

ORIGINAL RESEARCH

KRT17 enhances carboplatin resistance in ovarian cancer through the AKT/mTOR pathway

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Abstract

Ovarian cancer has gradually evolved into a killer of women's health. *Keratin 17 (KRT17)* belongs to keratin family. The role of *KRT17* in ovarian cancer is not yet clear. Carboplatin-resistant cell lines of ovarian cancer were established, and the effect of carboplatin on the cell viability of SKOV3-CBP, A2780-CBP (carboplatin-resistant cell lines) and SKOV3, A2780 cells were investigated by MTT assay; the expression of *KRT17* was predicted and verified by Gene Expression Profiling Interactive Analysis (GEPIA) and western blot (WB) to compare ovarian cancer and normal tissues; *KRT17* was successfully knocked down in carboplatin-resistant cell lines, the effect of carboplatin on the cell viability of carboplatin-resistant cell lines and non-drug-resistant cell lines was studied by MTT assay, combined with colony formation assay to study the effect of knockdown of *KRT17* on cell clonality; flow cytometry and WB to study the effect of knockdown of *KRT17* on cell cycle and apoptosis, and targeted signaling pathway. The results showed that as for the expression of *KRT17*, compared to normal tissues, ovarian cancer was higher than that; SKOV3-CBP and A2780-CBP were not highly sensitive to drugs and had strong drug resistance. After knocking down *KRT17*, the drug resistance decreased, the cell cycle was arrested in G0/G1, the protein expression levels of Cyclin D1 and cyclin dependent kinase 4 (CDK4) decreased, the apoptosis level increased and the protein expression level increased; *KRT17* mainly plays a regulatory role by targeting AKT/mammalian (or mechanistic) target of rapamycin (AKT/mTOR). This study found that *KRT17* could increase the carboplatin resistance of ovarian cancer cells by regulating the AKT/mTOR pathway, promoting the cycle process of ovarian cancer cells, and inhibit apoptosis.

Keywords

Ovarian cancer; AKT/mTOR; Carboplatin; Drug resistance

1. Introduction

The mortality rate of ovarian cancer is dominating in women [1]. While age-standardized ratios have remained stable or declined in most high-income countries, age-standardized ratios are rising in many low- and middle-income countries [2]. Ovarian cancer is often associated with several risk factors, such as a woman's ovulation frequency, medication, obesity index, and more. In addition, it may also be related to genetic factors. Because ovarian cancer is often diagnosed late, it consists of several subtypes with different biological and molecular properties, and the availability and accessibility of treatments are inconsistent. Currently, the treatment of ovarian cancer has become more diverse and more efficient, an important issue in the treatment of tumors with compounds is the development of drug resistance and side effects. Therefore, it is necessary to investigate the molecular mechanism of tumor resistance [3]. Physiologically, AKT/mTOR not only plays an important role in biological homeostasis *in vivo*, but also functions as a regulator when a pathological state occurs.

This includes not only functions of proliferation, but also functions such as cell survival or apoptosis, and regulation of metabolism. In tumors in particular, these processes are constitutively activated. Abnormal activation of signaling pathways can promote tumor resistance [4].

Keratin is an important unit of cytoskeleton and is usually expressed in epithelial cells. Many keratin proteins together form intermediate fibers. So far, 54 functional keratins have been found in humans. Keratin 17 (*KRT17*, CK17) is a type I keratin with a molecular weight of 48 kDa and consists of intermediate fibers about 10 nm in length. Although *KRT17* is abundantly expressed in epithelial cells, it is not highly expressed in all tissues. For example, its expression level in mature epithelial tissue is relatively low, but it has been found to be selectively expressed in epithelial stem cells. Furthermore, a similar situation occurs in reserve cells. These cells are characterized as proliferatively active cells. Also, its expression was observed in adenomyotic cells. Notably, *KRT17* is also present when cancer occurs, and its expression

can be observed in cancer tissues. Now studies have found that this gene can already be used as a biomarker for clinical diagnosis, for example, promoting non-small cell lung cancer growth and invasion [5]. It is highly expressed in pancreatic cancer tissues and promotes the proliferation, migration and invasion of pancreatic cancer cells [6].

KRT17 not only participates in the biological processes of cancer cells such as cell migration, but also promotes drug resistance in cancer cells [7]. Knockdown of *KRT17* enhanced cisplatin sensitivity in bladder cancer cells. *KRT17* is critical for bladder cancer development, and activation of the AKT and extracellular signal-regulated kinases (ERK) pathways may be related to the mechanism [8]. Knockdown of *KRT17* reduces the expression of p-AKT and p-mTOR in osteosarcoma cells and inhibits cell proliferation and glycolysis [9]. It was reported that elevated expression of *KRT17* may contribute to poor prognosis in epithelial ovarian cancer [10]. The Gene Expression Profiling Interactive Analysis (GEPIA) database shows that *KRT17* is highly expressed in ovarian cancer, but the role of *KRT17* in ovarian cancer is less studied, and the related mechanism is not clear. This paper aims to demonstrate the *KRT17* protein participation in carboplatin resistance in ovarian cancer through the AKT/mTOR pathway.

2. Methods

2.1 Cells

IOSE80 human normal ovarian epithelial cells, human ovarian cancer cell lines SKOV3 and A2780 were all from Central China Normal University (Wuhan, China). Roswell Park Memorial Institute Medium (RPMI1640) medium (Solarbio, Beijing, China) containing 10% fetal bovine serum (FBS) (Corning Inc., Corning, NK, USA) was used to culture the above cells, and 1% Penicillin-Streptomycin (Gibco, Waltham, MA, USA) was added for each use. Cells were cultured at 37 °C in a cell incubator containing 5% Carbon dioxide (CO₂).

2.2 Prediction using the TCGA database

The TCGA-based GEPIA database (<http://gepia2.cancer-pku.cn/#analysis>) was used to predict the expression of *KRT17* in normal tissues and ovarian cancer tissues. In the database, we set a *p* value of 0.001 and a fold change of 1.5.

