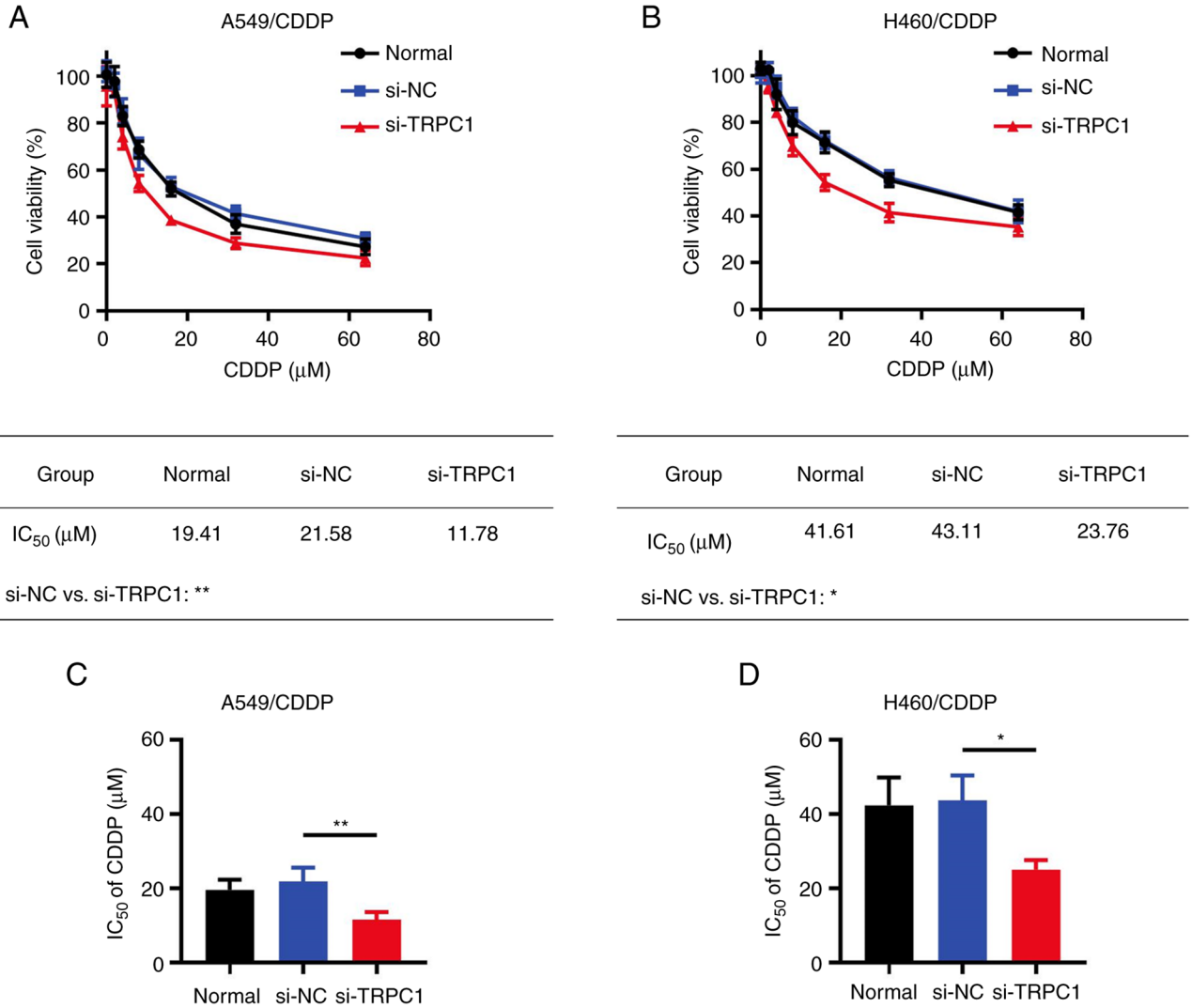


Figure 2. Transfection efficiency of siRNAs. Relative TRPC1 mRNA expression in the untransfected, si-NC, and si-TRPC1 transfected (A) A549/CDDP and (B) H460/CDDP cells. (C) Representative western blots of TRPC1 expression in the untransfected, si-NC, and si-TRPC1 transfected NSCLC cells. (D) Densitometry analysis of TRPC1 protein expression in the untransfected, si-NC, and si-TRPC1 transfected A549/CDDP and (E) H460/CDDP cells. *P<0.05, **P<0.01, ***P<0.001. NS, not significant; TRPC1, transient receptor potential canonical 1; CDDP, cis-Diamminedichloroplatinum; si, small interfering; NC, negative control; NSCLC, non-small cell lung cancer.

12.96 μM; P<0.05; Fig. 7A). Similar results were observed in the H460/CDDP cells (44.41 vs. 21.60 μM; P<0.01; Fig. 7B). Finally, the number of spheres formed was increased in the TRPC1-knockdown A549/CDDP (P<0.05; Fig. 7C and E) and H460/CDDP cells (P<0.05; Fig. 7D and E) treated with 740 Y-P.

Discussion

TRPC1, a member of the calcium channel family of proteins, modulates several cellular functions including cell proliferation, survival, and migration (18). The role of TRPC1 in cancer has been gradually uncovered (19-23). More specifically, a



Group	Normal	si-NC	si-TRPC1
IC_{50} (μM)	19.41	21.58	11.78
si-NC vs. si-TRPC1: **			

Group	Normal	si-NC	si-TRPC1
IC_{50} (μM)	41.61	43.11	23.76
si-NC vs. si-TRPC1: *			

Figure 3. Effect of si-TRPC1 on chemoresistance. The dose-response curves of CDDP in (A) untransfected, si-NC, and si-TRPC1 transfected A549/CDDP and (B) H460/CDDP cells. Comparison of CDDP IC_{50} between untransfected, si-NC, and si-TRPC1 transfected (C) A549/CDDP and (D) H460/CDDP cells. *P<0.05, **P<0.01. TRPC1, transient receptor potential canonical 1; CDDP, cis-Diamminedichloroplatinum; si, small interfering; NC, negative control.

