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Tumor necrosis factor receptor-associated protein 1 promotes aerobic glycolysis and cisplatin resistance by regulating the Wnt/β-catenin signaling pathway in lung cancer

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Summary. In this study, we investigated the effects of tumor necrosis factor receptor-associated protein 1 (TRAP1) on aerobic glycolysis in cisplatin-resistant lung cancer cells and explored the underlying mechanism. TRAP1 expression levels were determined in cisplatin-resistant lung cancer tissues and A549/CDDP cells. Subsequently, TRAP1 expression in A549/CDDP cells was silenced via small interfering RNA transfection. Moreover, changes in lactate content, glucose consumption, expression levels of lactate dehydrogenase A (LDHA), hexokinase 2 (HK2), and pyruvate kinase M2 (PKM2), and sensitivity to cisplatin were analyzed. Specifically, the Wnt/β-catenin signaling pathway was examined using the Wnt/β-catenin activator, BML-284. TRAP1 expression levels were higher in cisplatin-resistant tissues and A549/CDDP cells than in cisplatin-sensitive tissues and A549 cells (p<0.05). Moreover, the lactate content, glucose consumption, LDHA, HK2, PKM2 expression levels, and half-maximal inhibitory concentration of cisplatin were all significantly decreased after *TRAP1* silencing (p<0.05). Compared with A549 cells, the Wnt/β-catenin pathway was activated in A549/CDDP cells, which was inhibited via *TRAP1* silencing. BML-284 reversed the effects of *TRAP1* silencing on the aerobic glycolysis and cisplatin sensitivity of A549/CDDP cells. Our findings suggest that TRAP1 affects the cisplatin resistance of lung cancer, possibly by regulating aerobic glycolysis via the Wnt/β-catenin pathway.

Key words: Tumor necrosis factor receptor-related protein 1, Aerobic glycolysis, Lung cancer, Cisplatin resistance, Wnt/β-catenin

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Introduction

Adjuvant chemotherapy based on platinum drugs, such as cisplatin, is generally administered after tumor resection for patients with lung cancer. However, drug resistance is the primary cause of chemotherapy failure and ineffectiveness. Previous studies have proposed various mechanisms for drug resistance, including enhanced detoxification, altered energy supply modes, and enhanced DNA repair (Wu et al., 2024; McWhorter and Bonavida, 2024). Aerobic glycolysis, also known as the "Warburg effect," is a typical energy source for cancer cells (Wu et al., 2020). Even under aerobic conditions, cancer cells still rely on aerobic glycolysis rather than mitochondrial oxidative phosphorylation (OXPHOS) for their energy supply, thereby enhancing their ability to survive in anoxic environments as well as their tolerance to chemoradiotherapy. Studies have shown that metabolic reprogramming is the main mechanism of drug resistance in cancer cells (Lin et al., 2023). Lung cancer treatment can be improved by regulating energy metabolism to inhibit drug resistance in tumor cells. Tumor necrosis factor receptor-associated protein 1 (TRAP1) is an important member of the heat shock protein 90 (HSP90) family and important component of mitochondria. Studies have proven that TRAP1 is closely related to the energy metabolism of tumor cells and participates in tumor progression by inhibiting apoptosis, promoting drug resistance, and maintaining the energy supply under nutrient-deprivation conditions (Matassa, et al., 2018). TRAP1 is associated with drug resistance in ovarian cancer (Amoroso et al., 2017), glioblastoma (Wang et al., 2021), and colon cancer (Bruno et al., 2024), however, its effect on the drug resistance of lung cancer remains unclear. Wnt/β-catenin is an important signaling pathway involved in tumor development, and its roles in tumor drug resistance and tumor metabolism have been demonstrated by many studies. Moreover, TRAP1

affects the activation of the Wnt/β-catenin pathway (Lettini et al., 2020). Therefore, it is speculated that TRAP1 may regulate aerobic glycolysis and cisplatin resistance by activating the Wnt signaling pathway. To verify this, this study aimed to explore the relationships between TRAP1 and energy supply pathways and cisplatin resistance in lung cancer.

Materials and methods

Samples

Thirty lung cancer tissue samples were obtained via biopsy from patients receiving cisplatin chemotherapy alone at the Henan Provincial Chest Hospital from March 2020 to March 2021. Based on the efficacy of chemotherapy, 14 patients were classified into the cisplatin-sensitive group and 16 into the cisplatin-resistant group, according to the World Health Organization "Response Evaluation Criteria in Solid Tumors." In addition, 20 paracancerous tissue samples obtained during surgery were used as controls. All general and clinical patient data are presented in Table 1. All tissue samples were used with the informed consent of the patients.

Immunohistochemistry (IHC)

Resected lung cancer and adjacent tissues were immediately cleaned with phosphate-buffered saline (PBS) (Beijing, China), followed by formalin fixation and paraffin embedding. Subsequently, tissue samples were sectioned into 4-μm slices for immunohistochemical staining. Tissue sections were successively dewaxed, rehydrated, and washed with PBS. This was followed by antigen retrieval using an ethylenediaminetetraacetic acid (EDTA) solution subjected to heating for 20 minutes and subsequent cooling, and then incubated with 2% H_2O_2 for 1h, followed by overnight incubation at 4°C with TRAP1 monoclonal antibody (1:200, Abcam, UK) and then incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:1000, Abcam, UK) for 2 hours. Ultimately, diaminobenzidine solution was added for color rendering, followed by redyeing with hematoxylin. Positive expression was determined using a semi-quantitative method. The final score was the sum of the staining intensity (0 for no staining; 1-3 for light to intense staining) and the percentage of positive cells (0 for <5%, 1 for 6-25%, 2 for 26-50%, 3 for 51-75%, and 4 for 76–100%). Scores: 0–1 indicated negative (–), 2–4 indicated weakly positive (+), and 5-7 indicated strongly positive (++) samples. The average of five fields was calculated for each sample.

