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Mre11-Dependent Degradation of Stalled DNA Replication Forks Is Prevented by BRCA2 and PARP1

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Abstract

PARP inhibitors are currently being used in clinical trials to treat BRCA1- or BRCA2-defective tumors, based on the synthetic lethal interaction between PARP1 and BRCA1/2-mediated homologous recombination (HR). However, the molecular mechanisms that drive this synthetic lethality remain unclear. Here, we show increased levels of Mre11, a key component of MRN (Mre11-Rad50-Nbs1) complex that plays a role in the restart of stalled replication forks and enhanced resection at stalled replication forks in BRCA2-deficient cells. BRCA2-deficient cells also showed hypersensitivity to the Mre11 inhibitor mirin. Interestingly, PARP1 activity was required to protect stalled forks from Mre11-dependent degradation. Resistance to PARP inhibition in *BRCA2*-mutant cells led to reduced levels of Mre11 foci and also rescued their sensitivity to mirin. Taken together, our findings not only show that Mre11 activity is required for the survival of *BRCA2* mutant cells but also elucidate roles for both the BRCA2 and PARP1 proteins in protecting stalled replication forks, which offers insight into the molecular mechanisms of the synthetic lethality between BRCA2 and PARP1. *Cancer Res; 72(11); 2814–21. ©2012 AACR.*

Introduction

Inherited mutations in either the *BRCA1* or *BRCA2* genes, involved in homologous recombination (HR), predispose to an increased risk for breast and ovarian cancer. Previously, we and others have shown that cells and tumors mutated in these genes are hypersensitive to inhibitors of PARP (1, 2). Such PARP inhibitors are currently being evaluated in clinical trials for cancers with *BRCA* mutations (3). This hypersensitivity to PARP inhibitors has been explained by a synthetic lethal interaction between BRCA and PARP. Initially, the molecular mechanism to explain this synthetic lethality was suggested to be accumulation of DNA single-strand breaks (SSB) in PARP-inhibited cells that produce substrates for HR at collapsed replication forks. However, more recent reports suggest that this model is incomplete and additional roles for PARP and BRCA proteins are likely required to fully explain this phenomenon (4–9). The PARP1 protein has, in addition to a role in SSB repair, an active role at stalled replication forks, mediating replication restart in conjunction with Mre11 (7, 10). Previously, it has been shown that proteins involved in HR, for example, RAD51, XRCC3, and BLM, are also involved in promoting restart of stalled replication forks (11, 12). Because the BRCA2 protein is also involved in HR, a complementary role between BRCA2 and PARP1 may exist at stalled replication forks.

Mre11 is a key component of MRN (Mre11-Rad50-Nbs1) complex, which has previously been suggested to have a role in the restart of stalled replication forks (7, 13). *In vitro* experiments have shown that Mre11 nuclease activity can process replication structures to form ssDNA gaps behind forks, particularly in the absence of protection from RAD51 (14). These results suggest that Mre11 may have a role at stalled replication forks in HR-defective cells.

In this study, we have investigated the functions of BRCA2 and PARP1, together with Mre11 at hydroxyurea-induced stalled replication forks. Our results highlight the crucial role of Mre11-mediated degradation of synthesized DNA behind stalled replication forks, which may be of importance in PARP inhibitor–induced lethality of BRCA2-defective cells.

Materials and Methods

Cell lines and reagents

V-C8 and V-C8+B2 cell lines were kindly provided by Malgorzata Z. Zdzienicka (N. Copernicus University, Bydgoszcz, Poland; ref. 15). PARP inhibitor–resistant clones, PIR-2B and PIR-1C, were isolated from V-C8 as described previously (6). All these Chinese hamster ovary (CHO) cell lines and a human osteosarcoma cell line, U2OS, were grown in Dulbecco's Modified Eagle's Medium (DMEM) with 10% FBS at 37°C under atmosphere containing 5% CO_2. Olaparib was purchased from Selleck Chemicals. Mre11 nuclease activity inhibitor mirin and DNA-protein kinase catalytic site (DNA-PKcs) inhibitor NU7026 were purchased from Sigma.

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Immunofluorescence

Cells were either left untreated or treated with olaparib (10 μmol/L) for 6 hours before fixation and staining as previously described (7). The primary antibody used was rabbit polyclonal antibody against Mre11 (Cell Signaling Technology). The secondary antibodies were AlexaFluor 555–conjugated goat anti-rabbit IgG (Molecular Probes). DNA was counterstained with 4′,6-diamidino-2-phenylindole (DAPI).

RNA interference

To knockdown human BRCA2, PARP1, and Mre11, we used siRNA duplex oligonucleotides directed against the BRCA2 target sequence (sense): AACAACAAUUACGAACCAAACUU (16); the PARP1 target sequence (sense): GGGCAAGCACAGUGUCAAA (17), and the Mre11 target sequence (sense): GCUAAUGACUCUGAUGAUA (18). Five thousand cells grown in 6-well plates overnight were transfected with 10 nmol/L siRNA by Oligofectamine (Invitrogen), according to manufacturer's instructions for U2OS cells. To measure cellular proliferation, cells transfected in multiple wells were harvested every second day from one well and counted by a hemocytometer as described elsewhere (19, 20). After 48 hours of transfection, cells were subjected to Western blotting or DNA fiber assay as described below.