study illustrated that TRPC1 inhibited cell proliferation and invasion in esophageal squamous cell carcinoma (19). Another study revealed that TRPC1 overexpression promoted the migration of human malignant glioma cells (20). Additionally, a previous study suggested that TRPC1 could exacerbate metastasis in gastric cancer via regulation of a circular RNA-7/microRNA-135a-5p axis (21). More importantly, the regulatory role of TRPC1 in NSCLC has also been investigated; TRPC1 was shown to interact with EGFR and correspondingly facilitate the proliferation of NSCLC cells (22). In addition, another study indicated that TRPC1 could promote the proliferation of NSCLC cells (23). However, the effect of TRPC1 on NSCLC chemoresistance and stemness has not been previously explored. Consequently, the present study is the first to explore the regulatory effect of TRPC1 on NSCLC chemoresistance and stemness, as well as to determine the underlying mechanism.

Regarding the role of TRPC1 on chemoresistance, it has been reported that TRPC1 modulates chemoresistance in several types of cancer (24,25). A previous study demonstrated that TRPC1 enhanced store-operated Ca^{2+} entry and was thus involved in the resistance of breast cancer cells to chemotherapeutic drugs, such as cisplatin, 5-fluorouracil and paclitaxel (24). Another study showed that TRPC1 could interact with stromal interaction molecule 1 and calcium release-activated calcium channel protein 1 to induce chemoresistance in hepatocellular carcinoma (25). However, its effect on chemoresistance in NSCLC cells remains unknown. In the current study, TRPC1 was upregulated in chemoresistant NSCLC cells, while TRPC1 knockdown restored chemosensitivity in CDDP-resistant NSCLC cells. The above effects could be due to the fact that TRPC1 knockdown could prevent autophagy, which in turn could inhibit tumor cell apoptosis mediated by chemotherapeutic drugs. Therefore, TRPC1 silencing may decrease chemoresistance in NSCLC cells (26,27). Secondly, TRPC1 knockdown could inhibit the activity of CDK1 and CyclinB1, which are involved in the G2 to the M phase transition of the cell cycle (28). Additionally, chemotherapeutic drugs, such as CDDP, bind with DNA primarily during the G2/M phase transition to exert a cytotoxic effect (29). Therefore, TRPC1 knockdown may enhance chemosensitivity in chemoresistant NSCLC cells.