Cell culture and treatment

Lung cancer (A549) and cisplatin-resistant lung cancer (A549/CDDP) cells were purchased from Procell (Wuhan, China). The cells were cultured in Roswell Park Memorial Institute-1640 medium (Gibco, USA) with 10% fetal bovine serum and 1% streptomycin and penicillin in a 37°C and 5% CO_2 incubator. A549/CDDP cells were divided into the TRAP1-small interfering RNA (siRNA; transfected with TRAP1-siRNA plasmid) and siNC (transfected with negative control plasmid, Shanghai Sangon Biotechnology Co., Ltd., China) groups. Cells at 1×10^5 cells/mL were seeded in a six-well plate and cultured for 24h. After replacing the medium with a serum-free medium and culturing for 2h, the plasmids were used to transfect cells with Lipofectamine 2000 (Thermo Fisher Scientific, USA), followed by continuous culture for 6h and replacement with a complete medium. To determine whether TRAP1 regulates the Wnt/β-catenin signaling pathway, a Wnt activator group was used for the rescue experiment. After transfecting cells with the TRAP1-siRNA for 12h, the Wnt activator, BML-284 (MedChem Express, Shanghai, China), was added at 0.7 μM.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

After culturing for 48h post-transfection, cells in each group were collected and Trizol lysis buffer (Thermo Fisher Scientific) was used to extract total RNA. cDNA was synthesized using the Quant cDNA kit (Tiangen, China) and SYBR Green SYBR Premix Ex Taq (Takara, Japan) for RT-qPCR. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as the internal reference gene. *TRAP1*: forward primer 5′-GCTCTGGGAGTACGACATG-3′ and reverse primer 5′-TGTTTGGAAGTGGAACCTCCC-3′; *GAPDH*: forward primer 5′-AGGTCGGTGTGAACGGATTC-3′ and reverse primer 5′-TGTAGACCATGTAGTT GAGGTCA-3′. Relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Western blotting (WB)

After 48h of transfection, the cells in each group were collected and lysed using the radioimmuno precipitation assay protein lysate (Beyotime, Shanghai, China), and the protein was collected after centrifugation. Protein concentrations were determined using the bicinchoninic acid method, and then 40 ug protein samples were extracted and loaded on the gel. Subsequently, proteins were separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride membranes. Membranes were blocked with bovine serum albumin, and then rabbit anti-human TRAP1 (1:10000, ab109323, Abcam, UK), lactate dehydrogenase A (LDHA, 1:5000, ab47010, Abcam, UK), hexokinase 2 (HK2, 1:1000, ab227198, Abcam, UK), pyruvate kinase M2 (PKM2, 1:5000, ab85555, Abcam, UK), Wnt3a (1:1000, ab219412, Abcam, UK), β-catenin (1:1000, ab246504, Abcam, UK), β-actin

(1:5000, ab8227, Abcam, UK), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:2000, ab9485, Abcam, UK), and tubulin (1:5000, ab52623, Abcam, UK) antibodies were added and incubated overnight at 4°C, followed by incubation with the HRP-conjugated secondary antibodies (1:10000, ab6721, Abcam, UK) for 2h at room temperature. An electrochemically luminescent solution was used for protein visualization. The protein levels were determined using a gel imaging system (Bio-RAD, USA).

Determination of the extracellular acidification rate (ECAR) and oxygen consumption rate (OCR)

After culturing for 48h, the cells were modulated into a cell suspension with a density of 1.25×10^5/mL, and each cell group was set with three compound pores. The experimental procedures were performed using the XF^e Glycolysis Stress Test Kit (Seahorse Bioscience, USA) and XF^e Cell Mitochondrial Stress Test Kit (Seahorse Bioscience, USA), according to the manufacturer's instructions. The ECAR and OCR of each group were measured using the Seahorse XFe96 cell energy metabolizer.

Determination of glucose consumption

After culturing for 48h post-transfection, the cell supernatant of each group was collected, and the glucose consumption (mmol/L) in each group was determined by using the glucose content detection kit (Solarbio, Beijing, China), according to the manufacturer's instructions.

Determination of lactic acid content

After culturing for 48h post-transfection, the cell supernatants of each group were collected, and the lactic acid content (mmol/L) of each group was determined referring to the instructions of the lactate content detection kit (Solarbio, Beijing, China).

Cell counting kit (CCK)-8

Half-maximal inhibitory concentration (IC50) values of cisplatin in A549 and A549/CDDP cells were determined via the CCK-8 assay. Various concentrations of cisplatin (0, 2, 4, 8, 16, 32, and 64 μg/mL) were used, and the IC50 values of cisplatin in A549 and A549/CDDP cells were used as the working concentrations in subsequent experiments. After culturing for 24h after transfection, the cells were mixed with cisplatin at final concentrations of 12.5 or 32.5 μg/mL, respectively. The cells were seeded in 96-well plates at a density of 5×10^4 cells/mL and cultured for 24, 48, or 72h. Then, 10 μL CCK-8 solution was added to each well, and cultured for 4h, and the optical density at 450 nm was measured using a microplate reader. Finally, the cell proliferation inhibition rate was calculated.

Flow cytometry (FCM)

Twenty-four hours after transfection, cisplatin was added to cells at a final concentration of 12.5 or 32.5 μg/mL and cultured for 48h. Then, cells from each group were collected to prepare single-cell suspensions. The apoptosis rate was determined via FCM (Millipore, USA) using the FITC-Annexin V/PI apoptotic cell detection kit (Thermo Fisher Scientific, USA), according to the manufacturer's instructions.

Statistical analysis

SPSS 20.0 was used for data analysis. All experiments were set with six repeat holes in each group and conducted three times. One-way Analysis of Variance (ANOVA) was used for comparisons between multiple groups, and pairwise comparison was conducted by *SNK-q*. The independent sample t-test was used for comparisons between two groups. Values of $p<0.05$ were considered statistically significant.

Ethics

This study was approved by the Ethics Committee of Henan Provincial Chest Hospital and conformed to the Helsinki Declaration of 1975, as revised in 2008.

