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

Ruijie Li
Henan Provincial Chest Hospital

Mengguo Li
Henan Provincial Chest Hospital

Juan Lv
Henan Provincial Chest Hospital

Qian Sun (sunqian_sun123@163.com)
Henan Provincial Chest Hospital

Research Article

Keywords: Tumor necrosis factor receptor-related protein 1, aerobic glycolysis, lung cancer, cisplatin resistance, Wnt/β-catenin

Posted Date: March 13th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2650279/v1

License: Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Objective: To investigate the effect of tumor necrosis factor receptor-associated protein 1 (TRAP1) on aerobic glycolysis in cisplatin-resistant lung cancer and its possible mechanism.

Methods: The expression of TRAP1 in cisplatin resistant lung cancer tissues and A549/CDDP cells were detected. The expression of TRAP1 in A549/CDDP cells were silenced by transfection siRNA. The changes of lactate content, glucose consumption and the expression of lactate dehydrogenase A (LDHA), hexokinase 2 (HK2) and pyruvate kinase M2 (PKM2) and sensitivity to cisplatin were detected. The change of Wnt/β-catenin signaling pathway was detected, and the Wnt/β-catenin activator BML-284 was used to conduct reverse expression.

Results TRAP1 expression was increased in cisplatin-resistant tissues and A549/CDDP cells compared with cisplatin-sensitive tissues and A549, respectively ($P < 0.05$); After silencing TRAP1, the lactic content, glucose consumption, LDHA, HK2, PKM2 expression, IC50 concentration of cisplatin were significantly increased ($P < 0.05$). Compared with A549, Wnt/β-catenin pathway was activated in A549/CDDP, and inhibited after TRAP1 silencing. BML-284 can reverse the effects of silencing TRAP1 on aerobic glycolysis and cisplatin sensitivity of A549/CDDP.

Conclusion TRAP1 is involved in the cisplatin resistance in lung cancer, and its mechanism is related to the regulation of aerobic glycolysis by regulating Wnt/β-catenin pathway.

Introduction

Adjuvant chemotherapy based on platinum drugs (such as cisplatin) is usually used after tumor resection for lung cancer patients. However, drug resistance is the main cause of chemotherapy failure or ineffectiveness. Previous studies have proposed various of mechanisms of drug resistance, including enhancing the detoxification, changing energy supply mode and enhancing DNA repair ability$^{[1,2]}$. Aerobic glycolysis, also known as the "Warburg effect", is a typical energy supply feature of cancer cells$^{[3]}$. Even under aerobic conditions, cancer cells still rely on aerobic glycolysis rather than mitochondrial oxidative phosphorylation(OXPHOS) to obtain energy, thereby enhancing their survival ability in anoxic environment, as well as the tolerance to chemoradiotherapy. Existing studies have pointed out that metabolic reprogramming is the main mechanism of drug resistance in cancer cells$^{[4]}$, so it is an important strategy to improve the therapeutic effect of lung cancer by regulating the energy metabolism to inhibit drug resistance of tumor cells.

Tumor necrosis factor receptor associated protein 1 (TRAP1) is an important member of the heat shock protein 90 (HSP90) family and an important part of the mitochondrial energy metabolism. Studies have shown that TRAP1 is involved in tumor progression by inhibiting apoptosis, promoting drug resistance and in maintaining energy supply under nutrient deprivation conditions$^{[5]}$. TRAP1 has been reported to be associated with the drug resistance of ovarian cancer$^{[6]}$, glioblastoma$^{[7]}$ and colon cancer$^{[8]}$ and so on,
but whether it is involved in the drug resistance mechanism of lung cancer is still unclear. Therefore, this study intends to explore the relationship between TRAP1 and the energy supply pathway of lung cancer and its cisplatin resistance.

**Materials And Methods**

1.1 **Samples** Thirty lung cancer tissue samples obtained by biopsy in the Henan Provincial Chest Hospital from March 2020 to March 2021 were collected, and all patients received cisplatin chemotherapy alone. According to the efficacy of chemotherapy, fourteen patients were divided into the cisplatin sensitive group and sixteen patients were cisplatin resistant group according to the WHO "Response evaluation criteria in solid tumors (RECIST)". In addition, twenty paracancerous tissues obtained by operation were collected as control group. The general and clinical data of all patients were shown in Table 1. All tissue samples were used with the patient's authorization.
### Table 1
General and clinical data of patients

