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TKTL1 modulates the response of paclitaxel-resistant human ovarian cancer cells to paclitaxel

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Transketolase-like 1 (TKTL1) plays an important role in the pentose phosphate pathway (PPP) branch. The main obstacle of ovarian cancer treatment is chemotherapeutic resistance. We investigated whether inhibiting TKTL1 in OC3/TAX300 cells could re-sensitize paclitaxel-resistant cells to paclitaxel and proposed a mechanism of action. Western blotting revealed that TKTL1 expression levels in OC3/Tax300 cells were significantly higher than those in OC3 cells. Inhibition of TKTL1 significantly decreased the cellular proliferation rate and IC50 for paclitaxel. Metabolomics revealed that NADPH levels were reduced in the si-TKTL1 group, whereas $NADP^+$ was increased compared with the level in the negative si-TKTL1 group. A 2.2-fold increase in the ROS level and an obvious increase in the cell apoptosis rate were observed in the si-TKTL1+paclitaxel group compared with those in the negative si-TKTL1+paclitaxel and OC3/Tax300 + paclitaxel groups. Western blotting revealed that Bax and Caspase 3 proteins were up-regulated, whereas Bcl-2 expression was down-regulated. Quantitative RT-PCR revealed no changes in gst-π or mrp1 gene expression in the three groups, whereas GSH levels were reduced in the si-TKTL1 group as verified by metabolomics. TKTL1 inhibition also reduced tumor growth in *vivo*. Collectively, TKTL1 down-regulation sensitized paclitaxel-resistant OC3/Tax300 ovarian cancer cells to paclitaxel.

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1. Introduction

Ovarian cancer is one of the most common malignancies among women worldwide and the primary reason of gynecologic cancer-related deaths [1]. A great number of ovarian cancer patients exhibit no significant symptoms; thus, approximately greater than two-thirds of ovarian cancer cases are diagnosed at an advanced stage [2]. Cytoreductive surgery and chemotherapy containing carboplatin and paclitaxel represent the major treatments for advanced stage epithelial ovarian cancer, and most patients obtain complete clinical remission [3].

Despite a promising response rate of 65–80% to first-line chemotherapeutic drugs, greater than 50% patients experience recurrent disease and the inevitable need for further treatment [4]. In addition, the clinical response to second-line chemotherapeutic drugs is significantly reduced by 15–35% due to drug resistance against chemotherapeutic agents [5]. The mechanisms of drug resistance in cancer cells may include removal of drugs by transporters, specific drug metabolism or detoxification, intracellular drug sequestration, changes associated with apoptosis and altered cell cycle [6]. Thus, there is an urgent need to develop novel targets for this deadly disease to reverse chemotherapeutic resistance.

In recent years, the paramount role of re-programming of metabolic pathways in the malignant tumor has been recognized. Particularly, the pentose phosphate pathway (PPP), which branches from glycolysis at the first committed step of glucose metabolism, is required for the synthesis of ribonucleotides and is a major source of NADPH [7]. Transketolase (TKT) is a rate-limiting enzyme involved in the non-oxidative portion of PPP. TKT, transketolase-like protein 1 (TKTL1) and transketolase-like protein 2 (TKTL2) constitute the transketolase gene family [8], but only TKTL1 is up-regulated in tumors [9,10]. Elevated TKTL1 expression has been reported in several types of cancers, including lung cancer [11,12], colon and urothelial cancer [10], uterine cervix cancer [13], gastric cancer and head and neck squamous cell carcinoma [14,15], and is tightly correlated with cancer occurrence, development and progression.

Data from previous proteomics in our laboratory revealed that TKTL1 is up-regulated in chemoresistant ovarian cancer specimens

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in *vitro*. Given the lack of reports on TKTL1 function in ovarian cancer, we investigated TKTL1 expression in ovarian cancer cell lines and preliminarily elucidated its underlying biological functions in ovarian cancer by knockdown of target genes in the hopes of reversing chemoresistance and providing an attractive new target for epithelial ovarian cancer treatments.

2. Materials and Methods

The parental human ovarian carcinoma cell line OC3 and paclitaxel-resistant human ovarian cancer cell line OC3/TAX300 were previously established, routinely maintained in our laboratory and have been used in several studies [16,17]. Quantitative RT-PCR and Western blotting was employed to compare any differences in TKTL1 expression between OC3/TAX300 and OC cells. Immediately before transduction, the culture medium was replaced with fresh RMPI 1640 containing 10 μg/ml polybrene. Then, lentivirus expressing siRNA against TKTL1 was added at a multiplicity of infection of 50. Metabolomics analysis was conducted to analyze the metabolic substance alternations after TKTL1 knockdown in OC3/Tax300 cells. The alterations in cell proliferation and IC50 for paclitaxel were assessed by MTT assay. Flow cytometry and electron microscopy were used to study apoptosis after TKTL1 knockdown in OC3/Tax300 cells and exposure to 2 μg/ml of paclitaxel for 24 h. In addition, cellular ROS was also assessed. Quantitative RT-PCR and Western blotting were employed to investigate the mechanism of cellular apoptosis induction and reversal of chemoresistance. The animal study was approved by the Animal Research Ethics Committee of Peking University Health Science Center under protocol LA2017220. For xenograft experiments, 2×10^{-7} OC3/Tax300 cells transfected with siRNA (EX group) or negative siRNA (NC group) and OC3/Tax300 (BC group) cells were harvested and injected subcutaneously into the flank of 4-week-old BALB/c female athymic mice (Vital River Company, China). Mice were intraperitoneally injected with paclitaxel (10 mg/kg, Nanjing Kang Hai Pharmaceutical Co. Ltd) twice a week after the tumors formed. Mice were examined twice a week for the development of tumors. Tumor samples from the three animal groups were harvested on the 28th day after euthanasia for Western blotting. All values investigated were analyzed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). The Student's *t*-test was used for statistical analysis. Calculated P-values < 0.05 were considered statistically significant.

