nature portfolio

Peer Review File

Nucleolytic processing of abasic sites underlies PARP inhibitor hypersensitivity in ALC1-deficient BRCA mutant cancer cells

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

In this paper, the authors explore the mechanism behind why ALC1 deficiency render BRCA1 mutant cells hyper-sensible to PARP inhibition. They build up on this previously published observation and using a genetic approach they either discard or confirm different possibilities. In general, the manuscript is well presented, and the authors take advantage of beautiful genetic experiments to support the model in which ALC1 is required to allow access hidden abasic sites. In its absence, they are exposed during replication, when they are subjected to APE1-mediated cleavage, creating replication-associated DSBs that can trap PARP when the enzyme is inhibited. Thus, and in contrast of the previous proposed model in which ALC1 directly remove PARP1 from chromatin, this hyper-sensibility is more depending on the role of ALC1 in BER. Albeit the model makes sense of the genetics observation, a further molecular analysis is missing. Considering that PARP inhibition kills BRCA1 cells more due to PARP1 trapping than PARP1 loss of function, one can expect that, if the model holds true, the lack of ALC1 will trap PARP only when APE1 is present. This will unequivocally show that the effect is, indeed, indirect through BER. Additionally, the biggest caveat of the study is that every observation bar one is done in a single BRCA1 deficient background. Considering the pleiotropic phenotypes of different BRCA1 mutation, together with the huge clonal variability due to the intrinsic genomic instability associated to BRCA1 lack of function, the experiments should be performed in several cell lines. Once these two issues are addressed, the paper will be ready for publication in Nat Comm.

As a minor note, why are the sensibility to KBrO3 and UVC in Figure 2 do not follow the same graphical representation of the rest of the showed sensibilities?

Reviewer #2 (Remarks to the Author):

In this paper, Ramakrisnan et al investigate the underlying mechanism through which the absence of ALC1 enhances the response to PARP inhibitors (PARPi) in cells with BRCA mutations. They identified a function of ALC1 in facilitating the repair of abasic sites within the chromatin structure. They demonstrate that in cells lacking ALC1, abasic sites are susceptible to cleavage by APE1 at replication forks devoid of nucleosomes, leading to DSBs, impeding fork progression, and consequently, increasing sensitivity to PARPi. They conclude that cleaving abasic sites represents a critical endogenous lesion that heightens the response to PARPi in BRCA mutant cells. I have only minor issues with this manuscript.

The authors should demonstrate the universality of the main findings in several BRCA1-deficient cells. Most experiments are done with (while UWB1.289 is used sparsely from Fig. 3).

The present study is hampered by the fact that they don’t know where DNA damage lands in the genome. If their model is true, they should recapitulate this using a modified Cas9 which nicks DNA at specific sites. Nicking should be quite similar to APE1 cleavage of AP site. Using this system, the authors should perform ChIP experiments (on PARP1, ALC1) at localized sites and see their order of recruitment/dependency of each other in the vinicity of nicked DNA (+/- PARP inhibitors). Nucleosome sliding can also be monitored by ChIP. Moreover, PARPi sensitivity in ALC1 deficient cells should be enhanced by Cas9, but not dead Cas9. I would expect that this should lead to DSBs channeled to HR/NHEJ which can be monitored with in vivo cassettes.

The model presented should be applicable to BRCA2-deficient cells. This should be tested.

Fig 2A. It would be important to test for other DNA damaging agents targeting BER, such as H2O2 which has been used to show that PARP-1 is not implicated in BER (Nucleic Acids Res. 2011 Apr; 39(8): 3166–3175). Moreover, the same study reported that BER kinetics is unaffected in PARP1 knockdown cells. This seems to go against the proposed model.

Model. The authors propose the following model. PARP-1
recruits ALC1. When the complex of PARP-1/APE1 recognizes the AP site, this binding, in turn, would stimulate PARP-1 activity and bring ALC1 to the damaged site and open chromatin structure in the vicinity of the AP site. In this model the authors should consider the publication of Liu et al

NAR(2017) Vol.15:45(22): 12834-12847. It is not clear in this reviewer's mind that PARP-1 activity is increased by binding to AP sites. PARP-1 will bind to AP sites but this will increase its activity very slightly. Thus ALC1
would bind to PAR only after the AP site has been cleaved by APE1, which would, in turn, increase the activity of PARP-1 greatly.

PARP inhibitor olaparib on AP-containing DNA increases the motility of PARP-1 (Nucleic Acids Res. 2017 Dec 15; 45(22): 12834–12847). This completely contradicts the proposed model.

Reviewer #3 (Remarks to the Author):

Previously, it has been shown that BRCA mutant cancer cells with ALC1 loss are hypersensitive to PARPi. However, the molecular basis of hypersensitivity in these cells remains unclear. In this manuscript, the authors propose that nucleolytic cleavage of abasic sites is one of the contributing factors to hypersensitivity in BRCA mutant cells with the loss of ALC1. The authors have used a combination of biochemical and cell biology methodologies to delineate the underlying mechanism for hypersensitivity in these cells. They have shown that recognition of abasic sites via APE1 is essential for causing hypersensitivity in ALC1 deficient BRCA mutant cancer cells. Furthermore, they have performed different epistasis analyses to demonstrate the potential role of the base repair factor APE1 in PARPi hypersensitivity. The conclusions made in this manuscript are sound and provide important insights to overcome the issue of PARPi resistance in BRCA mutant cancer cells.

Major:
• The authors propose a model suggesting that since ALC1 facilitates the accessibility of abasic sites to APE1, the loss of ALC1 would lead to the accumulation of abasic sites in chromatin due to inefficient repair. Furthermore, these abasic sites would be accessible to APE1 at the replication fork when nucleosomes are removed. Do the authors have any insights or observations on how bifunctional glycosylase compensates for APE1 function when these abasic sites accumulate?
• The authors mentioned that nucleolytic cleavage of abasic sites at replication forks via APE1 results in double-strand breaks and fork stalling, probably contributing to PARPi hypersensitivity. However, the authors have not actually monitored the homologous recombination (HR) activity in BRCA proficient and deficient cells with APE1 and ALC1 settings.
• In Figure 5e, the authors have shown that the loss of ALC1 causes a reduction in APE1 localization to chromatin. However, the reduction is not very drastic, so could the authors shed some light on other possible ways APE1 still localizes to chromatin?
• For complementation-based experiments, the authors have used cDNA expression for wild-type APE1 and mutant constructs. I wonder why they have not used stable instead of transient expression.