2.3 RNAi

KRT17 knockdown plasmids were obtained from Hanheng Biology (Beijing, China), and these plasmid particles were cloned into expression plasmids. Subsequently, with the help of packaging plasmid (10 μg) and transfer plasmid (15 μg), expression plasmid (20 μg) was co-transfected into 293T cells using Lipofectamine® 2000 (Solarbio, Beijing, China). 293T were cultured at 37 °C and 5% CO₂ for 48 h and 72 h. The supernatant was the virus released from 293T. The virus could be used to infect the target cells to reduce the expression of *KRT17*. Then the group with higher knockdown efficiency was selected as the group for subsequent knockdown.

2.4 Cell treatment

SKOV3, A2780 were exposed to increasing carboplatin concentrations and cultured for 6 months to establish drug-resistant cell lines SKOV3-CBP, A2780-CBP. When detecting the expression of *KRT17* in cells, the grouping scheme was: IOSE80, SKOV3, SKOV3-CBP, A2780, A2780-CBP. When examining the effect of *KRT17* in SKOV3-CBP and A2780-CBP cell lines, the grouping scheme was as follows: si-NC group, si-*KRT17*#1 group and si-*KRT17*#2 group. The two drug-resistant cell lines were seeded on six-well plates, and then 50 μL of lentiviral particles containing si-NC, si-*KRT17*#1 and si-*KRT17*#2 were used to infect the two cells (Multiplicity of infection (MOI) = 10) per well, 5 μg/mL puromycin was used to select transduced cells at 72 h.

2.5 MTT assay

Cell suspensions were seeded into 96-well plates at a concentration of 4×10^3 cells/mL in 100 μL. MTT assay (Labeled, Beijing, China) was used to detect cell viability. Cells were treated with increasing concentrations (4, 8, 16, 32, 64, 128, 256 and 512 μM) of carboplatin for 72 h. Cells were then treated with 10 μL of MTT and 90 μL of fresh medium for 4 h. Formazan crystals will then form. Then carefully aspirate the supernatant, add 110 μL of formazan solution to each well, and place it on a shaker to shake at low speed for 10 minutes to fully dissolve the crystal. The absorbance of cells at 490 nm was measured separately using a microplate reader (SpectraMax i3X, Molecular Devices, San Jose, CA, USA). Calculate Half-maximal inhibitory concentration (IC₅₀) based on the read value. The experiment was repeated three times.

2.6 Colony formation assay

72 h after transfection, SKOV3-CBP and A2780-CBP cells were prepared into a cell suspension of 500 cells/well, and then seeded into 6-well plates after pipetting and mixing. The culture conditions were the same as above and cultured for one week. Cells were then washed with Phosphate-buffered saline (PBS), then fixed with 95% ethanol for about 10 minutes, and 0.1% crystal violet was prepared and used to stain cells for about 20 minutes. Finally, sterile water was used to rinse the cells three times, followed by single clones larger than 1.5 mm in diameter were counted.

2.7 Flow cytometry

1×10^6 /mL cells were resuspended. To evaluate the apoptosis rates and the proportion of each stage of cell cycle of cells, a BD Accuri™ C6 plus flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) was employed to flow cytometry. An Annexin A5 (Annexin V) - Phycoerythrin (PE) (Apoptosis Detection Kit) (Solarbio, Beijing, China) was applied for detecting the proportions of cell cycle and cell apoptosis, then analyzed results according to manufacturer's protocols. The ratio of cell apoptosis was computed and calculated using Fluorescence activated cell sorting (FACS) scan software (BD, San Jose, CA, USA).

2.8 Western Blot (WB)

Total protein was obtained using Radio-Immunoprecipitation Assay (RIPA) buffer (Cat no. 89901; Thermo Fisher, Waltham, MA, USA), followed by centrifuging at $16,000 \times g$, 4°C for 15 minutes. Bicinchoninic acid assay (BCA) protein assay kit (Thermo Fisher, Waltham, MA, USA) was performed to quantify the protein concentration. Denaturation of total protein was processed in a metal bath at 95°C for 5 minutes, and then run Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (7.5%), $20 \mu\text{g}$ of protein were separated per lane. Afterwards, proteins were wet transferred to Polyvinylidene Fluoride (PVDF) membranes (Solarbio, Beijing, China), which were blocked by 5% skimmed milk dissolved in Tris Buffered Saline with Tween (TBST), shaking on a shaker at room temperature for 2 h. Related primary antibodies were applied to incubate with membranes.

The primary antibodies were including *KRT17* (1:2000) (Cat no. 17516-1-AP; Proteintech, Shanghai, China), CyclinD1 (1:2000) (Cat no. 60186-1-Ig; Proteintech, Shanghai, China), CDK4 (1:2000) (Cat no. 11026-1-AP; Proteintech, Shanghai, China), Bax (1:2000) (Cat no. 50599-2-Ig; Proteintech, Shanghai, China), Bcl-2 (1:2000) (Cat no. 12789-1-AP; Proteintech, Shanghai, China), Cleaved-caspase3 (1:2000) (Cat no. 19677-1-AP; Proteintech, Shanghai, China), p-AKT(1:2000) (Cat no. 66444-1-Ig; Proteintech, Shanghai, China), AKT (Cat no. 60203-2-Ig; Proteintech, Shanghai, China), p-mTOR(1:2000) (Cat no. 67778-1-Ig; Proteintech, Shanghai, China), mTOR(1:2000) (Cat no. 66888-1-Ig Proteintech, Shanghai, China), p-ERK(1:2000) (Cat no. 28733-1-AP; Proteintech, Shanghai, China), ERK(1:2000) (Cat no. 11257-1-AP; Proteintech, Shanghai, China), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:2000) (Cat no.5174; Cell Signaling Technology, Danvers, MA, USA). Then, the membranes were incubated with the horseradish peroxidase (HRP)-conjugated secondary antibody (1:1000) (Cat. no. 7074; Cell Signaling Technology, Inc.) for 2 h at 37°C . The Easysee Western Blot Kit (Beijing TransGen Biotech, Beijing, China) was used to measure chemiluminescence signals. ImageJ version 1.53 (National Institutes of Health, Bethesda, MD, USA) was used to analyze the expression of proteins in each group.