Results

TRAP1 levels are upregulated in drug-resistant lung cancer tissues

Clinical data revealed no significant differences in the sex, age, smoking pattern, tumor diameter, pathological type, tumor stage, differentiation degree, and lymphatic metastasis between the cisplatin-sensitive and cisplatin-resistant groups (Table 1). PCR and WB showed that the relative expression levels of TRAP1 mRNA and protein in the cisplatin-resistant group (0.88±0.27 and 0.89±33, respectively) were significantly higher than those in the cisplatin-sensitive group (0.72±0.33 and 0.63±0.23, respectively) and those in both groups were significantly higher than those in the control group (0.44±0.22 and 0.41±0.29, respectively) (Fig. 1A,B). IHC revealed that TRAP1 was mainly expressed in the cytoplasm of lung cancer cells, and the proportion and degree of positively stained cells in lung cancer tissues were higher than those in paracancerous tissues and more obvious in the cisplatin-resistant group than in the cisplatin-sensitive group (Fig. 1C).

TRAP1 levels are upregulated in A549/CDDP cells

The results showed that the relative mRNA and protein expression levels of TRAP1 in A549/CDDP cells (1.04±0.05 and 1.05±0.17, respectively) were significantly higher than those in A549 cells (3.22±0.49 and 2.29±0.38, respectively). Meanwhile, the expression

of TRAP1 significantly decreased in the TRAP1-siRNA group (0.37±0.05 and 0.32±0.04, respectively) compared with that in the siNC group (3.15±0.51 and 2.23±0.29, respectively), and there was no difference between the siNC and A549/CDDP groups (Fig. 2A,B). These results demonstrate that TRAP1 expression is increased in drug-resistant lung cancer cell lines.

Downregulation of TRAP1 inhibits the Warburg effect in A549/CDDP cells

High lactate content and glucose consumption are characteristic of the Warburg effect. OCR and ECAR are indicators of the glycolysis and oxidative phosphorylation capacity of cells, and LDHA, HK-2, and PKM2 are the maker proteins. Therefore, we examined the changes in lactate content, glucose consumption, ECAR, and OCR in A549/CDDP cells with downregulation of TRAP1. The results showed that the ECAR, lactate content (6.73±0.61), and glucose consumption (3.71±0.40) of A549/CDDP cells were significantly higher than those of A549 cells (4.21±0.05 and 2.30±0.32, respectively), while the OCR of A549/CDDP cells decreased. With TRAP1 downregulation, the ECAR, lactate content (4.32±0.57), and glucose consumption (2.32±0.34) of A549/CDDP cells decreased significantly, while OCR decreased (Fig. 3A-D). WB revealed that the expression levels of LDHA (0.77±0.07), HK2 (1.05±0.07), and PKM2 (0.61±0.06) were significantly higher in A549/CDDP cells than in A549 cells (0.59±0.07, 0.83±0.08, and 0.45±0.04, respectively). However, the expression levels of LDHA (0.61±0.04), HK2 (0.86±0.06), and PKM2 (0.47±0.05) were significantly decreased after TRAP1 down-regulation in A549/CDDP cells (Fig. 3E), suggesting that the downregulation of TRAP1 reduces the Warburg effect in A549/CDDP cells.

Downregulation of TRAP1 enhances the cisplatin sensitivity of A549/CDDP cells

After treating cells with different concentrations of cisplatin, the CCK-8 results showed that, compared with A549 cells, the IC50 of A549/DDP was significantly increased (Fig. 4A). Also, the proliferation inhibition rates of the TRAP1-siRNA and TRAP1-siRNA+12.5/32.5 μg/mL groups were significantly higher than those of the siNC and siNC+12.5/32.5 μg/mL groups (Fig. 4B). The results of FCM also showed that the apoptosis rates of TRAP1-siRNA+12.5/32.5 μg/mL cisplatin (23.69±3.00 and 58.83±4.88) were all higher than those of the siNC+12.5/32.5 μg/mL group (8.29±1.39 and 34.48±2.58) (Fig. 4C), which is consistent with the results of CCK-8 assay. These results indicated that the downregulation of TRAP1 enhances the sensitivity of A549/CDDP cells to cisplatin.

Table 1. General and clinical data of patients.

Data n	Paracancerous 20	Cisplatin-sensitive 14	Cisplatin-resistant 16	t/χ^2	*p*
Age (years)	64.25±4.08	64.86±4.47	64.69±3.95	0.099	0.906
Gender [case (%)]					
Male	11 (55.00)	8 (57.14)	9 (56.25)	0.016	0.992
Female	9 (45.00)	6 (42.86)	7 (43.75)		
Smoking [case (%)]					
Yes	12 (60.00)	8 (57.14)	10 (62.50)	0.089	0.956
No	8 (40.00)	6 (42.86)	6 (37.50)		
Pathological type [case (%)]					
Adenocarcinoma	—	7 (50.00)	7 (43.75)		
Squamous carcinoma	—	5 (35.71)	6 (37.50)	0.158	0.924
Alveolar cell carcinoma	—	2 (14.29)	3 (18.75)		
Stage [case (%)]					
I/II	—	4 (28.57)	5 (31.25)	0.026	0.873
III/IV	—	10 (71.43)	11 (68.75)		
Differentiation degree [case (%)]					
High	—	6 (42.86)	5 (31.25)	0.433	0.510
Moderate/Low	—	8 (57.14)	11 (68.75)		
Lymphatic metastasis [case (%)]					
Yes	—	6 (42.86)	10 (62.50)	1.158	0.282
No	—	8 (57.14)	6 (37.50)		
TRAP1 (IHC) [case (%)]					
-	10 (50.00)	2 (14.29)	1 (6.25)		
+	8 (40.00)	8 (57.14)	6 (37.50)	14.697	0.005
++	2 (10.00)	4 (28.57)	9 (56.25)		

Note: TRAP1: tumor necrosis factor receptor-associated protein 1; IHC: Immunohistochemistry.