Minor:
• The authors have utilized both wild-type (WT) and endonuclease-dead mutant variants of APE1 to demonstrate that APE1-mediated cleavage of abasic sites promotes hypersensitivity in these cells. However, in the discussion, they have not to emphasize that cleaved abasic sites, not the intact ones, contribute to hypersensitivity.
• Abbreviations used in the manuscript should be spelled out when first mentioned.
• Ensure that scientific claims are appropriately referenced. In a few places in the results sections, the authors have not cited previously made discoveries.
• The authors should include representative images for APE1 localization in Edu-positive and Edu-negative cells, along with quantification of APE1 intensity (Fig 5E).

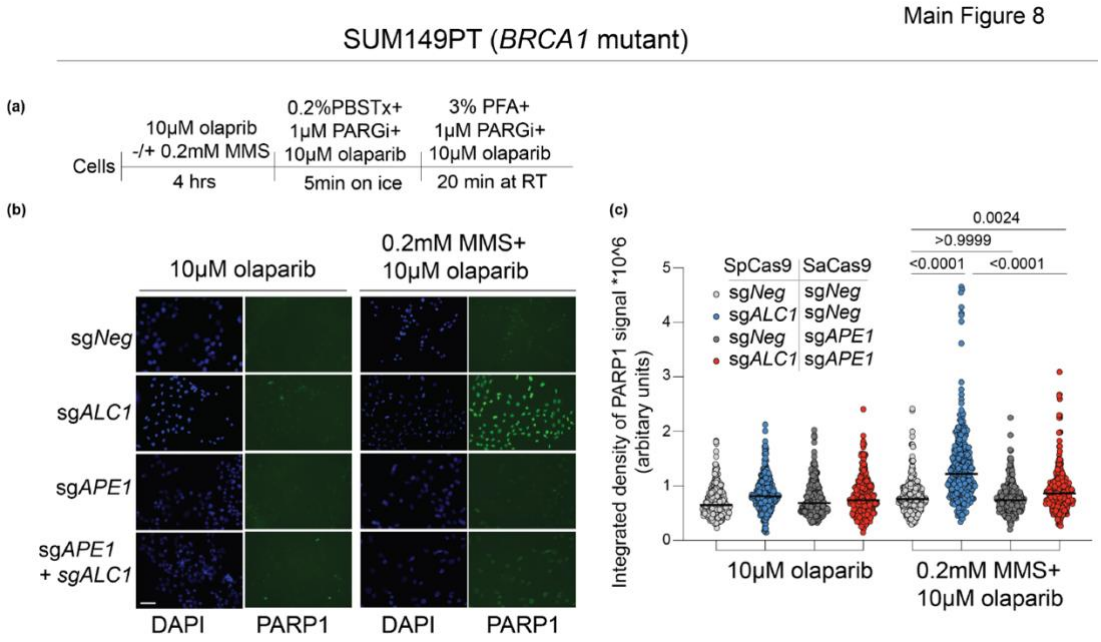
Point-by-point rebuttal is presented below. Reviewer's comments are in BLACK, author's response is in BLUE and figure numbers corresponding to the manuscript are in RED.

Report 1

In this paper, the authors explore the mechanism behind why ALC1 deficiency render BRCA1 mutant cells hyper-sensible to PARP inhibition. They build up on this previously published observation and using a genetic approach they either discard or confirm different possibilities. In general, the manuscript is well presented, and the authors take advantage of beautiful genetic experiments to support the model in which ALC1 is required to allow access hidden abasic sites. In its absence, they are exposed during replication, when they are subjected to APE1-mediated cleavage, creating replication-associated DSBs that can trap PARP when the enzyme is inhibited.

- Thus, and in contrast of the previous proposed model in which ALC1 directly remove PARP1 from chromatin, this hyper-sensibility is more depending on the role of ALC1 in BER. Albeit the model makes sense of the genetics observation, a further molecular analysis is missing. Considering that PARP inhibition kills BRCA1 cells more due to PARP1 trapping than PARP1 loss of function, one can expect that, if the model holds true, the lack of ALC1 will trap PARP only when APE1 is present. This will unequivocally show that the effect is, indeed, indirect through BER.

We thank the reviewer for suggesting this insightful experiment, the results of which helped to validate our proposed model. Using quantitative immunofluorescence assay, we now show that ALC1 loss results in increased PARP1 trapping by the PARP inhibitor Olaparib, and that this PARP1 trapping is lost in APE1/ALC1 knockout cells (Fig. 8a-c). This is consistent with our model that PARPi hypersensitivity in ALC1-deficient cells is due to increased trapping of PARP1 enzymes at APE1 generated DNA lesions (e.g. incised AP-site).

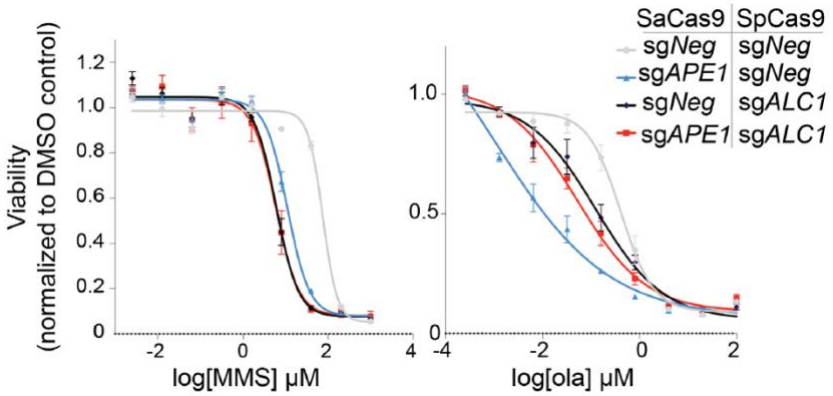


APE1 promotes PARPi mediated-PARP1 trapping and hypersensitivity in ALC1-deficient BRCA mutant cells. ***(8a)*** *Schematic of the assay.* ***(8b)*** *Representative images of the chromatin bound PARP1 in indicated conditions. Scale bar 50 microns.* ***(8c)*** *Quantification of the chromatin bound signal for PARP1 signal. Each dot represents a single cell. Median is indicated. P-value determined by Kruskal-Wallis test was derived from 300 cells sampled over three biologically independent experiments.*

