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PLK1 overexpression suppresses homologous recombination and confers cellular sensitivity to PARP inhibition

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The overexpression of Polo-like kinase 1 (PLK1) is associated with poor clinical outcomes in various malignancies, making it an attractive target for anticancer therapies. Although recent studies suggest PLK1's involvement in homologous recombination (HR), the impact of its overexpression on HR remains unclear. In this study, we investigated the effect of PLK1 overexpression on HR using bioinformatics and experimental approaches. Analyzing The Cancer Genome Atlas (TCGA) and Cancer Cell Line Encyclopedia (CCLE) datasets with the Homologous Recombination Deficiency (HRD) score, we found a positive correlation between PLK1 expression and HRD score, indicating that increased PLK1 expression suppresses HR. To validate these findings, we performed cell line-based experiments, demonstrating that PLK1 overexpression attenuates RAD51 focus formation and HR, as measured by ASHRA in T47D cells. Since HR-deficient cells are hypersensitive to PARP inhibitors, we further confirmed that PLK1 overexpression increases sensitivity to PARP inhibitors, both in CCLE dataset analysis and experiments using T47D cells. Additionally, we found that the effects of PLK1 overexpression on HR suppression and increased PARP inhibitor sensitivity were mitigated by either a PLK1 kinase inhibitor or the kinase-dead mutant [T210A]. This suggests that PLK1's impact on HR and PARP inhibitor sensitivity is mediated through its kinase activity. Moreover, analysis of clinical ovarian cancer samples revealed that higher PLK1 expression correlates with increased sensitivity to PARP inhibitors. Our results suggest that PLK1 overexpression suppresses homologous recombination, leading to enhanced sensitivity to PARP inhibition, presenting a potential therapeutic strategy for targeting cancers with overexpression of PLK1.

Keywords PLK1, Overexpression, Cancer, Homologous recombination, PARP inhibition

PLK1 is a serine/threonine kinase involved in cell cycle regulation and carcinogenesis[1]. PLK1 is often overexpressed in many types of malignancies. Overexpression of PLK1 is linked to shorter survival in certain types of malignancies such as non-small cell lung cancer, head and neck squamous cancer, melanoma, breast

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cancer, ovarian cancer, neuroblastoma, colorectal cancer and cervical cancer[2–7]. With this understanding, PLK1 is an attractive target for establishing anti-cancer treatments. Indeed, several PLK1 inhibitors have been established and some of them have been in clinical trials[1,8]. The first among these classes is BI 2536, a small molecule inhibitor of the kinase domain of PLK1. BI 2536 failed in the phase II trial for both leukemia (acute myeloid leukemia: AML) and solid tumors such as lung and pancreatic cancers[8]. The PLK1 inhibitor that is in the most advanced stage of clinical development is Volasertib. Volasertib shows anti-tumor effects in ovarian cancer, non-small cell lung cancer, and breast cancer in early phase studies, both in combination and as a single agent. However, since PLK1 expression levels do not correlate with Volasertib sensitivity[8], developing alternative strategies for treating PLK1-overexpressing cancers is crucial.

Poly(ADP–ribose) polymerase (PARP) inhibitors (PARPis) are a class of drugs that target the key repair enzyme PARP1, which is known to play a role in several DNA repair pathways by binding to stretches of single-stranded DNA[9]. PARP inhibitors (PARPis) were initially developed as radiosensitizers in the 1970s, but have gained significant attention in the last decade following a pivotal observation: cells with mutations in the BRCA1 and BRCA2 genes, which cause homologous recombination (HR) defect, display elevated sensitivity to these agents[10]. This sensitivity of BRCA mutant cells to PARP inhibition is thought to result from a 'synthetic lethality' of cells with defective HR-mediated DNA repair to PARP inhibition[11]. Germline BRCA1/2 mutations, however, only account for a small subset of epithelial cancers, and it is thought that other mechanisms may lead to a phenotypic suppression of homologous recombination in sporadic cancers[12]. In addition, when certain genes related to HR, including RAD51, RAD54, DSS1, RPA1, NBS1, ATR, ATM, CHK1, CHK2, FANCD2, FANCA, or FANCC are not functional in cells, they become hypersensitive to PARP inhibitor[13]. Recent reports have revealed that PLK1 plays a role in homologous recombination. PLK1 regulates DNA resection, an initial step of HR, through the phosphorylation of CtIP[14,15]. Furthermore, PLK1 regulates the activity of RAD51, a recombinase, by phosphorylating it with CHK1[16]. These findings prompted us to investigate the effect of overexpression of PLK1 on HR and its sensitivity to PARP inhibition.

The genomic instability is a consequence of loss of heterozygosity (LOH), telomeric allelic imbalance (TAI), and large-scale state transitions (LST). The degree of HR deficiency (HRD) can be measured by adding up the LOH, TAI, and LST. Indeed, the HRD score is associated with the functional status of BRCA1/2, which are well-known HR regulators[17]. The TCGA and the CCLE datasets include genomic data, which allows for the calculation of HRD in every sample within the dataset. This study investigates the impact of PLK1 overexpression on HR using bioinformatics. The findings from bioinformatics analysis are verified through experiments conducted on cell lines. Furthermore, the effect of PLK1 overexpression on the sensitivity to PARP inhibitors is evaluated with both cell lines and clinical samples.

Results

Increased expression of PLK1 suppresses HR in the TCGA dataset

Before investigating the effect of PLK1 expression on HR, we examined PLK1 expression patterns in various cancers using the TCGA dataset. PLK1 expression patterns vary depending on the type of cancer (Fig. 1). This finding suggests that the threshold of PLK1 overexpression in one type of malignancy may not be the same as overexpression in other tumor types. Consequently, we investigated the correlation between PLK1 expression and HRD.