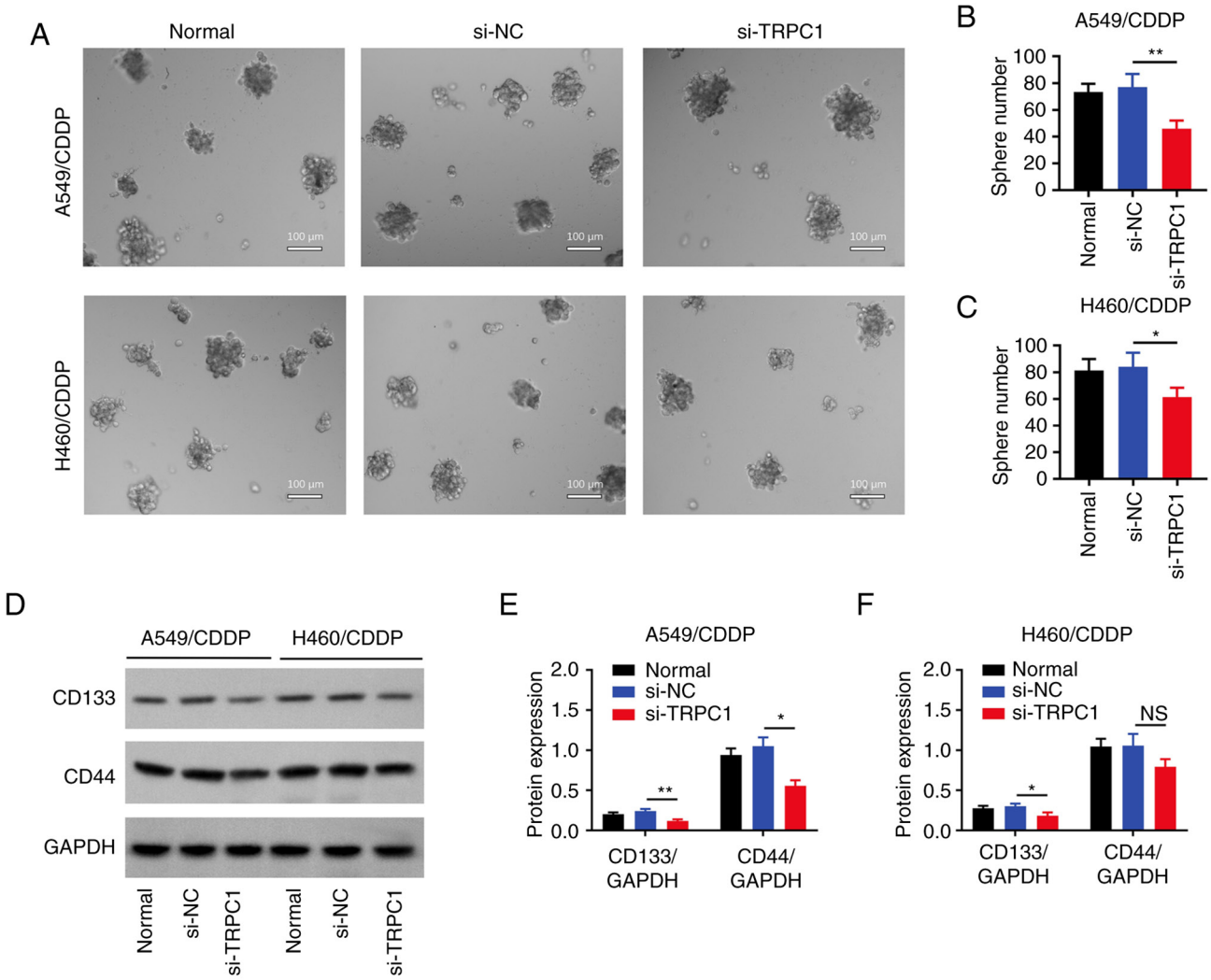


Figure 4. Effect of si-TRPC1 on stemness. (A) Representative images of the sphere formation assay in the untransfected, si-NC, and si-TRPC1 transfected NSCLC cells. Number of spheres formed in the untransfected, si-NC, and si-TRPC1 transfected (B) A549/CDDP and (C) H460/CDDP cells. (D) Representative blots of CD133, CD44, and GAPDH expression in the untransfected, si-NC, and si-TRPC1 transfected NSCLC cells. Densitometry analysis of CD133 and CD44 expression in the untransfected, si-NC, and si-TRPC1 transfected (E) A549/CDDP and (F) H460/CDDP cells. *P<0.05, **P<0.01. TRPC1, transient receptor potential canonical 1; CDDP, cis-Diamminedichloroplatinum; si, small interfering; NC, negative control.

