OncoTargets and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Inhibition of ATM with KU-55933 Sensitizes Endometrial Cancer Cell Lines to Olaparib

Anqing Zhang[1], Liqin Zhang[2], Xia Xie[1], Dan Liu[2]

[1]Gynaecology Department, Sunshine Union Hospital, Weifang, People's Republic of China; [2]Gynaecology Department, Affiliated Hospital of Weifang Medical University, Weifang, People's Republic of China

Correspondence: Dan Liu, Gynaecology Department, Affiliated Hospital of Weifang Medical University, Yuhe Road 2428#, Weifang, 261041, People's Republic of China, Email kaiyan0417@126.com

Background: Endometrial cancer (EC) is one of the most prevalent gynecologic cancers, which poses a serious threat to women's health worldwide. Olaparib, the first FDA-approved PARP inhibitor for the treatment of BRCA-mutated breast, ovarian and pancreatic cancers, triggers apoptosis of cancer cells through synthetic lethality by inhibiting PARP1/2 enzymatic activity and BRCA1/2-dependent homologous recombination (HR) repair deficiency. However, the synergistic lethal effects between Olaparib and inhibitors of other DNA damage response proteins, such as ATM, PTEN and RAD51, are still unknown.

Aim: Exploring the synergistic lethal effect between Olaparib and KU-55933 on EC.

Methods: The GEPIA database was used to test EC patient survival rate. CCK8 was used for cell viability assays. Western blot was used for examining gene levels. The wound healing assay was used to detect cell migration ability. Flow cytometry was used for detecting the apoptosis rate. All experimental conditions were repeated independently in triplicate and analyzed in three separate experiments.

Results: In this study, we discovered that the frequency of ATM alterations in endometrial cancer reaches nearly 20% and that there is a positive correlation between ATM alterations and prognosis. Furthermore, we discovered that endometrial cells with low expression levels of ATM are sensitive to Olaparib. Treatment with KU-55933, a specific inhibitor of ATM, significantly enhanced the sensitivity of endometrial cancer cells to Olaparib, as evidenced by colony formation, cell migration and apoptosis assay. Further analysis revealed that KU-55933 potentiates Olaparib-induced cell apoptosis by inhibiting ATM phosphorylation.

Conclusion: Our study demonstrates that inhibiting ATM could enhance the sensitivity of endometrial cancer to Olaparib, thereby providing a potential alternative treatment for the clinical treatment of endometrial cancer.

Keywords: endometrial cancer, ATM inhibitor, PARP inhibitor, homologous recombination, synthetic lethality

Background

Endometrial cancer (EC) is a type of epithelial malignant tumors occurring in the endometrium.[1] It is one of the most prevalent gynecologic cancers, which poses a serious threat to women's health and lines the fourth after mammary glands cancer, lung cancer and rectum cancer.[2,3] EC can be classified basically into two types based on Bokhman's classification complemented with detailed genetic, biochemical and histopathological studies. Type I EC represents high expression of estrogen and progesterone receptors, low histological differentiation and presenting as an early-stage disease. Type II EC represents a highly aggressive and invasive cancer with lower expression of estrogen and progesterone receptors than type I, commonly presenting as an advanced-stage disease.[4–6] Compared to other malignancies, endometrial cancer often presents with early clinical symptoms, leading to early diagnosis in most cases.[7] After systemic therapy, most endometrial cancer patients have a good prognosis and a low recurrence rate. However, if the cancer does recur, the prognosis is often poor.[1] Surgical excision is an effective therapeutic means for early EC. But for recurrent EC, chemotherapy is the preferred option.[8] Paclitaxel combined with carboplatin is more effective and better tolerated than triple therapy with doxorubicin and cisplatin for patients at the time of first recurrence.[9] In addition to the above-mentioned chemotherapeutic agents, topotecan and docetaxel have also shown activity in recurrent endometrial cancer, although response rates have been disappointingly low.[10]

Received: 21 June 2023
Accepted: 7 December 2023
Published: 19 December 2023

A complex network of proteins is involved in DNA damage response (DDR) via detecting and repairing DNA damages. DDR sensor proteins identify damaged DNA area and control the subsequent cellular responses. For double-strand breaks (DSBs), the Ku70/Ku80 protein heterodimer and MRN complex (MRE11-RAD50-NBS1) are the predominant sensors. Ku7/Ku80 binds DNA DSBs within seconds of them being generated and serves as a platform for the subsequent recruitment of classical non-homologous end-joining (NHEJ) proteins. The MRN complex plays key roles in triggering activation of the DNA-damage signaling kinase Ataxia-telangiectasia mutated (ATM), the initiation of DNA end-resection and promotion of repair by HR. Chromatin context, transcriptional status, cell cycle stage and extent of end-resection are all factors that contribute to the selection of DNA repair, either by HR or NHEJ.[11] Poly(ADP-ribose) polymerase (PARP) is an important sensor for DNA single-strand breaks (SSBs). Seventeen PARP family members have been identified in human cells, of which PARP-1 has a predominant role in DNA repair. PARP1 is best known for its role in DNA base excision repair (BER) and repair of DNA single-strand breaks (SSBs),[12] although it also has a less well-defined role in DNA double-strand break (DSB) repair by alternative non-homologous end-joining (alt-NHEJ).

Olaparib, also named by the chemical name of 4-[3-(4-cyclopropylcarbonylpiperazin-1-carbonyl)-4-fluorobenzyl]-2H-phthalazin-1-one, is the Federal Drug Agency (FDA) approved PARP (Poly ADP-ribose polymerase)-inhibitor for clinical use in BRCA1/2 mutated ovarian cancer.[13] It mainly acts as a competitive inhibitor of NAD+ at the catalytic site of PARP1 and PARP2.[14] PARP enzymes are responsible for DNA single-strand break (SSBs) repair.[15] By inhibiting PARP, olaparib disrupts SSBs repair and induces synthetic lethality in cells that have mutations in BRCA1/2.[16–19] BRCA1/2 are involved in homologous recombination (HR), which is critical for double-stranded DNA break repair.[20,21] The BRCA1/2 genes play a crucial role in ensuring the proper assembly and disassembly of HR proteins at the site of DNA damage. Mutations in these genes can lead to defective HR, which increases the risk of cancer development, and also increases the sensitivity to genotoxic agents including ionizing radiation and chemotherapeutic drugs that damage DNA such as platinum agents and PARP inhibitors.[22] Olaparib can inhibit PARP catalytic activity, and trap PARP1 and PARP2 on DNA lesions that block replication machinery and prevent fork protection, fork reversal, and fork restart.[23] This results in double-strand breaks (DSBs) that need to be repaired by HR.[23,24] As this damage can only be accurately repaired by HR, cancer cells that are deficient in BRCA1/2 or other HR genes will be more sensitive to Olaparib.[17,25] Although Olaparib is currently the only clinically approved treatment for patients with BRCA1/2 mutations, recent studies have repeatedly shown that cells with defects in other HR genes, including ATM (Ataxia-telangiectasia mutated), PTEN and RAD51, are also sensitive to Olaparib.[26,27]