2.9 RT-PCR

Total RNA was extracted from cells through a RNA extraction kit (Agbio, Hunan, China). Complementary DNA (cDNA) was then synthesized by reverse transcription kit (Agbio, Hunan, China). SYBR Green Master Mix (BioRad, Hercules, CA, USA) was used for Real-Time Quantitative Reverse Transcription PCR (qRT-PCR) and β -actin was used as an internal control. Target gene transcription was normalized to GAPDH expression (internal control) using the $2^{-\Delta\Delta C_t}$ method to calculate fold induction of target mRNA. All primers used are listed in Table 1.

2.10 Statistical analysis

All data were presented as the mean \pm standard deviation (SD) for experiments performed in triplicate. Unpaired or paired

Student's *t*-test or one-way analyses of variance (ANOVA) was utilized to analyze results followed by Tukey's test to compare each group. Data were analyzed and graphs were plotted by using GraphPad Prism version 6.0 software (GraphPad Software, Inc., La Jolla, CA, USA). $p < 0.05$ indicated a significant difference.

3. Results

3.1 *KRT17* is highly expressed in carboplatin-resistant cells

Cells were exposed to carboplatin concentrations, and after 6 months, drug-resistant cell lines SKOV3-CBP, A2780-CBP were successfully established, during which drug resistance and stability were checked monthly. MTT was used to test the drug resistance of cell lines to carboplatin. The results showed that the cell viability of all cells decreased gradually with the increase of carboplatin concentration. Among them, under the treatment of drugs, the cell viability of SKOV3-CBP and A2780-CBP was higher than that of KOV3 and A2780. The corresponding IC50 of carboplatin in the SKOV3, SKOV3-CBP, A2780 and A2780-CBP was 81.39 ± 5.76 , 236.8 ± 20.17 , 49.37 ± 2.99 and 169.3 ± 14.8 , respectively (Fig. 1A). Subsequently, the expression difference of *KRT17* in ovarian cancer and normal tissues was analyzed on the TCGA-based GEPIA platform, and ovarian cancer had a higher expression level (Fig. 1B). To verify this result, WB and RT-PCR was performed in five cell lines of IOSE80, SKOV3, SKOV3-CBP, A-2780 and A2780-CBP. The results consistently showed that the other four cell lines had significant protein and mRNA expression levels of *KRT17* compared to IOSE80. In addition, the protein expression level of *KRT17* in SKOV3-CBP was higher relative to non-resistant cells SKOV3; the protein expression level of *KRT17* in A2780-CBP was higher relative to A2780 (Fig. 1C–D). These results preliminarily indicate that *KRT17* is highly expressed in carboplatin-resistant cells.

3.2 Knockdown of *KRT17* enhances the sensitivity of carboplatin-resistant cells

To investigate the role of *KRT17* in carboplatin-resistant cells, an siRNA capable of knocking down carboplatin-resistant cells in carboplatin-resistant cells was designed. mRNA and WB was used to measure knockdown efficiency. It was found that both si-*KRT17*#1 and si-*KRT17*#2 significantly reduced the protein expression level of *KRT17* (48 kDa) in both cell lines compared with the si-NC group (Fig. 2A–B). This indicated that the knockdown of *KRT17* was successful. To study the drug sensitivity of carboplatin-resistant cells, MTT assay was used to detect cell viability and calculate IC50 values. The results showed that the cell viability decreased gradually with the increase of carboplatin concentration. Among them, under the treatment of drugs, the cell viability of the *KRT17* knockdown group was lower than that of the si-NC group, and the drug resistance was also reduced, and the corresponding IC50 was shown in Fig. 1C. Subsequently, colony formation assays were used to examine cell proliferation. Under the microscope, the number of purple crystals in the si-NC group was more (263 per field in SKOV3-CBP, 40 per field in A2780-

TABLE 1. The sequence of primers.

| Gene | Sense | Antisense |
|--------------|--------------------------|--------------------------|
| <i>KRT17</i> | CGGAGACAGAGAACCGCTAC | CACAATGGTACGCACCTGAC |
| <i>GAPDH</i> | GTGAAGGTCGGTGTGAACGGATTT | CACAGTCTTCTGAGTGGCAGTGAT |

KRT17: Keratin 17; *GAPDH*: Glyceraldehyde 3-phosphate dehydrogenase.