We analyzed the correlation between PLK1 expression and HRD in malignancies associated with shorter survival when PLK1 is overexpressed[18]. The Pearson correlation coefficient was used to analyze the correlation

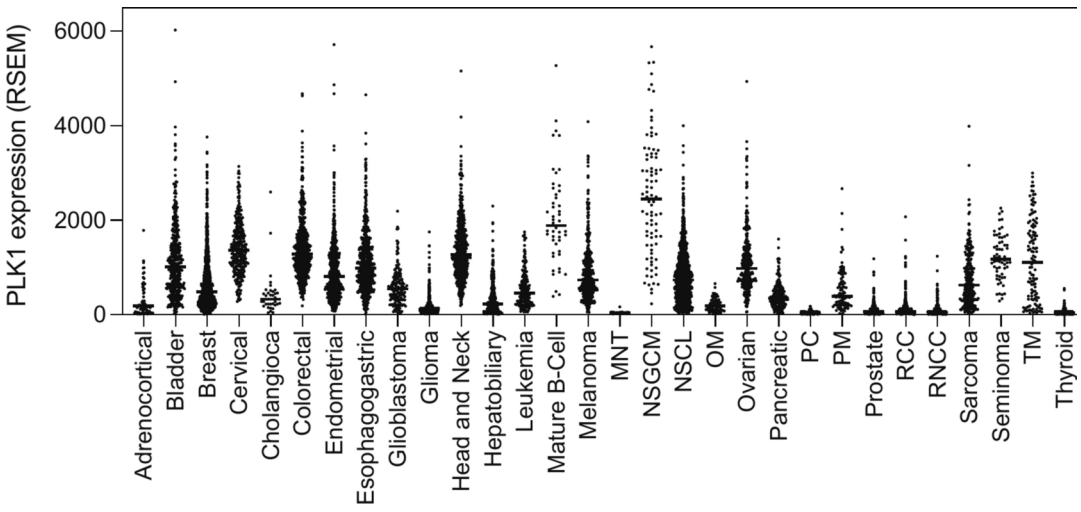

Fig. 1. The expression of PLK1 in various malignancies. PLK1 expression in different types of malignancies in the TCGA dataset is shown. MNT: Miscellaneous Neuroepithelial Tumor, NSGCT: Non-Seminomatous Germ Cell Tumor, NSCL: Non-Small Cell Lung carcinoma, OM: Ocular Melanoma, PC: Pheochromocytoma, PM: Pleural Mesothelioma, RCC: Renal Clear Cell, RNCC: Renal Non-Clear Cell, TM: Thymic Epithelial.

between PLK1 expression levels and HRD scores. While a positive correlation between PLK1 expression and HRD scores was observed, the strength of the correlation varied among cancer types. Notably, breast cancer exhibited the strongest correlation (Fig. 2). However, for many other cancer types, including colorectal and cervical cancers, the correlation was weak or nonsignificant. These variations in correlation strength could be influenced by the limitations of sample size in certain cancer types. To address this possibility, we conducted a

