



KIAA1529 regulates RAD51 expression to confer PARP inhibitors resistance in ovarian cancer

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ABSTRACT

PARP inhibitors (PARPi) are currently used as first-line therapy for advanced and recurrent ovarian cancer, but the clinical efficacy is limited by drug resistance. We aimed to investigate the role of KIAA1529 in PARPi resistance in ovarian cancer. The expression of KIAA1529 was determined in ovarian cancer cells using qRT-PCR and western blotting. Immunohistochemistry was used to examine the expression of KIAA1529 in primary ovarian cancer and recurrent ovarian cancer tissues. The effects of KIAA1529 on PARPi resistance were evaluated by knocking down KIAA1529 expression in ovarian cancer cells and assessing cell viability by CCK8 assays, apoptosis by flow cytometry, and homologous recombination (HR) repair by immunofluorescence analysis. The interaction between KIAA1529 and RAD51 was examined by western blotting. KIAA1529 was confirmed to be expressed in all ovarian cancer cell lines, and high expression of KIAA1529 was observed in recurrent ovarian cancer tissues. Inhibiting KIAA1529 expression increased the sensitivity of ovarian cancer cells to PARPi treatment. Furthermore, KIAA1529 increased the expression of the downstream effector RAD51 via Aurora-A, and HR was restored in ovarian cancer cells. This study demonstrates that KIAA1529 regulates RAD51 expression through Aurora-A to restore HR, which confers resistance to PARPi in ovarian cancer cells. These findings could provide a novel therapeutic target to overcome PARPi resistance in ovarian cancer.

Introduction

Ovarian cancer has the highest mortality rate of gynaecological malignant tumours, accounting for 4.7% of all female tumour-related deaths [1]. The nature of heterogeneity in ovarian cancer is part of the reason for the delay in effective screening and diagnostic strategies [2]. Mainstay of treatment consists of surgical debulking with complete macroscopic resection and platinum-based chemotherapy, but the 5-year survival rate remains approximately 45%. Drug resistance is one of the main reasons leading to treatment failure [3]. 20% of early-stage and 80% of advanced-stage ovarian cancer patients eventually develop resistance to traditional chemotherapy drugs, leading to disease progression [4]. Poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi) have changed the treatment landscape of epithelial ovarian cancer. PARPi were approved as targeted therapy for ovarian cancer and for maintenance therapy and drug therapy for patients with relapses in 2014. In recent years, several clinical studies have shown that PARPi significantly prolong progression-free survival in ovarian cancer [5,6]. PARPi are widely used as first-line treatments for advanced and

recurrent ovarian cancer. Although the use of PARPi is a major step forward, it is apparent that patients will eventually become resistant to PARPi [7,8]. Therefore, understanding the molecular mechanism of PARPi resistance is helpful for increasing the sensitivity of ovarian cancer to PARPi and to develop clinical treatment benefits for patients with recurrent ovarian cancer.

An *in vitro* transcription/translation system and a computer-based method based on GenBank analysis were used to predict and examine the coding potentials of large proteins in the KIAA family. Understanding the function of KIAA1529, a member of the KIAA family [9], has been very limited to date. Fei *et al.* used a genome-wide association study to identify the relationship between the single-nucleotide polymorphism (SNP) rs2061634 located on the KIAA1529 gene and Behcet's disease [10]. However, few studies have examined the relationship between KIAA1529 and cancers.

In this study, we investigated whether KIAA1529 plays a role in PARPi resistance and how KIAA1529 modulates ovarian cancer sensitivity to PARPi. We performed immunohistochemistry (IHC) and qRT-PCR in ovarian cancer cell lines and ovarian cancer tissues. We found for

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the first time that KIAA1529 was sufficient to promote PARPi resistance in ovarian cancer. The regulation of PARPi resistance by KIAA1529 is mediated by Aurora-A and affects the expression of RAD51, which is an important protein for homologous recombination repair. This study identifies novel mechanisms that may aid in the development of strategies to overcome PARPi resistance in ovarian cancer.

Materials and methods

Cell culture

The human epithelial ovarian adenocarcinoma cell line SKOV3 and other human epithelial ovarian cancer cell lines, such as A2780, C13K and OV2008, were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The SKOV3 cell line was cultured in McCoy's 5A medium containing 10% fetal bovine serum (FBS). The A2780 cell line was cultured in DMEM containing 10% FBS. The C13K and OV2008 cell lines were cultured in RPMI-1640 medium containing 10% FBS. The cells were maintained in a humidified incubator with 5% CO₂ at 37°C.