<table>
<thead>
<tr>
<th>Data</th>
<th>Control</th>
<th>Cisplatin sensitive</th>
<th>Cisplatin resistant</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>14</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age(years old)</td>
<td>64.25 ± 4.08</td>
<td>64.86 ± 4.47</td>
<td>64.69 ± 3.95</td>
<td>0.099</td>
<td>0.906</td>
</tr>
<tr>
<td>Gender[case(%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11(55.00)</td>
<td>8(57.14)</td>
<td>9(56.25)</td>
<td>0.016</td>
<td>0.992</td>
</tr>
<tr>
<td>Female</td>
<td>9(45.00)</td>
<td>6(42.86)</td>
<td>7(43.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoke[case(%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12(60.00)</td>
<td>8(57.14)</td>
<td>10(62.50)</td>
<td>0.089</td>
<td>0.956</td>
</tr>
<tr>
<td>No</td>
<td>8(40.00)</td>
<td>6(42.86)</td>
<td>6(37.50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor diameter (cm)</td>
<td></td>
<td></td>
<td></td>
<td>0.110</td>
<td>0.913</td>
</tr>
<tr>
<td>Pathological type[case(%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td></td>
<td>7(50.00)</td>
<td>7(43.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous carcinoma</td>
<td></td>
<td>5(35.71)</td>
<td>6(37.50)</td>
<td>0.158</td>
<td>0.924</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>2(14.29)</td>
<td>3(18.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage[case(%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>/</td>
<td></td>
<td>4(28.57)</td>
<td>5(31.25)</td>
<td>0.026</td>
<td>0.873</td>
</tr>
<tr>
<td>/</td>
<td></td>
<td>10(71.43)</td>
<td>11(68.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Differentiation degree[case(%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td></td>
<td>6(42.86)</td>
<td>5(31.25)</td>
<td>0.433</td>
<td>0.510</td>
</tr>
<tr>
<td>Moderate/Low</td>
<td></td>
<td>8(57.14)</td>
<td>11(68.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphatic metastasis[case(%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>6(42.86)</td>
<td>10(62.50)</td>
<td>1.158</td>
<td>0.282</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>8(57.14)</td>
<td>6(37.50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRAP1(IHC)[case(%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10(50.00)</td>
<td>2(14.29)</td>
<td>1(6.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>8(40.00)</td>
<td>8(57.14)</td>
<td>6(37.50)</td>
<td>14.697</td>
<td>0.005</td>
</tr>
<tr>
<td>++</td>
<td>2(10.00)</td>
<td>4(28.57)</td>
<td>9(56.25)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.2 Immunohistochemistry (IHC)

Lung cancer or paracancerous tissues were washed with phosphate buffer saline (PBS) immediately after excision and preserved after paraffin embedding. Take the preserved lung cancer or paracancerous tissue sections, dewaxed, rehydrated, washed with PBS, added with ethylene diamine tetraacetic acid antigen repair solution and cooled after heating for 20 minutes, and incubated with 2% H$_2$O$_2$ for 1h followed by incubation with TRAP1 primary antibody (1:1000) overnight at 4°C and secondary antibody for 2h. Finally, diaminobenzidine solution was added for color rendering followed by redyeing with hematoxylin. Positive expression was defined by semi-quantitative method. The final score was the sum of staining intensity (0 for no staining, 1–3 from light to deep) and percentage of positive cells (0 for less than 5%, 1 for 6–25%, 2 points for 26–50%, 3 for 51–75%, 4 for 76–100%). 0 ~ 1 was negative(-), 2 ~ 4 was weak positive(+), 5 to 7 were strongly positive(++) . The average of 5 fields is taken for each sample.

1.3 Cell culture and treatment

Lung cell line A549 and cisplatin-resistant cell line A549/CDDP were purchased from Procell(Wuhan, China). The cells were cultured in RPMI-1640 cell culture medium (Gibco, USA) with 10% fetal bovine serum (FBS) and 1% streptomycin and penicillin in a 37°C and 5% CO2 incubator. A549/CDDP cells were divided into the TRAP1-siRNA group (transfected with TRAP1-siRNA plasmid) and siNC group (transfected with negative control plasmid, Shanghai Sangon Biotechnology Co., Ltd, China): Cells at 1×10$^5$ cells/mL were seeded in a 6-well plate and cultured for 24 h, 2 mL per well. After being replaced with serum-free medium and cultured for 2h, the plasmids were transfected using Lipofectamine 2000 (Thermo Fisher, USA) followed by continued culture for 6 h and replacement with complete medium. In order to prove that the regulation of Wnt/β-catenin signaling pathway is the mechanism of TRAP1, a Wnt activator group was used for rescue experiment: after the cells were transfected with TRAP1-siRNA for 12h, the wnt activator BML-284 (MedChem Express, Shanghai, China) was added in (0.7µM).