Western blotting

Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer in the presence of 1× Protease Inhibitor Cocktail (Sigma). An aliquot of 50 μg total protein was run on an SDS-PAGE gel and transferred to Hybond ECL membrane (Amersham Pharmacia). This membrane was immunoblotted with rabbit polyclonal antibody against Mre11 (Cell Signaling), mouse monoclonal antibody against BRCA2 (Merck Chemicals), mouse monoclonal antibody against PARP1 (Santa Cruz Biotechnology), and mouse monoclonal antibody against actin (Sigma) in 5% milk overnight. Immunoreactive proteins were visualized with ECL reagents (Roche), following the manufacturer's instructions.

Toxicity assay

Two hundred cells were plated in duplicate into 6-well plates overnight before the addition of indicated doses of olaparib or mirin for a continuous treatment. Seven to 10 days later, when colonies could be observed, cells were fixed and stained with methylene blue in methanol (4 g/L). Colonies consisting of more than 50 cells were subsequently counted.

DNA fiber assay

Cells were pulse-labeled with 25 μmol/L 5-chloro-2-deoxyuridine (CldU) and 250 μmol/L 5-iodo-2-deoxyuridine (IdU) as indicated; hydroxyurea, olaparib, and mirin were added either between or after these 2 bromodeoxyuridine (BrdUrd) analogues. Labeled cells were harvested and DNA fiber spreads prepared as described earlier (21). For immunodetection of CldU -labeled tracts, acid-treated fiber spreads were incubated with rat anti-BrdU monoclonal antibody (AbD Serotec) that recognizes CldU, but not IdU, for 1 hour at room temperature. Slides were fixed with 4% formaldehyde and incubated with an AlexaFluor 555–conjugated goat anti-rat IgG (Molecular Probes) for 1.5 hours at room temperature. IdU-labeled patches were detected by a mouse anti-BrdU monoclonal antibody (Becton Dickinson) that recognizes IdU, but not CldU, overnight at 4°C, followed by an AlexaFluor 488–conjugated goat anti-mouse $F(ab')_2$ fragment (Molecular Probes) for 1.5 hours at room temperature. Fibers were examined with a BIO-RAD Radiance confocal microscope using a ×60 (1.3NA) lens. The lengths of CldU-labeled (AF 555, red) and IdU-labeled (AF 488, green) patches were measured using the ImageJ software, and micrometer values were converted into kilobytes using the conversion factor 1 μm = 2.59 kb (21). Replication structures were quantified using the Cell Counter Plug-in for ImageJ (Kurt De Vos, University of Sheffield, Sheffield, UK).

Statistical analysis

The paired one-tailed Student *t* test was used for statistical analyses ($P \geq 0.05$, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Results

Mre11 activity is required for survival in BRCA2-defective cells

We have previously reported that PARP activity is hyperactivated in BRCA2 and other HR-defective cells (6). In this current study, we sought to further understand the molecular mechanism explaining this observation. Because it has been established that PARP can mediate recruitment of Mre11 to replication forks (7, 13), we wanted to test whether the number of Mre11 foci is higher in BRCA2-defective cells. In accordance with this hypothesis, we found a higher level of spontaneous Mre11 foci in CHO V-C8 cells defective for BRCA2 (Fig. 1A and B), despite no noticeable change in Mre11 protein expression (Supplementary Fig. S1A). Next, we wanted to determine whether the increased Mre11 foci is a consequence of the upregulated PARP activity in these cells. Therefore, we transiently treated cells with the PARP inhibitor olaparib, which was able to selectively kill BRCA2-deficient cells (1, 2). Interestingly, we could not detect a significant decrease in the number of Mre11 foci in the PARP-inhibited BRCA2-defective cells (Supplementary Fig. S1B), suggesting that Mre11 foci are upregulated independently of PARP activity in these untreated cells. In addition, the induction of Mre11 foci was evident in the presence of a DNA-PKcs inhibitor and thus independent of nonhomologous end joining (NHEJ) pathway (Supplementary Fig. S2).

Localization into nuclear foci often reflects the decreased mobility of the active protein. To test whether localization of the MRN complex to sites of damage is dependent on the nuclease activity, we investigated the levels of Mre11 foci in BRCA2-defective V-C8 cells in the presence of the Mre11 nuclease inhibitor mirin. We observed a decreased number of Mre11 foci in BRCA2-defective cells (Fig. 1B), suggesting that the activity of the protein explains the spontaneous formation of Mre11 foci in these cells.