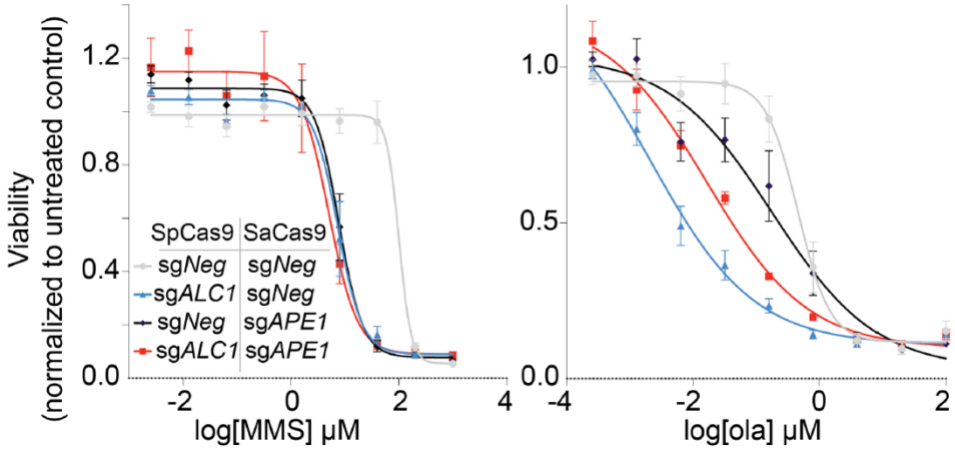
- Additionally, the biggest caveat of the study is that every observation bar one is done in a single BRCA1 deficient background. Considering the pleiotropic phenotypes of different BRCA1 mutation, together with the huge clonal variability due to the intrinsic genomic instability associated to BRCA1 lack of function, the experiments should be performed in several cell lines. Once these two issues are addressed, the paper will be ready for publication in Nat Comm.

We agree with the reviewer that performing the experiments across multiple cell lines would improve the generalizability of our findings. To demonstrate that APE1 loss confers PARP inhibitor resistance in ALC1-deficient BRCA-mutant cancer cells, we have now confirmed our results across multiple different *BRCA1* mutant cell lines and a *BRCA2* deleted cell line from various tissues and genetic backgrounds:

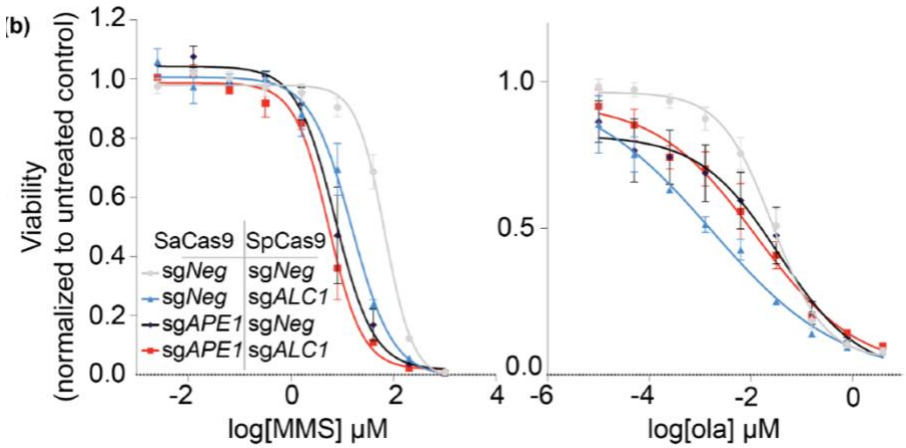
(i) SUM149PT: TNBC cell line which expresses BRCA1 with exon 11 mutation (Fig.3h)



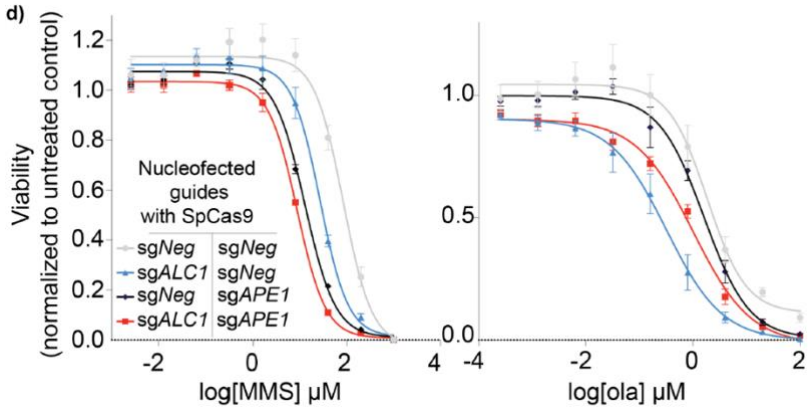
(ii) UWB1.289: HGSOC cell lines which expresses BRCA1 with exon 11 mutation (Supp. Fig.2e).



(iii)MDA-MB-426: TNBC cell line with *BRCA1* 5396+1G > A mutation that results in complete loss of BRCA1 protein expression (Fig.4b).



In addition, we have also confirmed our findings in OVSAHO, a HGSOC cell line with homozygous BRCA2 deletion (Fig.4d).



- As a minor note, why are the sensibility to KBrO3 and UVC in Figure 2 do not follow the same graphical representation of the rest of the showed sensibilities?

All drug assays that were performed in a 96-well plate provided enough data points to be fitted to a dose-response curve. In contrast, all clonogenic assays were performed using 2-3 different doses of genotoxin and hence are plotted as a bar graph. By plotting the data from 96-well viability and 6-well clonogenic assays using different graph styles, we aim to make it easier for readers to understand that two different assays have been used for assessing viability.