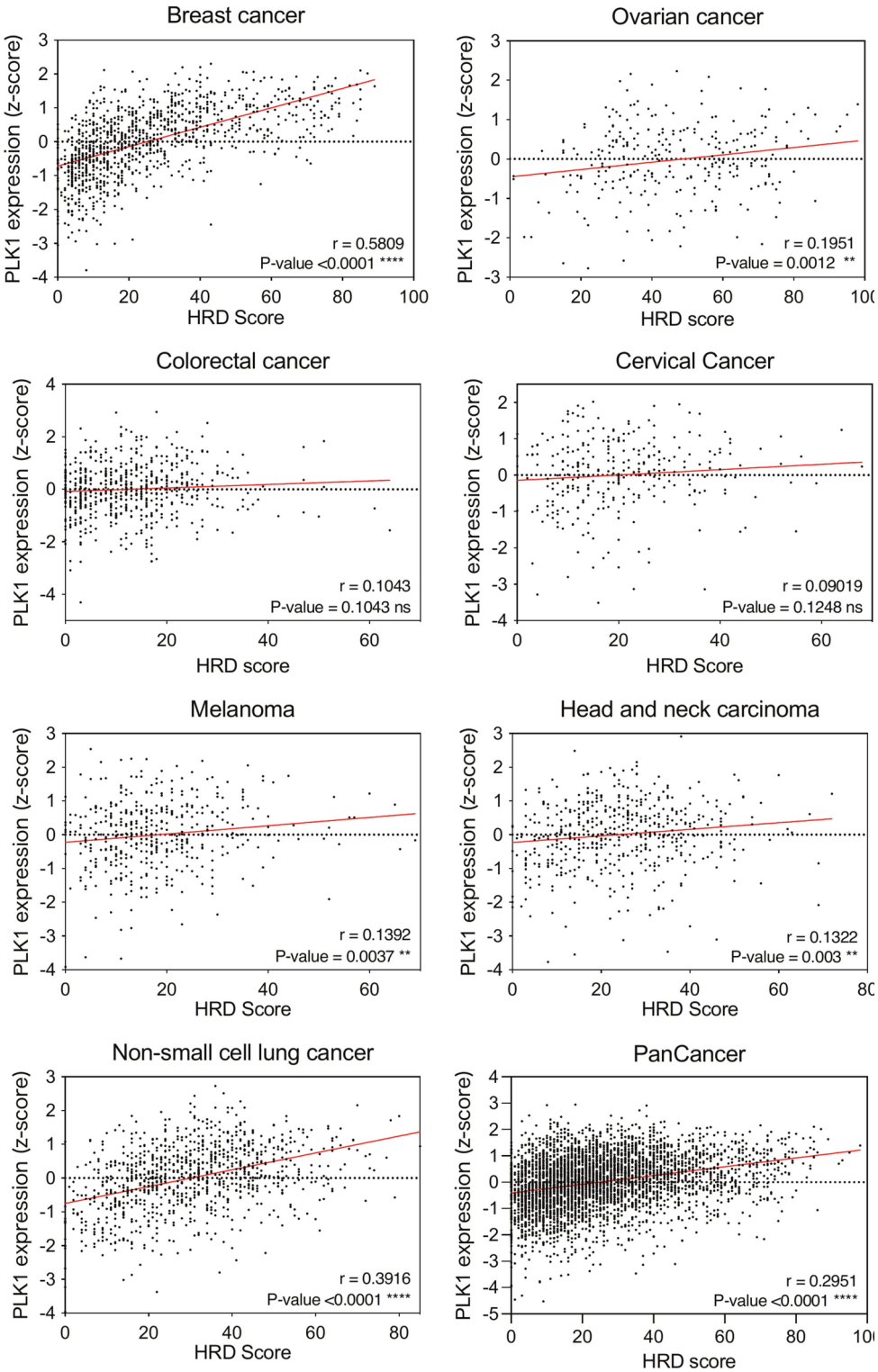

Fig. 2. Positive correlation between PLK1 expression and HRD score. Scatterplot of PLK1 expression level shown as z-score versus HRD score among individual malignancies or pancancer. Pearson's correlation (r) values are presented in each graph.

pan-cancer analysis, which provided further evidence for the association between increased PLK1 expression and elevated HRD scores, with significant differences observed across multiple malignancies (Fig. 2).

The impact of PLK1 expression on HR and the sensitivity to PARP inhibition in the CCLE dataset

To ensure the accuracy and reliability of the results obtained from the TCGA dataset analyses, we also examined the relationship between PLK1 expression and HRD score in the Cancer Cell Line Encyclopedia (CCLE) dataset. The CCLE dataset comprises genetic information from 1,739 cancer cell lines[19]. PLK1 expression and HRD score data are available for 930 cancer cell lines in the CCLE dataset[20]. Using the Pearson correlation coefficient analysis, we found a positive correlation between PLK1 expression and HRD score (Fig. 3A).

The CCLE dataset also includes drug sensitivity data. For 566 cell lines, both PLK1 expression and sensitivity to Olaparib, a commonly used PARP inhibitor in clinical settings are available. Given that HR defects result in increased sensitivity to PARP inhibition, we examined the effect of PLK1 expression on sensitivity to PARP inhibitors. In the analysis of these 566 cell lines, there is a negative correlation between PLK1 expression and Olaparib Ic50 value. This suggests that increased PLK1 expression is associated with heightened sensitivity to the PARP inhibitor (Fig. 3B).

Increased expression of PLK1 suppresses HR in cell-based experiments

To validate the reproducibility of the bioinformatics results, we performed cell line-based experiments using T47D cells, comparing the effects on HR between parental, vector-control (VC) and PLK1-overexpressing cells. Since successful HR requires the recruitment of Rad51 to DNA damage sites by BRCA2[21], we investigated whether Rad51 focus formation was affected by overexpression of PLK1. PLK1 expression does not influence the induction of γH2AX, a marker of DNA double-strand breaks, following PARP inhibitor treatment (Fig. 4A,B). However, cells overexpressing PLK1 show a marked decrease in Rad51 focus formation under the same conditions (Fig. 4C). These findings indicate that while PLK1 overexpression does not interfere with the generation of DNA double-strand breaks or the subsequent cellular signaling pathways leading to γH2AX focus formation, it does inhibit homologous recombination[22].

The efficiency of HR has traditionally been measured using the DR-GFP system, where HR restores a functional promoter followed by a GFP sequence in response to an I-SceI restriction enzyme treatment. This results in a detectable GFP signal in HR-proficient cells[23]. However, it is known that the DR-GFP system often does not correlate well with sensitivity to genotoxic agents, including PARP inhibitors, in cells with certain gene mutations, such as BRCA1 missense mutations[24].

Recently, a new system called ASHRA has been developed to measure the integration of a nucleic acid sequence of interest, such as a GFP sequence, into an endogenous locus using the CRISPR/Cas9 system. The efficiency of HR measured with ASHRA is highly correlated with cellular sensitivity to genotoxic agents, suggesting that ASHRA is the best method for this study, which investigates the effect of PLK1 overexpression on both HR efficiency and cellular sensitivity to PARP inhibition[24]. We used a donor sequence to integrate the GFP sequence into the *ß-actin* genome (ACTB) using the CRISPR/Cas9 nuclease, targeting the *ß-actin* locus. This system was designed to create a fusion transcript of *ß-actin* followed by in-frame integration of the GFP sequence in HR-proficient cells. To assess the effects of PLK1 overexpression on HR, we compared HR activity among parental T47D cells, vector-control (VC) cells and PLK1-overexpressing cells. Both parental and VC cells showed the induction of the fusion transcript of β-actin-GFP with gRNA targeting ACTB, indicating successful integration of the GFP sequence into the β-actin genome, as compared to samples expressing non-target scramble gRNA (Fig. 4D). In contrast, PLK1-overexpressing cells did not exhibit the induction of β-actin-GFP production with either ACTB-targeting or non-targeting gRNA (Fig. 4D). This suggests that the overexpression of PLK1 suppresses HR. It is known that cells with impaired HR are sensitive to PARP inhibition[13]. Strikingly, T47D cells overexpressing PLK1 also display increased sensitivity to PARP inhibition (Fig. 4E).