1.4 qRT-PCR

After culturing for 48h after transfection, cells in each group were collected and Trizol lysis buffer (Thermo Fisher, USA) was added to extract total RNA. cDNA was synthesized by Quant cDNA kit (Tiangen, China) and SYBR Green SYBR Premix Ex Taq (Takara, Japan) was used for quantitative real-time polymerase chain reaction (qRT-PCR). GAPDH was used as an internal reference gene. TRAP1 forward primer: 5'-GCTCTGGGAGTACGACATG-3', reverse primer: 5'-TGTTTGGAAGTGGAACCTCCC-3'. GAPDH forward primer: 5'-AGGTCGGTGTGAACGGAACCTCCC-3', reverse primer: 5'-TGTAGACCATGTAGTTGGAGTCA-3'. Relative mRNA expression was calculated by 2$^{−ΔΔCt}$ method.

1.5 Western blot (WB)

Being cultured for 48 h after transfection, cells in each group were collected and lysed with RIPA protein lysate (Beyotime, Shanghai, China), and the protein was collected after centrifugation. Protein concentrations were determined by BCA method. Then the proteins were separated by SDS-PAGE and
transferred to polyvinylidene fluoride membranes. After blocking with bovine serum albumin, rabbit anti-human TRAP1, lactate dehydrogenase A (LDHA, 1:5000, Abcam), hexokinase 2 (HK2, 1:1000, Abcam), pyruvate kinase M2 (PKM2, 1:5000, Abcam), Wnt3a(1:1000, Abcam), β-catenin(1:5000, Abcam), GAPDH(1:2000, Abcam) and tublin(1:5000, Abcam) antibodies were added overnight at 4°C, followed by incubation of HRP-conjugated secondary antibodies(1:10000, Abcam, UK) for 2h at room temperature. Electrochemical luminescence solution was used for protein visualization. The protein levels were analyzed by gel imaging system (Bio-RAD, USA).

### 1.6 Glucose consumption detection

After being cultured for 48 h after transfection, the cell supernatant of each group were collected, and the glucose consumption (mmol/L) of each group was determined according to the instructions of the glucose content detection kit (Solarbio, Beijing, China).

### 1.7 Lactic acid content

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

### 1.8 CCK-8

The IC50 concentration of cisplatin in A549 and A549/CDDP cells was detected by CCK8 assay and the concentration of cisplatin was 0, 2, 4, 8, 16, 32, 64 µg/mL, respectively, and the IC50 concentration of A549 and A549/CDDP was used as the working concentration of cisplatin in subsequent experiments. After being cultured for 24h after transfection, the cells were added with cisplatin with final concentrations of 12.5 µg/mL or 34.5µg/mL dose, respectively. The cells were seeded in 96-well plates at a density of 5×10^4 cells/mL and were cultured for 24, 48 or 72 h, respectively, 10 µL CCK-8 solution was added to each well, and after culturing for 4 h, the optical density value at 450nm was measured by a microplate reader, and the cell proliferation inhibition rate was calculated.

### 1.9 Flow cytometry (FCM)

24h after transfection, cisplatin was added with a final concentration of 6.25µM or 12.5µM, and cultured for 48h, cells from each group were collected to prepare single cell suspension. According to the instructions of FITC-Annexin V/PI apoptotic cell detection kit (Thermo Fisher, USA), after adding FITC-Annexin V and PI stain, the apoptosis rate was determined by flow cytometry (MILLIPORE, USA).

### 1.10 Statistical analysis

SPSS 20.0 was used for data analysis. All experiments were set with 6 repeat holes in each group, and conducted for three times. Statistical differences among multiple groups were analyzed by one-way ANOVA and pairwise comparison was conducted by SNK-q. Statistical differences between two independent groups were analyzed by independent samples t test. Statistical significance was indicated by P< 0.05.
Results

2.1 TRAP1 is up-regulated in the drug-resistant lung cancer tissues

The clinical data showed that there was no significant difference in gender, age, smoke, tumor diameter and pathological type, tumor stage, differentiation degree, lymphatic metastasis between the cisplatin sensitive group and cisplatin resistant group (Table 1), and the results of PCR and WB showed that the relative expression levels of TRAP1 mRNA and protein in cisplatin resistant group were significantly higher than those in cisplatin sensitive group, and both the two groups were significantly higher than those in control group (Fig. 1A, B). The results of IHC showed that TRAP1 mainly expressed in cytoplasm of lung cancer, and the proportion and degree of positive staining cells in lung cancer tissues were higher than those in paracancerous tissues, and more obvious in cisplatin resistant group than cisplatin sensitive group (Fig. 1C).