Report 2

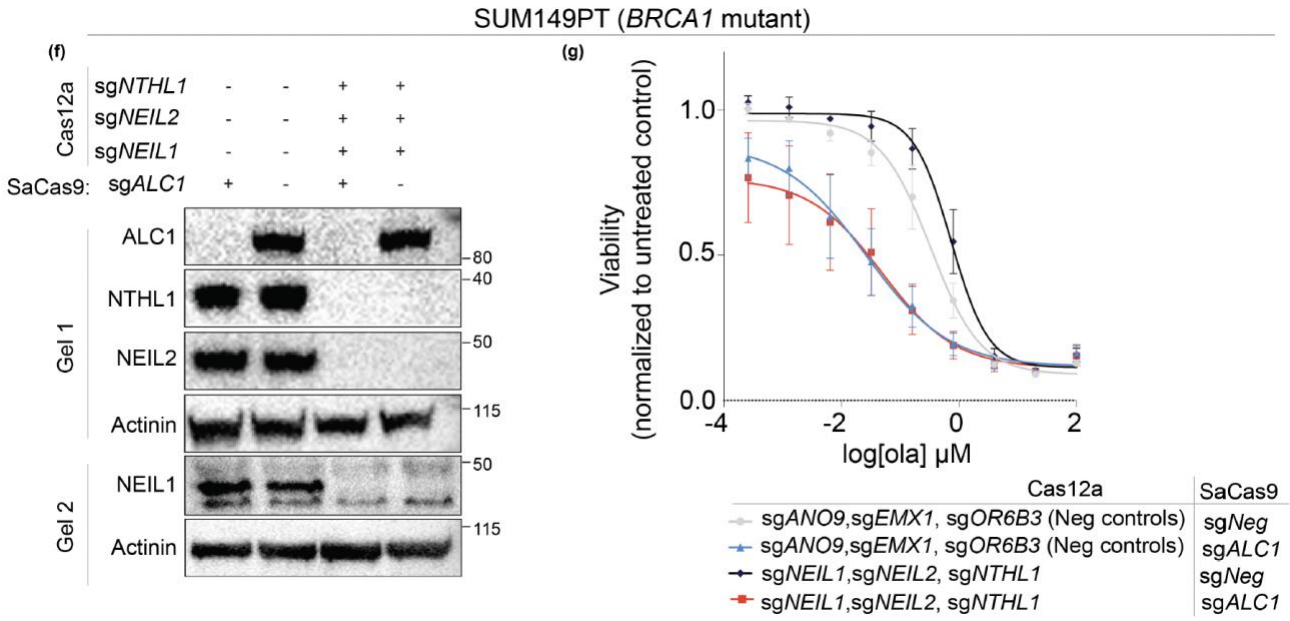
Previously, it has been shown that BRCA mutant cancer cells with ALC1 loss are hypersensitive to PARPi. However, the molecular basis of hypersensitivity in these cells remains unclear. In this manuscript, the authors propose that nucleolytic cleavage of abasic sites is one of the contributing factors to hypersensitivity in BRCA mutant cells with the loss of ALC1. The authors have used a combination of biochemical and cell biology methodologies to delineate the underlying mechanism for hypersensitivity in these cells. They have shown that recognition of abasic sites via APE1 is essential for causing hypersensitivity in ALC1 deficient BRCA mutant cancer cells. Furthermore, they have performed different epistasis analyses to demonstrate the potential role of the base repair factor APE1 in PARPi hypersensitivity. The conclusions made in this manuscript are sound and provide important insights to overcome the issue of PARPi resistance in BRCA mutant cancer cells.

Major:

- The authors propose a model suggesting that since ALC1 facilitates the accessibility of abasic sites to APE1, the loss of ALC1 would lead to the accumulation of abasic sites in chromatin due to inefficient repair. Furthermore, these abasic sites would be accessible to APE1 at the replication fork when nucleosomes are removed. Do the authors have any insights or observations on how bifunctional glycosylase compensates for APE1 function when these abasic sites accumulate?

This was an insightful experiment suggested by the reviewer. Amongst the bifunctional glycosylases, OGG1 has been reported to primarily function as a monofunctional glycosylase *in vivo* (PMID: 11139613,30043138). We therefore focused on depletion of NTHL1, NEIL1 and NEIL2 glycosylases, which function on oxidized bases. Given the substrate redundancy of these glycosylases, we used a Cas12a based approach to simultaneously deplete all three enzymes and examined their epistasis with ALC1 for olaparib response. We observed that NTHL1/NEIL1/NEIL2 deficiency did not have an impact on olaparib sensitivity in either ALC1 proficient or deficient settings. This observation suggests that substrates of NTHL1/NEIL1/NEIL2 may not be present in *BRCA* mutant cancer cells at a high enough frequency to contribute to olaparib sensitivity (Fig.S2f-g). Of note, we were not able to deplete APE1 in NTHL1/NEIL1/NEIL2-deficient cells and hence we cannot exclude the possibility that the AP lyase activity of bifunctional glycosylases can contribute to PARPi sensitivity in APE1/ALC1-deficient *BRCA* mutant cells. The lethal genetic interaction between APE1 and

NTHL1/NEIL1/NEIL2 could be due to some level of functional redundancy between two or more of these abasic site processing enzymes.

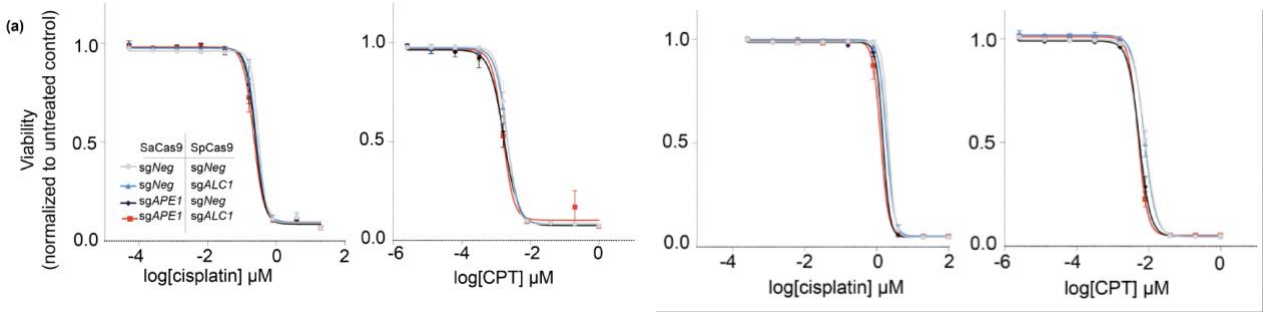


Epistasis between ALC1 and bifunctional glycosylases in response to olaparib in BRCA1 mutant SUM149PT cells. ***(Supp. Fig. 2f)*** *Immunoblot showing depletion of NTHL1, NEIL2, NEIL1, ALC1 in SUM149PT.* ***(Supp. Fig. 2g)*** *Sensitivities of the indicated SUM149PT cell lines to olaparib. Data are presented as mean ± s.e.m from three biologically independent experiments.*