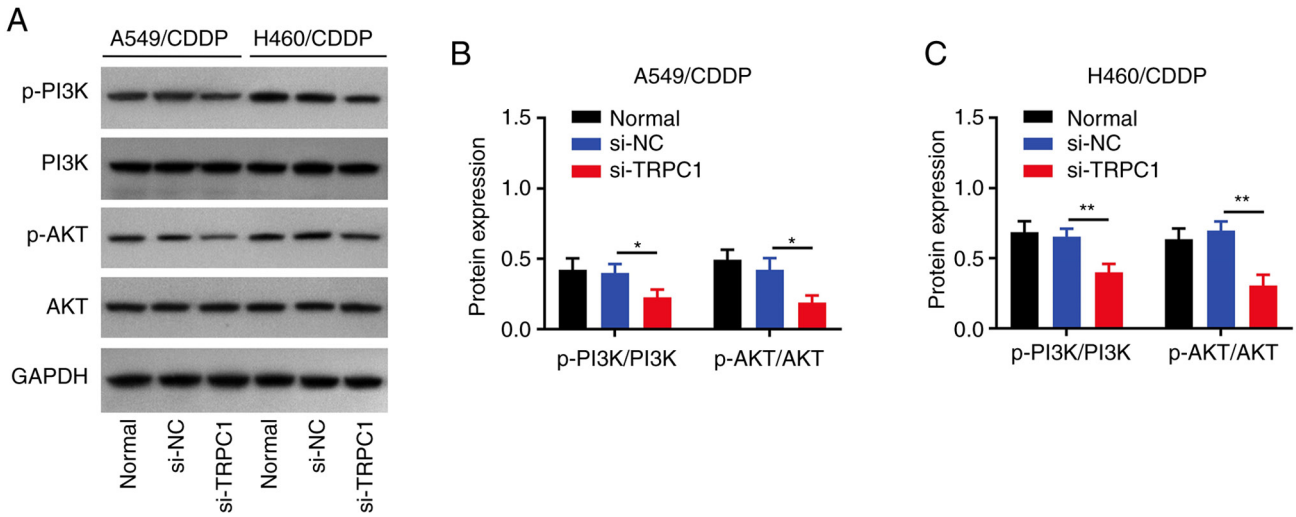


Figure 5. Effect of si-TRPC1 on PI3K/AKT signaling. (A) Representative blots of p-PI3K, PI3K, p-AKT, AKT, and GAPDH expression in the untransfected, si-NC, and si-TRPC1 transfected NSCLC cells. Densitometry analysis of the p-PI3K/PI3K and p-AKT/AKT ratios in the untransfected, si-NC, and si-TRPC1 transfected (B) A549/CDDP and (C) H460/CDDP cells (C). *P<0.05, **P<0.01. TRPC1, transient receptor potential canonical 1; CDDP, cis-Diamminedichloroplatinum; si, small interfering; NC, negative control; p-, phospho; NSCLC, non-small cell lung cancer.