2.2 TRAP1 is up-regulated in A549/CDDP cells

The results showed that the relative mRNA level and protein expression level of TRAP1 in A549/CDDP cells were significantly higher than those in A549 cells. Meanwhile, the expression of TRAP1 significantly decreased in TRAP1-siRNA group compared with siNC group, and there was no difference between siNC group and A549/CDDP (Fig. 2A, B). The results demonstrated that TRAP1 expression in drug-resistant lung cancer cell lines was increased.

2.3 Down-regulation of TRAP1 inhibited the Warburg effect of A549/CDDP

High lactate content and glucose consumption are characteristics of the Warburg effect, and LDHA, HK−2 and PKM2 are the maker proteins. Therefore, the changes of lactate content and glucose consumption in A549/CDDP cells after down-regulating TRAP1 were detected. The results showed that the lactate content and glucose consumption of A549/CDDP cells were significantly higher than those of A549 cells. After down-regulation of TRAP1, the lactate content and glucose consumption of A549/CDDP cells decreased significantly (Fig. 3A, B). WB results showed that the relative expressions of LDHA, HK−2 and PKM2 proteins in A549/CDDP cells were significantly higher than those in A549 cells, and those in TRAP1-siRNA were all decreased significantly than siNC group (Fig. 3C), suggesting that down-regulation of TRAP1 reduced the Warburg effect of A549/CDDP cells.

2.4 Down-regulation of TRAP1 enhanced cisplatin sensitivity of A549/CDDP cells

After treating cells with different concentrations of cisplatin, CCK8 results showed that compared with A549, the IC50 of A549/DDP was significantly increased (Fig. 4A). And the proliferation inhibition rates of TRAP1-siRNA and TRAP1-siRNA + 12.5/32.5 µg groups were significantly higher than those of siNC and
siNC + 12.5/32.5 µg groups, respectively (Fig. 4B). The results of FCM also showed that the apoptosis rates of siRNA+12.5/32.5 µg cisplatin were all higher than those of siNC+12.5/32.5 µg group (Fig. 4C), which is consistent with the results of CCK8 assay. The results above indicated that down-regulation of TRAP1 enhanced the sensitivity of A549/CDDP cells to cisplatin.

### 2.5 TRAP1 promoted the Warburg effect by regulating the Wnt/β-catenin pathway

Wnt/β-catenin is an important signal pathway involved in tumor genesis and development, and its role in tumor drug resistance has also been confirmed by various of studies. Several studies have reported that TRAP1 has a certain effect on the activation of Wnt/β-catenin pathway, so it is speculated that TRAP1 may regulate aerobic glycolysis by activating Wnt signaling pathway. Therefore, the expression of Wnt pathway-related proteins were detected. The results showed that compared with A549 cells, the expressions of Wnt3a and β-catenin in A549/CDDP cells were significantly increased, and compared with siNC group, Wnt3a and β-catenin expressions were decreased in TRAP1-siRNA group. Then the rescue experiments were performed by Wnt activators BML-284 to verify the relationship of TRAP1 and wnt/β-catenin. The results showed that the expression levels of Wnt3a and β-catenin in the BML-284 group were significantly higher than those in TRAP1-siRNA group (Fig. 5).

The results of CCK8 assay showed that the proliferation activity of BML-284 + 32.5 µg was increased (Fig. 6A, B) and the apoptosis rate decreased compared with TRAP1-siRNA + 32.5 µg group (Fig. 6C). Meanwhile, the inhibition of TRAP1-siRNA on glucose consumption, lactic acid production and Warburg effect-related proteins expression were weakened by BML-284 (Fig. 6D,E,F). These results demonstrated that the agonist of Wnt/β-catenin BML-284 reversed the inhibition of TRAP1-siRNA on Warburg effect of A549/CDDP cells, which means that TRAP1 may promote the Warburg effect and enhance cisplatin resistance in lung cancer through regulating Wnt/β-catenin pathway.