- The authors mentioned that nucleolytic cleavage of abasic sites at replication forks via APE1 results in double-strand breaks and fork stalling, probably contributing to PARPi hypersensitivity. However, the authors have not actually monitored the homologous recombination (HR) activity in BRCA proficient and deficient cells with APE1 and ALC1 settings.

We thank the reviewer for suggesting this important control experiment. To address whether loss of APE1 impacts homologous recombination activity in ALC1-deficient *BRCA*-proficient and deficient cells we performed two complementary experiments:

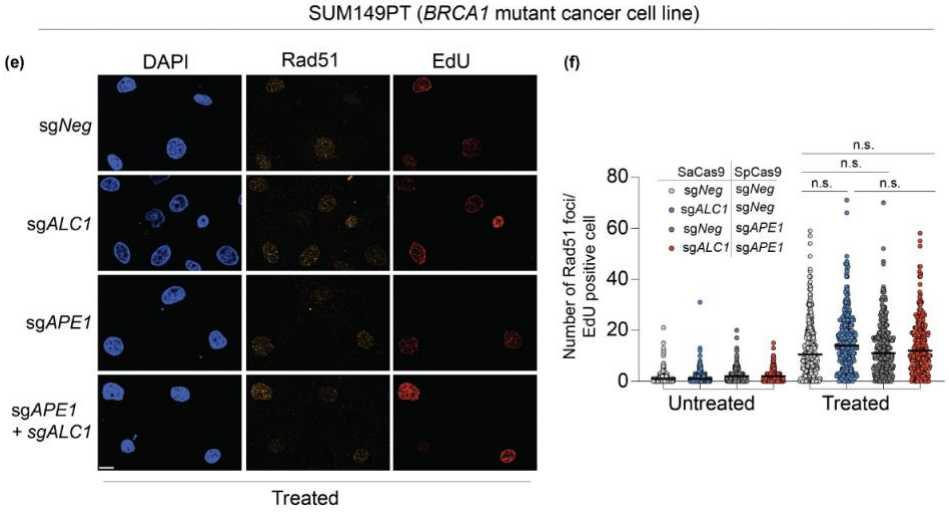
(i) Sensitivity to platinum and camptothecin: Both platinum and camptothecin induce double-strand breaks that rely on HR to resolve the damage. As a read-out of HR, we assessed the sensitivity of ALC1, APE1- and ALC1/APE1-deficient cells to platinum and camptothecin. This experiment was performed in *BRCA1* mutant SUM149PT cells (Supp. Fig.3a) and *BRCA1* proficient DLD1 cells (Supp. Fig.3b, c). Loss of these base damage repair proteins did not have any impact on platinum and camptothecin sensitivity. This highlights that ALC1-, APE1- or ALC1-/APE1- do not impact HR repair.



SUM149PT (*BRCA1* mutant cells) DLD1 WT (*BRCA1* WT cells)

Loss of ALC1 or APE1 do not impact Platinum or Camptothecin sensitivity in either BRCA1 mutant or WT cells.

(ii) Rad51 foci: As another readout for HR, we quantified chromatin associated Rad51in BRCA-mutant SUM149PT cells. These cells express a hypomorphic BRCA1-del11q protein, which partially retains the ability to load Rad51. Therefore, Rad51 foci can be visualized in these *BRCA* mutant cells when treated with high levels of DNA damage. We observe that loss of ALC1 and APE1 alone or in combination did not impact the ability of these cells to form cisplatin induced-Rad51 foci (Fig.4e, f). Together these data suggest that ALC1 or APE1 do not contribute to HR.



Loss of ALC1 or APE1 do not impact Rad51 foci formation. ***(4e)*** *Representative immunofluorescence image of Rad51 foci in indicated SUM149PT cells.* ***(4f)*** *Quantification of the chromatin bound for Rad51 foci/EdU positive cells. Each data represents a signal cell. Median is indicated. P-value determined by Kruskal-Wallis test was derived from 300 cells sampled over three biologically independent experiments.*

• In Figure 5e, the authors have shown that the loss of ALC1 causes a reduction in APE1 localization to chromatin. However, the reduction is not very drastic, so could the authors shed some light on other possible ways APE1 still localizes to chromatin?

The IF assay monitors any APE1 molecule bound to DNA. Based on our current studies and previous *in vitro* experiments (PMID: 36104361), APE1 needs ALC1 to access abasic sites in the nucleosome that are occluded by the histone octamer. In contrast, APE1 should be able to access any abasic sites in the nucleosome that are solvent-exposed or in nucleosome-free DNA without the aid of ALC1 remodeling. This could perhaps account for a modest reduction in APE1 chromatin localization upon ALC1 loss.

• For complementation-based experiments, the authors have used cDNA expression for wild-type APE1 and mutant constructs. I wonder why they have not used stable instead of transient expression.

We indeed used lentivector to stably express the cDNA for the WT and mutant protein. We also ensured that the expression level of the APE1 constructs matched the endogenous protein level. We have now elaborated the figure labeling to make it clear to the readers (Fig.5b).

Minor:

• The authors have utilized both wild-type (WT) and endonuclease-dead mutant variants of APE1 to demonstrate that APE1-mediated cleavage of abasic sites promotes hypersensitivity in these cells. However, in the discussion, they have not to emphasize that cleaved abasic sites, not the intact ones, contribute to hypersensitivity.