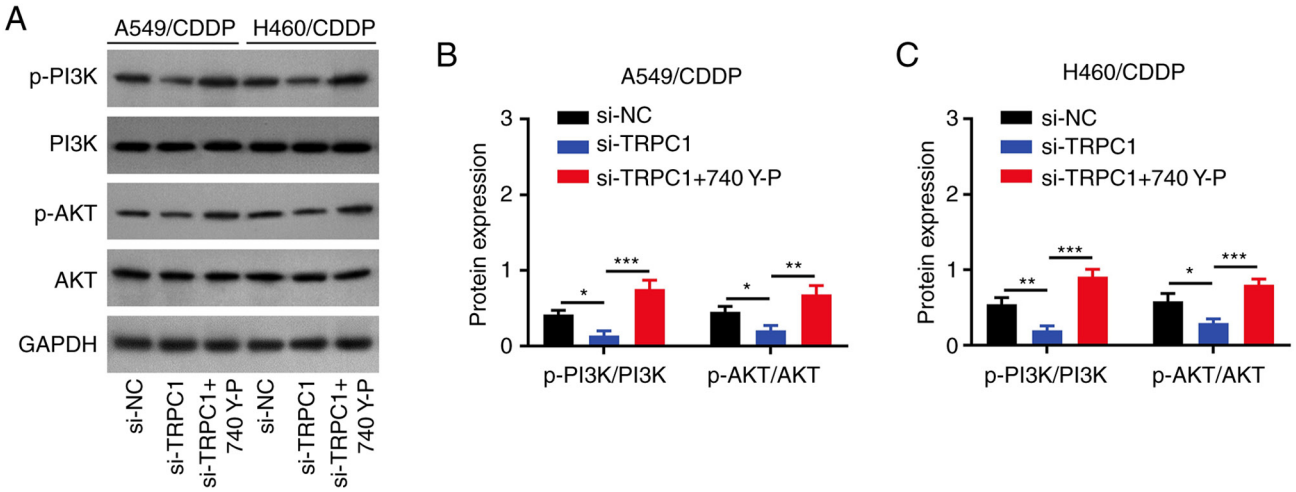
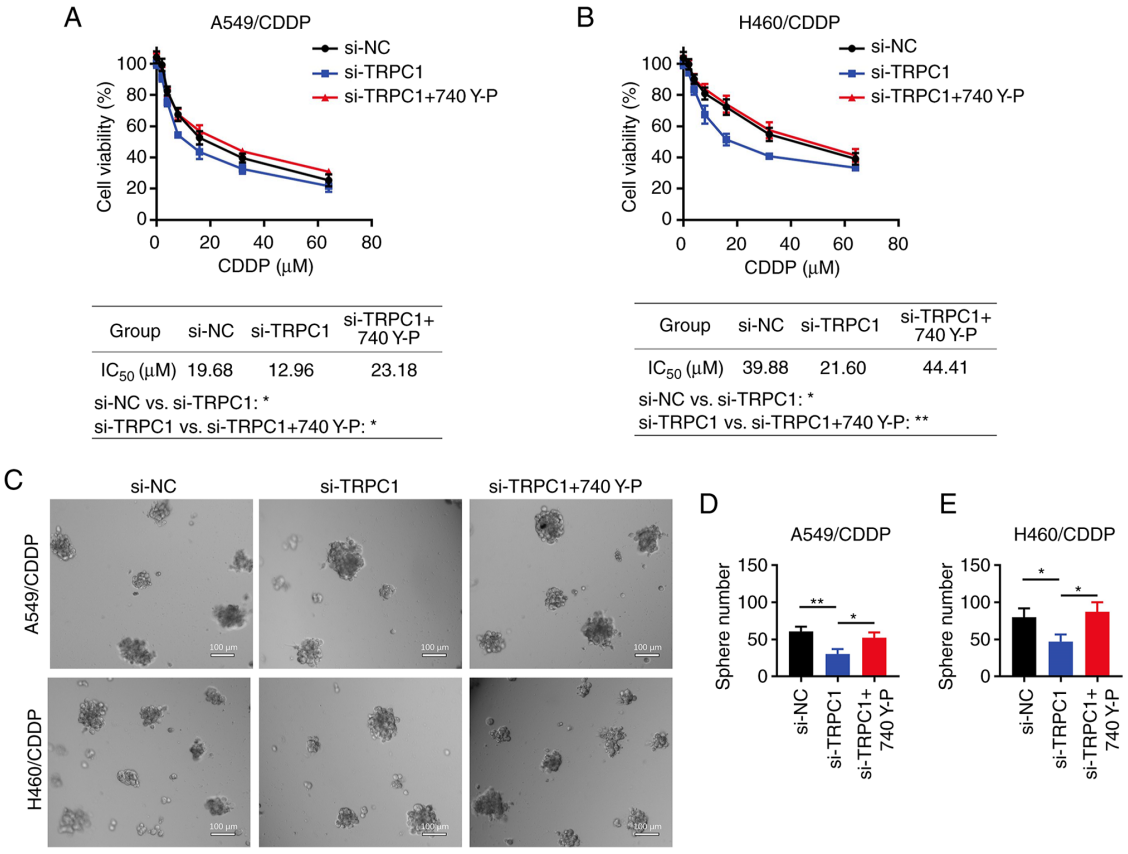