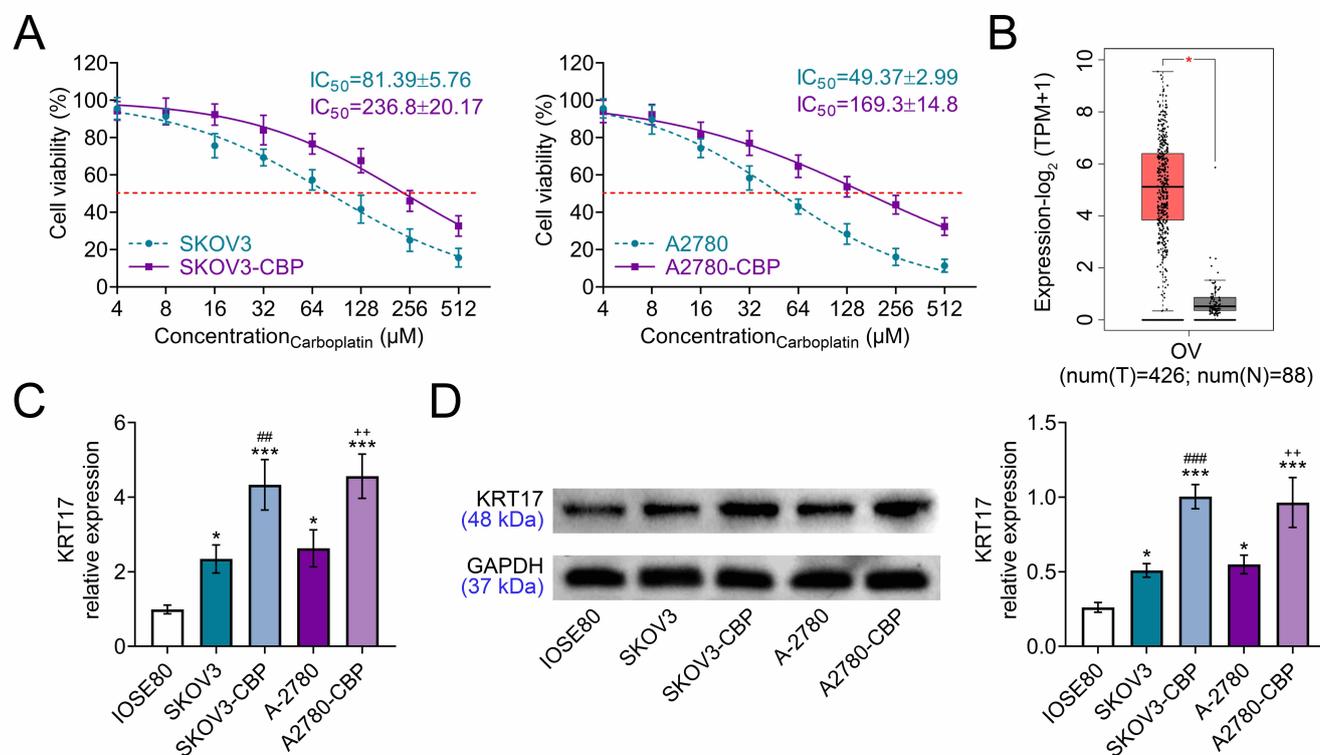


FIGURE 1. The effect of carboplatin on the cell viability of drug-resistant and non-resistant cells and the difference of *KRT17* expression in normal tissues and ovarian cancer. (A) Changes in cell viability of carboplatin-induced drug-resistant cell lines SKOV3-CBP and A2780-CBP, SKOV3 and A2780 in the face of increasing concentrations of carboplatin. (B) Differential expression of *KRT17* in normal tissues and ovarian cancer was analyzed based on GEPIA. (C) Differences in mRNA expression levels of *KRT17* in five cell lines of IOSE80, SKOV3, SKOV3-CBP, A-2780 and A2780-CBP. (D) Differences in protein expression levels of *KRT17* in five cell lines of IOSE80, SKOV3, SKOV3-CBP, A-2780 and A2780-CBP. $N = 3$, * $p < 0.05$ vs. the Normal or IOSE80 group, *** $p < 0.001$ vs. the IOSE80 group; ### $p < 0.001$ vs. the IOSE80 group; ++ $p < 0.01$ vs. the IOSE80 group. *KRT17*: Keratin 17. *GAPDH*: Glyceraldehyde 3-phosphate dehydrogenase.

CBP), occupying most of the field of view, while the number of purple crystals observed under the microscope in the *KRT17* knockdown group was relatively small, and the difference was significant (124/133 per field in SKOV3-CBP, 19/26 per field in A2780-CBP) (Fig. 2D). In this experiment, it was indicated that knockdown of *KRT17* enhanced carboplatin-resistant cell sensitivity.

3.3 Knockdown of *KRT17* induces carboplatin-resistant cell cycle arrest

Further, the effect of *KRT17* on the cell cycle of carboplatin-resistant cells piqued our interest. Flow cytometry was used to examine the proportions of the various phases of the cell cycle. The results are shown in Fig. 3A, knockdown of *KRT17* makes the cells blocked in G0/G1 phase, and the ratio is significantly higher than that in the si-NC group, and the proportion of

cells in the S phase is also much higher than that in the si-NC group. After detection of the cyclins CyclinD1 (34 kDa) and CDK4 (34 kDa), it was found that the expression levels of these two proteins that promote cell cycle progression in carboplatin-resistant cells were significantly lower than those in the si-NC group (Fig. 3B). This suggested that knockdown of *KRT17* induces carboplatin-resistant cell cycle arrest.

3.4 Knockdown of *KRT17* promotes carboplatin-induced apoptosis in drug-resistant cells

To study the effect of *KRT17* on apoptosis, flow cytometry was first used. Compared with the si-NC group, the apoptosis rate of *KRT17* knockdown was significantly increased (Fig. 4A). Subsequently, the protein expression levels of Bax (21 kDa), Bcl-2 (26 kDa) and cleaved-caspase3 (32 kDa) were detected.