ATM kinase belongs to a family of serine/threonine phosphatidylinositol 3-kinase-like-protein-kinases (PIKK) and plays a vital role in cellular DNA damage response.[28,29] In the resting state, ATM mainly resides in the nucleus as an inactive dimer and transfers to chromatin to undergo auto-phosphorylation and dissociate into active monomers in response to DNA double-strand breaks.[30,31] Once activated, ATM phosphorylates downstream effector proteins, including P53, CHK2, H2AX, among others, to regulate cell cycle and death.[29]

Due to its critical role in DSB repair, similar to BRCA1/2, ATM is a promising therapeutic target for cancer treatment. Zhang et al reported that a patient with gallbladder carcinoma harboring an ATM-inactivating mutation responded well to Olaparib, with a progression-free survival of 13 months.[32] Another study suggests that ATM inhibition could enhance the effects of Olaparib in the cell lines of lung, gastric and breast cancer. Moreover, the efficacy of Olaparib was significantly improved in two patient-derived triple-negative breast cancer (TNBC) xenograft models.[29] These findings indicate that combining ATM inhibition and Olaparib can effectively enhance tumor killing and overcome acquired resistance.

In this study, we investigated the therapeutic potential of combining ATM inhibition with Olaparib in endometrial cancer cell lines. Specifically, we examined the effects of ATM inhibitor KU-55933, both alone and in combination with Olaparib, in Ishikawa and Hec-108 cell lines. Our results demonstrated that KU-55933 not only inhibits colony formation and cell migration but also promotes apoptosis induced by Olaparib. These findings provide valuable insights that could be used to guide more comprehensive in vitro and in vivo follow-up studies, with the ultimate goal of informing the design of clinical trials focused on enhancing the therapeutic index and improving clinical outcomes for patients with endometrial cancer.

Methods

Cell Lines and Reagents

This study used six human endometrial cancer cell lines, Ishikawa (type I), Hec-1B (type II), AN3CA (type I), KLE (type II) and RL952 (type I) which were purchased from Procell Life Science & Technology Co., Ltd., and Hec-108 (type I) was obtained from Shanghai Huiying biological technology Co., Ltd. Ishikawa and Hec-108 were cultured in DMEM (SH30243.01, Hyclone), while others were maintained in DMEM/F12 (SH30023.01, Hyclone). Both were supplemented with 10% FBS (FB15015, Clark) and 1% penicillin/streptomycin (100 U/mL). Cell cultures were incubated at 37 °C in a humidified incubator under 5% CO2.

Olaparib (HY-10162) and ATM inhibitor, KU55933 (HY-12016), were purchased from MedChemExpress, dissolved in 50 mM stocks in DMSO and stored at −20 °C. The antibodies used in this study, including ATM (92356), Phospho-ATM (5883), PARP (9532), Caspase-3 (9662), cleaved Caspase-3 (9664), b-actin (3700) and horseradish peroxidase (HRP)-linked anti-mouse (7076)/rabbit (7074) IgG, were all purchased from Cell Signaling Technology.

ATM Expression and Prognostic of Endometrial Cancer Analysis

The GEPIA database (http://gepia.cancer-pku.cn/index.html) was used to analyze the transcription level of the ATM gene in various human cancers and its association with prognostic endometrial cancer by retrieving expression levels of ATM mRNA (log2-transformed) in human cancers vs normal tissues for statistical comparison. The cBioPortal database (http://www.cbioportal.org/) was used to evaluate the frequency of changes in ATM mutations in endometrial cancer. Moreover, we also analyzed the prognostic value of ATM in endometrial cancer through cBioPortal.

Drug Treatment and Cell Viability Assays

For measurement of sensitivity to Olaparib, cells were seeded in six-well plates at a concentration of 10^4 per well and treated with various concentrations of drug after 24 hr. Medium and drug were replaced every 3 days. After 10 days, Cell Counting Kit-8 (CCK8, C0039 and Beyotime) working solution was added into the well and incubated for 4 hr. For measurement of sensitivity to KU-55933, cells were treated with various concentrations of drug for 72 hr. The incubation time of Olaparib single treatment for sensitivity detection was in accordance with the reports of Aki Miyasaka et al.[20] The incubation time of KU-55933 and the combination treatment with Olaparib was according to the reports of Ian Hickson et al.[33] CCK8 tetrazolium salt is reduced by cellular dehydrogenases to an orange formazan product that is soluble in tissue culture medium. The absorbance at 460nm was measured using a microplate spectrometer. Each condition is performed in three replicated wells, and the data represent three independent experiments.

Western Blot Analysis

Equal amounts of cell lysate were loaded onto SDS-PAGE electrophoresis and transferred onto a PVDF membrane (BSP0161, Pall). The membrane was subsequently exposed to primary antibodies against ATM (92356), Phospho-ATM (5883), PARP (9532), Caspase-3 (9662), cleaved Caspase-3 (9664), and b-actin. Next, the membranes were incubated with corresponding secondary antibodies, and the bands were detected using enhanced chemiluminescence (1810212, Clinx).

Colony Formation Assay

Triplicate wells of 6-well plates were seeded with 600 Ishikawa or Hec-108 cells and maintained in a complete medium with Olaparib/KU-55933 alone or in combination for 72 hr. After that, treated cultures were incubated for additional 2 weeks in a drug-free medium to allow colony formation. Cells were washed with cold PBS and fixed at room temperature for 30 min with a 4% neutral paraformaldehyde solution. Cell colonies were stained with 0.5% (w/v) crystal violet for 30 min, and visible colonies (≥50 cells) were then counted. GraphPad Prism 5.0 was used for statistical analyses of the quantitative data.

Cell Migration Assay

For the cell migration assay, 1×10^5 / cells of Ishikawa or Hec-108 were seeded in each well of 6-well plates. Once the cells reached 100% confluence, sterile pipette tips were used to scratch the wound uniformly. The culture medium was then replaced with a serum-free medium with KU-55933 or/and Olaparib. The cell migration ability was assessed at 0, 24, 48, and 72 hr using a light microscope to capture images. Each experiment was conducted in triplicate.