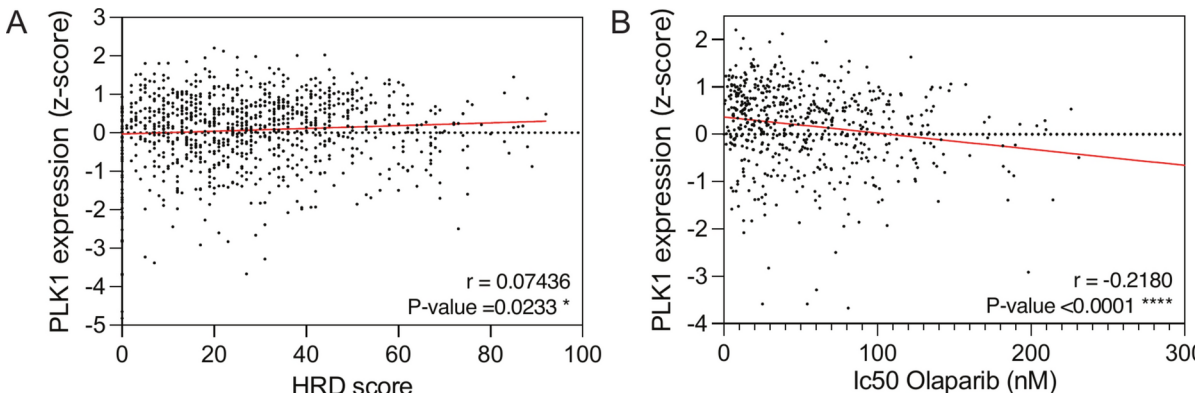

Fig. 3. Expression of PLK1 is positively correlated with both HRD score and sensitivity to PARP inhibition. The scatterplot shows the correlation of PLK1 expression shown as z-score and HRD score (**A**) and Ic50 value to Olaparib (**B**). Pearson's correlation (r) values are shown in each graph.

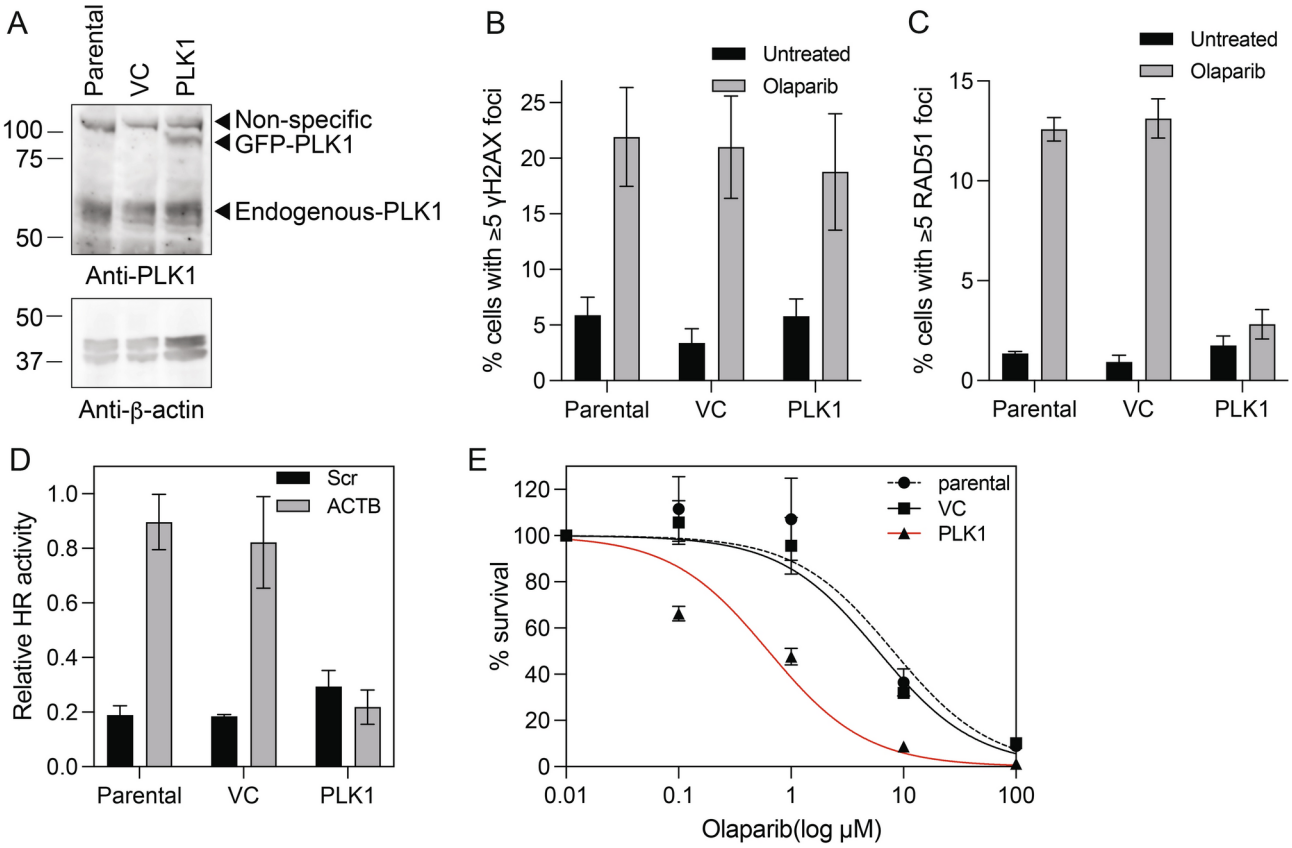

Fig. 4. PLK1 overexpression results in increased sensitivity to PARP inhibitor through suppression of homologous recombination. (**A**) Cell lysates from parental T47D cells, T47D cells transfected with GFP vector control (VC) and T47D cells transfected with GFP-PLK1 were subjected to western blot with indicated antibodies. The transferred membranes were cut before being exposed to the primary antibody. The figure shows cropped data, and the full-length images are available in the Supplementary Figure S1. (**B**) Immunofluorescence analysis of γH2AX foci in GFP-positive T47D cells treated with PARP inhibitor (1 μM Olaparib) for 24 h. Untreated cells were also analyzed as a control. The histogram shows the percentage of GFP-positive cells with more than 5 γH2AX foci. The error bar shows the standard error of three independent experiments. (**C**) Cells were stained with anti-Rad51 antibody in the same condition as the experiment shown in (**B**). The histogram shows the percentage of GFP-positive cells with more than 5 Rad51 foci. The error bar shows the standard error of three independent experiments. (**D**) The bar graph shows HR activity, indicated by the amount of β-actin-GFP products, as measured by RT-PCR, in parental, vector-control (VC) and PLK1-overexpressing T47D cells. The Error bars show the standard error of four independent experiments. (**E**) Line chart shows sensitivity to PARP inhibitor (Olaparib) in parental, vector-control (VC) and PLK1-overexpressing T47D cells (PLK1). The error bar shows the standard error of three independent experiments.