TRAP1 promotes the Warburg effect by regulating the Wnt/β-catenin pathway

Wnt/β-catenin is an important signaling pathway involved in tumor genesis and development, and its role in tumor drug resistance has also been confirmed by various studies. Several studies have reported that TRAP1 has a certain effect on the activation of the

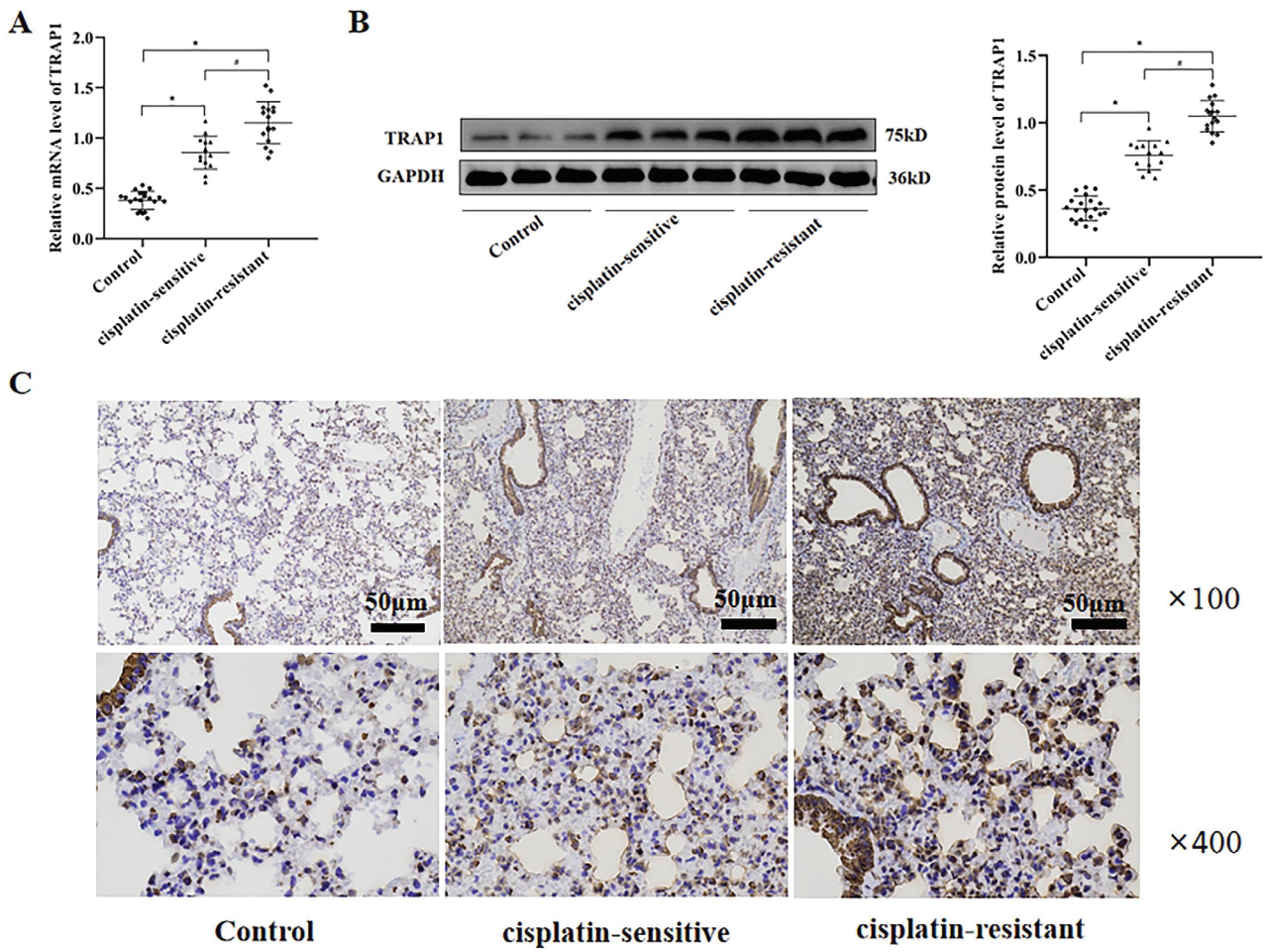

Fig. 1. TRAP1 expression is upregulated in cisplatin-resistant lung cancer tissue (n=1). TRAP1: tumor necrosis factor receptor-associated protein 1(n=1). GAPDH: Glyceraldehyde-3-phosphate dehydrogenase. **A.** RT-qPCR analysis of TRAP1 expression in lung tissues (paracancer, cisplatin-sensitive and cisplatin-resistant). **B.** WB and statistical analysis of TRAP1 expression in different lung tissues. **C.** Positive expression of TRAP1 in different lung tissues checked by IHC. 20 cases in paracancerous group, 14 cases in cisplatin-sensitive group, and 16 cases in cisplatin-resistant group. *p<0.05 *versus* control group. $^{\#}p$<0.05 *versus* cisplatin-sensitive group.

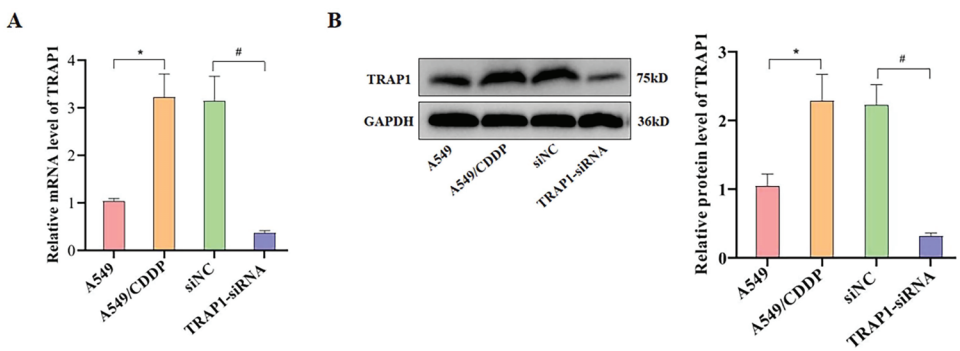

Fig. 2. TRAP1 expression is upregulated in A549/CDDP cells (n=3). TRAP1: tumor necrosis factor receptor-associated protein 1. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase. **A.** RT-qPCR analysis of TRAP1 mRNA expression in A549 cells and A549/CDDP cells (untreated, and transfected with siNC plasmid and TRAP1-siRNA plasmid). **B.** WB and statistical analysis of TRAP1 protein expression in A549 cells and A549/CDDP cells from different treatment groups. *p<0.05 *versus* A549 group. $^{\#}p$<0.05 *versus* siNC group.