Figure 6. Effect of 740 Y-P and TRPC1 siRNA on PI3K/AKT signaling. (A) Representative blots of p-PI3K, PI3K, p-AKT, AKT, and GAPDH expression in the si-NC transfected NSCLC cells as well as si-TRPC1 transfected NSCLC cells with and without 740 Y-P treatment. Densitometry analysis of the p-PI3K/PI3K and p-AKT/AKT ratios in the (B) si-NC and si-TRPC1 transfected A549/CDDP cells with and without 740 Y-P treatment, and (C) in the si-NC transfected and si-TRPC1 transfected H460/CDDP cells with and without 740 Y-P treatment. *P<0.05, **P<0.01, ***P<0.001. TRPC1, transient receptor potential canonical 1; CDDP, cis-Diamminedichloroplatinum; si, small interfering; NC, negative control; p-, phospho; NSCLC, non-small cell lung cancer.



Group	si-NC	si-TRPC1	si-TRPC1+ 740 Y-P
IC_{50} (μM)	19.68	12.96	23.18

si-NC vs. si-TRPC1: *
si-TRPC1 vs. si-TRPC1+740 Y-P: *

Group	si-NC	si-TRPC1	si-TRPC1+ 740 Y-P
IC_{50} (μM)	39.88	21.60	44.41

si-NC vs. si-TRPC1: *
si-TRPC1 vs. si-TRPC1+740 Y-P: **

Figure 7. Effect of 740 Y-P and TRPC1 siRNA on chemoresistance and stemness. (A) The dose-response curve of CDDP in si-NC and si-TRPC1 transfected A549/CDDP cells with and without 740 Y-P treatment, and (B) in the si-NC and si-TRPC1 transfected H460/CDDP cells with and without 740 Y-P treatment. (C) Representative images of sphere formation in the si-NC and si-TRPC1 transfected NSCLC cells with and without 740 Y-P treatment. (D) Number of spheres formed in the si-NC and si-TRPC1 transfected A549/CDDP cells with and without 740 Y-P treatment, and (E) in si-NC and si-TRPC1 transfected H460/CDDP cells with and without 740 Y-P treatment. *P<0.05, **P<0.01. TRPC1, transient receptor potential canonical 1; CDDP, cis-Diamminedichloroplatinum; si, small interfering; NC, negative control; p-, phospho; NSCLC, non-small cell lung cancer.