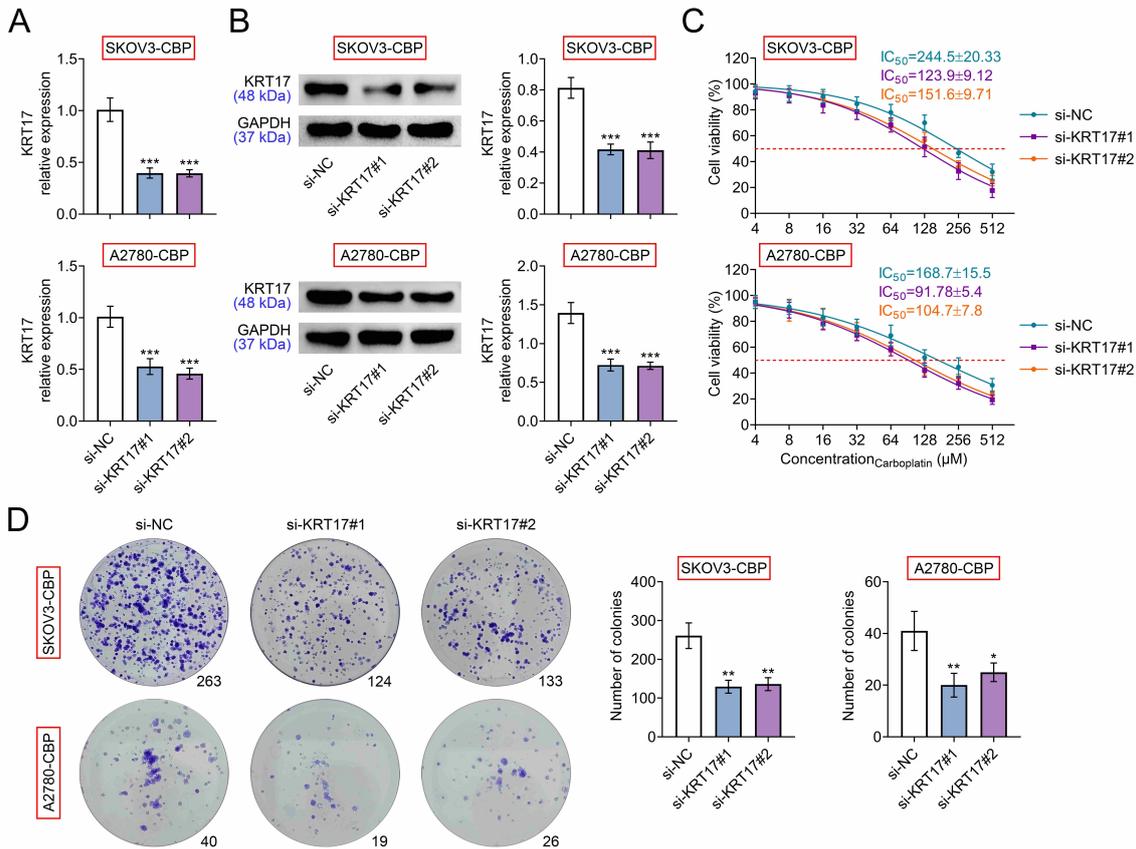


FIGURE 2. *KRT17* protein expression level, cell viability and cloning ability in carboplatin-resistant cells after *KRT17* knockdown. (A) The effect of knockdown of *KRT17* on the mRNA expression level of cellular *KRT17*. (B) The effect of knockdown of *KRT17* on the protein expression level of cellular *KRT17*. (C) The effect of *KRT17* knockdown on cell viability and IC₅₀ of carboplatin, the red dotted line is the corresponding drug concentration at 50% cell viability. (D) The effect of knockdown of *KRT17* on the ability of cells to form colonies, purple represents the monoclonal cell mass. N = 3, ***p* < 0.01 vs. the si-NC group, ****p* < 0.001 vs. the si-NC group. IC₅₀: Half-maximal inhibitory concentration. Si-NC: SiRNA-non coding.

The results are shown in Fig. 4B. Compared with the si-NC group, the protein expression levels of Bax and cleaved-caspase3 in *KRT17* knockdown cells were higher than that, the protein expression trend of Bcl-2 is opposite. This indicated that knockdown of *KRT17* activated carboplatin-induced apoptosis of drug-resistant cells.

3.5 Knockdown of *KRT17* inhibits the AKT/mTOR pathway

To study the signaling pathway regulated by *KRT17*, WB was used to study the protein expression levels of p-AKT (60–62 kDa), AKT (56 kDa), p-mTOR (289 kDa), mTOR (289 kDa), p-ERK (38–43 kDa) and ERK (43 kDa). The results are shown in Fig. 5. Compared with the si-NC group, knockdown of *KRT17* significantly reduced the phosphorylation levels of AKT, mTOR and ERK, indicating that after *KRT17* was knocked down, these proteins were activated, thereby triggering the AKT/mTOR pathway. Thus, knockdown of *KRT17* will inhibit the AKT/mTOR pathway.

4. Discussion

In this study, carboplatin-resistant cell lines SKOV3-CBP and A2780-CBP were first established. *KRT17* plays an important role in cellular processes and its aberrant activation has been observed in many types of cancer cells. It is widely reported to be closely associated with a variety of cancers. In a recent cohort analysis, *KRT17* was identified as a prognostic biomarker for stage II colorectal cancer, which is consistent with the regulatory role of *KRT17* found in this study [11]. Other studies have reported that *KRT17* can affect the proliferation, migration and invasion of pancreatic cancer by regulating the mTOR/S6k1 pathway [6]. However, existing studies have not elucidated the biological mechanism of *KRT17*'s promoting effect in ovarian cancer. At present, chemotherapy still plays an important role in the treatment of ovarian cancer, but drug resistance is an urgent problem to be solved in tumor treatment. Our results showed that SKOV3-CBP and A2780-CBP had lower drug sensitivity and higher IC₅₀ of carboplatin, the decreased expression of *KRT17* greatly increased the sensitivity of cells to carboplatin, significantly decreased IC₅₀ of carboplatin, and decreased the clonogenic ability of cells accordingly. In addition, after knockdown of *KRT17*, a large number of cells were arrested in G₀/G₁ phase, and the