Drug treatments and cell viability assay

Olaparib was purchased from Selleckchem. SKOV3 cells were seeded at a density of 5×10^3 cells/well in 96-well plates in 150 µl of media. After overnight incubation, the original medium was removed, and 100 µl of media containing different concentrations of olaparib (20, 40, and 80 µmol/L) was added to each well. Cell viability was measured after 72 h. Cell viability was determined by CCK8 assay according to the manufacturer's instructions (Dojindo Molecular Technologies, Inc., Shanghai, China). CCK8 solution (10 µl) was added and incubated for 2 h in the dark. The absorbance was measured with a Bio-Rad 550 microplate reader at 450 nm. The effect of olaparib on cell growth was normalized to that of the untreated control. Each data point was generated in triplicate, and each experiment was performed three times.

Cell transfection

siRNA duplexes were synthesized by Invitrogen, USA, to target the KIAA1529 sequence 5'-CAUCCUGAAUGAGGAGAAUGUGAA-3' and to target the Aurora-A sequence 5'-AUGCCUGUCUUACUGUCATT-3'. For the control experiments, cells were transfected with an siRNA scrambled duplex (Invitrogen, USA). The siRNA was cloned into the PLV-MU6-[shRNA]-EF1α lentiviral vector and transfected into H293T cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Briefly, H293T cells were plated in 10 cm dishes at 30% confluence in DMEM containing 10% FBS. For each dish, 7.5 µg of lentiviral vector, 2.5 µg of pVSVg, 2.5 µg of pPLP1 and 2.5 µg of pPLP2 were mixed and transfected into H293T cells. Lentiviruses were collected after 48 h by filtration through a 0.45 µm strainer. The lentivirus suspension and fresh medium containing 20% FBS were mixed at a ratio of 4:1, and polybrene was added to achieve a final concentration of 8 mg/ml. A total of 1×10^6 SKOV3 cells were inoculated into a 10 cm cell culture plate, and 10 ml of the prepared mixture was added and cultured under conventional conditions for 3-4 days to infect the cells. Transduced cells and the transfection efficiency were examined by western blotting.

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from each group of cells using TRIzol reagent according to the manufacturer's protocol. cDNA was synthesized from total RNA using a First Strand cDNA Synthesis Kit (Transgen, China). We used 1 µl of cDNA in the PCR with KIAA1529 or GAPDH primers mixed with Power SYBR Green PCR mix in a final volume of 20 µl. The reactions were performed in a Bio-Rad system using Real-time PCR Syber Mix (DBI). The primers used were as follows:

KIAA1529 : forward5- ' ATGGGGTTCAGTGCATGGATT - 3'

reverse5- ' ACTCTGCCGATTGATTTAGGC - 3';

β - actin : forward5- ' CATGTACGTTGCTATCCAGGC - 3',

reverse5- ' CTCCTTAATGTCACGCACGAT - 3'.

The conditions for the reaction were as follows: 1 cycle at 95°C for 2 min and 40 cycles at 95°C for 10 s and 60°C for 30 s. The fold amplification for each gene was calculated using the 2-ΔΔCt method. Each sample was tested in triplicate.

Western blot analysis

Cells were harvested and subjected to protein immunoblotting. Cells were washed twice in ice-cold PBS and lysed in RIPA lysis buffer (Beyotime, China). After 30 min of cooling on ice, the cells were centrifuged at 12000 r/min for 30 min at 4°C. Protein concentrations were determined. Approximately 50 µg of protein was separated in a 10% SDS-PAGE gel, and then the proteins were transferred to a PVDF membrane. After being blocked with TBS buffer (10 mM Tris-HCl, pH 7.5, 150 mM sodium chloride) containing 0.05% Tween-20 and 5% nonfat milk, the blots were incubated with primary antibodies against KIAA1529 (ab107675, Abcam, 1:100), Aurora-A (AA921, Beyotime, 1:500), RAD51 (14961-1-AP, Proteintech, 1:500), and GAPDH (60004-1-Ig, Proteintech, 1:8000) overnight at 4°C, which was followed by incubation with horseradish peroxidase-conjugated secondary antibodies (Abmart, 1:4000) for 1 h at 37°C. The immunoreactive bands were visualized using an ECL chemiluminescence system.