Because the presence of Mre11 foci in BRCA2-defective cells requires the activity of the enzyme, we reasoned that

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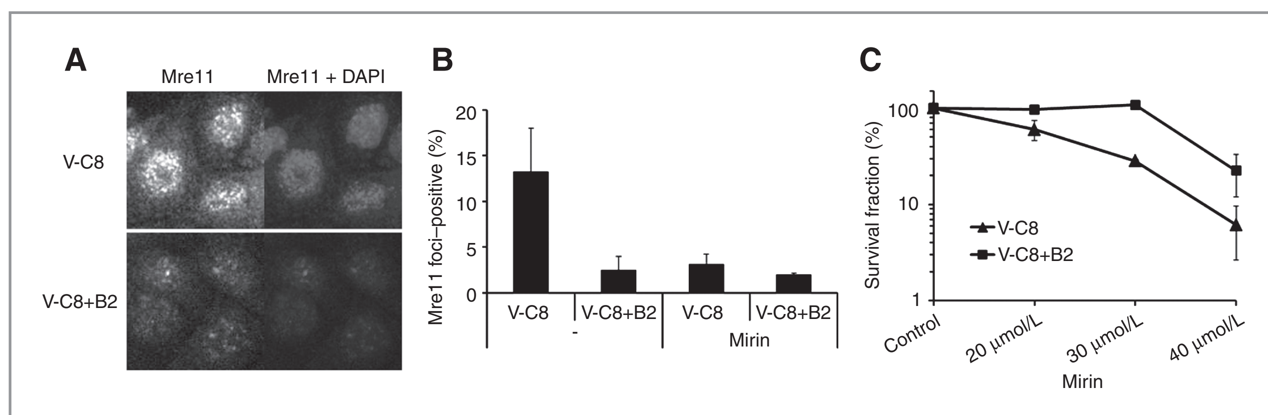

Figure 1. Increased Mre11 activity in BRCA2-deficient cells is required for survival. A, immunofluorescent staining for Mre11 foci formation in BRCA2-deficient V-C8 cells (top) and BRCA2-proficient V-C8+B2 cells (bottom). B, quantification of Mre11 foci in V-C8 and V-C8+B2 cells in the presence or absence of mirin. Error bars, ±SD. C, clonogenic survival in V-C8 and V-C8+B2 cells following continuous treatment with increasing concentrations of the Mre11 inhibitor mirin. Error bars, ±SD. The averages and error bars from at least 3 experiments are depicted.

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potentially toxic Mre11 substrates may accumulate in these cells. We next wanted to investigate whether the nuclease activity is required for survival in BRCA2-defective cells. To test this, we used increasing doses of mirin (22) and conducted clonogenic survival assays. We found that mirin selectively killed BRCA2-defective cells when compared with the same cells expressing the functional *BRCA2* gene (Fig. 1C).

BRCA2 protects from Mre11-dependent degradation of stalled replication forks

Previously, we reported that PARP1, Mre11, RAD51, and the RAD51 paralogue XRCC3 are all involved in restart of transiently stalled replication forks (7, 11). Because increased Mre11 foci occur in BRCA2-defective cells, we wanted to assess whether this is associated with a defect in the restart of stalled replication forks in BRCA2-defective cells. To test this, we used the DNA fiber technique in V-C8 and in BRCA2-complemented V-C8+B2 cell lines. Ongoing replication forks were stalled with hydroxyurea for 2 to 6 hours and then restart efficiency, as well as new origin-firing frequency, were determined by analyzing fiber tracts that had incorporated the first base analogue only (stalled replication forks) or the second analogue only (newly fired origins). We saw no defect in replication restart and new origin firing in BRCA2-defective cells (Supplementary Fig. S3), in line with a recent report (9). These data suggest that RAD51 and XRCC3 have a function at stalled replication forks that is mechanistically distinct from BRCA2.

It has been reported previously that BRCA2 is required for stabilization of stalled replication forks (9, 23). To confirm and extend this observation, we visualized DNA fibers at hydroxyurea-stalled replication forks and examined the length of the labeled replication tracts to assess their stability. We found that the stalled fiber tracts exhibited a decrease in length only in BRCA2-defective cells (Fig. 2A–C), in line with recently reported data (9). Furthermore, tract length decreased with longer periods of hydroxyurea exposure (Fig. 2A and C), suggesting that an active exonuclease process is responsible. The Mre11 protein is known to degrade synthesized DNA strands at replication forks (9, 14), which could potentially explain the increase in Mre11 foci in BRCA2-defective cells. Here, we found that the decrease in fiber length was reverted by the administration of mirin, that is, by inhibiting Mre11 nuclease activity (Fig. 2D). Next, we measured fibers that retain only the first label following degradation of nascent stalled forks in BRCA2-defective cells and found that the number of fibers with complete loss of secondary labeling were decreased in the presence of mirin (Fig. 2E), in line with recently reported data (9). Furthermore, inhibition of Mre11 reduced the average number of replication protein A (RPA) foci (Supplementary Fig. S4), suggesting a reduction in ssDNA formed by resection in these cells. Altogether, these observations confirm a recently reported role for BRCA2 in protection from Mre11-dependent degradation of stalled replication forks (9).

BRCA2 and PARP1 protect stalled replication forks

As the recruitment of Mre11 to stalled replication forks in BRCA2-defective cells is unaffected by PARP inhibition, we reasoned that there may be distinct functions of PARP1 and BRCA2 at stalled replication forks, potentially explaining the strong synthetic lethality. To test this, we treated cells with the PARP inhibitor olaparib and investigated the degradation rate of hydroxyurea-stalled replication forks, as outlined above. Interestingly, we found an increase in the degradation of replication tract after addition of the PARP inhibitor (Fig. 3A–C). Furthermore, we also observed an increase in the degradation of labeled fibers in BRCA2-proficient V-C8+B2 cells after administration of PARP inhibitor (Fig. 3B), suggesting that inactivation of PARP alone in wild-type BRCA2 function cells can induce degradation of stalled replication forks.