We have now emphasized this new finding from our study in the discussion.

• Abbreviations used in the manuscript should be spelled out when first mentioned.

We have corrected this.

• Ensure that scientific claims are appropriately referenced. In a few places in the results sections, the authors have not cited previously made discoveries.

We have included the relevant citations in the revised manuscript.

• The authors should include representative images for APE1 localization in Edu-positive and Edu-negative cells, along with quantification of APE1 intensity (Fig 5E).
Included as Fig.6d.

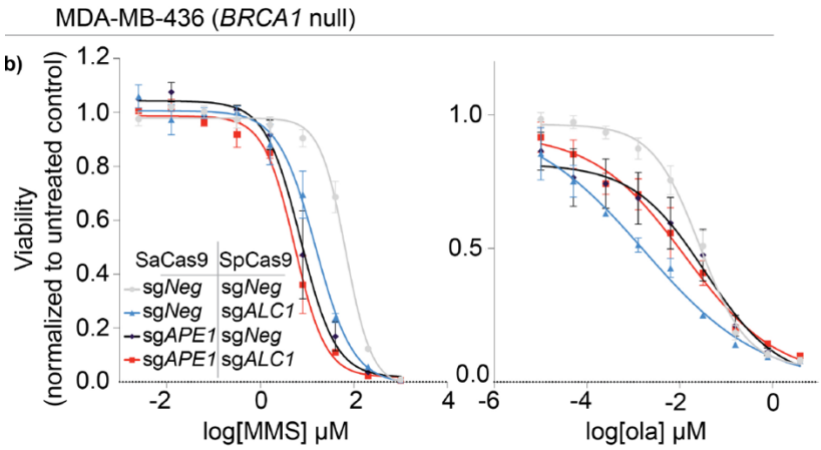
Report 3

In this paper, Ramakrishnan et al investigate the underlying mechanism through which the absence of ALC1 enhances the response to PARP inhibitors (PARPi) in cells with BRCA mutations. They identified a function of ALC1 in facilitating the repair of abasic sites within the chromatin structure. They demonstrate that in cells lacking ALC1, abasic sites are susceptible to cleavage by APE1 at replication forks devoid of nucleosomes, leading to DSBs, impeding fork progression, and consequently, increasing sensitivity to PARPi. They conclude that cleaving abasic sites represents a critical endogenous lesion that heightens the response to PARPi in BRCA mutant cells. I have only minor issues with this manuscript.

We thank the reviewer for the constructive feedback and are glad to learn that the reviewer has "**only minor issues with this manuscript**."

The authors should demonstrate the universality of the main findings in several BRCA1-deficient cells. Most experiments are done with (while UWB1.289 is used sparsely from Fig.3).

Beyond using SUM149PT and UWB1.289 we have now performed experiments in the *BRCA1* mutant cell line MDA-MB-436 (Fig.4a, b). SUM149PT and UWB1.289 cells express hypomorphic BRCA1 with an exon 11 mutation. In contrast, MDA-MB-436 has a *BRCA1* 5396+1G > A mutation that results in complete loss of BRCA1 protein expression. Epistasis analysis between ALC1 and APE1 for olaparib and MMS were consistent across all three cell lines, highlighting our key findings hold across multiple different *BRCA1* mutant cell lines from various tissues and genetic backgrounds.

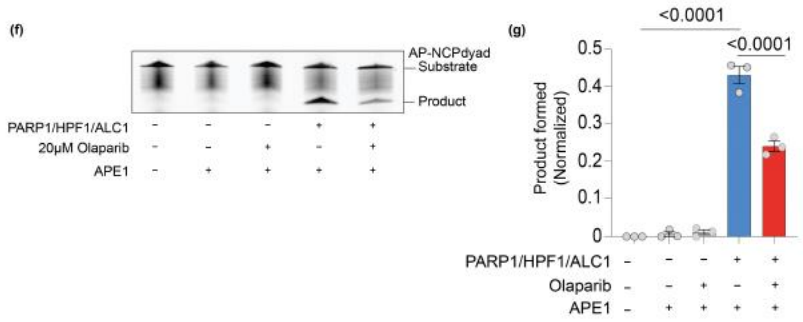


Epistasis analysis between ALC1 and APE1 for MMS and olaparib response in BRCA1-null MDA-MB-436 (Fig.4a, b).

- The present study is hampered by the fact that they don't know where DNA damage lands in the genome. If their model is true, they should recapitulate this using a modified Cas9 which nicks DNA at specific sites. Nicking should be quite similar to APE1 cleavage of AP site. Using this system, the authors should perform ChIP experiments (on PARP1, ALC1) at localized sites and see their order of recruitment/dependency of each other in the vinicity of nicked DNA (+/- PARP inhibitors). Nucleosome sliding can also be monitored by ChIP. Moreover, PARPi sensitivity in ALC1 deficient cells should be enhanced by Cas9, but not dead Cas9. I would expect that this should lead to DSBs channeled to HR/NHEJ which can be monitored with in vivo cassettes.

We would like to clarify that our model predicts that ALC1 acts at abasic sites and NOT nicks. Hence a nicking Cas9-based assay will not be a suitable experimental system for the study. Also, there is substantial evidence in the literature that demonstrates that nucleosomes are a barrier to efficient Cas9 cleavage (PMID: 26987018, PMID: 30201707, PMID: 30413470, PMID: 30189348) posing technical limitations to the experiment requested

by the reviewer. To map the location repaired by PARP1/ALC1, one would need to engineer a cell line with a site-specific abasic site buried in the nucleosome. While potentially interesting, the design of this new system and performing ChIP studies warrants a new study of its own. To circumvent the complexity of the cell-based system, we have used an *in-vitro* system in the original manuscript that provides direct evidence for the ability of ALC1 to promote cleavage of nucleosome-buried abasic site in a PAR-dependent manner (Fig.6f, g).