PLK1 overexpression suppresses HR and sensitizes cells to PARP inhibition through its kinase activity

Next, we explored whether the suppression of HR and the increased sensitivity to PARP inhibitors in PLK1-overexpressing cells are mediated by its kinase activity. To address this, we examined RAD51 foci formation and performed ASHRA assays under conditions of PLK1 wild-type overexpression, with or without treatment with BI2536 (a PLK1 kinase inhibitor), PLK1 T210A (a kinase-dead mutant)[25], and Aurora A, whose increased expression promotes PLK1 kinase[26] (Fig. 5A). Overexpression of the PLK1 T210A mutant, as well as wild-type PLK1 treated with BI2536, failed to suppress RAD51 foci formation, suggesting that PLK1-mediated HR suppression is dependent on its kinase activity (Fig. 5B). Moreover, overexpression of Aurora A also led to a reduction in RAD51 foci formation (Fig. 5B), further supporting the notion that PLK1 kinase activity is essential for HR suppression. Similarly, ASHRA assays showed that while overexpression of wild-type PLK1 blocked β-actin-GFP integration, neither PLK1 T210A overexpression nor PLK1 inhibition with BI2536 affected HR efficiency (Fig. 5D). Additionally, Aurora A overexpression suppressed β-actin-GFP integration (Fig. 5D), aligning with the effect of PLK1 kinase activity. Furthermore, while both wild-type PLK1 and Aurora A overexpression increased cellular sensitivity to the PARP inhibitor, neither the kinase-dead T210A mutant nor BI2536 treatment altered PARP inhibitor sensitivity (Fig. 5E). These findings demonstrate that the effects of PLK1 on HR suppression and PARP inhibitor sensitivity are kinase-dependent.