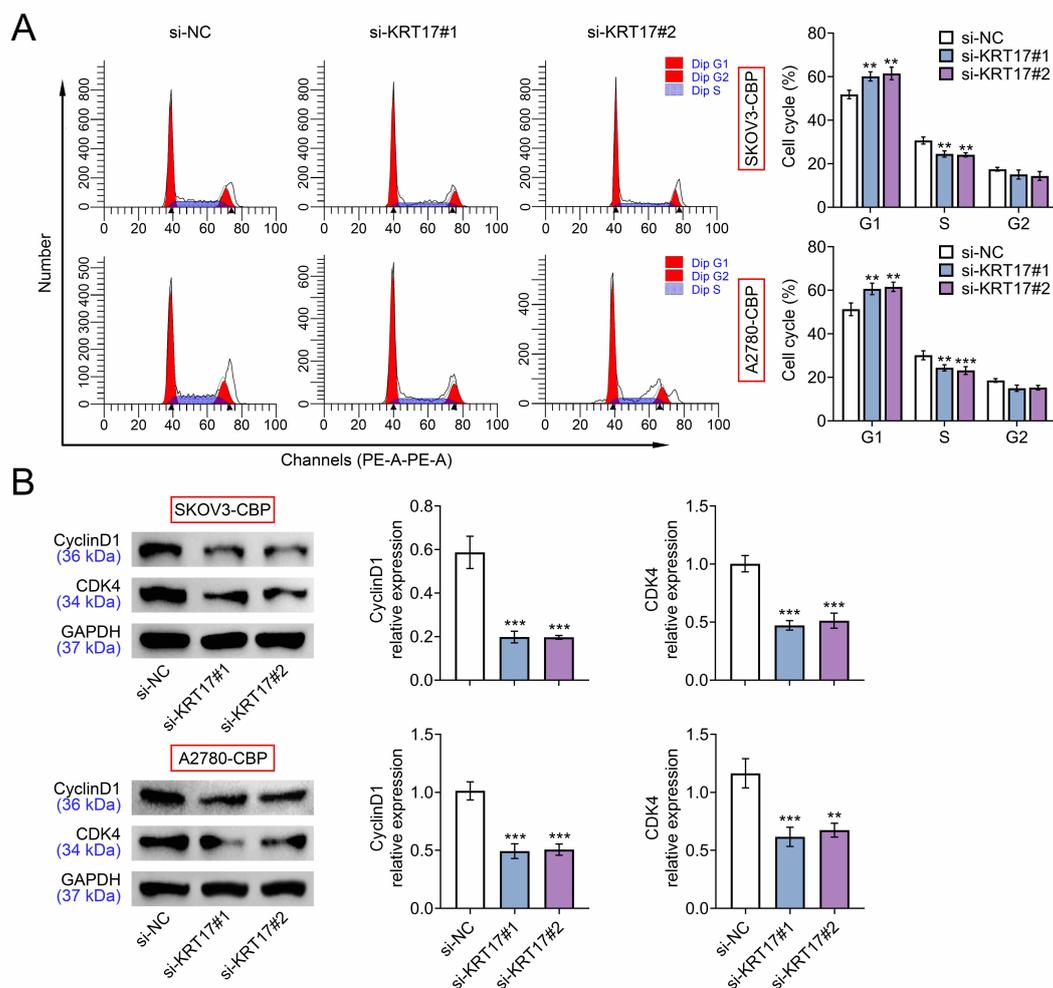


FIGURE 3. After *KRT17* knockdown, the proportion of cells in each stage of the cell cycle of carboplatin-resistant cells and the changes of cyclins. (A) Flow cytometry shows the effect of *KRT17* knockdown on cells at various stages of the cell cycle in cells, with the number of cells on the ordinate and the channel on the abscissa. And the proportion of carboplatin-resistant cells at various stages of the cell cycle. (B) The effect of carboplatin-resistant cells on the protein expression levels of cyclins CyclinD1 and CDK4. N = 3, ** $p < 0.01$ vs. the si-NC group, *** $p < 0.001$ vs. the si-NC group. N = 3, ** $p < 0.01$ vs. the si-NC group, *** $p < 0.001$ vs. the si-NC group.

expressions of CyclinD1 and CDK4, which promote cell cycle progression, were also decreased. Activation of the apoptotic process was also observed. This suggested drug resistance reactivated *KRT17* in cells leads to the development of cancer cells. Studying the signaling pathways in tumor activation is beneficial to the development of clinical drugs. At present, AKT/mTOR is a widely reported signaling pathway that promotes tumor resistance, but its relationship with *KRT17* after drug resistance in ovarian cancer is still unclear. Our results suggest that *KRT17* knockdown can inhibit the AKT/mTOR pathway. This suggests that knocking down *KRT17* may be an effective strategy for the treatment of ovarian cancer.

Ovarian cancer is often associated with several risk factors, such as a reduced risk of ovarian cancer in women who ovulate less frequently in their lifetime relative to women who ovulate more frequently [12]. In addition, studies have shown that postmenopausal women who have undergone hormone therapy, have taken fertility drugs, or have a higher Body mass index (BMI) may increase the risk of ovarian cancer

[13, 14]. About 10% of cases are related to family history [15]. According to literature reports, a large number of genetic mutations exist when ovarian cancer develops. Among them, the mutation of Breast Cancer gene 1 (*BRCA1*) or Breast Cancer gene 2 (*BRCA2*) gene will have up to 50% chance to induce the occurrence of ovarian cancer [16]. These indicate that early screening of molecular markers of cancer is very important. In this study, *KRT17* was found to be able to regulate the AKT/mTOR signaling pathway, and many studies have also reported its association with cancer. For example, Lastwika *et al.* [17] found that the activation of the AKT-mTOR pathway in non-small cell lung cancer promoted the occurrence of cancer; Liu *et al.* [18] found that the proliferation, invasion and cloning of gastric cancer cells were related to the activation of the AKT/mTOR signaling pathway by methylation. In addition, it has been reported that *KRT17* can also regulate the development of bladder cancer through the activation of AKT and ERK pathways. The elucidation of the role of this pathway in bladder cancer is also very meaningful. These pathways