Wnt/b-catenin pathway. Therefore, it is reasonable to speculate that TRAP1 may regulate aerobic glycolysis by activating the Wnt signaling pathway. Therefore, we examined the expression of Wnt pathway-related proteins. The results showed that, compared with A549 cells (0.79±0.05 and 0.74±0.05), the expression of Wnt3a (1.05±0.10) and β-catenin (1.04±0.08) in A549/CDDP cells were significantly increased; compared with the siNC group (1.03±0.07 and 0.94±0.07), Wnt3a and β-catenin expression were decreased in the TRAP1-siRNA group (0.77± 0.05,0.75±0.07). Next, rescue experiments were performed with the Wnt activator BML-284 to verify the relationship between TRAP1 and wnt/β-catenin. The results showed that the expression levels of Wnt3a (1.32±0.07) and β-catenin (1.30±0.07) in the BML-284 group were significantly higher than those in the TRAP1-siRNA group (Fig. 5).

The CCK-8 assay showed that the proliferation activity of BML-284+32.5 μg/mL was increased (Fig. 6A,B) and the apoptosis rate (38.40±3.27) decreased compared with the TRAP1-siRNA+32.5 μg/mL group (59.09±4.97) (Fig. 6C). In addition, inhibition of TRAP1-siRNA on glucose consumption (3.60±0.45), lactic acid production (2.02±0.30), and Warburg effect-related PKM2, HK2, and LDH protein expression (0.56±0.05, 0.98±0.08, and 0.71±0.06, respectively) were weakened by BML-284 (Fig. 6D-F). These results demonstrated that the agonist of Wnt/β-catenin, BML-284, reversed the inhibition of TRAP1-siRNA on the Warburg effect in A549/CDDP cells. This means that TRAP1 may promote the Warburg effect and enhance cisplatin resistance in lung cancer by regulating the Wnt/β-catenin pathway.

Discussion

TRAP1 is a mitochondrial protein that interacts with the respiratory chain complex of the HSP90 family, protects cells from oxidative stress-induced injury, and maintains mitochondrial integrity (Lettini et al., 2020; Serapian et al., 2021). TRAP1 also has the functions of anti-apoptosis, regulating cell proliferation and metabolism, and maintaining protein balance (Zhang et al., 2021; Joshi et al., 2020; Kang and Kang 2022). Studies have reported that TRAP1 is highly expressed in colorectal cancer (Maddalena et al., 2017), breast cancer (Vartholomaiou et al., 2017), gastric cancer (Han et al., 2016), and other cancers and is involved in drug resistance in rectal cancer, glioblastoma, and other tumors (Maddalena et al., 2020; Perez Gonçalves et al., 2021). Palladino et al. (Palladino et al., 2016) found that TRAP1 silencing inhibited cell cycle progression in thyroid cancer, leading to cell necrosis, and promoting the sensitivity of cells to chemotherapeutic drugs. Therefore, the inhibition of TRAP1 expression may be an important target for antitumor therapy. However, there have been few reports on the correlation between TRAP1 expression and drug resistance in lung cancer. Zhang et al. (2021a,b) showed that the expression of TRAP1 in A549/DDP and H1299/DDP cells was significantly higher than that in A549 and H1299 cells;

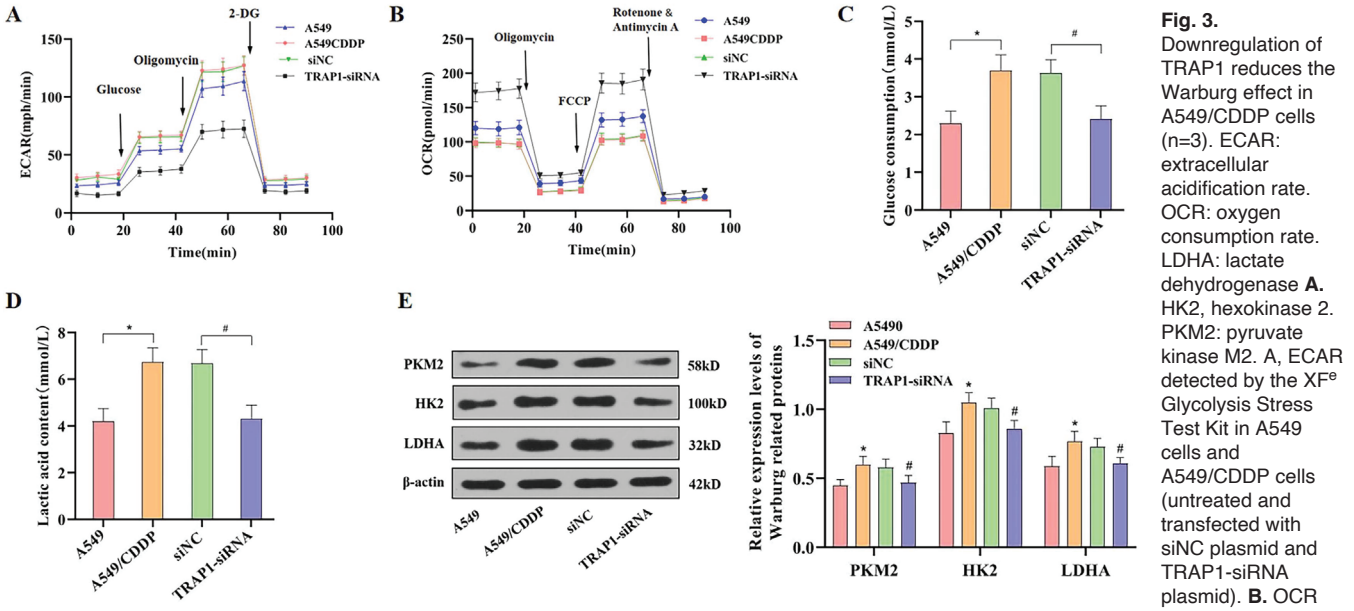

Fig. 3. Downregulation of TRAP1 reduces the Warburg effect in A549/CDDP cells (n=3). ECAR: extracellular acidification rate. OCR: oxygen consumption rate. LDHA: lactate dehydrogenase **A.** HK2, hexokinase 2. PKM2: pyruvate kinase M2. A, ECAR detected by the XF^e Glycolysis Stress Test Kit in A549 cells and A549/CDDP cells (untreated and transfected with siNC plasmid and TRAP1-siRNA plasmid). **B.** OCR detected by the XF^e Cell Mitochondrial Stress Test Kit in A549 cells and A549/CDDP cells from different treatment groups. **C.** Glucose consumption detected by the glucose content detection kit in A549 cells and A549/CDDP cells from different treatment groups. **D.** Lactic acid content detected by the lactate content detection kit in A549 cells and A549/CDDP cells from different treatment groups. **E.** WB and statistical analysis of Warburg effect-related protein (KM2, HK2 and LDHA) expression in A549 cells and A549/CDDP cells from different treatment groups.*p<0.05 *versus* A549 group, $^{\#}p$<0.05 *versus* siNC group.