Cancer stemness plays a critical role in the pathology of chemoresistance. It has been reported that the microenvironment of cancer stem cells can promote chemoresistance through several factors (30). Regarding the effect of TRPC1 on cancer stemness, a previous study reported that TRPC1 was associated with stemness in dental pulp stem cells (31). Herein, TRPC1

knockdown decreased sphere formation and reduced the expression levels of CD133 and CD44 in chemoresistant NSCLC cells. A possible explanation could be that TRPC1 silencing could attenuate EMT via inhibiting the PI3K/AKT signaling pathway. It has been reported that EMT is associated with cancer stemness in several types of cancer (32,33), suggesting that TRPC1 knockdown could reduce cancer stemness in NSCLC cells. In addition, another interesting finding of the present study was that there was no difference in CD44/GAPDH expression between the si-NC transfected H460/CDDP cells and si-TRPC1 transfected H460/CDDP cells, whereas CD44/GAPDH was lower in the si-TRPC1 transfected A549/CDDP cells compared with si-NC transfected A549/CDDP cells; a possible explanation for this could be that: TRPC1 has a limited effect on CD44 expression in H460 cells compared with A549/CDDP cells. Hence, TRPC1 has a limited effect on CD44 expression in H460/CDDP cells compared with that in A549/CDDP cells; however, this effect requires further validation.

The effect of TRPC1 on PI3K/AKT signaling has been previously investigated. TRPC1 promotes hypoxia-associated EMT via activation of the PI3K/AKT signaling pathway in breast cancer cells (11). Another study showed that TRPC1 could enhance the resistance of colon cancer cells to drugs by regulating PI3K/AKT signaling (29). However, whether these mechanisms occur in NSCLC cells has not been determined. Herein, it was shown that TRPC1 knockdown inhibited PI3K/AKT signaling. However, cell treatment with 740 Y-P promoted PI3K/AKT signaling, chemoresistance, and stemness in TRPC1-depleted chemoresistant NSCLC cells. The above finding could be due to an increase in the influx of Ca^{2+} from the extracellular environment, which in turn may reverse the TRPC1 knockdown-mediated inhibition of downstream AKT phosphorylation. That is, TRPC1 knockdown attenuated the PI3K/AKT signaling pathway, further enhancing chemosensitivity and attenuating the stemness of chemoresistant NSCLC cells (34).

The current study has some limitations: i) There are no data to show whether this mechanism is observed *in vivo*; ii) Since TRPC1 expression was relatively high in CDDP-resistant NSCLC cells, a TRPC1 overexpression plasmid may not exert any notable effects on CDDP-resistant NSCLC cells, thus overexpression plasmids were not used in the current study. However, experiments where TRPC1 expression is overexpressed following knockdown may have value to show the necessity and sufficiency of TRPC1 expression; iii) although some previous studies have already investigated the regulatory role of TRPC1 on the proliferation and differentiation of NSCLC cells (22,23), the absence of these experiments to evaluate the effect of TRPC1 on these phenotypes in NSCLC cells is a limitation of the present study; iv) the absence of non-cancerous cell lines as a negative control is a major limitation of the present study.

Collectively, the results of the current study suggested that targeting TRPC1 could attenuate NSCLC stemness and chemoresistance via inactivation of PI3K/AKT signaling; however, further studies are required to determine if this effect is observed *in vivo*.

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Availability of data and materials

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

Authors' contributions

JG contributed to the conception and design of the study. JJ and XY performed the experiment. JH was responsible for the interpretation of data for the work. YZ, HZ, KZ and YW contributed to the data acquisition, data analysis and data interpretation. All authors contributed to the drafting/revising of article. JH and JG confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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