Next, we wanted to determine whether the increased degradation rate was concomitant with an increase in hydroxyurea-induced Mre11 foci formation. Consistent with this possibility, we found a slight increase in Mre11 foci, not statistically significant, when inhibiting PARP in the hydroxyurea-treated cells (Fig. 3D). However, we found that the high level of degradation of hydroxyurea-stalled forks in PARP-inhibited BRCA2-defective cells was fully reversed in the presence of mirin (Fig. 3E and F). These data suggest that

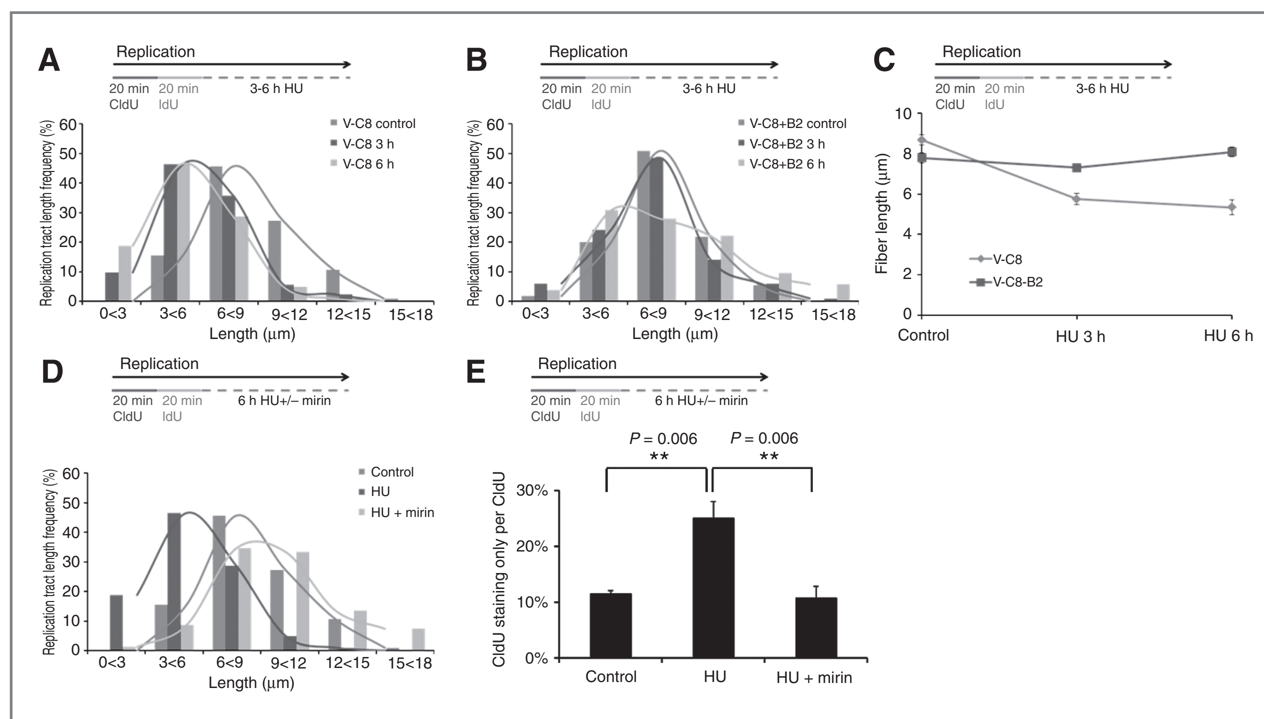

Figure 2. BRCA2 protects from Mre11-mediated degradation of stalled replication forks. A, replication tract length distributions from DNA fibers from BRCA2-deficient V-C8 cells in the presence (replication stalling) or absence (unperturbed replication) of hydroxyurea (HU) for indicated times. B, replication tract length distributions from DNA fibers from BRCA2-proficient V-C8+B2 cells in the presence (replication stalling) or absence (unperturbed replication) of HU for indicated times. C, average remaining fiber length in V-C8 or V-C8+B2 cells treated with HU for indicated times. Error bars, ±SEM. D, replication tract length distributions from DNA fibers from BRCA2-deficient V-C8 cells after 6 hours of HU treatment with or without mirin. E, quantification of fiber tract with complete loss of secondary IdU labeling in V-C8 cells after 6 hours of HU treatment with or without mirin. Error bars, ±SD. The averages and error bars from at least 3 experiments are depicted.

Mre11 activity is also required for degrading hydroxyurea-stalled forks in BRCA2-defective cells after PARP inhibition.