further studies showed that downregulating TRAP1 induced apoptosis and G2/M cell cycle arrest in A549/DDP and H1299/DDP cells, thereby increasing cell sensitivity to cisplatin. Therefore, TRAP1 may play an important role in cisplatin resistance in lung cancer. Previous studies have indicated that TRAP1 is highly expressed in lung cancer tissues and cells (Liu et al., 2019; Zhang et al., 2021a,b), and its expression was elevated in various drug-resistant cells (Kuchitsu et al., 2020; Mathieu et al., 2019). In the present study, mRNA and protein level results showed that the expression of TRAP1 in lung cancer tissues was significantly higher than that in paracancerous tissues, and higher in the cisplatin-resistant than in the cisplatin-sensitive group; the same conclusion was obtained with the IHC results. Concurrently, the expression of TRAP1 in A549/CDDP cells was significantly upregulated compared to that in A549 cells, however, after silencing TRAP1, the expression level of TRAP1 was significantly decreased in A549/CDDP cells. These results are consistent with those of previous studies showing that TRAP1 may induce drug resistance by blocking drug-induced apoptosis in tumor cells (Agorreta et al., 2014). These results suggest that TRAP1 may be a key factor in cisplatin resistance in lung cancer cells.

Aerobic glycolysis, also known as the "Warburg

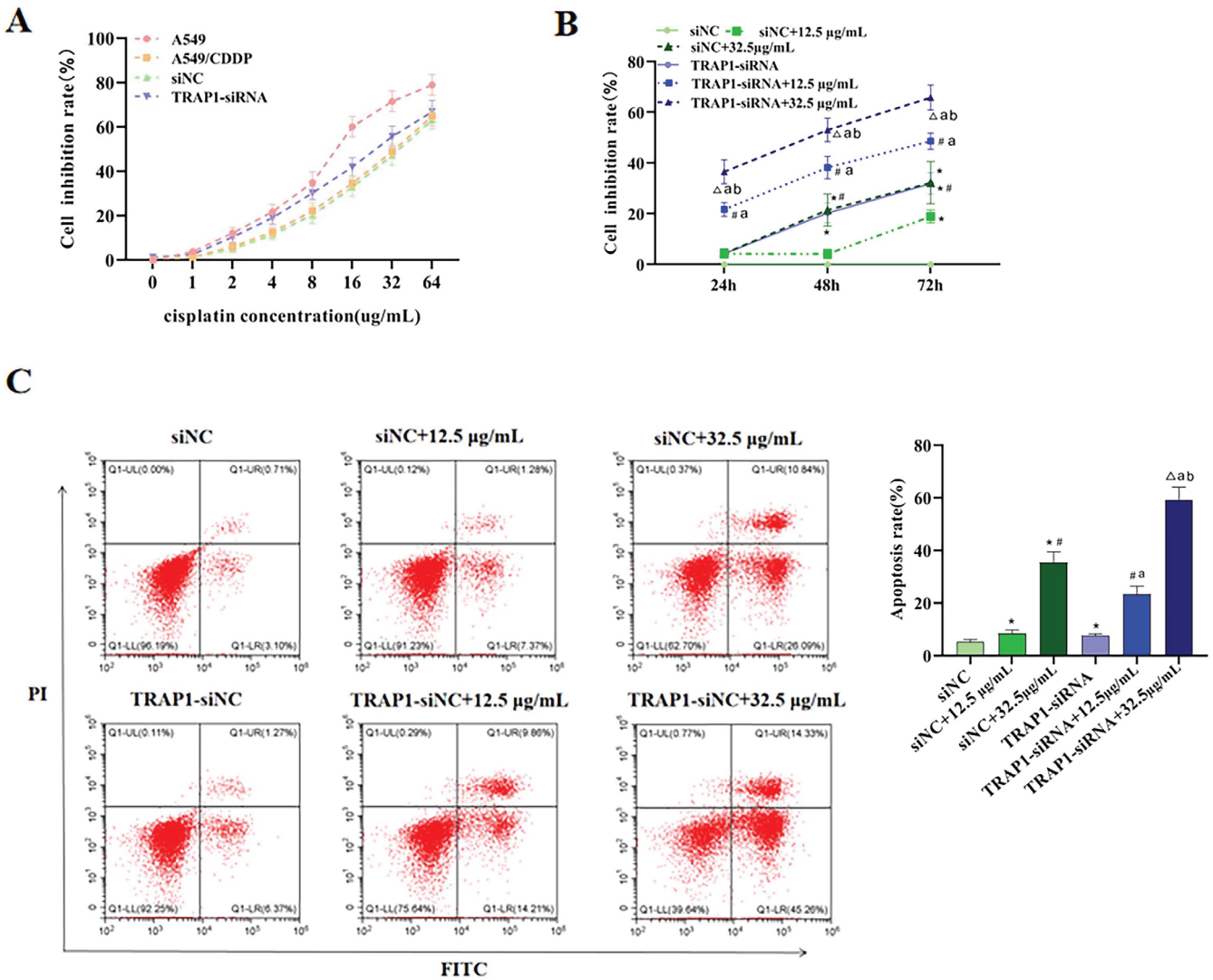

Fig. 4. Downregulation of TRAP1 enhances the cisplatin sensitivity of A549/CDDP cells. TRAP1: tumor necrosis factor receptor-associated protein 1. **A.** CCK8 analysis of IC50 concentration of cisplatin in A549 cells and A549/CDDP cells (untreated and transfected with siNC plasmid and TRAP1-siRNA plasmid) (n=6). **B.** Cell proliferation inhibition rate measured by CCK-8 in A549/CDDP cells transfected with siNC plasmid and TRAP1 siRNA plasmid and treated with cisplatin (0 μg/mL,12.5 μg/mL and 32.5μg/mL). (n=3). **C.** Flow cytometry and statistical analysis of apoptosis rate in A549/CDDP cells from different treatment groups (n=3). *p<0.05 *versus* siNC group. $^{\#}p$<0.05 *versus* siNC+12.5 μg/mL group. $^{\triangle}p$<0.05 *versus* siNC+32.5μg/mL group. ^{a}p<0.05 *versus* TRAP1-siRNA group. ^{b}p<0.05 *versus* TRAP1-siRNA+12.5 μg/mL group.

effect," is an important characteristic of tumor metabolic reprogramming and is the most important energy supply mode of tumors (Vaupel et al., 2019). It can transfer Adenosine Triphosphate (ATP) faster, accelerate the continuous proliferation and malignant progression of tumors, and induce tumor resistance (Icard et al., 2018). Therefore, inhibition of the Warburg effect is considered an important strategy to reduce drug resistance in tumors (Liu et al., 2024). TRAP1 is a key regulatory factor involved in tumor cell metabolism and the regulation of OXPHOS in the Warburg effect transformation; however, its expression and roles vary in different tumor tissues. For example, the Warburg effect is the main metabolic pattern in colorectal cancer, breast cancer, lung cancer, and other tumors with high TRAP1 expression, whereas OXPHOS is the main metabolic mode in ovarian and cervical cancers with low TRAP1 expression (Amoroso et al., 2016; Lettini et al., 2017).

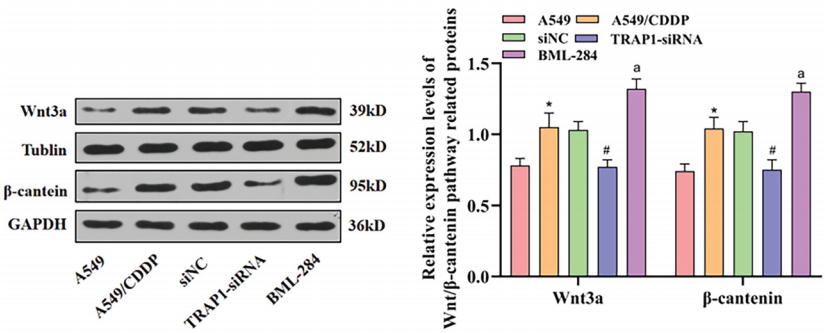

Fig. 5. Downregulation of TRAP1 reduces the activation of the Wnt/β-catenin pathway in A549/CDDP cells (n=3). TRAP1: tumor necrosis factor receptor-associated protein 1. WB and statistical analysis of Wnt3a and β-catenin protein expression in A549 cells and A549/CDDP cells (untreated and transfected with siNC plasmid TRAP1-siRNA plasmid and Wnt activators BML-284 plasmid). **p*<0.05 *versus* A549 group. #P<0.05 *versus* siNC group. [a]*p*<0.05 *versus* TRAP1-siRNA group.