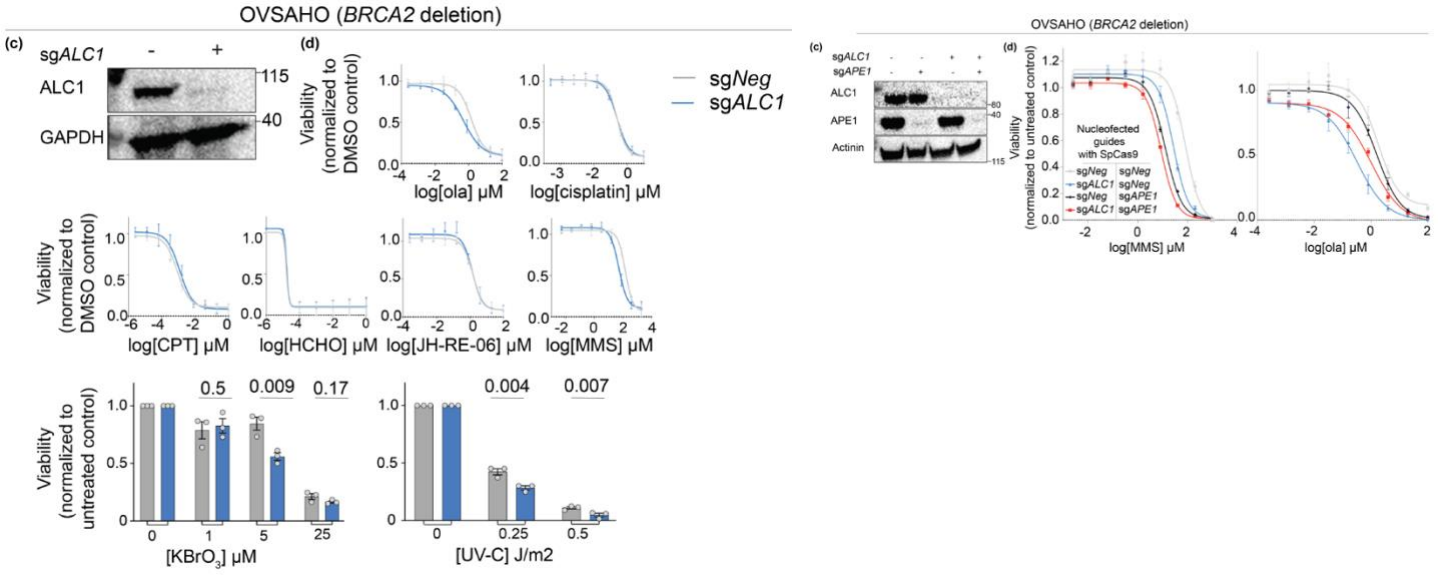


ALC1 promotes the repair of abasic sites by APE1 at the chromatin. ***(6f)*** *Representative gel for APE1 single turnover kinetic experiments with AP-NCP dyad substrate and product bands detected using the 6-FAM label.* ***(6g)*** *Quantification of the AP-NCP-6 product formation assays for the indicated reactions. The data shown are the mean ± s.e.m from the three independent experiments. P values derived by one-way ANOVA.*

We would also like to refer the reviewer to our previous cell line-based study (PMID: 33462394) where we showed that both PAR-binding and histone-interaction activity of ALC1 is critical for PARP inhibitor response. These results highlight that ALC1 is recruited in a PARP1/2 dependent manner and its nucleosome interaction ability contributes to PARPi response.

The model presented should be applicable to BRCA2-deficient cells. This should be tested.

This is an important point that enhances the impact of our study. We have now confirmed both the drug sensitivity of ALC1-deficient cells (Fig.2c, d) and epistasis interaction between ALC1 and APE1 (Fig.4c, d) in OVSAHO, which is a BRCA2-deleted high-grade serous ovarian cancer cell line.



ALC1 genotoxic and genetic interactions are consistent in BRCA1- and BRCA2-deficient cells. Left ***(2c)*** *Immunoblot showing depletion of ALC1 in BRCA2 deleted OVSAHO cells.* ***(2d)*** *Sensitivities of the indicated OVSAHO cell lines to various genotoxins. Data are presented as mean ± s.e.m from three biologically independent experiments. P values derived by unpaired two-tailed t-test. (Right)* ***(4c)*** *Immunoblot showing depletion of ALC1 and APE1 in OVSAHO cells.* ***(4d)*** *Sensitivities of the indicated OVSAHO cell lines to MMS and olaparib. Data are presented as mean ± s.e.m from three biologically independent experiments.*

- Fig 2A. It would be important to test for other DNA damaging agents targeting BER, such as H2O2 which has been used to show that PARP-1 is not implicated in BER (Nucleic Acids Res. 2011 Apr; 39(8): 3166–3175). Moreover, the same study reported that BER kinetics is unaffected in PARP1 knockdown cells. This seems to go against the proposed model.

We would like to emphasize important distinctions in the nature of damage and the repair pathway elicited by alkylating agents (e.g. MMS), versus strong ROS-producing oxidizing agents (e.g. H_2O_2). MMS primarily results in base damage and is repaired by the classical BER pathway. In contrast, H_2O_2 predominantly oxidizes the sugar, resulting in direct disintegration of the oxidized sugar and channeling repair to single-strand break repair, mitigating the need for BER (PMID: 35643889). Hence, it is likely that H_2O_2 induced damage is primarily dependent on SSBR rather than BER. While we could not find studies directly comparing the ROS levels induced by H_2O_2 and $KBrO_3$, $KBrO_3$ has been shown to result in generation of damaged base 8-oxoG, necessitating reliance on BER (PMID: 7859366).

To further clarify the reviewer's point of utilizing **other agents targeting BER**, we would like to draw attention to a recent publication (PMID: 33833118) which showed that ALC1 and PARP1 deficiency renders sensitivity to hmdC, which results in misincorporation of hmdUTP and hence necessitates BER repair initiated by SMUG1.

- Model. The authors propose the following model. PARP-1 recruits ALC1. When the complex of PARP-1/APE1 recognizes the AP site, this binding, in turn, would stimulate PARP-1 activity and bring ALC1 to the damaged site and open chromatin structure in the vicinity of the AP site. In this model the authors should consider the publication of Liu et al NAR (2017) Vol.15:45(22): 12834-12847. It is not clear in this reviewer's mind that PARP-1 activity is increased by binding to AP sites. PARP-1 will bind to AP sites, but this will increase its activity very slightly. Thus ALC1would bind to PAR only after the AP site has been cleaved by APE1, which would, in turn, increase the activity of PARP-1 greatly.