Cross-resistance to Mre11 and PARP inhibitors in BRCA2-reverted cells

It has been reported that BRCA2-defective cells can develop PARP inhibitor resistance by accumulating additional mutations in the *BRCA2* gene that restores the far C-terminal domain in the BRCA2 protein, often lacking the ssDNA domain (24, 25). Previously, we isolated PARP inhibitor–resistant (PIR) V-C8 clones that had reverted through a similar mechanism (6). Although these cells also developed resistance to cisplatin, they remained sensitive to 6-thioguanine, which can induce DNA double-strand breaks (DSB) that are repaired by HR (16). We wanted to determine whether PARP inhibitor resistance in BRCA2-defective cells is associated with resistance to the Mre11 inhibitor mirin. The reverted clones (PIR-2B and PIR-1C), which had lost their sensitivity to PARP inhibitor olaparib (Fig. 4A), were also found to be resistant to the Mre11 inhibitor mirin (Fig. 4B). To test whether the PIR clones were also resistant to Mre11-mediated degradation of stalled replication forks, the DNA fiber assay was used in PIR-2B and PIR-1C cells. No changes to the synthesized DNA were observed in either PIR-2B or PIR-1C clones following hydroxyurea treatment for 6 hours (Supplementary Fig. S5). Furthermore, elevated Mre11 foci in V-C8 cells were also reverted to a background level in the PIR clones (Fig. 4C). Altogether, these data suggest that resistance to Mre11-dependent degradation of stalled replication forks in V-C8 cells may be corelated to the resistance to PARP inhibition.

Mre11 mediates proliferation in PARP1 and BRCA2 siRNA–depleted cells

Our data suggest that PARP1 and BRCA2 protect against Mre11-mediated degradation of stalled replication forks. As these conclusions are drawn from the use of inhibitors to PARP and Mre11, we wanted to test whether similar results are obtained when removing the proteins using siRNA depletion in U2OS cells (Fig. 5A). Indeed, siRNA depletion of BRCA2 alone increased degradation of the stalled fork and additional PARP1 siRNA depletion further augmented the degradation (Fig. 5B), altogether suggesting that inhibition or siRNA depletion give similar results.

Next, we wanted to determine how Mre11 can affect cell proliferation in relation to PARP and BRCA2. We combined depletion of PARP1, BRCA2, and Mre11 and determined cell proliferation by measuring population doublings over 7 days (Fig. 5C). Although siRNA depletion of BRCA2 alone reduced cell proliferation, combined knockdown of BRCA2 and Mre11 reduced proliferation to the same extent as observed in BRCA2 and PARP1 codepleted cells. Interestingly, the

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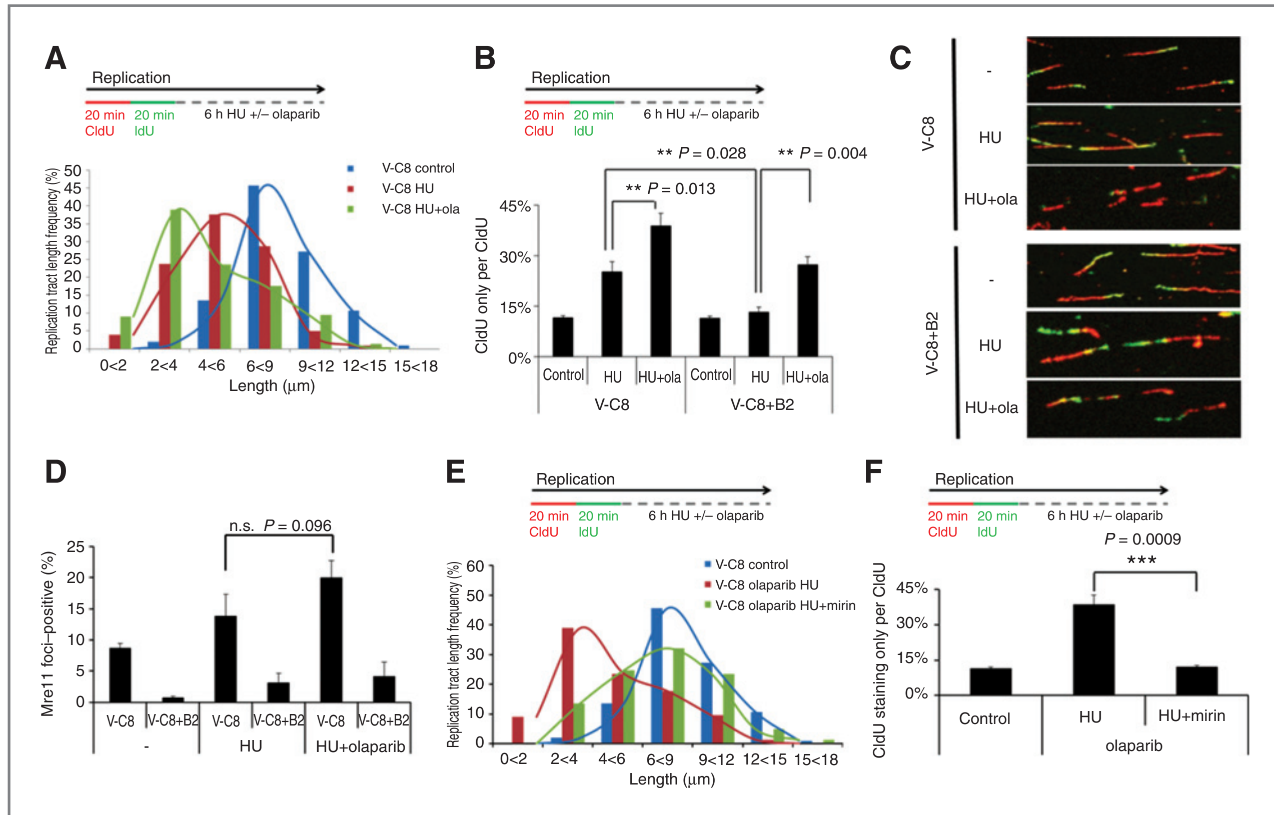