Patients

23 surgically resected ovarian serous adenocarcinomas between 2017 and 2021 were retrospectively reviewed at the Department of Pathology, Zhongnan Hospital of Wuhan University. Among these, 16 were patients with ovarian cancer who underwent primary surgery and 7 were patients with recurrent ovarian cancer who underwent secondary surgery were selected. All study participants or their legal guardians provided informed written consent prior to study enrolment.

Immunohistochemical staining and interpretation

For immunohistochemical analysis of KIAA1529, tissue sections were paraffinized in xylene and rehydrated in a series of diluted alcohols. Antigen retrieval was performed in boiling 10 mM citrate buffer (pH 6.0) for 10 min. After blocking endogenous peroxidase activity with 0.3% H₂O₂ in PBS for 15 min and blocking nonspecific binding with 10% normal goat serum for 1 h at 37°C, the tissue sections were incubated with anti-KIAA1529 antibodies (ab107675, Abcam, 1:100) overnight at 4°C. After three washes in PBS buffer, the sections were incubated with biotinylated secondary antibodies at 37°C for 1 h, washed three times in PBS, and then incubated in Vectastain ABC-HRP solution (Vector Labs) at room temperature for 30 min according to the manufacturer's instructions. The signal of the target protein was developed using diaminobenzidine (DAB) substrate (Vector Labs). Finally, the sections were counterstained with haematoxylin. The stained slides were reviewed by two pathologists who were blinded to the clinicopathological characteristics according to the Features German Immunoreactive Score [11]. The final IHC score was the average score of all scores of each tumour.

Immunofluorescence microscopy

SKOV3 cells were cultured on glass coverslips. After the treatments, the cells were fixed with 4% paraformaldehyde for 15 min at room temperature. The cell membranes were permeabilized with 0.1% Triton X-100 for 10 min, and then the cells were blocked with 5% BSA for 30 min. Mouse anti-RAD51 antibodies (14961-1-AP, Proteintech, 1:500)

were added and incubated for 2 h at 37°C. After three washes in PBS, the cells were incubated with FITC-conjugated anti-mouse secondary antibodies for 1 h at 37°C. After being incubated with the appropriate fluorescent secondary antibody, the cells were stained with Hoechst-33342 and immediately observed under a fluorescence microscope.

Analysis of apoptosis using flow cytometry

An annexin V-FITC apoptosis kit (KeyGEN Biotech, Nanjing, China) was used to determine the number of apoptotic cells according to the manufacturer's instructions. Briefly, cells were grown in six-well plates and treated with olaparib. The cells were harvested at the indicated time, washed twice with ice-cold PBS and resuspended in 500 μ l of binding buffer. Then, 5 μ l of annexin V-FITC and 10 μ l of PI were added, and the mixture was incubated in the dark at 4°C for 15 min. A total of 10^6 events per sample were analysed by using a BD FACScan flow cytometer. Apoptotic cells are presented as the percentage of Annexin V+PI+ cells.

Statistical analysis

All experiments were repeated at least three times. All values are presented as the mean \pm standard deviation (SD). Statistical analysis was

performed with the SPSS 19.0 software package. Relationships between two variables were analysed with Student's *t*-tests. A probability value of 0.05 was selected as the significance level.

Results

KIAA1529 is expressed in ovarian cancer cell lines and is associated with the recurrence of ovarian cancer

Knowledge of the function of the KIAA1529 protein is very limited. Consequently, this study examined the expression of KIAA1529 and showed that the KIAA1529 gene and protein were significantly expressed in ovarian cell lines (Fig. 1A, 1B). Moreover, we also examined the expression of KIAA1529 in 23 ovarian serous adenocarcinoma tissues and found that the expression of KIAA1529 in recurrent ovarian cancer tissues was significantly higher than that in ovarian cancer at initial treatment, indicating that KIAA1529 is related to the recurrence of ovarian cancer (Fig. 1C, 1D).