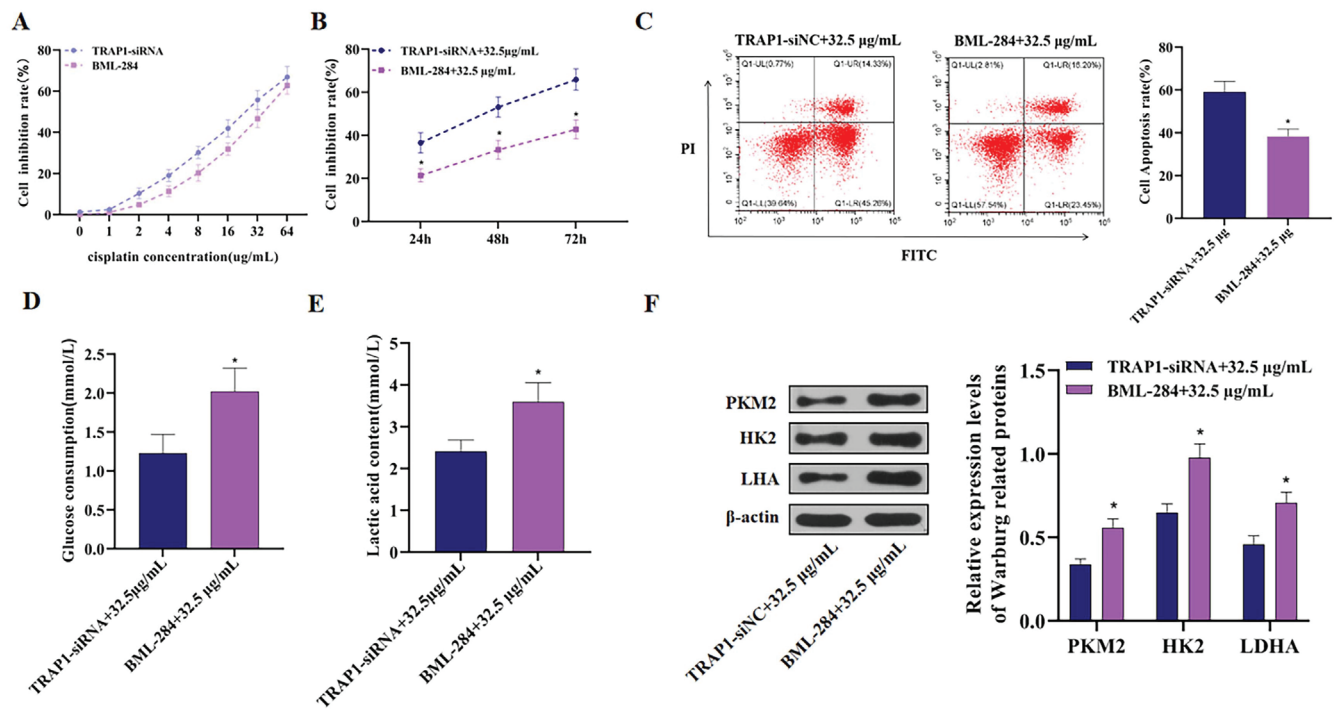

Fig. 6. TRAP1 promotes the Warburg effect and increases cisplatin resistance by regulating the Wnt/β-catenin pathway. TRAP1: tumor necrosis factor receptor-associated protein 1. LDHA: lactate dehydrogenase **A.** HK2: hexokinase 2. PKM2: pyruvate kinase M2. A, CCK8 analysis of IC50 concentration of cisplatin in A549/CDDP cells transfected with TRAP1-siRNA plasmid and Wnt activators BML-284 plasmid. BML-284 increased the IC50 concentration of cisplatin(n=6). **B.** CCK8 analysis of Cell proliferation inhibition rate in A549/CDDP cells from different treatment groups (n=3). **p*<0.05 *versus* TRAP1-siRNA+32.5 μg/mLgroup. **C.** Flow cytometry and statistical analysis of apoptosis rate in A549/CDDP cells from different treatment groups (n=3). **D.** Glucose consumption measured by the glucose content detection kit in A549/CDDP cells from different treatment groups (n=3). **p*<0.05 *versus* TRAP1-siRNA+32.5 μg/mLgroup. **E.** Lactic acid content detection by the lactate content detection kit in A549/CDDP cells from different treatment groups (n=3). **F.** WB and statistical analysis of Warburg effect-related protein (KM2, HK2 and LDHA) expression in A549/CDDP cells from different treatment groups (n=3). BML-284 enhanced the Warburg effect-related protein expression (n=3). **p*<0.05 *versus* TRAP1-siRNA+32.5 μg/mLgroup.

These results suggest that high TRAP1 expression might facilitate the metabolic transformation of tumors via the Warburg effect, rather than OXPHOS (Guzzo et al., 2014). Indeed, TRAP1 inhibits mitochondrial respiration through the interaction of respiratory chain complexes to enhance the expression of glucose transporters, thereby promoting glycolysis and increasing glucose uptake and lactate production (Sciacovelli et al., 2013; Im, 2016), which means that TRAP1 promotes glycolysis to remodel metabolism by downregulating OXPHOS. Yoshida et al. (2013) showed that TRAP1 deletion increased mitochondrial oxygen consumption, resulting in an increase in tricarboxylic acid cycle products, such as ATP and ROS levels, thus inhibiting the Warburg effect; high expression of TRAP1 had the opposite effect. Silencing TRAP1 can also reduce the Warburg effect and decrease lactate production in glioblastomas (Wu et al., 2016). Previous studies have shown that decreasing TRAP1 levels in drug-resistant cells increases OCR and decreases glycolysis compared with drug-sensitive cell lines (Matassa et al., 2016). Maddalena et al. (2020) illustrated that TRAP1 can enhance Warburg metabolism in colorectal cancer cells by regulating the activity of phosphofructokinase and increasing cell drug resistance, whereas targeting TRAP1 could enhance cell sensitivity to drugs. These results suggest that TRAP1 is involved in the regulation of energy metabolism in tumor cells and may improve the response of tumor cells to drugs by regulating the Warburg effect. The present study showed that the Warburg effect in A549/CDDP cells was more obvious than that in