Figure 3. Inhibition of PARP induces instability of stalled replication forks. A, replication tract length distributions from DNA fibers from BRCA2-deficient V-C8 cells after 6-hour hydroxyurea (HU) treatment with or without PARP inhibitor olaparib. B, quantification of fiber tract with complete loss of secondary IdU (green) labeling after 6-hour HU treatment with or without olaparib (ola). Error bars, ±SD. C, representative images of replication tract from V-C8 and V-C8+B2 cells after release into 6-hour HU treatment with or without olaparib. D, quantification of Mre11 foci–positive cells in V-C8 and V-C8+B2 following treatment with HU and olaparib for 6 hours. Error bars, ±SD. E, replication tract length distributions from DNA fibers from BRCA2-deficient V-C8 cells after 6-hour treatment with HU and olaparib in the presence or absence of mirin. F, quantification of fiber tract with complete loss of secondary IdU (green) labeling in V-C8 cells after 6-hour treatment with olaparib, HU, and mirin as indicated. Error bars, ±SD. The averages and error bars from at least 3 experiments are depicted.

growth of cells with triple knockdown of BRCA2, Mre11, and PARP1 was almost completely abolished, showing that Mre11 mediates cell proliferation also in PARP1- and BRCA2-depleted cells.

To test whether the decreased proliferation is also related to the activity of proteins, we carried out similar experiments using Mre11 and PARP inhibitors, that is, mirin and olaparib, in V-C8 and V-C8+B2 cells. As expected, inhibition of Mre11

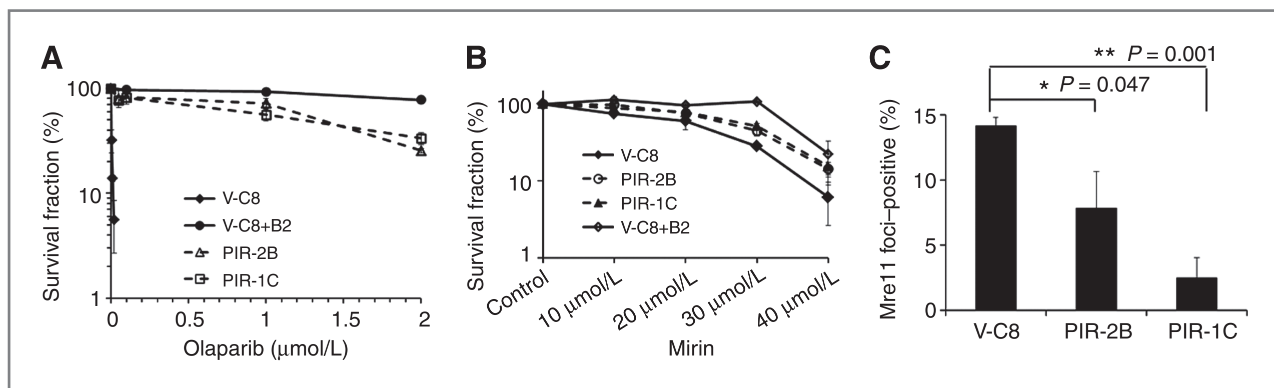

Figure 4. Resistance to PARP inhibitor correlates with stabilization of DNA forks at stalled replication. A, clonogenic survival in V-C8, V-C8+B2, PIR-2B, and PIR-1C cells following continuous treatment with increasing concentrations of olaparib. Error bars, ±SD. B, clonogenic survival in V-C8, V-C8+B2, PIR-2B, and PIR-1C cells following continuous treatment with increasing concentrations of mirin. Error bars, ±SD. C, quantification of Mre11 foci–positive cells in V-C8 and PIR-2B, PIR-1C. Error bars, ±SD. The averages and error bars from at least 3 experiments are depicted.