### Discussion

TRAP1 is the only mitochondrial protein that interacts with the respiratory chain complex in the HSP90 family, and protects cells from oxidative stress injury and maintains mitochondrial integrity. TRAP1 also has the functions of anti-apoptosis, regulating cell metabolism and maintaining protein balance, etc. Studies have reported that TRAP1 is highly expressed in colorectal cancer, breast cancer, gastric cancer and other cancers, and is involved in the drug resistance of rectal cancer, glioblastoma and other tumors. Giuseppe et al. found that silencing TRAP1 could inhibit the progression of cell cycle of thyroid cancer, leading to cell necrosis and promote the sensitivity of cells to chemotherapeutic drugs. Therefore, targeted inhibition of TRAP1 expression can be an important target for anti-tumor. At present, there are few studies on relationship of TRAP1 and drug resistance in lung cancer. Some studies have shown that TRAP1 expressed highly in lung cancer. In this study, the results showed that the expression of TRAP1 in lung cancer tissues was significantly higher than that in paracancerous tissues,
and higher in drug resistance group than sensitive group; Concurrently, the expression of TRAP1 in A549/CDDP cells was significantly up-regulated than A549 cells, which is consistent with the results of previous studies.

Warburg effect is the most typical energy supply method for tumor metabolism \[^{[21]}\], which can deliver ATP faster, accelerate the continuous proliferation and malignant progression, and induce tumor resistance\[^{[22]}\]. Therefore, inhibition of Warburg effect has been considered as an important means to reduce drug resistance of tumor \[^{[23]}\]. TRAP1, as a key regulatory factor involved in tumor cell metabolism, is involved in the regulation of OXPHOS to Warburg effect transformation, but its expression and roles vary in different tumor tissues. For example, in colorectal cancer, breast cancer, lung cancer and other tumors with high expression of TRAP1, Warburg effect is the main metabolic pattern, while in ovarian cancer and cervical cancer with low expression of TRAP1, OXPHOS is the main metabolic pattern \[^{[24]}\], indicating that high expression of TRAP1 is conducive to the metabolic transformation of tumors into Warburg rather than OXPHOS \[^{[25]}\]. In fact, 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 \[^{[26, 27]}\], which means TRAP1 promotes glycolysis to remodel metabolism by downregulating OXPHOS. Oichiro Yoshidaa et al.\[^{[28]}\] showed that TRAP1 deletion increased the mitochondrial oxygen consumption, resulting in the increase of tricarboxylic acid cycle products, such as ATP and ROS levels, and thus inhibited Warburg effect, and high expression of TRAP1 had the opposite effect. In glioblastoma, silencing TRAP1 could also reduces the Warburg effect and decrease lactate production \[^{[29]}\]. Maddalena et al.\[^{[16]}\] illustrated that TRAP1 can enhance Warburg metabolism of colorectal cancer cells by regulating the activity of phosphofructokinase and increase cell drug resistance, while targeting TRAP1 could enhance cell sensitivity to drugs. These results above indicate that TRAP1 may improve the response of tumor cells to drugs by regulating Warburg effect. The results of this study also showed that the Warburg effect of A549/CDDP cells was more obvious than A549 cells, and silencing TRAP1 resulting in the decrease of lactate content, glucose consumption and protein expression of LDHA, HK2 and PKM2 in A549/CDDP cells, which illustrating that down-regulation of TRAP1 reduced the Warburg effect of A549/CDDP cells. Meanwhile, the IC50 concentration, cell proliferation and apoptosis under different concentrations of cisplatin were also detected, and the results showed that down-regulation of TRAP1 could significantly reduce the IC50 concentration of cisplatin, proliferation activity and promote cell apoptosis of A549/CDDP cells, indicating that down-regulation of TRAP1 increased the sensitivity of cells to cisplatin. Then it was concluded that TRAP1 was related to cisplatin resistance of lung cancer and the reason may be related to the promotion on Warburg effect under cisplatin.