Flow Cytometry Detection of Apoptosis

To detect cell apoptosis, the FITC Annexin V apoptosis detection kit I (556547, BD Biosciences) was used. Ishikawa and Hec-108 cells were cultured to 30–50% confluence and then treated with Olaparib (50 μM) alone or in combination with KU-55933 (10 μM) for 24 hr. The cells were then harvested, washed with cold PBS and stained with PI and FITC-Annexin V. Apoptosis was detected by CytoFLEX (Beckman Coulter) and analyzed by CytExpert software.

Statistical Analysis

Data were expressed as the means ± standard deviations of three independent experiments. The significance of differences between the two samples was analyzed using Student's *t*-test. $P<0.05$ was considered to denote a statistically significant difference. The synergistic effect was calculated by SynergyFinder (https://synergyfinder.fimm.fi/) using Bliss model.[34]

Results

Endometrial Cancer Cells Exhibit Low Expression and High Mutation Frequency of ATM

To investigate the role of ATM in human cancers, we utilized the GEPIA database to analyze the transcription level of ATM genes in various cancer tissues compared to normal. As shown in Figure 1A, ATM transcription was downregulated in all five gynaecological tumors including breast invasive carcinoma (BRCA), cervical and endocervical cancers (CESC), ovarian serous cystadenocarcinoma (OV), uterine corpus endometrial carcinoma (UCEC) and uterine carcinosarcoma (UCS). In addition to BRCA, the expression level of ATM in the other four gynecological tumors was significantly inhibited (Figure 1B). Then, we further evaluated the alteration of ATM in various human cancers through the cBioPortal database. The results showed that the mutation frequency of ATM in UCEC was much higher than that in other cancers and reached nearly 20% (Figure 1C). Although the expression level of ATM had nothing to do with prognosis (Figure 1D), further analysis showed that the alteration of ATM was significantly associated with disease-specific survival (DSS), disease-free survival (DFS), progress-free survival (PFS) and overall survival (OS) (Figure 1E–H). Specifically, ATM alteration is related to improved survival of patients with uterine endometrioid carcinoma, but not uterine serous carcinoma or uterine papillary sarcoma (Figure S1 and S2). Furthermore, mutation and deep deletion are the main ATM alteration in patients with endometrioid carcinoma, but four ATM alteration types exist in patients with uterine serous carcinoma or uterine papillary sarcoma (Figure S3A). Meanwhile, the mRNA level was not different between patients in these two groups (Figure S3B) and the genome alteration did not change the ATM mRNA level (Figure S3C). These results indicate that ATM may be a potential therapeutic target for endometrial cancer.

Endometrial Cells with Low Expression Levels of ATM are Sensitive to Olaparib

HR-based synergistic lethal effect is the main mechanism of tumor killing by Olaparib. However, as an important regulator of HR, the mutation frequency of ATM in endometrial cancer is highest among all analyzed tumor types in Figure 1C. Therefore, we conducted a CCK8 assay to test the sensitivity of six endometrial cancer cell lines to Olaparib. Although the sensitivity varied, Olaparib was capable of killing all six cell lines (Figure 2A). To investigate whether the sensitivity was relevant to ATM, we detected the expression level of ATM in six cell lines using Western blot. Interestingly, the two most sensitive cell lines, KLE and RL952, expressed the lowest ATM, while the least sensitive cells, Ishikawa and Hec-108, showed the highest ATM (Figure 2B). These findings suggest that ATM negatively regulated the sensitivity of endometrial cancer cells to Olaparib.