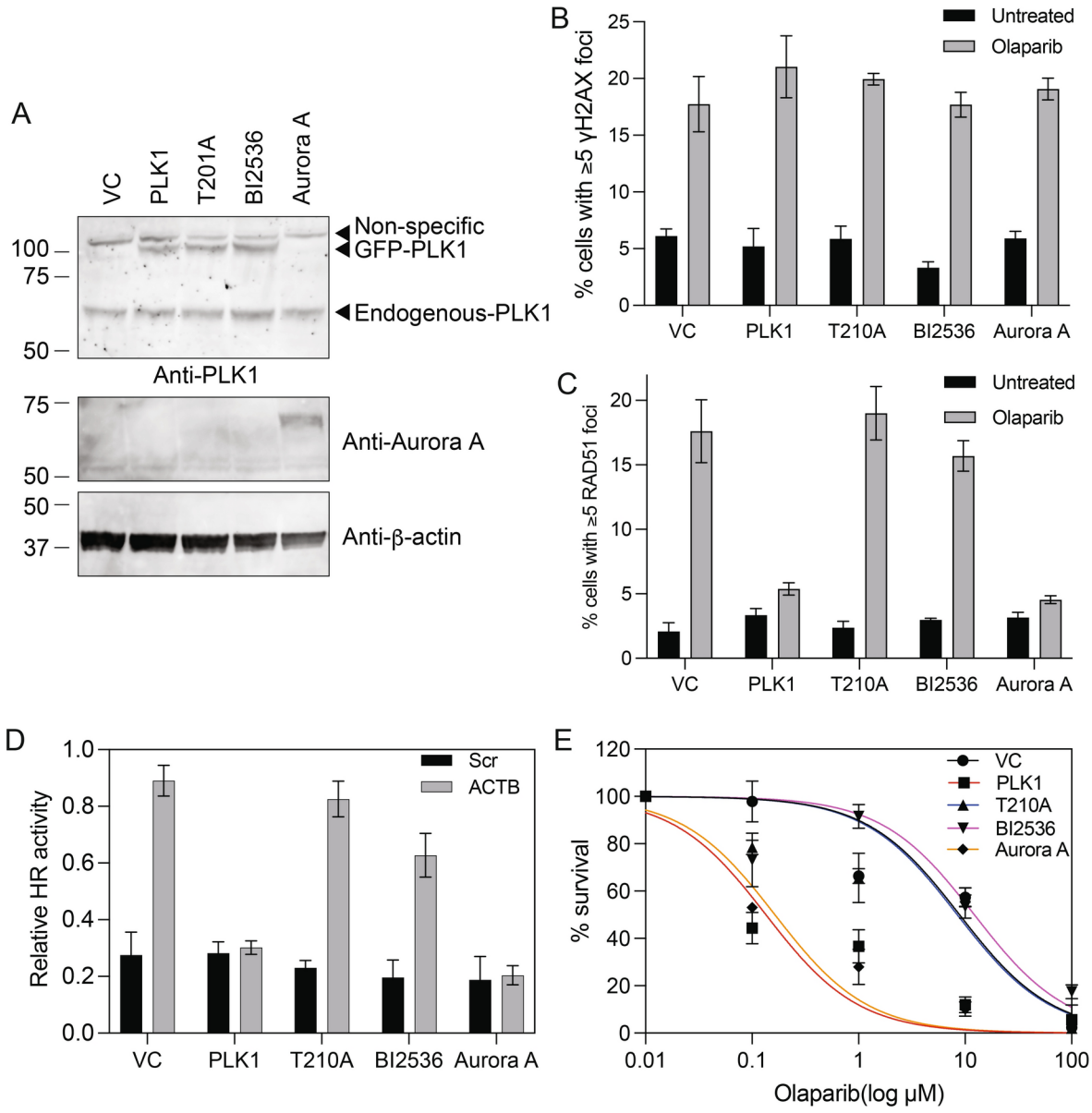

Fig. 5. PLK1 kinase activity influences homologous recombination and sensitivity to PARP Inhibition in T47D cells. (**A**) Western blot analysis of T47D cell lysates transfected with GFP vector control (VC), GFP-PLK1 wild type (PLK1), PLK1 T210A mutant (T210A), GFP-PLK1 wild type treated with BI2536, and GFP-Aurora A (Aurora A). Expression levels of PLK1 and the effects of kinase inhibition or mutation are shown using the indicated antibodies. Membranes were cut before exposure to primary antibodies. Cropped images are shown, and full-length blots are available in Supplementary Figure S2. (**B**) Immunofluorescence analysis of γ-H2AX foci in GFP-positive T47D cells transiently transfected as described in (**A**). Cells were treated with PARP inhibitor (1 μM Olaparib) either with or without BI2536 (25 μM) for 24 h. The histogram shows the percentage of GFP-positive cells with more than 5 γ-H2AX foci. Error bars represent the standard error from three independent experiments. (**C**) Immunofluorescence analysis of Rad51 foci in GFP-positive T47D cells under the same conditions as Fig. 5B. The histogram displays the percentage of GFP-positive cells with more than 5 Rad51 foci. Error bars represent the standard error from three independent experiments. (**D**) Homologous recombination (HR) efficiency measured by β-actin-GFP transcript levels, as determined by RT-PCR, in T47D cells transfected with GFP vector control, GFP-PLK1, GFP-PLK1 T210A mutant, GFP-PLK1 treated with BI2536, and GFP-Aurora A. Error bars represent the standard error from four independent experiments. (**E**) Sensitivity to PARP inhibitor (Olaparib) in T47D cells transfected with GFP vector control, GFP-PLK1, GFP-PLK1 T210A mutant, GFP-PLK1 treated with BI2536 (2 μM), and GFP-Aurora A. Cell viability was assessed after PARP inhibitor treatment, with error bars representing the standard error from three independent experiments.

Age	Median (range)	54 (41–77)
Pathological Stage	I	6
	II	0
	III	5
	IV	7
Pathological diagnosis	Serous adenocarcinoma	8
	Mucinous adenocarcinoma	1
	Clear cell carcinoma	3
	Endometrioid adenocarcinoma	4
	Carcinosarcoma	2

Table 1. Baseline characteristics.

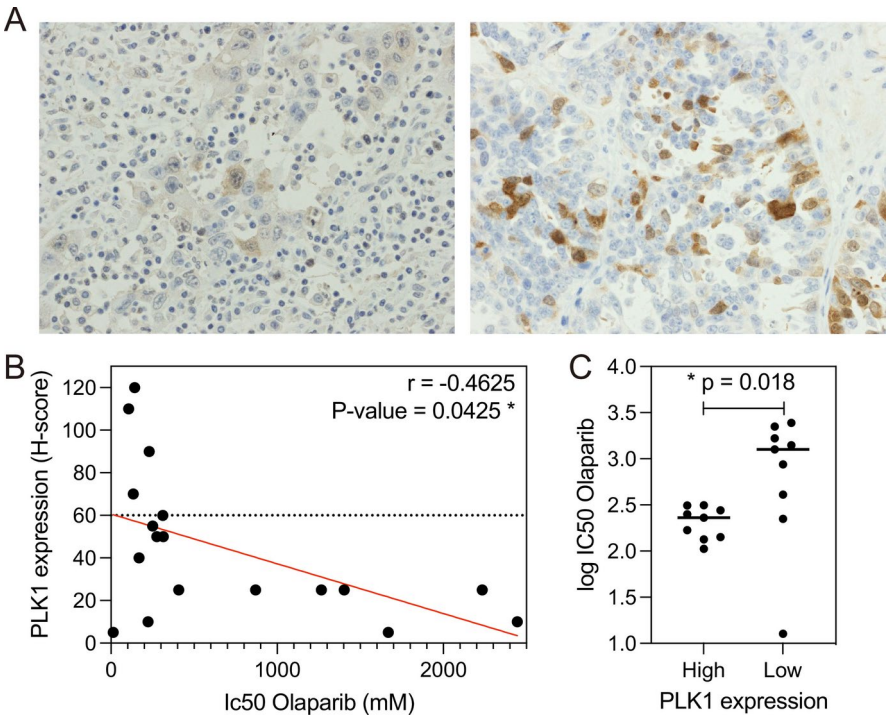

Fig. 6. PLK1 overexpression is a predictive factor for high sensitivity to PARP inhibitors in ovarian cancer. (**A**) Representative images of patient-derived tumor samples stained with PLK1 are shown. The left panel shows an example of a low level of PLK1 and the right panel shows an example of a high level of PLK1. (**B**) The scatterplot shows the correlation of PLK1 expression shown as H-score and Ic50 value to Olaparib. Spearman's correlation (r) value is shown. The break line indicates a median value of PLK1 expression in the cohort. (C) The plot shows the log IC50 values for cells with high and low PLK1 expression, divided by the median. The p-value was calculated using the Mann–Whitney U-test.