At present, the molecular mechanism of TRAP1 on cisplatin resistance in lung cancer is still unclear, which requires further discussion. Wnt/\(\beta\)-catenin pathway is an important pathway involved in embryo development, tissue regeneration, cell proliferation and tumor genesis, development and drug resistance\[^{[30, 31]}\]. Studies have shown that TRAP1 can activate the Wnt/\(\beta\)-catenin pathway, thus regulating a variety of biological process\[^{[32]}\]. Based on previous research, it was speculated that TRAP1
might play a role in regulating aerobic glycolysis by activating Wnt/β-catenin signaling pathway. Therefore, the expression of Wnt/β-catenin pathway-related proteins were detected and reversal experiment using Wnt activators was performed. The results showed that silencing TRAP1 significantly reduced the protein expression levels of Wnt3a and β-catenin, which suggested that TRAP1 activate the Wnt/β-catenin pathway; And after adding with Wnt/β-catenin agonist BML–284, the expression of Wnt3a and β-catenin was significantly up-regulated, the cell proliferation ability and expressions of aerobic glycolysis related proteins HK2, PFKM, LDHA were up-regulated, and the apoptosis rate was down-regulated, which showed that Wnt/β-catenin agonist reverse the effect of silencing TRAP1 on A549/CDDP cells. Then, it was concluded that TRAP may promote the Warburg effect and cisplatin resistance of lung cancer by activating the Wnt/β-catenin pathway.

**Conclusion**

This study proved that TRAP1 is highly expressed in lung cancer tissues especially cisplatin resistance lung cancer tissues, and promotes cisplatin resistance by enhancing Warburg effect and changing energy supply mode of lung cancer cells. The mechanism is related to its activation effect on Wnt/β-catenin signaling pathway.

**Declarations**

**Data availability statement**

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

**Funding statement**

This study was supported by the Medical Science and technology project of Henan Province (No.LHGJ2018020557).

**Conflict of interest disclosure**

The authors declare that they have no competing interests.

**Author Contributions**

Ruijie Li and Qian Sun wrote the main manuscript text and Menguo Lv, Juan Li prepared all figures and table and all authors reviewed the manuscript.

**Ethics approval statement**

This study has approved by the ethical committee of the Henan Provincial Chest Hospital, and the study was conducted in accordance with the Declaration of Helsinki.
Patient consent statement

The human tissues involved in this study have been authorized by the patients for scientific use.

References


**Figures**

**Figure 1**

The expression of TRAP1 was up-regulated in cisplatin-resistant lung cancer. (A) TRAP1 mRNA expression in different lung tissues detected by qRT-PCR. B: TRAP1 protein expression in different lung tissues detected by Western blot; C: TRAP1 expression in different lung tissues detected by IHC. *P<0.05,* compared with control group, *#P<0.05,* compared with cisplatin-sensitive group.

**Figure 2**

The expression of TRAP1 was up-regulated in A549/CDDP cells. A: TRAP1 mRNA in different cells detected by qRT-PCR. B: TRAP1 protein expression in different cells detected by Western blot. *P<0.05,* compared with A549, *#P<0.05,* compared with siNC.
Figure 3

Downregulation of TRAP1 reduced the Warburg effect of A549/CDDP cells. A: Glucose consumption detection. B: Lactic acid content detection. C: Warburg effect-related proteins expression detected by Western blot. *$p<0.05$, compared with A549, #$p<0.05$, compared with siNC.
Figure 4

Down-regulation of TRAP1 enhanced cisplatin sensitivity of A549/CDDP cells. A: IC50 concentration of cisplatin detected by CCK8. B: Cell proliferation inhibition rate measured by CCK-8. C: Cell apoptosis rate detected by flow cytometry. *P<0.05, compared with siNC. #P<0.05, compared with siNC+12.5 μg. △P<0.05, compared with siNC+32.5 μg. aP<0.05, compared with TRAP1-siRNA. bP<0.05, compared with TRAP1-siRNA+12.5.
Figure 5

Down-regulation of TRAP1 reduced the activation of Wnt/β-catenin pathway in A549/CDDP cells. A: Wnt/β-catenin pathway protein expression detected by Western blot. *P<0.05, compared with A549. **P<0.05, compared with siNC. aP<0.05, compared with TRAP1-siRNA.

Figure 6
TRAP1 promotes Warburg effect and increases cisplatin resistance by regulating Wnt/β-catenin pathway. A: BML-284 increased the IC50 concentration of cisplatin. B: BML-284 promoted the proliferation of A549/CDDP cells. C: BML-284 inhibited the apoptosis of A549/CDDP cells promoted by silencing TRAP1. D,E: BML-284 enhanced the glucose consumption and lactic acid content. F: BML-284 enhanced the Warburg effect-related proteins expression. *P<0.05, compared with TRAP1-siRNA+32.5μg.