Inhibiting KIAA1529 expression can increase the sensitivity of ovarian cancer cells to PARP inhibition

In our study, we used shRNA to inhibit KIAA1529 expression in

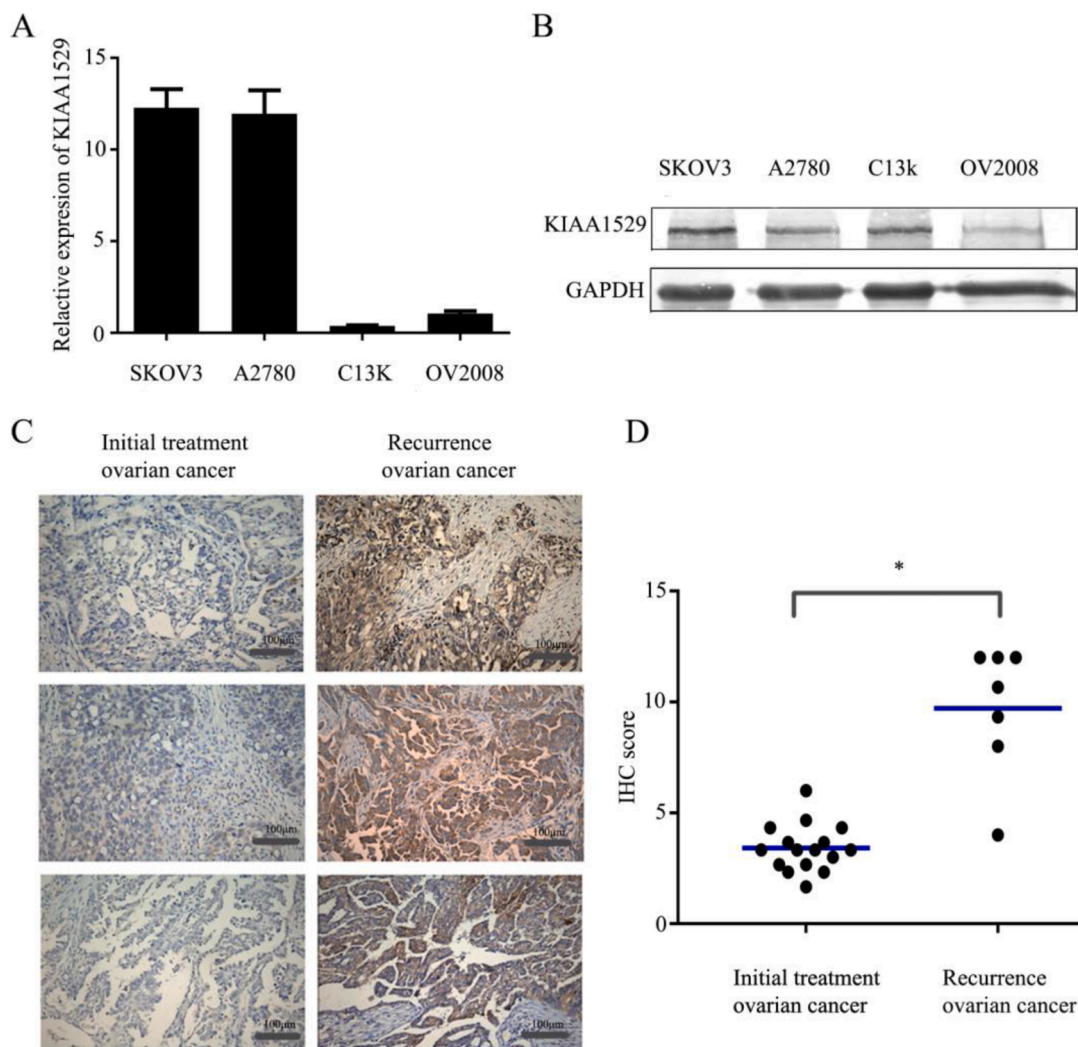


Fig. 1. KIAA1529 is expressed in ovarian cancer cell lines and is associated with the recurrence of ovarian cancer. A-B. KIAA1529 expression was first examined in ovarian cancer cell lines by qRT-PCR and western blotting. C-D. KIAA1529 expression was examined in 23 surgically resected ovarian serous adenocarcinoma tissues, and the expression of KIAA1529 in recurrent ovarian cancer tissues was significantly higher than that in ovarian cancer at initial treatment (**p* < 0.05).