PLK1 expression correlates with increased sensitivity to PARP inhibitor ex-vivo, in clinical samples of ovarian cancer

To expand the clinical significance of our findings, we assessed the relationship between PLK1 levels and PARP inhibitor sensitivity in clinical samples, using an ex vivo drug sensitivity analysis method[27,28]. Fresh biopsies of ovarian cancers (n = 18) were obtained from participating patients at St. Marianna University Hospital, between September 2012 and August 2014 (Table 1). We dissociated ovarian cancer cells through collagenase treatment of the biopsy sample and subjected them to drug sensitivity analysis in vitro[29]. In parallel, we also performed immunostaining in these samples to measure the protein expression level of PLK1. PLK1 expression level was measured by a H-score, a scoring system widely used in medicine[30] (Fig. 6A).

There was a negative correlation between PLK1 expression and sensitivity to PARP inhibitor treatment ex vivo (Fig. 6B). In this analysis, Spearman's correlation was used due to a limited sample number, and the correlation was statistically significant. Moreover, samples with high PLK1 expression, defined by expression levels above the median, were sensitive to PARP inhibition (Fig. 6C). The effect of PLK1 expression levels

remained significant in a multivariate analysis using a Gaussian model to account for other possible factors like age, staging or pathological diagnosis (Table 2).

Discussion

Recent reports have revealed the involvement of PLK1 in HR, especially in DNA resection and RAD51 loading[14–16]. The significant clinical implication of PLK1 is its overexpression, which is linked to poorer clinical outcomes. However, the impact of PLK1 overexpression on HR was previously unknown. Our study has revealed that the overexpression of PLK1 suppresses homologous recombination via its kinase activity. However, the mechanism by which the overexpression of PLK1 suppresses HR remains unclear. The kinase activity of PLK1 affects HR through the phosphorylation of CtIP to promote DNA resection. These facts suggest that overexpression of PLK1 may suppress HR by impairing DNA resection due to potentially abnormal phosphorylation of CtIP. PLK1 plays a pivotal role in cell cycle progression[31,32] and carcinogenesis[33]. HR suppression by PLK1 overexpression might be mediated through interactions with molecules involved in the cell cycle or carcinogenesis. To fully understand the clinical implications and the precise mechanics of HR, further studies are required.

PARP inhibitors are attractive anti-cancer agents due to their clinical efficacy and limited adverse events. However, their use is currently limited to specific indications. Our study suggests that PLK1 overexpression can be a biomarker predicting high sensitivity to PARP inhibition. This finding could expand the use of PARP inhibitors, particularly in poor prognosis malignancies with PLK1 overexpression. Continuous treatment with PARP inhibitors often leads to acquired resistance. Our results indicate that the PLK1 expression level may affect sensitivity to PARP inhibition, suggesting that decreased PLK1 expression could contribute to acquired resistance. Also, defects in the kinase activity of PLK1 may affect HR efficiency as defects of 53BP1 restore HR in BRCA1 deficient cells[34]. Additionally, our results imply that PLK1 inhibitors, potential anti-cancer agents, might attenuate the effects of PARP inhibitors, potentially contributing to acquired resistance to PARP inhibition treatments. Therefore, our study may provide insights into mechanisms of acquired resistance to PARP inhibitors and emphasize caution in combination anticancer therapies.

PLK1 overexpression is linked to poorer clinical outcomes in many types of malignancies. The mechanism behind this association is not well understood. HR deficiency is linked to poorer clinical outcomes in prostate cancer[35], suggesting that PLK1 overexpression may lead to poor outcomes through HR suppression. If this is the case, treatment with PARP inhibitors could significantly improve clinical outcomes in malignancies with PLK1 overexpression. Nonetheless, further studies are needed to validate these findings.

Methods

Cell line

T47D cells were purchased from ATCC (#HTB-133). The cells were passaged and used for no more than 3 months.

Cell culture

T47D cells were cultured in RPMI-1640 medium with 10% fetal bovine serum and 1% penicillin–streptomycin at 37 °C with a 5% CO2 atmosphere.

Transfection procedure

Transfections were performed using the following plasmids:

pBS-ACTB-C200_GFP (Addgene #169798).
LentiCRISPR V2-ACTB-C1 (Addgene #169796).
LentiCRISPR V2-scr (Addgene #169795).
pEGFP-AuroraA (Addgene #198402).
PLK1 T210A (Addgene #132965).
PLK1-GFP

pEGFP-vector control

Transfections were conducted using PEI MAX™-Transfection Grade Linear Polyethylenimine Hydrochloride (MW 40,000) (Polysciences). To form complexes, 15 µg of total plasmid DNA was combined with PEI MAX in OPTI-MEM at a DNA ratio of 1:2 and incubated at room temperature for 15–20 min. Cells were incubated for 48 h for qPCR analysis and 72 h for Western blot analysis at 37 °C in a 5% CO_2 incubator.

	Crude		Adjusted	
	OR	p-value	OR	p-value
PLK1 expression	1.04E−05	*1.04E−05	2.6E−08	*1.56E−02
Age	5.27E+06	4.53E−01	1.55E+06	4.33E−01
Stage	8.78E+22	5.73E−01	2.40E+55	8.14E−01
Pathology	1.14E+19	6.22E−01	2.15E−65	1.60E−01

Table 2. Gaussian multivariate analysis for clinical samples. OR: odds ratio. C.I.: confidence interval. Sensitivity (Ic50), PLK1 expression, age and stage were evaluated as a continuous variable.

Cell viability assay

Clonogenic assay: Clonogenic assays were performed as described previously[36].

Clinical samples: Cells were plated into 96-well plates at a density of 3000 cells per well. Different doses of drugs were added and the cells were incubated at 37 °C for a week. Cell Titer-Blue reagent (Promega) was used to assay for cell viability according to the manufacturer's guidelines. Each experiment was done in quadruplicate.