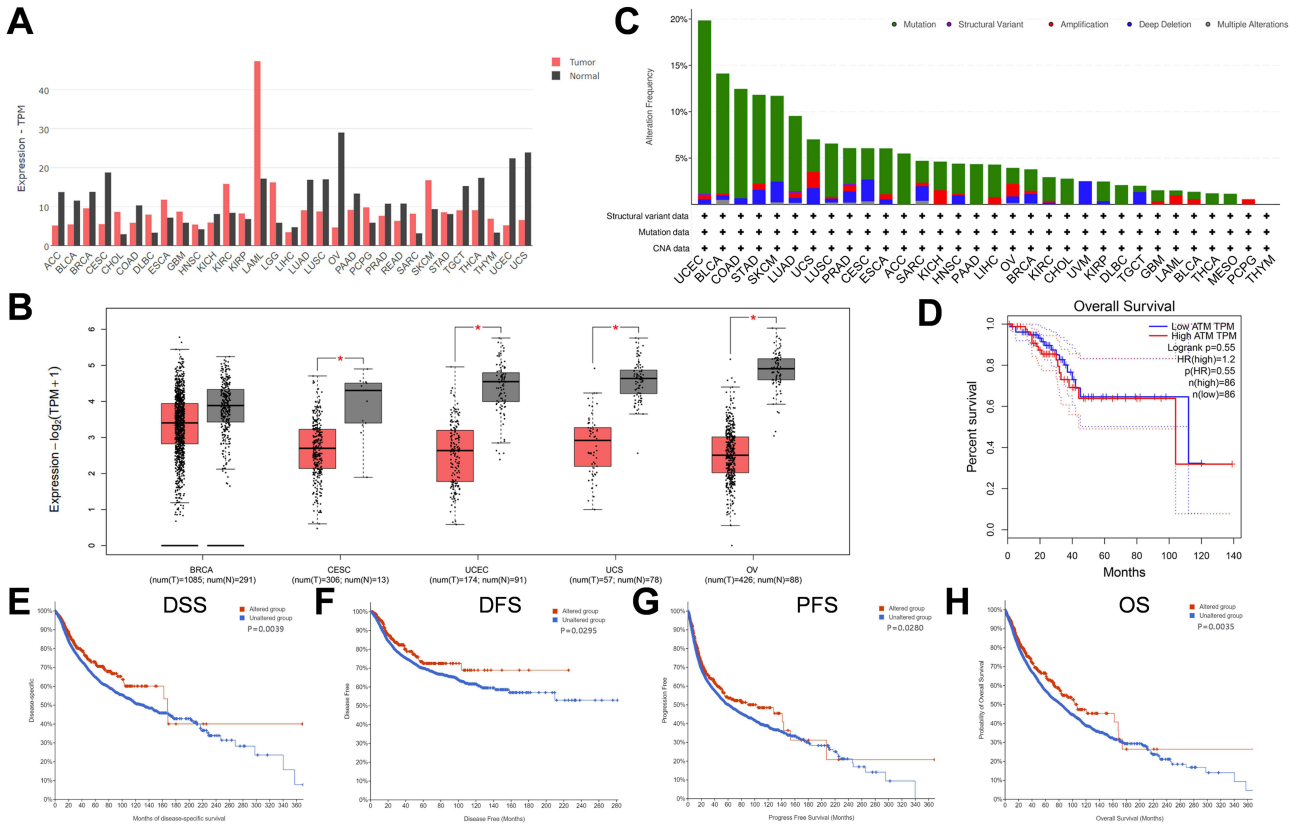

Figure 1 Endometrial cancer cells exhibit low expression and high mutation frequency of ATM (**A** and **B**) The GEPIA database verified that ATM gene expression was significantly downregulated in endometrial cancer tissues (UCEC) (n = 174) compared with normal endometrial tissues (n = 91). *, P < 0.05. (**C**) This graph generated by the cBioPortal database indicated the alteration frequency of ATM in various cancer types. (**D**–**H**) Prognostic significances of ATM gene expression in patients with endometrial cancer were shown based on the GEPIA (**D**) and cBioPortal (**E**–**H**) databases. DSS, disease specific survival; DFS, disease free survival; PFS, progression free survival; OS, overall survival.

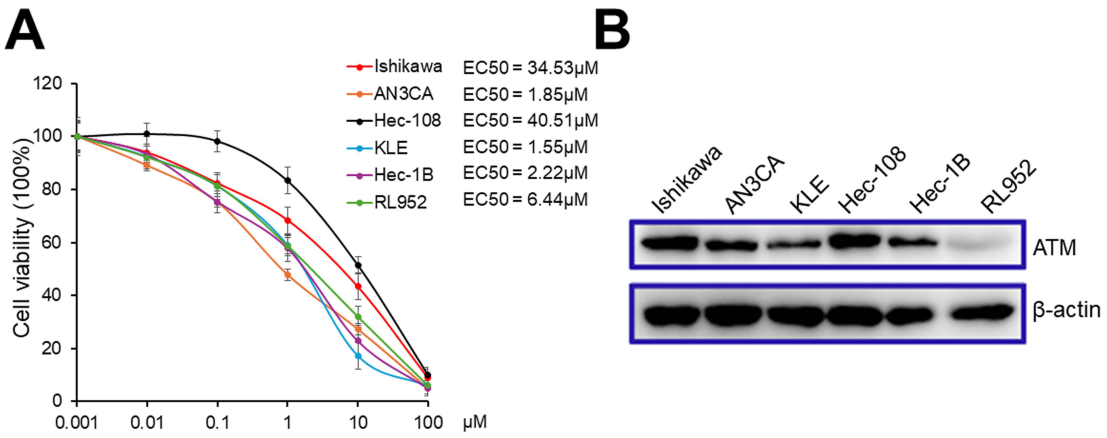

Figure 2 Endometrial cells with low expression levels of ATM are sensitive to Olaparib. (**A**) Endometrial cancer lines were treated with Olaparib in different concentrations from 0.001 μM to 100 μM. After a certain time, cell viability was determined by CCK-8 assay. (**B**) Expression levels of ATM in different endometrial cancer cell lines were detected by Western blot.

KU-55933 Potentiates the Effects of Olaparib

To assess the biological function of ATM in endometrial cancer cells, we utilized KU-55933, a specific inhibitor of ATM, to interfere with its expression. The sensitivity to KU-55933 of these six cell lines was examined by CCK8 assay to determine the dose for combination treatment (Figure S4). As shown in Figure 3A and B, the sensitivity of Ishikawa and