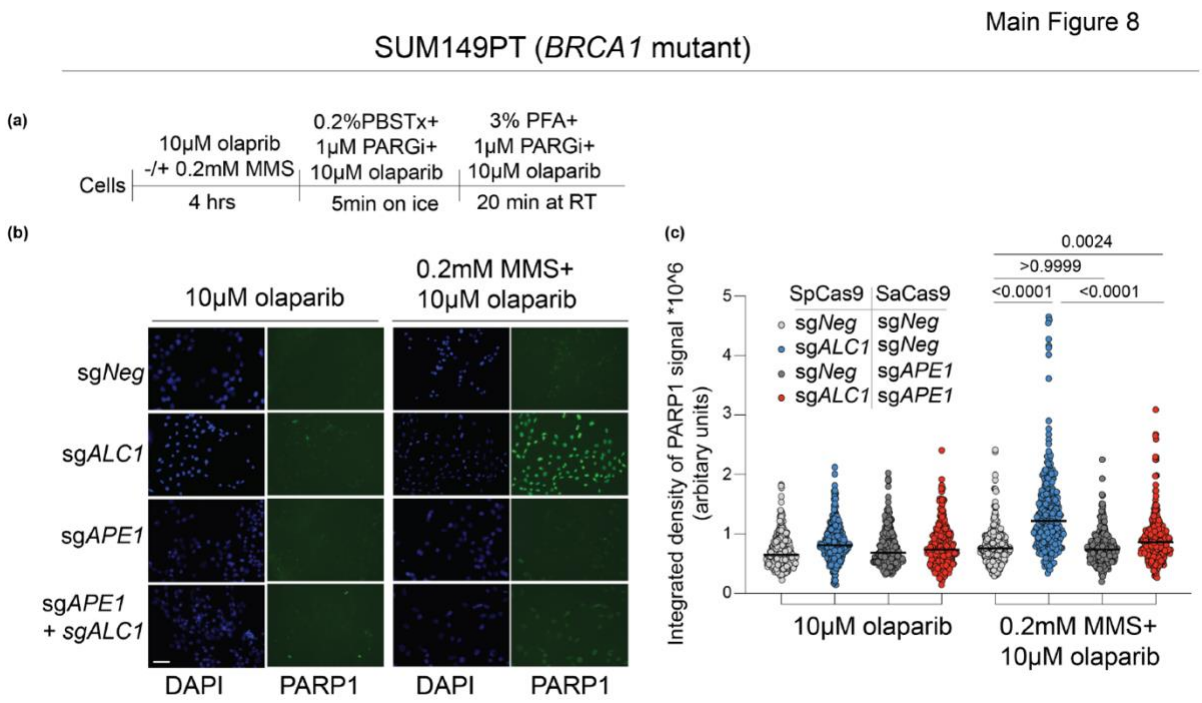
The reviewer brings up a very important concept here. ALC1 is a unique PAR-dependent chromatin remodeler, and the PAR-binding macro domain of ALC1 has a 10 nM binding affinity to PAR-chain (PMID: 29220653). This unusually high affinity of the macrodomain to PAR chains allows this remodeler to bind to very low levels of PARylation. This low level of PARylation would be sufficiently generated by the "very slight increase in activity" for AP-sites as noted by the reviewer. In fact, our previous studies (PMID: 33462394) demonstrated that a high dose of 20 µM olaparib was required to completely abrogate ALC1-localization to damaged chromatin. In summary, even a mild stimulation of PARP activity in the presence of AP-sites should suffice for the recruitment of a high-affinity PAR binding proteins such as ALC1, but perhaps not sufficient for recruitment of other proteins that require increased levels of PARP1-mediated PARylation. We have elaborated on this concept in the discussion of the revised manuscript.

PARP inhibitor olaparib on AP-containing DNA increases the motility of PARP-1 (Nucleic Acids Res. 2017 Dec 15; 45(22): 12834–12847). This completely contradicts the proposed model.

We thank the reviewer for referring this paper which supports our studies. Our model predicts that PARP1 trapping will occur when APE1 has incised the AP-containing DNA and generates a break. Our data shows that sgAPE1 cells (where AP-sites would be intact) are less sensitive to PARPi compared to sgALC1 cells (where AP-sites will be cleaved) (Fig.3h, 4b,4d and Supp. Fig.2e). Hence our data and the resulting model does not support that PARP-1 trapping occurs on intact AP-containing DNA, which is consistent with the manuscript referred by the reviewer.

To further support that trapping occurs upon APE1 incision, we performed PARP1 trapping experiments in APE1-proficient and -deficient ALC1-depleted cells. We show that ALC1 loss (when abasic sites will be cleaved) results in increased PARP1 trapping by PARP inhibitor. Concomitant loss of APE1 and hence a lack of AP site cleavage reduced PARP1 trapping by PARPi (Fig. 8a-c). These data establish that PARP1 is predominantly trapped at incised AP sites and not at intact AP sites.

Main Figure 8



APE1 promotes PARPi mediated-PARP1 trapping and hypersensitivity in ALC1-deficient BRCA mutant cells. ***(8a)*** *Schematic of the assay.* ***(8b)*** *Representative images the chromatin bound PARP1 in indicated conditions. Scale bar 50microns.* ***(8c)*** *Quantification of the chromatin bound for PARP1 signal. Each data represents a signal cell. Median is indicated. P-value determined by Kruskal-Wallis test was derived from 300 cells sampled over three biologically independent experiments.*

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have succesfully addressed all teh points I raised in my previous revision. therefore, I am happy to support the publciation of the manuscript in Nat Comm.

Reviewer #2 (Remarks to the Author):

I have looked twice at the revised version of the manuscript. The author's responses to my comments are adequate and I am supportive of publication.

Reviewer #3 (Remarks to the Author):

The authors have addressed all the concerns point by point in the revised manuscript.

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Reviewer #3 (Remarks to the Author):

The authors have addressed all the concerns point by point in the revised manuscript.

Author's response:

We are glad to learn that all three reviewers unanimously support the publication of our manuscript.