Immunofluorescence

Cells were cultured in 96 well plastic plates (BD Falcon). Once cells attached, cells were treated with 1 mM of PARP inhibitor for 24 h. Untreated cells were also prepared with the same condition. The following procedures were performed as described previously[36]. Cells were fixed with 4% paraformaldehyde (PFA) in PBS for 15 min, washed with PBS, permeabilized with 0.2% Triton-X100 for 5 min, and blocked with 3% BSA in PBSt for 15 min. After blocking, cells were incubated with primary antibodies for 1 h at room temperature, washed three times with PBSt and then incubated with secondary antibodies for 30 min at room temperature. Nuclei were stained with DAPI. Immunofluorescence images were acquired with an All-in-One Fluorescence Microscope BZ-X800 (Keyence). Image analysis was conducted using CellProfiler version [4.2.8]. Nuclei were identified using the *IdentifyPrimaryObjects* module based on DAPI staining, and GFP-positive cells were detected using the *IdentifySecondaryObjects* module for GFP channel. Foci were enhanced using the *EnhanceOrSuppressFeatures* module to improve the contrast, then identified using the *IdentifyPrimaryObjects* module on the Alexa-594 channel. The percentage of GFP-positive cells with more than five foci was calculated.

Antibodies

The antibodies and dilution used in this study were: Anti-γH2AX (Ser139) antibody (Millipore, 05–636, 1:1000); Anti-Rad51 antibody (abcam, ab133534, 1:2000); Anti-PLK1 antibody (Thermo Scientific, PL6/PL2, 1:1000 for WB and Cell Signaling, 208G4, 1:50 for IHC); Anti-Aurora A antibody (Cell Signaling Technology, #91,590, 1:1000); Anti-β-actin (SIGMA, AC-15, 1:1000); and secondary antibodies (Alexa Flour, 1:1000).

Bioinformatic analysis

TCGA: RNA-sequencing data from TCGA (mRNA expression z-scores for all samples: log RNA Seq V2 RSEM) were downloaded from cBioPortal (https://www.cbioportal.org/). The HRD scores associated with the TCGA data were obtained from the Genomic Data Commons website (https://gdc.cancer.gov/node/998) [18]. The mRNA expression data and HRD scores were combined using R (ver. 3.6.1).

CCLE: RNA-sequencing and drug sensitivity data from the Cancer Cell Line Encyclopedia (mRNA expression z-scores: log RNA Seq RPKM; Olaparib-1 treatment response: IC50) were downloaded from cBioPortal (https://www.cbioportal.org/). The HRD scores relevant to the CCLE data were accessed from https://github.com/shirotak/CellLine_HRD_DrugRes [20]. The mRNA and drug sensitivity data, along with the HRD scores, were combined using R (ver. 3.6.1).

Statistical analysis

To compare drug sensitivity to a PARP inhibitor in cells with high or low PLK1 expression, the Mann–Whitney U test was performed using GraphPad Prism. For Correlation analysis, Pearson's correlation was performed. When the sample size is small, such as < 20 cases, Spearman's correlation analysis was performed. Both analyses were conducted using GraphPad prism. For multivariate analysis, the Poisson regression model was performed using a generalized linear model (glm) package in R.

Study approval

The experiments using clinical samples were performed with the approval of the Clinical Ethics Committee of St. Marianna University. Approval number: 2623. Informed consent was obtained from all participants and all experiments were performed following the guidelines and regulations.

Patients

Patients who were suspected of having ovarian cancer and underwent surgery at St. Marianna University Hospital between September 2012 and August 2014 were considered for this study. Only those whose resected specimens were pathologically diagnosed as ovarian cancer by two pathologists were included in this study.

Immunohistochemistry (IHC) and measurement of protein expression

IHC was performed as described previously[37]. Paraffin tissue sections were cut onto coated slides (3 μm) and deparaffinized by routine techniques. Endogenous peroxidase activity was blocked with a 3% H2O2 in PBS for 5 min. Prior to PLK1 staining, antigen retrieval was performed with Antigen Retrieval Solution (pH 9.0) (Nichirei bioscience) at 95 °C in a steamer for 40 min, followed by the sections were incubated with an anti-PLK11 antibody for 60 min. Labeling was detected with the Histofine Simple Stain, MULTI (Nichirei Bioscience), following the protocol suggested by the manufacturer, and all sections were counter stained with hematoxylin. For measurement of protein expression, the percentage of positive cells was determined by counting about 500 cells within five high-resolution fields.

HR activity by ASHRA

Cells were cultured in a 10 cm dish and transfected with a donor vector, either LentiCRISPRv2-scr or LentiCRISPRv2-ACTB-C1 (constructed by VectorBuilder, ID: VB010000-9355sqw or VB210304-1065msq, respectively). After 72 h of incubation, genomic DNA was extracted using the ReliaPrep Cell Miniprep System (Promega). Quantitative PCR was performed on a StepOne™ Real-Time PCR System (Thermo Fisher) using

PowerUp SYBR Green master mix (Applied Biosystems). The primer sequences used in this analysis were 5'-GTCCTGCTGGAGTTCGTGACCG-3' and 5'-GTGCAATCAAAGTCCTCGGC-3' for the knocked-in allele and 5'-AGTTGCGTTACACCCTTTCTTG-3' and 5'-GTGCAATCAAAGTCCTCGGC-3' for the control allele. The relative quantity of the knocked-in allele was calculated by the Ct method.

Data availability

Data is provided within the manuscript.

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Author contributions

K.S. and A.S.S. conceived the study. S.P., A.S.S., R.S., S.J.A., T.O., M.I., K.M., R.A., F.G., Z.C., Z.M.M.J., E.S., A.Y.,

R.M., K.P., I.M., O.T., N.S., K.Y. and J.A.B. performed the experiments. A.S.S., J.A.B. and K.S. analyzed the data and wrote the manuscript.

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