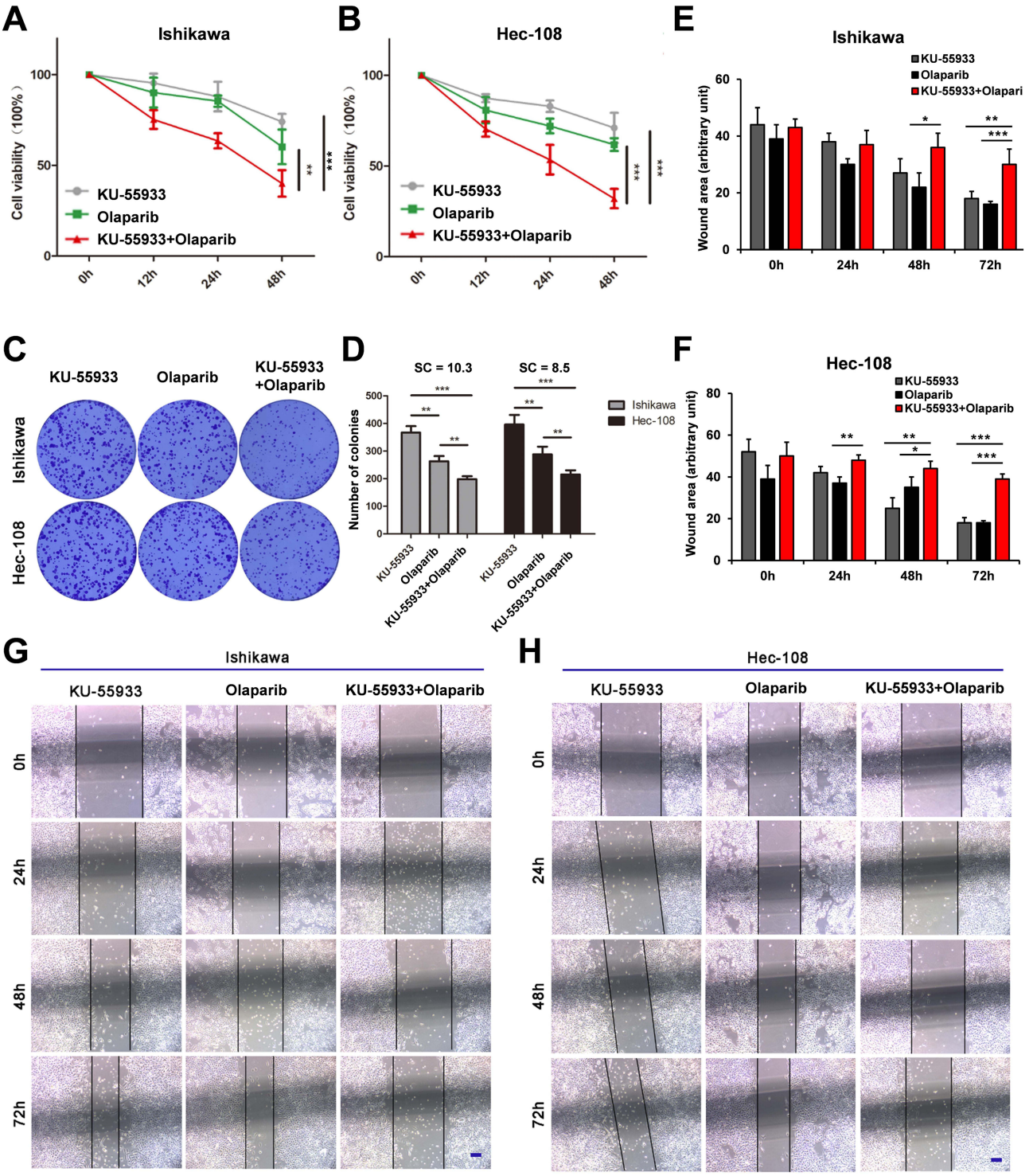

Figure 3 KU-55933 potentiates the effects of Olaparib. (**A** and **B**) Cell viability of Ishikawa (**A**) and Hec-108 (**B**) cells treated with Ku-55933 (10 μM) and Olaparib (50 μM) alone or together was evaluated with CCK8 assay, respectively. (**C** and **D**) Colony formation assay of Ishikawa and Hec-108 cells treated with Ku-55933 and Olaparib alone or together. (**E**–**H**) Representative images of Cell migration assay performed in Ishikawa (**E** and **G**) and Hec-108 (**F** and **H**) cells treated with Ku-55933 and Olaparib alone or together. Bar=100 μm. Data were presented as mean±SD. NS, nonsignificance, *, P < 0.05, **, P < 0.01, ***, P < 0.001. The independent biological experiments were repeated at least three times.

Hec-108 cells to Olaparib was significantly improved by treatment with KU-55933. Furthermore, combination treatment with Olaparib and KU-55933 resulted in a significant reduction in clone size and number compared to treatment with KU-55933 or Olaparib alone in Ishikawa and Hec-108 cells (Figure 3C and D). In addition, KU-55933 also markedly

boosted Olaparib to inhibit the migration capabilities of Ishikawa and Hec-108 cells (Figure 3E–H). These results demonstrate that Olaparib in combination with KU-55933 may be an effective treatment means for endometrial cancer with high-level ATM.

KU-55933 Promotes the Cell Death Induced by Olaparib by Inhibiting the Phosphorylation of ATM

To investigate the potential of combining Olaparib and KU-55933 in endometrial cancer treatment, we detected the apoptosis-inducing ability of these drugs in Ishikawa and Hec-108 cells using PI and Annexin V double staining. Although KU-55933 or Olaparib individual could induce about 20% and 50% cell apoptosis, respectively, the two-drug combination treatment could dramatically elevate the apoptosis rate to over 80% (Figure 4A and B). However, the underlying molecular mechanism is unknown. Since ATM activation depends on its phosphorylation modification, we