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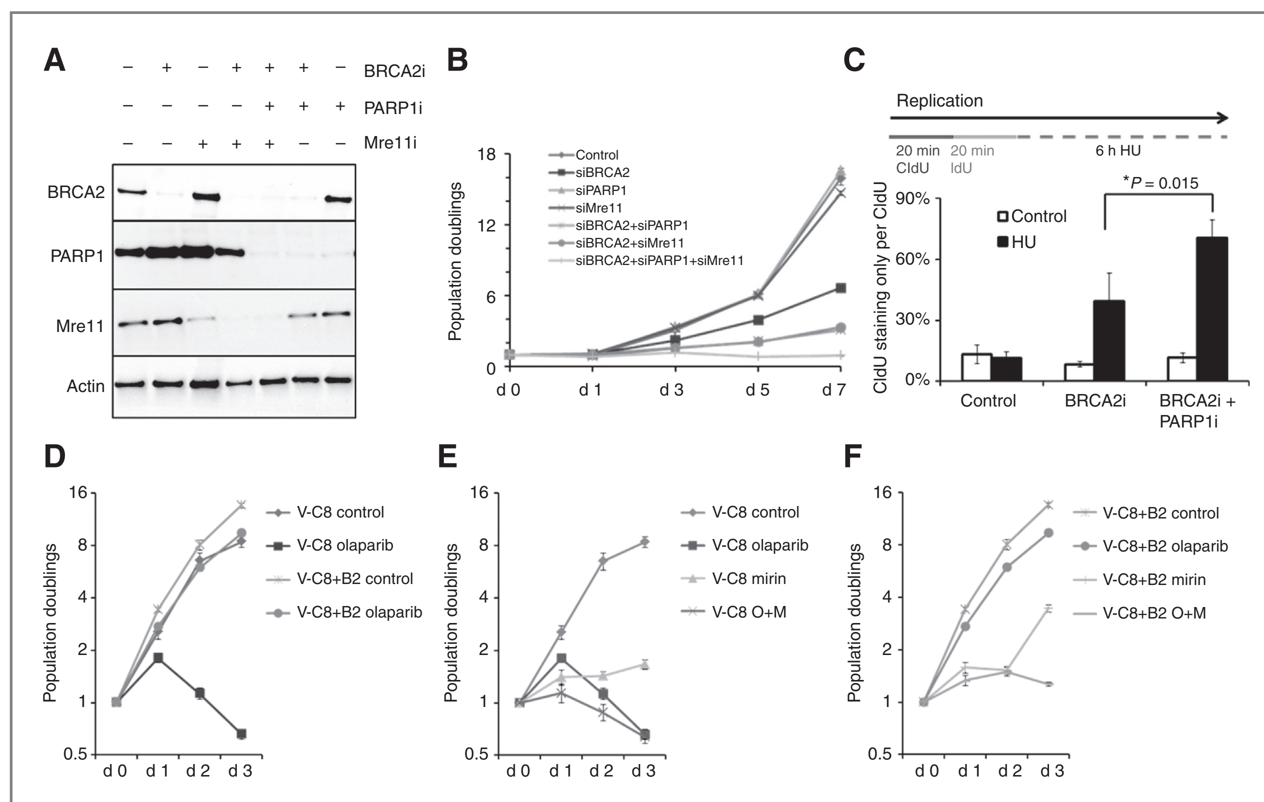

Figure 5. Proliferation arrest in BRCA2, PARP1, and Mre11 triple knockdown cells. A, Western blot analysis of BRCA2, PARP1, Mre11, and actin in U2OS cells transfected with indicated siRNA. B, growth curve of depleted cells in A. C, quantification of fiber tracks with complete loss of secondary IdU labeling after 6-hour treatment with hydroxyurea (HU) in U2OS cells transfected with indicated siRNA. D, growth curve of V-C8 and V-C8+B2 cells following treatment of olaparib (10 μmol/L). E, growth curve of V-C8 cells following treatment with olaparib (10 μmol/L) and mirin (M; 30 μmol/L). F, growth curve of V-C8+B2 cells following treatment with olaparib (10 μmol/L) and mirin (30 μmol/L). Error bars, ±SD. The averages and error bars from at least 3 experiments are depicted.

nuclease activity further arrested proliferation of cells in the presence of PARP inhibitor in both BRCA2-proficient and -deficient cells (Fig. 5D–F).

Discussion

In this study, we report elevated levels of spontaneous Mre11 foci in BRCA2-defective cells, and the likely explanation for this is increased resection at stalled replication forks, presumably occurring from instability in BRCA2-defective cells. Such a heightened degree of resection can be explained by a novel function for BRCA2 in protecting stalled replication forks from being processed by Mre11, a function that has recently been shown to be distinct from the role of BRCA2 in HR (9). Our finding that levels of RPA foci, binding to tracts of ssDNA, decrease after inhibition of Mre11 nuclease activity supports this notion. Such resection seems important to promote survival, as BRCA2-defective cells are hypersensitive to the Mre11 inhibitor mirin, and siRNA depletion of Mre11 substantially reduces viability in BRCA2-depleted cells. The molecular mechanism behind our observation that Mre11 activity is important for BRCA2-defective cells is not entirely clear. In speculation, replication fork intermediates may accumulate in BRCA2-defective cells, which require Mre11 activity to avoid production of toxic intermediates at stalled replication forks.

Previously, we reported that a portion of Mre11 foci require PARP1 to relocate to replication forks stalled with hydroxyurea for 24 hours, which are slowly repaired and associated with DSBs (7, 11). Here, we do not observe PARP activity required to relocate Mre11 to transiently hydroxyurea-stalled forks, which may be explained by that these are easily repaired and not associated with DSBs (7, 11). Instead, we find increased Mre11-dependent degradation of hydroxyurea-stalled replication forks in the presence of a PARP inhibitor. Our interpretation of these data is that PARP relocates early to stalled forks; first, to protect the stalled replication fork and mediate restart (Fig. 6). After severe stalling, associated with replication collapse and DSB formation, PARP has a role in mediating RAD51-dependent recombination repair, as shown earlier (7, 11).