SKOV3 ovarian cancer cells (Fig. 2A). To determine whether inhibiting KIAA1529 alters the sensitivity of SKOV3 cells to PARPi, we used CCK8 assays to measure cell viability after treatment with PARPi. Cell viability was markedly decreased when KIAA1529 was inhibited in cancer cells treated with olaparib (20, 40, and 80 $\mu\text{mol/L}$) for 72 h (Fig. 2B). Consistently, flow cytometry showed that inhibiting the expression of KIAA1529 increased the number of apoptotic cells in response to 40 $\mu\text{mol/L}$ PARPi treatment (Fig. 2C). Collectively, these data suggest that inhibiting KIAA1529 expression can increase the sensitivity of ovarian cancer cells to PARPi.

KIAA1529 affects the expression of RAD51 through Aurora-A

The ELM database was used to predict that the KIAA1529 protein might have a domain that interacts with Aurora-A in our previous study. Therefore, we used western blotting to analyse the relationship between KIAA1529 and Aurora-A and to examine the relationship with RAD51, which is an important HR protein. The results showed that inhibiting KIAA1529 expression increased Aurora-A expression and inhibited RAD51 expression (Fig. 3A). Furthermore, to study the correlation between the expression of Aurora-A and RAD51, we decreased the expression of Aurora-A and found that the expression of RAD51 was restored (Fig. 3B). In summary, KIAA1529 in ovarian tumour cells could increase the protein expression of RAD51 by inhibiting the expression of Aurora-A.

Inhibiting Aurora-A can restore HR capacity and maintain the resistance of ovarian cells to PARP inhibitors

RAD51 is also one of the most important functional proteins associated with HR and could restore HR capacity in ovarian cancer cells. Finally, we investigated RAD51 expression in the nucleus by immunofluorescence analysis to demonstrate changes in the HR capacity of ovarian cancer cells in the presence of Aurora-A inhibition. When the expression of Aurora-A was inhibited, the proportion of SKOV3 cells

expressing RAD51 in the nucleus was increased (Fig. 4A, 4B). Furthermore, inhibiting Aurora-A significantly increased the viability of ovarian cancer cells treated with PARPi compared to cells in the control group. These results indicated that inhibiting Aurora-A could decrease the sensitivity of ovarian cancer cells to PARPi (Fig. 4C).

Discussion

While it is the fifth most common gynaecological malignancy, ovarian cancer is the leading cause of death due to gynaecological malignancies [12]. The very recent milestone and most intriguing new therapeutic option consists of PARPi treatment. There have been many recent clinical trials assessing the efficacy of PARPi in patients with recurrent or primary ovarian cancer. The results of those trials showed that PARPi significantly prolongs progression free survival (PFS) as a maintenance treatment after response to platinum-based chemotherapy [5,13,14].

"Synthetic lethality" is the theoretical basis of PARPi therapy [15]. DNA damage caused by chemotherapy drugs and other factors can be recognized, and DNA damage can be repaired through the DNA damage response (DDR). Poly ADP-ribose polymerase (PARP) is a key protein in the DDR that can recognize single-strand DNA breaks. When PARP activity is suppressed, single-strand DNA breaks accumulate and become double-strand breaks that rely on HR for repair. Homologous recombination deficiency (HRD) in cancer cells may result in the death of tumour cells [16–18]. The mechanism of PARPi resistance in ovarian cancer is still unclear. Some scholars hypothesized that it might be related to reversal of the HRD phenotype, stabilization of the DNA replication fork, increased PARylation activity and the removal of the inhibitor from the cell by efflux transporters [19,20]. Reversal of the HRD phenotype was the first resistance mechanism to be observed. Secondary mutations in BRCA negate truncating mutations and restore an open reading frame. This effect was first observed in laboratory studies and then in clinical samples from patients after platinum-based therapy [21, 22]. Reversion mutations are similarly found in other