A549 cells. Silencing of TRAP1 resulted in a decrease in ECAR and an increase in OCR in A549/CDDP cells, thereby decreasing lactate content, glucose consumption, and expression of LDHA, HK2, and PKM2 proteins. This indicated that the down-regulation of TRAP1 in A549/CDDP cells initiated metabolic reprogramming, with a reduced Warburg effect in A549/CDDP cells. IC50 values, cell proliferation, and apoptosis under different concentrations of cisplatin were also detected, and the results showed that downregulation of TRAP1 significantly reduced the IC50 values of cisplatin, proliferation activity, and promoted cell apoptosis in A549/CDDP cells, indicating that TRAP1 downregulation increased the sensitivity of cells to cisplatin. Thus, it was concluded that TRAP1 is related to cisplatin resistance in lung cancer, which may be related to the promotion of the Warburg effect by cisplatin.

Currently, the molecular mechanism underlying the role of TRAP1 in cisplatin resistance in lung cancer remains unclear and requires further investigation. The wnt/β-catenin pathway is an important pathway involved in embryo development, tissue regeneration, cell proliferation and tumor genesis, development, and drug resistance (Wang et al., 2020; Zhu et al., 2021). Studies have shown that TRAP1 can activate the Wnt/β-catenin pathway, thus regulating a variety of biological processes (Lettini et al., 2020). Based on previous research, it was speculated that TRAP1 might play a role in regulating aerobic glycolysis by activating the Wnt/β-catenin signaling pathway. Therefore, the expression of Wnt/β-catenin pathway-related proteins was detected, and a reversal experiment using Wnt activators was performed. The results showed that TRAP1 silencing significantly reduced the protein expression levels of Wnt3a and β-catenin, which suggested that TRAP1 activates the Wnt/β-catenin pathway. In addition, after adding the Wnt/β-catenin agonist BML-284, the expression of Wnt3a and β-catenin was significantly upregulated, the cell proliferation ability and expression of aerobic glycolysis-related proteins HK2, PKM2, LDHA were upregulated, and the apoptosis rate was downregulated. This showed that the Wnt/β-catenin agonist reversed the effect of TRAP1 silencing in A549/CDDP cells. Then, we concluded that TRAP1 may promote the Warburg effect and cisplatin resistance in lung cancer by activating the Wnt/β-catenin pathway. In addition, there are some shortcomings in this study, e.g., that other lung cancer cell lines were not used to verify the results, which is also the direction of our future research.

Conclusion

In this study, TRAP1 was highly expressed in lung cancer tissues, especially cisplatin-resistant lung cancer tissues, and promoted cisplatin resistance by enhancing the Warburg effect and altering the energy supply of lung cancer cells. Our findings suggest that the underlying mechanism may involve the activation of the Wnt/β-catenin signaling pathway.

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