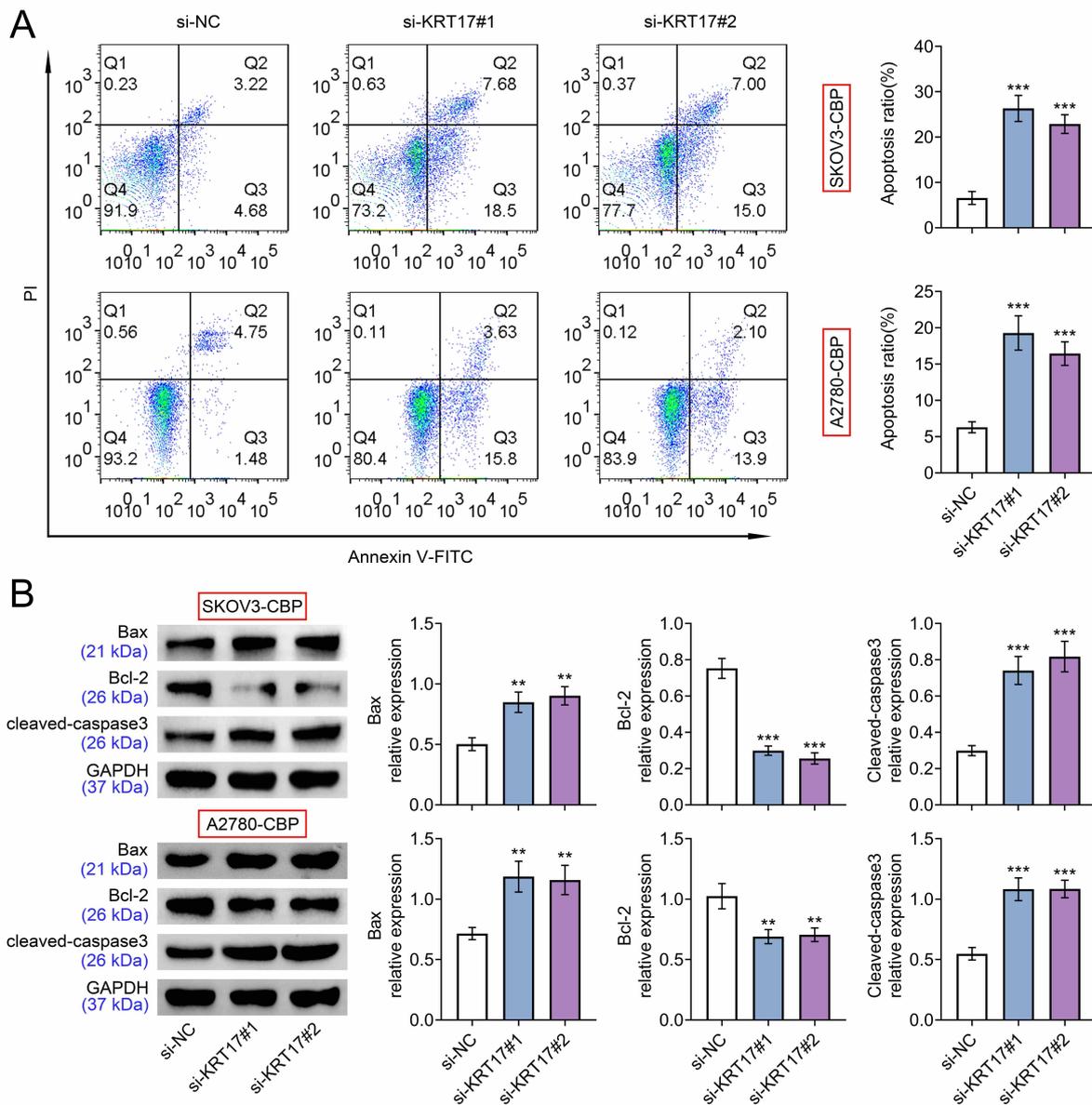


FIGURE 4. Carboplatin-resistant cells undergo apoptosis after *KRT17* knockdown. (A) Flow cytometry showing the proportion of apoptosis in carboplatin-resistant cells after *KRT17* knockdown. (B) The effect of knockdown of *KRT17* on the expression level of apoptotic proteins in carboplatin-resistant cells. N = 3, ** $p < 0.01$ vs. the si-NC group, *** $p < 0.001$ vs. the si-NC group.

may serve as new therapeutic targets for ovarian cancer [8]. These findings are consistent with this study, indicating that the AKT/mTOR signaling pathway plays an important role in ovarian cancer.

However, this study has flaws. First, the *KRT17* gene, as an activated gene in cancer, must interact with other genes in the family, and this study did not involve the relationship between *KRT17* and other genes; second, although this study knocked down *KRT17* to study its effect on carboplatin resistance. However, it may trigger a series of compensatory responses, and these compensatory responses may affect normal cell biological processes, which have not been covered in this study.

5. Conclusion

In conclusion, this study found that *KRT17* can increase the carboplatin resistance of ovarian cancer cells by regulating the AKT/mTOR pathway and promote the progression of ovarian cancer cell cycle and inhibit apoptosis.

AUTHOR CONTRIBUTIONS

CXY—designed the experiments and QY carried them out. JL—analyzed and interpreted the data, JZ—prepared the manuscript with contributions from all co-authors.