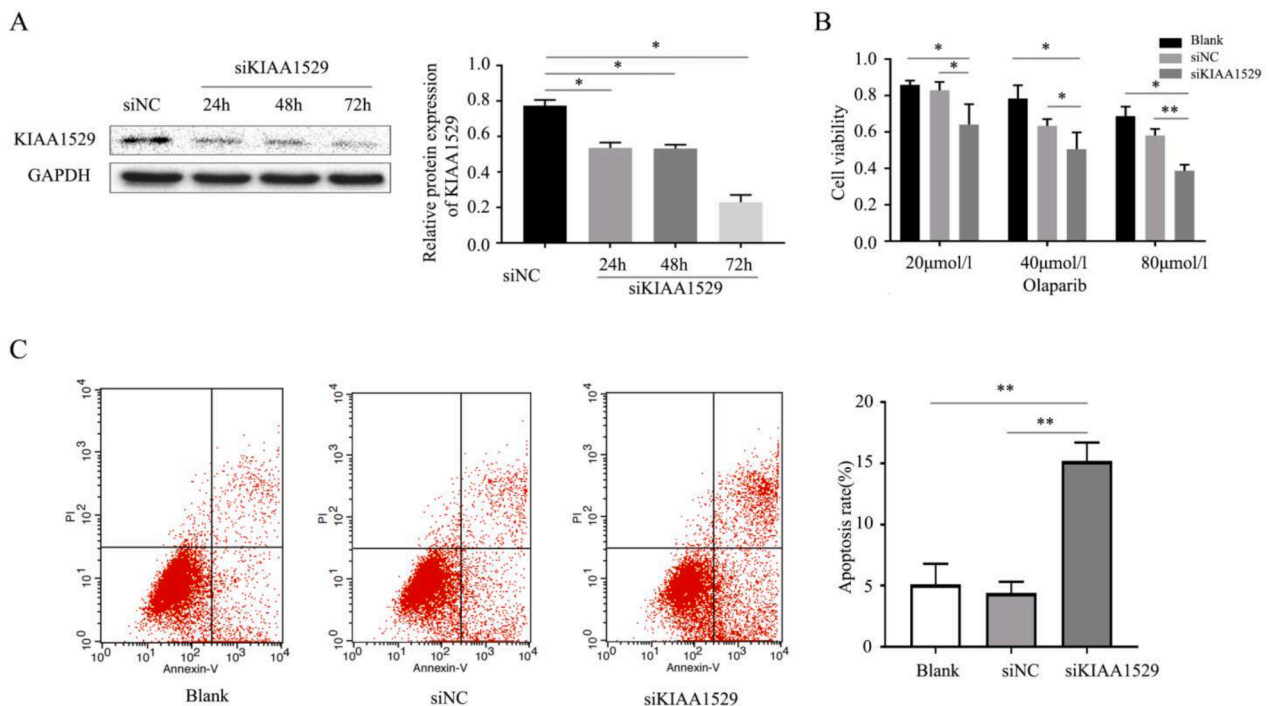


Fig. 2. Inhibiting KIAA1529 expression can increase the sensitivity of ovarian cancer cells to PARP inhibition. A. Western blot analysis of KIAA1529 expression in SKOV3 cells after transfection with siKIAA1529 for 24 h, 48 h and 72 h. B. SKOV3 cells were transfected with siKIAA1529 to inhibit the expression of KIAA1529 and were treated with olaparib (20, 40, and 80 $\mu\text{mol/L}$) for 72 h. Cell viability was examined by CCK8 assays. C. SKOV3 cells were transfected with siKIAA1529 to inhibit the expression of KIAA1529 and treated with olaparib 40 $\mu\text{mol/L}$ for 72 h. The apoptotic rates were determined by flow cytometry (* $p < 0.05$, ** $p < 0.01$).