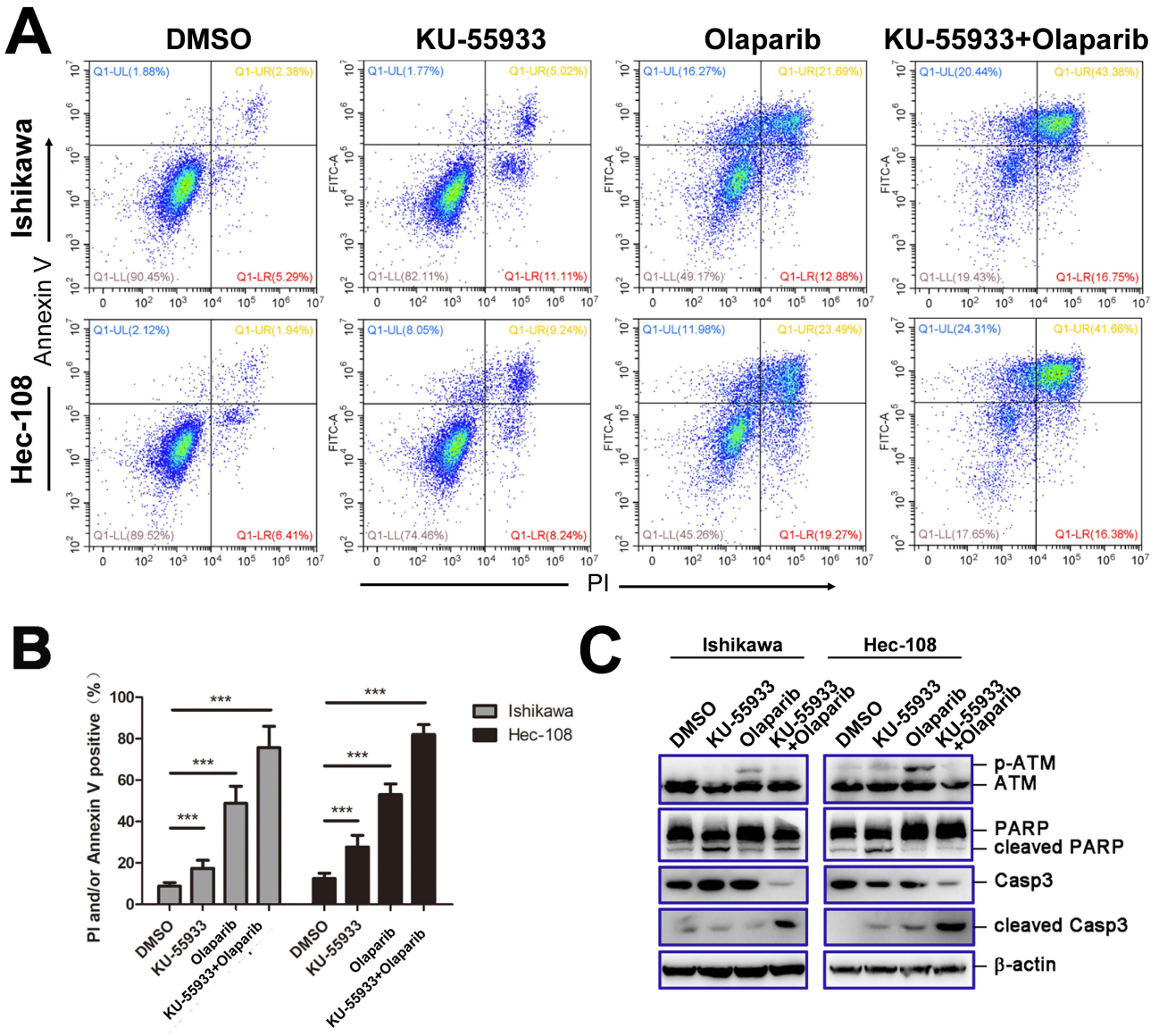

Figure 4 KU-55933 promotes cell death induced by Olaparib by inhibiting the phosphorylation of ATM. (**A** and **B**) Apoptosis induced by Ku-55933 (10 μM) and Olaparib (50 μM) alone or together was detected by flow cytometry (**A**), the percentages of stained cells (PI+/Annexin V-+ PI-/Annexin V++ PI+/Annexin V+) were shown (**B**). Data were presented as mean±SD. ***, $P < 0.001$. The independent biological experiments were repeated at least three times. (**C**) The activation and expression level of ATM, PARP and caspase-3 in Ishikawa and Hec-108 cells treated with Ku-55933 and Olaparib alone or together were detected by Western blot.

detected the phosphorylation level of ATM in cells that were treated with KU-55933 and Olaparib alone or together. The results showed that KU-55933 significantly inhibit the phosphorylation of ATM induced by Olaparib (Figure 4C). Simultaneously, the cleavage of caspase-3 was heavily increased in cells treated with KU-55933 and Olaparib (Figure 4C). During caspase-dependent apoptosis, PARP1 is cleaved by caspases 3 and 7. We observed that KU-55933 treatment could increase the cleavage of PARP1. However, the combination of KU-55933 and Olaparib did not increase the level of cleavage of PARP1. These findings suggest that KU-55933 promoted the apoptosis of Ishikawa and Hec-108 cells induced by Olaparib by inhibiting the phosphorylation of ATM.

Discussion

Endometrial cancer is one of the most common gynecological cancers worldwide. With risk factors including advanced age, obesity and prolonged unopposed estrogen exposure, the incidence of endometrial cancer is rising.[35–37] As most patients are diagnosed with early-stage disease, surgical removal of the uterus and adnexa is generally the treatment of choice.[35] Chemotherapy, locoregional radiotherapy, or a combination of both is usually used as an adjuvant treatment based on the risk of locoregional recurrence or metastasis. However, even getting the optimal surgical and adjuvant treatment, 7~15% of early-stage (I–II) patients have recurring disease.[38,39] Despite significant progress in endometrial cancer treatment in recent years, the challenges in curing this disease have not been fully addressed.

Pharmacological inhibition of compensatory DDR pathway components, such as PARP, in cells with functional genetic defects in an HR-related gene can induce irreversible genomic instability, mitotic catastrophe and cell death.[40] Although BRCA1/2 play key roles in HR repair, the relationship between other tumor suppressor genes and HR repair is still controversial. Previous studies have demonstrated that ATM has the potential to be a therapeutic target for mantle cell lymphoma[13,41,42] and colorectal cancer[43] based on synthetic lethality with PARP inhibitors. In fact, PARP inhibition has been granted as a "breakthrough therapy designation" for prostate cancer carrying ATM mutations.[44] In this study, we found that ATM expression was significantly downregulated in endometrial cancer (Figure 1A and B). What was intriguing was that the downregulated ATM did not affect the prognosis (Figure 1D), and the ATM mRNA level was similar in Type I and Type II EC tumors (Figure S3B). As previous studies have reported that the most common causes of homologous recombination deficiency (HRD) are BRCA mutations,[45] we then analyze the mutation of ATM in various human cancers, and the results showed that the alteration frequency of ATM gene reached nearly 20% in endometrial cancer, much more than that of prostate cancer (about 7%) (Figure 1C). Patients with Type I EC have a higher ATM mutation rate (22.81%), but patients with Type II EC have increased amplification, deep deletion and structural variation rate in ATM coding region, suggesting that Type II ECs carry more complex genomic variations (Figure S3A). More importantly, this mutation is closely related to the good prognosis (Figure 1E–H). This promoted us to study the potential of inhibiting ATM to kill endometrial cancer.

Olaparib is the first FDA-approved PARP inhibitor for patients with advanced ovarian, breast and pancreatic cancer, particularly those with BRCA1/2 deficiencies. However, the sensitivity of endometrial cancer to Olaparib is still unknown. Through CCK8 assays and Western blot analysis of six endometrial cancer cell lines, we found that the sensitivity of the cells to Olaparib had a reverse correlation with the expression level of ATM (Figure 2A and B). This sensitivity was strengthened by the ATM inhibitor, KU-55933, treatment (Figure 3A and B). Further studies indicated that ATM inhibitor and PARP inhibitor together could inhibit colony formation and cell migration more significantly than those induced by KU-55933 or Olaparib alone (Figure 3C-H). PI and Annexin V double staining assay also demonstrated that the two inhibitors combination could immediately kill the endometrial cancer cells effectively (Figure 4A and B).

There are still limits of this study: (1) We identified the downregulation of ATM in ECs, but the factors that contribute to ATM downregulation remain unclear. ATM expressions are regulated by various factors at both transcriptional and post-transcriptional levels.[46] ATM level can even be regulated by virus coded miRNAs.[47] Therefore, the mechanism of ATM dysregulation in ECs may be complex and needs to be further investigated; (2) The functional study of this research was processed using Ishikawa and Hec-108 cells. These two cell lines are both Type I EC cells, and whether the synergistic function of Olaparib and KU-55933 still work in type II ECs remains unclear; (3) Increased cell death would expect increased cleaved PARP. However, this does not occur in the presence of Olaparib. Similar results were reported by Ruoxi Hong et al in MCF7 cells.[48] The binding of Olaparib to PARP1 may inhibit the binding between PARP1 and caspases, and the cleavage of PARP1, which needs to be further confirmed.

In summary, our study has proven that ATM inhibitors could be a potential means to enhance the sensitivity of endometrial cancer to Olaparib. Furthermore, we provide a new idea and alternative for the clinical treatment of endometrial cancer. Of course, there are still some limitations in our research. First is that the underlying molecular mechanism of endometrial cancer cell apoptosis induced by ATM inhibitor and PARP inhibitor combination is still not clear. Given that ATM is involved in multiple functions in DNA repair and checkpoints, the mechanisms responsible for the cytotoxicity associated with ATM inhibitors are much more complex.[41] We have validated that KU-55933 improves the sensitivity of endometrial cancer cells to Olaparib by inhibiting the phosphorylation of ATM, but we are not sure if there are other mechanisms. Second, we just analyze the therapeutic efficacy of ATM inhibitor and PARP inhibitor combination at the cellular level. Many more animal trials will be needed before it can be tested on people.