We show that inhibition of PARP1 in cells with either wild-type functional BRCA2 or mutational *BRCA2* further increases degradation of stalled replication forks. This suggests that PARP1 and BRCA2 both function in protecting stalled replication forks. The role of BRCA2 in protecting stalled replication forks is mediated through its ability to stabilize RAD51 filaments (9), a process that does not involve PARP1 (1, 2, 26).

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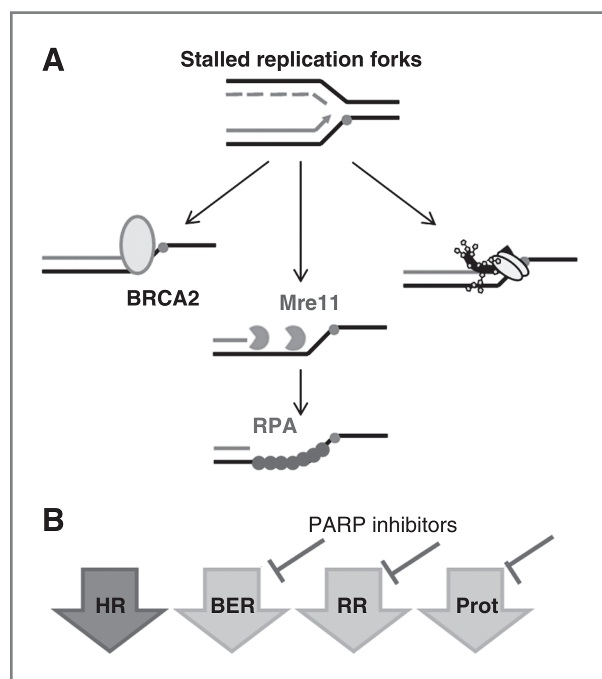

Figure 6. Model for BRCA2- and PARP1-mediated protection of stalled replication forks from Mre11-dependent degradation. A, when replication stalls, various actions may take place to protect replication forks, including: (i) BRCA2 loads RAD51 to protect forks; (ii) PARP1 is binding to and protecting stalled replication forks by formation of PAR polymers. In absence of BRCA2, PARP activity is upregulated (6), and in absence of PARP activity, RAD51 foci are upregulated (26), potentially to compensate for the loss of the other pathway. In absence of either or both pathways, Mre11 is activated to mediate degradation of stalled forks, leading to long tract of ssDNA formation that may promote alternative replication repair pathways. B, the synthetic lethality between PARP inhibitors and BRCA2 is suggested to involve inhibition of base excision repair (BER; ref. 4), replication restart (RR; ref. 7, 10), and fork protection (Prot).

Hence, we suggest the presence of PARP1 and formation of PAR polymers at sites of stalled forks prevents resection as a distinct mechanism (Fig. 6).

There is a synthetic lethal interaction between PARP1 and BRCA2, which is not fully understood at the molecular level. A synthetic lethality may arise if 2 complementary pathways, either of which is required for survival, are inactivated, such as 2 different DNA repair pathways, for example, SSB repair and HR. However, a synthetic lethality may also arise when an essential function is catalyzed by 2 distinct mechanisms. Here, we find that PARP1 and BRCA2 both protect stalled replication forks from degradation, potentially with distinct mechanisms. An increased PARP1 activity is observed in BRCA2-defective S-phase cells (6). Here, we suggest that this increased PARP activity is related to an important role for PARP to protect replication forks in BRCA2-defective cells. Also, RAD51 foci are elevated in PARP-inhibited or -defective cells (26), which may reflect a BRCA2-mediated compensatory pathway in absence of PARP protection of replication forks. We suggest that the synthetic lethality between PARP1 and BRCA2 is mediated also through protecting replication forks, which may be distinct from the role of PARP inhibitors impairing replication restart and base excision repair (Fig. 6).

We find that knockdown of Mre11 further reduces growth in PARP1 and BRCA2 siRNA–depleted cells, suggesting that indeed Mre11-mediated resection at replication forks in BRCA2-defective cells is important for survival. The reason resection is important for survival in absence of replication fork protection remains unclear but may involve additional pathways for fork reactivation (27).

In conclusion, we report that Mre11 is hyperactivated in BRCA2-defective or PARP-inhibited cells, owing to failure to protect stalled replication forks. The Mre11 activity is required to improve survival, which may open new routes to targeting cancer using Mre11 inhibitors and is important for understanding the underlying mechanisms for synthetic lethality between PARP1 and BRCA2.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Ying, T. Helleday
Development of methodology: S. Ying, T. Helleday
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Ying
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Ying, T. Helleday
Writing, review, and/or revision of the manuscript: S. Ying, F.C. Hamdy, T. Helleday
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S. Ying, T. Helleday
Study supervision: F.C. Hamdy, T. Helleday

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