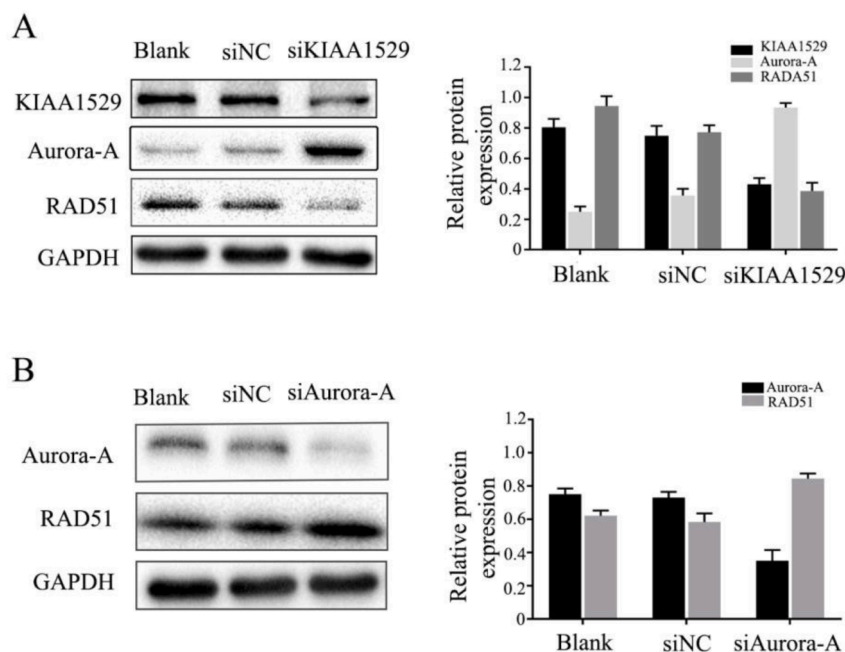


Fig. 3. KIAA1529 affects the expression of RAD51 through Aurora-A. **A.** Western blot analysis of the expression of RAD51 and Aurora-A in SKOV3 cells transfected with siKIAA1529 and treated with 40 μmol/L olaparib for 72 h. **B.** Western blotting was used to analyse the expression of RAD51 when SKOV3 cells were transfected with siAurora-A and treated with 40 μmol/L olaparib for 72 h.

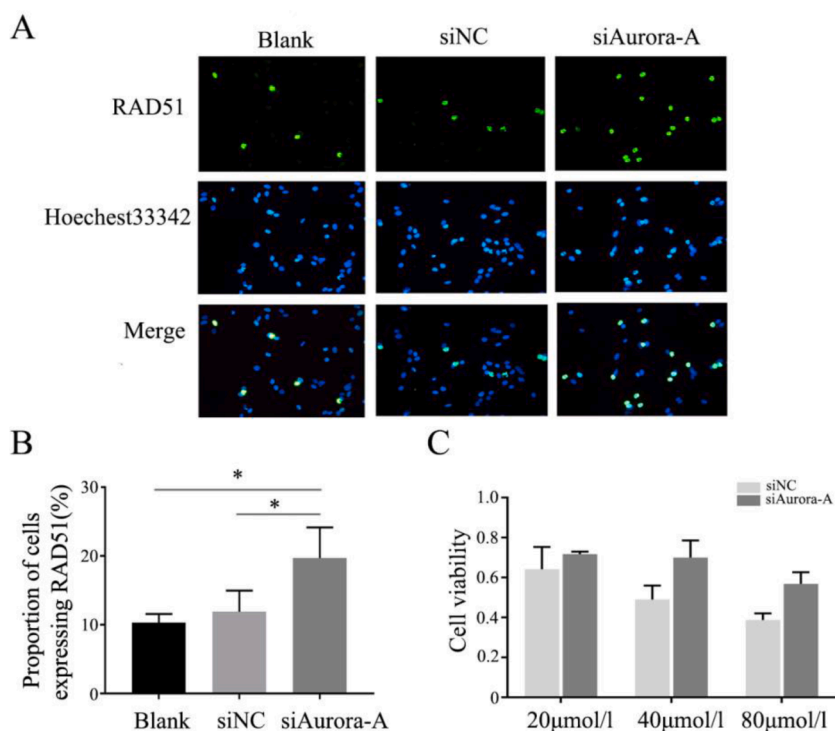


Fig. 4. Inhibiting Aurora-A can restore HR capacity and maintain the resistance of ovarian cells to PARP inhibitors. **A.** RAD51 expression in the nucleus of SKOV3 cells was examined by immunofluorescence analysis in the presence of Aurora-A inhibition and exposure to olaparib. **B.** The proportion of SKOV3 cells expressing RAD51 in the nucleus in the presence of Aurora-A inhibition and exposure to olaparib (**p* < 0.05). **C.** SKOV3 cells were transfected with siAurora-A to inhibit the expression of Aurora-A and treated with olaparib (20, 40, and 80 μmol/L) for 72 h. Cell viability was examined by CCK8 assays.

altered HR-associated proteins. For example, reversion mutations in RAD51C and RAD51D have been described in association with acquired resistance to PARPi [23].

KIAA1529 is located on 9q22.33 and has a length of 6269 bp. The protein is encoded at LOC5765 and has a molecular weight of 191 kDa [9]. Research on the correlation between KIAA1529 and cancers has shown that high expression of KIAA1529 is associated with a poor prognosis of prostate cancer [24]. Our study revealed that the expression of KIAA1529 in recurrent ovarian cancer tissues was significantly higher

than that in ovarian cancer at initial treatment. This indicated that KIAA1529 could be related to the sensitivity of drug therapy for ovarian cancer.