Conclusions

KU-55933, as the specific inhibitor of ATM, significantly improves the sensitivity of endometrial cancer cells to Olaparib by promoting cell apoptosis induced by Olaparib by inhibiting the phosphorylation of ATM.

Abbreviations

FDA, Federal Drug Agency of United States; PARP, Poly ADP-ribose polymerase; SSB, DNA single-strand break; HR, homologous recombination; ATM, Ataxia-telangiectasia mutated; PIKK, phosphatidylinositol 3-kinase-like-protein-kinases; DSB, Double strands break; TNBC, triple negative breast cancer; BRCA, breast invasive carcinoma; CESC, cervical and endocervical cancers; OV, ovarian serous cystadenocarcinoma; UCEC, uterine corpus endometrial carcinoma; HRD, homologous recombination deficiency; DSS, disease-specific survival; DFS, disease free survival; PFS, progress-free survival; OS, overall survival.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Consent for Publication

All the authors agreed to publish this work.

Acknowledgments

Thanks to all the colleagues in Affiliated Hospital of Weifang Medical University who helped with this work.

Funding

This study is supported by soft science program of Weifang Municipal Science and Technology Bureau: 2020RKX085.

Disclosure

All the authors declared that there is no competing interest.

References

1. Li Y, Yang D, Yang S. Analysis of Prognostic Factors and Treatment Modes of Patients with Recurrent Endometrial Carcinoma. *Evid Based Complement Alternat Med*. 2021;2021:8793187. doi:10.1155/2021/8793187
2. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;136(5):E359–86. doi:10.1002/ijc.29210
3. Wartko P, Sherman ME, Yang HP, Felix AS, Brinton LA, Trabert B. Recent changes in endometrial cancer trends among menopausal-age U.S. women. *Cancer Epidemiol*. 2013;37(4):374–377. doi:10.1016/j.canep.2013.03.008
4. Bansal N, Yendluri V, Wenham RM. The molecular biology of endometrial cancers and the implications for pathogenesis, classification, and targeted therapies. *Cancer Control*. 2009;16(1):8–13. doi:10.1177/107327480901600102
5. Fong P, Meng LR. Effect of mTOR inhibitors in nude mice with endometrial carcinoma and variable PTEN expression status. *Med Sci Monit Basic Res*. 2014;20:146–152. doi:10.12659/MSMBR.892514
6. Husing A, Dossus L, Ferrari P, et al. An epidemiological model for prediction of endometrial cancer risk in Europe. *Eur J Epidemiol*. 2016;31(1):51–60. doi:10.1007/s10654-015-0030-9