A complete description of the Materials and Methods is included in the supplemental material and methods. The siRNA sequence is listed in Table 1, and primer pairs for quantitative RT-PCR are listed in Table 2.

Table 1
Primer pairs for quantitative RT-PCR.

Gene name	Primer sequence
TKTL1	F 5′- CTGCTCTTTCTCTCGGCATTG -3′
	R 5′- TCCTCCTGAGCGCAAGTACTC -3′
Bcl-2	F 5′- GGATTGTGGCCTTCTTTGAGTTC -3′
	R 5′- CGGTTCAGGTACTCAGTCATCCA -3′
Bax	F 5′- TGGAGCTGCAGAGGATGATTG -3′
	R 5′- CCAGTTGAAGTTGCCGTCAGA -3′
Caspase-3	F 5′- ATACCAGTGGAGGCCGACTTC -3′
	R 5′- CAAAGCGACTGGATGAACCA -3′
GST-π	F 5′- TGCTCACATAGTTGGTGTAGATGAGGGATA -3′
	R 5′- TGACCGAGGCTACATTCAGATGACACC -3′
MRP1	F 5′- TGCAGAAGGCGGGGAGAACCTC -3′
	R 5′- GTCGTCCGTTTCCAGGTCCACG -3′
actin	F 5′-TCCTCCTGAGCGCAAGTACTC-3′
	R 5′-GACTCGTCATACTCCTGCTTGCT-3′

F, forward primer; R, reverse primer.

Table 2
SiRNA sequences.

genes	sequence
TKTL1-siRNA-1#	TGGAATTACTGTGTATGAA
TKTL1-siRNA-2#	GACCAGAAACTATGGTTAT
TKTL1-siRNA-3#	GATCATTACAGTGGAGGAT
TKTL1-siRNA-NC	TTCTCCGAACGTGTCACGT

3. Results

3.1. Quantitative RT-PCR and western blotting analysis reveal that TKTL1 is up-regulated in paclitaxel-resistant ovarian carcinoma cells

Quantitative RT-PCR and Western blotting also demonstrated that TKTL1 was up-regulated in paclitaxel-resistant ovarian cancer cells (OC3/Tax300) compared with its expression in OC3 cells (Fig. 1A–C).

3.2. Establishment of TKTL1 knockdown cells

As revealed by quantitative RT-PCR in Fig. 1E and by Western blotting in Fig. 1F–G, TKTL1-siRNA2# significantly inhibited TKTL1 expression at the mRNA and protein levels. Therefore, TKTL1-si2# was used in subsequent experiments as the experimental (EX) group.

3.3. Inhibition of TKTL1 expression reverses paclitaxel resistance and reduces cell proliferation

TKTL1 knockdown significantly suppressed cell growth in the EX group compared to that in the negative control (NC) group ($P < 0.05$, Fig. 2A). The IC50 for paclitaxel in the EX group (4.38 μg/ml) was significantly lower than that in the NC group (15.17 μg/ml; $P < 0.001$) and blank control (BC) group (15.25 μg/ml; $P < 0.001$; Fig. 2B). The IC50 values exhibited no significant differences between the NC and BC groups ($P > 0.05$). Cell growth inhibition rate curves were constructed for paclitaxel using the following concentration gradient: 0, 1, 2, 4, 8, 16 and 32 μg/ml (Fig. 2C).

3.4. TKTL1 knockdown decreased the effect of TKTL1 on the pentose phosphate pathway

NADPH and $NADP^+$ levels in the EX and NC groups were determined by LC-MS/MS-based analysis of metabolites. NADPH levels were reduced, whereas $NADP^+$ levels were increased based on calculations of the peak area under the curve (Fig. 2D–E). In addition, glutamine levels were also reduced (Fig. 2F). This finding suggests that TKTL1 may promote aerobic glycolysis via enhanced metabolite production and cellular NADPH redox equivalents.

3.5. TKTL1 knockdown increased cellular ROS levels

ROS are involved in the cellular response to stress and associated with apoptosis via mitochondrial DNA damage [18]. The level of intracellular ROS was examined based on ROS fluorescence, and an approximately 2.2-fold increased ROS level was observed in the EX group after treatment with paclitaxel compared to that in the NC and BC groups (Fig. 2G).

3.6. TKTL1 knockdown following paclitaxel treatment induced cell apoptosis

We assessed cell apoptosis based on flow cytometry analysis after TKTL1 inhibition and treatment with 2 μg/ml paclitaxel for 2

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