In our previous study on paclitaxel resistance in ovarian cancer, KIAA1529 was identified in the AKT2 signalling pathway using proteomic methods. In the present study, we further found that inhibiting KIAA1529 expression in ovarian cancer cells significantly increased the sensitivity of tumour cells to PARPi. These results indicate that KIAA1529 is involved in the mechanism of PARPi resistance in ovarian

cancer cells. In our previous study, the ELM database was used to perform KIAA1529 protein structure analysis, and it was found that the KIAA1529 protein might have a domain that interacts with Aurora-A. This study also confirmed that KIAA1529 had a negative regulatory effect on Aurora-A expression.

Aurora-A is a member of the Aurora family of serine/threonine kinases. Aurora-A can phosphorylate itself at Thr288 and binds to tubulin TPX2 to regulate mitosis by controlling centriole and spindle formation [25]. In addition, Aurora-A regulates NF- κ B [26, 27], Hippo [28], PI3K/Akt/mTOR [29] and other molecular signalling pathways to affect tumour proliferation, invasion and metastasis [30]. Aurora-A also play a role in DNA repair and function as a DNA repair modulator to control cancer cell chemosensitivity. The TPX2/Aurora-A heterodimer plays a previously unrecognized role in DNA damage repair and replication fork stability by counteracting 53BP1 function [31]. CDK1/Aurora-A can mediate the phosphorylation of the DNA double-strand break repair factor CtIP at serine 327, triggering CtIP binding to the PLK1 polo-box domain to promote an error-prone DNA repair mechanism [32]. Our findings demonstrated that PARPi treatment can stimulate the protein expression of KIAA1529 in ovarian tumour cells and inhibit the expression of Aurora-A. When KIAA1529 was downregulated, Aurora-A expression was elevated, and the sensitivity of ovarian cancer cells to PARP inhibitors was increased.

RAD51 is an ATPase that promotes the formation of nucleoprotein structures on single-stranded DNA [33]. RAD51 can effectively bind to both single- and double-stranded DNA, and it is a key protein in HR repair. In the context of DNA damage, RAD51 can effectively bind with BRCA2 to form homologous fragments embedded in homologous chromosomes, which can be used as templates for accurate DNA repair and maintaining genomic stability in cells [34]. Thus, RAD51 is one of the most important functional proteins associated with HR. Several studies have shown that reversions or secondary mutations can be identified not only in BRCA1 and BRCA2 but also in RAD51 [23,35,36]. Thus, HR can be (partially) restored, leading to resistance to chemotherapy or PARPi. Waks AG et al. revealed that RAD51 foci acquired post-PARPi resistance in breast cancer patients with genomic reversion, which was consistent with the reconstitution of HR [37]. Feng Y et al. reported that high RAD51 expression indicated unfavourable survival outcomes and resistance to platinum, taxane, and PARP inhibitors in patients with ovarian cancer [38]. These results are consistent with our study. We further found that KIAA1529 regulated the expression and localization of RAD51 in ovarian cancer cells through Aurora-A. These results indicated that KIAA1529 could restore the DNA HR repair. Taken together, these data suggest that KIAA1529 ultimately affects the protein expression of RAD51, causing ovarian cancer cells to regain HR capability and ultimately leading to resistance to PARPi treatment.

Conclusion

We provide the first evidence that elevated expression of KIAA1529 may serve as a critical point leading to PARPi resistance in ovarian cancer. We described for the first time that KIAA1529 was positively correlated with RAD51 expression in patients with ovarian cancer through Aurora-A. The KIAA1529/Aurora-A/RAD51 signalling pathway mediates the recovery of HR and PARPi resistance in ovarian cancer cells. Therefore, targeting KIAA1529 may improve the sensitivity of ovarian cancer to PARPi.

Data Availability

All the data in the current study are included in the article or were uploaded as supplementary information.

Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee Zhongnan

Hospital of Wuhan University of China (No. 2022121K). This paper has not been published elsewhere in whole or in part. All authors have read and approved the content and agree to submit it for publication. Informed consent was obtained from all individual participants included in the study.

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Author contributions

Y. Qiao and X.C. Yu were responsible for the study design and performed the experiments. B. Zhou was responsible for clinical data collection. K. Zhang and J.Y. Huang were responsible for data analysis. J. Liao designed the study. Y. Qiao and J. Liao wrote the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2022.101497.

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