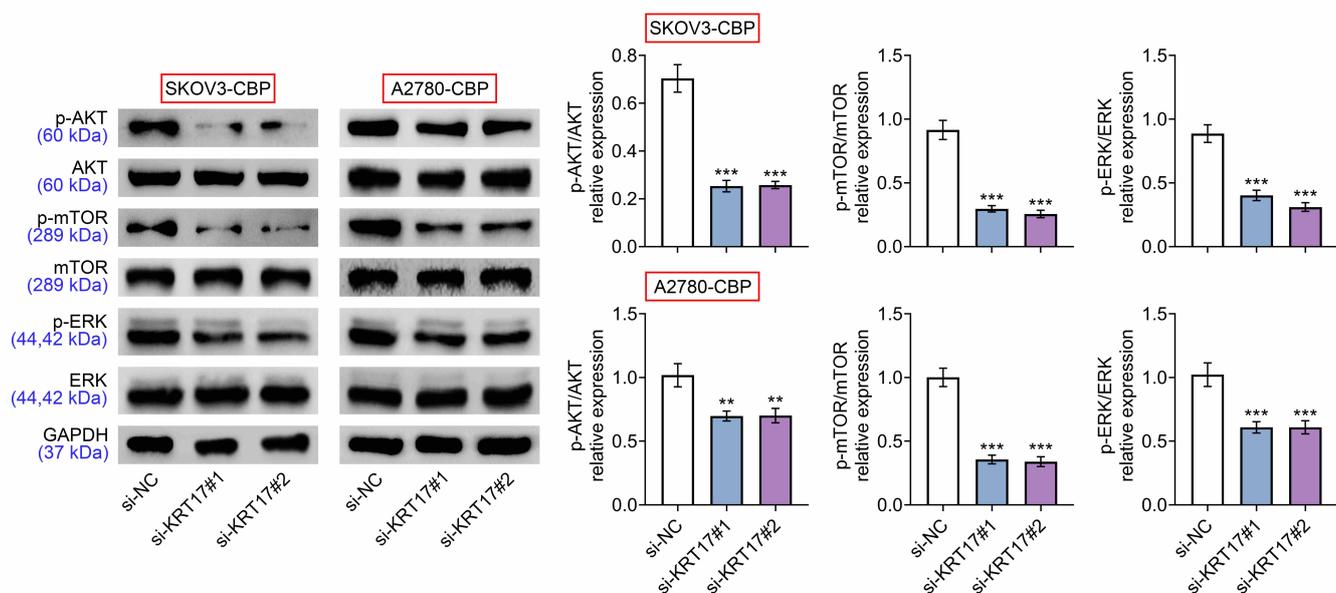


FIGURE 5. Effect of knockdown of *KRT17* on the protein expression levels of p-AKT, AKT, p-mTOR, mTOR, p-ERK and ERK in carboplatin-resistant cells. N = 3, ** $p < 0.01$ vs. the si-NC group, *** $p < 0.001$ vs. the si-NC group. AKT: AKT serine/threonine kinase 1; mTOR: mammalian target of rapamycin; ERK: extracellular signal-regulated kinases.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ACKNOWLEDGMENT

Not applicable.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Arndt T, Taube ET, Deubzer HE, Rothe K, Calaminus G, Sehouli J, *et al.* Management of malignant dysgerminoma of the ovary. *European Journal of Gynaecological Oncology*. 2022; 43: 353–362.
- Kaser EC, Lequio M, Zhu Z, Hunzeker ZE, Heslin AJ, D’mello KP, *et al.* Ovarian cancer immunotherapy en route: IL9 inhibits growth of ovarian cancer and upregulates its expression of Ox40L and 4-1BBL. *European Journal of Gynaecological Oncology*. 2022; 43: 163–168.
- Zhou J, Jiang Y, Chen H, Wu Y, Zhang L. Tanshinone I attenuates the malignant biological properties of ovarian cancer by inducing apoptosis and autophagy via the inactivation of PI3K/AKT/mTOR pathway. *Cell Proliferation*. 2020; 53: e12739.
- Jiang X, Cheng Y, He Y, Cong S, Sun L, Wu D, *et al.* LNC00115 mediates cisplatin resistance by regulating the miR-7/ERK signalling pathway in ovarian cancer. *Cancer Management and Research*. 2021; 13: 3817–3826.
- Wang Z, Yang MQ, Lei L, Fei LR, Zheng YW, Huang WJ, *et al.* Overexpression of *KRT17* promotes proliferation and invasion of non-small cell lung cancer and indicates poor prognosis. *Cancer Management and Research*. 2019; 11: 7485–7497.
- Li D, Ni XF, Tang H, Zhang J, Zheng C, Lin J, *et al.* *KRT17* functions as a tumor promoter and regulates proliferation, migration and invasion in pancreatic cancer via mTOR/S6k1 pathway. *Cancer Management and Research*. 2020; 12: 2087–2095.
- Li J, Chen Q, Deng Z, Chen X, Liu H, Tao Y, *et al.* *KRT17* confers paclitaxel-induced resistance and migration to cervical cancer cells. *Life Sciences*. 2019; 224: 255–262.
- Li C, Su H, Ruan C, Li X. Keratin 17 knockdown suppressed malignancy and cisplatin tolerance of bladder cancer cells, as well as the activation of AKT and ERK pathway. *Folia Histochemica et Cytobiologica*. 2021; 59: 40–48.
- Lu Z, Yang H, Cao H, Huo C, Chen Y, Liu D, *et al.* Forsythoside a protects against lipopolysaccharide-induced acute lung injury through up-regulating microRNA-124. *Clinical Science*. 2020; 134: 2549–2563.
- Wang Y, Lang H, Yuan J, Wang J, Wang R, Zhang X, *et al.* Overexpression of keratin 17 is associated with poor prognosis in epithelial ovarian cancer. *Tumor Biology*. 2013; 34: 1685–1689.
- Ujiiie D, Okayama H, Saito K, Ashizawa M, Thar Min AK, Endo E, *et al.* *KRT17* as a prognostic biomarker for stage II colorectal cancer. *Carcinogenesis*. 2020; 41: 591–599.
- McGuire S. World cancer report 2014. Geneva, Switzerland: world health organization, international agency for research on cancer, who press, 2015. *Advances in Nutrition*. 2016; 7: 418–419.
- Menon U, Karpinskyj C, Gentry-Maharaj A. Ovarian cancer prevention and screening. *Obstetrics & Gynecology*. 2018; 131: 909–927.
- Camilloni A, Nati G, Maggolini P, Romanelli A, Carbone G, Giannarelli D, *et al.* Chronic non-cancer pain in primary care: an Italian cross-sectional study. *Signa Vitae*. 2021; 17: 54–62.
- Samadder NJ, Giridhar KV, Baffy N, Riegert-Johnson D, Couch FJ. Hereditary cancer syndromes—a primer on diagnosis and management. *Mayo Clinic Proceedings*. 2019; 94: 1084–1098.
- Moschetta M, George A, Kaye SB, Banerjee S. BRCA somatic mutations and epigenetic BRCA modifications in serous ovarian cancer. *Annals of Oncology*. 2016; 27: 1449–1455.
- Lastwika KJ, Wilson W, Li QK, Norris J, Xu H, Ghazarian SR, *et al.* Control of PD-L1 expression by oncogenic activation of the AKT–mTOR pathway in non-small cell lung cancer. *Cancer Research*. 2016; 76: 227–238.
- Liu X, Xiao M, Zhang L, Li L, Zhu G, Shen E, *et al.* The m6a

methyltransferase METTL14 inhibits the proliferation, migration, and invasion of gastric cancer by regulating the PI3K/AKT/mTOR signaling pathway. *Journal of Clinical Laboratory Analysis*. 2021; 35: e23655.

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