7. Creasman WT, Odicino F, Maisonneuve P, et al. Carcinoma of the corpus uteri. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. *Int J Gynaecol Obstet*. 2006;95 Suppl 1:S105–S143. doi:10.1016/s0020-7292(06)60031-3
8. Davidson BA, Moss HA, Arquiette J, Kamal AH. Top Ten Tips Palliative Care Clinicians Should Know When Caring for Patients with Endometrial Cancer. *J Palliat Med*. 2018;21(6):857–861. doi:10.1089/jpm.2018.0053
9. Homesley HD, Filiaci V, Gibbons SK, et al. A randomized Phase III trial in advanced endometrial carcinoma of surgery and volume directed radiation followed by cisplatin and doxorubicin with or without paclitaxel: a Gynecologic Oncology Group study. *Gynecol Oncol*. 2009;112 (3):543–552. doi:10.1016/j.ygyno.2008.11.014
10. Barrington DA, Dilley SE, Smith HJ, Straughn JM. Pembrolizumab in advanced recurrent endometrial cancer: a cost-effectiveness analysis. *Gynecol Oncol*. 2019;153(2):381–384. doi:10.1016/j.ygyno.2019.02.013
11. Lemaitre C, Soutoglou E. Double strand break (DSB) repair in heterochromatin and heterochromatin proteins in DSB repair. *DNA Repair*. 2014;19:163–168. doi:10.1016/j.dnarep.2014.03.015
12. Caldecott KW. DNA single-strand break repair. *Exp Cell Res*. 2014;329(1):2–8. doi:10.1016/j.yexcr.2014.08.027
13. Weston VJ, Oldreive CE, Skowronska A, et al. The PARP inhibitor olaparib induces significant killing of ATM-deficient lymphoid tumor cells in vitro and in vivo. *Blood J Am Soc Hematol*. 2010;116(22):4578–4587.
14. Amé JC, Spenlehauer C, De Murcia G. The PARP superfamily. *Bioessays*. 2004;26(8):882–893.
15. Satoh MS, Lindahl T. Role of poly(ADP-ribose) formation in DNA repair. *Nature*. 1992;356(6367):356–358. doi:10.1038/356356a0
16. Philip CA, Laskov I, Beauchamp MC, et al. Inhibition of PI3K-AKT-mTOR pathway sensitizes endometrial cancer cell lines to PARP inhibitors. *BMC Cancer*. 2017;17(1):638. doi:10.1186/s12885-017-3639-0
17. Farmer H, McCabe N, Lord CJ, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature*. 2005;434 (7035):917–921. doi:10.1038/nature03445
18. Amin O, Beauchamp MC, Nader PA, et al. Suppression of Homologous Recombination by insulin-like growth factor-1 inhibition sensitizes cancer cells to PARP inhibitors. *BMC Cancer*. 2015;15:817. doi:10.1186/s12885-015-1803-y
19. Audeh MW, Carmichael J, Penson RT, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. *Lancet*. 2010;376(9737):245–251. doi:10.1016/s0140-6736(10)60893-8
20. Miyasaka A, Oda K, Ikeda Y, et al. Anti-tumor activity of olaparib, a poly (ADP-ribose) polymerase (PARP) inhibitor, in cultured endometrial carcinoma cells. *BMC Cancer*. 2014;14:179. doi:10.1186/1471-2407-14-179
21. Ren Y, Song Z, Rieser J, et al. USP15 Represses Hepatocellular Carcinoma Progression by Regulation of Pathways of Cell Proliferation and Cell Migration: a System Biology Analysis. *Cancers*. 2023;15(5):1371. doi:10.3390/cancers15051371
22. Perkhofer L, Gout J, Roger E, et al. DNA damage repair as a target in pancreatic cancer: state-of-The-art and future perspectives. *Gut*. 2021;70 (3):606–617.
23. Murai J, Huang SY, Das BB, et al. Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors. *Cancer Res*. 2012;72(21):5588–5599. doi:10.1158/0008-5472.CAN-12-2753
24. Lloyd RL, Wijnhoven PWG, Ramos-Montoya A, et al. Combined PARP and ATR inhibition potentiates genome instability and cell death in ATM-deficient cancer cells. *Oncogene*. 2020;39(25):4869–4883. doi:10.1038/s41388-020-1328-y
25. Bryant HE, Schultz N, Thomas HD, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature*. 2005;434(7035):913–917. doi:10.1038/nature03443
26. Forster MD, Dedes KJ, Sandhu S, et al. Treatment with olaparib in a patient with PTEN-deficient endometrioid endometrial cancer. *Nat Rev Clin Oncol*. 2011;8(5):302–306. doi:10.1038/nrclinonc.2011.42
27. Min A, Im SA, Yoon YK, et al. RAD51C-deficient cancer cells are highly sensitive to the PARP inhibitor olaparib. *Mol Cancer Ther*. 2013;12 (6):865–877. doi:10.1158/1535-7163.Mct-12-0950
28. Kastan MB, Lim DS. The many substrates and functions of ATM. *Nat Rev Mol Cell Biol*. 2000;1(3):179–186. doi:10.1038/35043058
29. Riches LC, Trinidad AG, Hughes G, et al. Pharmacology of the ATM Inhibitor AZD0156: potentiation of Irradiation and Olaparib Responses Preclinically. *Mol Cancer Ther*. 2020;19(1):13–25. doi:10.1158/1535-7163.Mct-18-1394
30. Uziel T, Lerenthal Y, Moyal L, Andegeko Y, Mittelman L, Shiloh Y. Requirement of the MRN complex for ATM activation by DNA damage. *EMBO j*. 2003;22(20):5612–5621. doi:10.1093/emboj/cdg541
31. Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature*. 2003;421 (6922):499–506. doi:10.1038/nature01368
32. Zhang W, Shi J, Li R, et al. Effectiveness of Olaparib Treatment in a Patient with Gallbladder Cancer with an ATM-Inactivating Mutation. *Oncologist*. 2020;25(5):375–379. doi:10.1634/theoncologist.2019-0498
33. Hickson I, Zhao Y, Richardson CJ, et al. Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. *Cancer Res*. 2004;64(24):9152–9159. doi:10.1158/0008-5472.CAN-04-2727
34. Ianevski A, He L, Aittokallio T, Tang J. SynergyFinder: a web application for analyzing drug combination dose-response matrix data. *Bioinformatics*. 2017;33(15):2413–2415. doi:10.1093/bioinformatics/btx162
35. Rütten H, Verhoef C, van Weelden WJ, et al. Recurrent Endometrial Cancer: local and Systemic Treatment Options. *Cancers (Basel)*. 2021;13(24). doi:10.3390/cancers13246275
36. Lortet-Tieulent J, Ferlay J, Bray F, Jemal A. International Patterns and Trends in Endometrial Cancer Incidence, 1978-2013. *J Natl Cancer Inst*. 2018;110(4):354–361. doi:10.1093/jnci/djx214
37. Onstad MA, Schmandt RE, Lu KH. Addressing the Role of Obesity in Endometrial Cancer Risk, Prevention, and Treatment. *J Clin Oncol*. 2016;34 (35):4225–4230. doi:10.1200/jco.2016.69.4638
38. Legge F, Restaino S, Leone L, et al. Clinical outcome of recurrent endometrial cancer: analysis of post-relapse survival by pattern of recurrence and secondary treatment. *Int J Gynecol Cancer*. 2020;30(2):193–200. doi:10.1136/ijgc-2019-000822
39. Del Carmen MG, Boruta DM, Schorge JO. Recurrent endometrial cancer. *Clin Obstet Gynecol*. 2011;54(2):266–277. doi:10.1097/GRF.0b013e318218c6d1
40. Pilié PG, Tang C, Mills GB, Yap TA. State-of-The-art strategies for targeting the DNA damage response in cancer. *Nat Rev Clin Oncol*. 2019;16 (2):81–104.

41. Mak JPY, Ma HT, Poon RYC. Synergism between ATM and PARP1 Inhibition Involves DNA Damage and Abrogating the G(2) DNA Damage Checkpoint. *Mol Cancer Ther*. 2020;19(1):123–134. doi:10.1158/1535-7163.Mct-19-0474
42. Williamson CT, Muzik H, Turhan AG, et al. ATM deficiency sensitizes mantle cell lymphoma cells to poly(ADP-ribose) polymerase-1 inhibitors. *Mol Cancer Ther*. 2010;9(2):347–357. doi:10.1158/1535-7163.Mct-09-0872
43. Wang C, Jette N, Moussienko D, Bebb DG, Lees-Miller SP. ATM-Deficient Colorectal Cancer Cells Are Sensitive to the PARP Inhibitor Olaparib. *Transl Oncol*. 2017;10(2):190–196. doi:10.1016/j.tranon.2017.01.007
44. Choi M, Kipps T, Kurzrock R. ATM Mutations in Cancer: therapeutic Implications. *Mol Cancer Ther*. 2016;15(8):1781–1791. doi:10.1158/1535-7163.Mct-15-0945
45. Ledermann JA, Drew Y, Kristeleit RS. Homologous recombination deficiency and ovarian cancer. *Eur J Cancer*. 2016;60:49–58. doi:10.1016/j.ejca.2016.03.005
46. Guo X, Yang C, Qian X, et al. Estrogen receptor alpha regulates ATM Expression through miRNAs in breast cancer. *Clin Cancer Res*. 2013;19(18):4994–5002. doi:10.1158/1078-0432.CCR-12-3700
47. Lung RW, Hau PM, Yu KH, et al. EBV-encoded miRNAs target ATM-mediated response in nasopharyngeal carcinoma. *J Pathol*. 2018;244(4):394–407. doi:10.1002/path.5018
48. Hong R, Ma F, Zhang W, et al. 53BP1 depletion causes PARP inhibitor resistance in ATM-deficient breast cancer cells. *BMC Cancer*. 2016;16(1):725. doi:10.1186/s12885-016-2754-7