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Mechanism for Synthetic Lethality in BRCA-Deficient Cancers: No Longer Lagging Behind

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Abstract

Two recent studies implicate PARP as sensors of incompletely processed Okazaki fragments, changing our view about how single-strand breaks arise in unperturbed cells. Unligated Okazaki fragments may trigger homologous recombination-mediated repair and underpin genome instability in BRCA1/BRCA2-deficient cancers.

Poly(ADP-ribose) polymerase (PARP) inhibitors are of great clinical interest due to their efficient killing of cells with homologous recombination (HR) defects, e.g., due to mutations in *BRCA1* or *BRCA2* (Bryant et al., 2005; Farmer et al., 2005). This effect is achieved through synthetic lethality, whereby loss of two complementary pathways in a cancer cell leads to cell death. Since healthy cells are spared the drug toxicity, synthetic lethality will be able to target and kill cancer cells more specifically and with fewer side effects than chemotherapy (Figure 1). While the PARP inhibitor olaparib is now in use for the treatment of BRCA-deficient cancers, the exact mechanism behind the efficacy of PARP inhibitors is not fully understood, in part due to lack of knowledge about the role(s) of PARP in unperturbed cells. Two recently published studies provide new insights into the role of PARP at the replication fork (Hanzlikova et al., 2018; Maya-Mendoza et al., 2018).

Mammalian cells contain two major PARP proteins: PARP1 and PARP2. Both enzymes function by post-translationally modifying themselves and target proteins by adding poly(ADP-ribose) molecules (PAR) in a process referred to as PARylation. The roles of PARP have mainly been defined in the context of exogenous stress. They were shown to localize to the sites of single-strand DNA breaks (SSBs), where PARylation recruits XRCC1, a critical SSB repair protein (El-Khamisy et al., 2003). It has been proposed that upon PARP inhibition, SSBs remain unrepaired and are converted into double-strand breaks (DSBs) when encountered by a replication fork (Bryant et al., 2005; Farmer et al., 2005). An extension of this model proposes that PARP is trapped at SSBs by the PARP inhibitor and subsequently poses a barrier to the replication fork (Murai et al., 2012). When replication forks stall or collapse as a result of PARP inhibition, HR is required for replication fork restart. BRCA-deficient cells will therefore be unable to rescue fork stalling, accumulate DNA damage, and eventually die (Bryant et al., 2005; Farmer et al., 2005).

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Mistakes that occur during unperturbed DNA replication are thought to drive genome instability, which is a hallmark of cancer (Tubbs and Nussenzweig, 2017). Hanzlikova et al. (2018) set out to study PARylation in the absence of exogenous DNA damage. The authors performed several elegant experiments in which PARylation was stabilized by inhibiting poly(ADP-ribose) glycohydrolase (PARG), which normally quickly degrades PAR. The authors almost exclusively detected PAR on chromatin in S-phase cells, and the signal localized with sites of DNA replication. The authors went on to show that this S-phase PARylation is not caused by DNA damage randomly encountered by the traveling fork; rather, it is associated with unprocessed Okazaki fragments, which are discontinuous DNA fragments synthesized on the lagging strand during DNA replication. Indeed, increased PARylation was detected in cells deficient in either flap endonuclease I (FEN1), which processes Okazaki fragments so they can be ligated together, or DNA ligase I (LIG1), which ligates Okazaki fragments. The authors also showed that PARylation leads to the recruitment of XRCC1, indicating that PARP attracts the SSB repair pathway to unligated Okazaki fragments when normal processing by FEN1 and LIG1 fails. In cells treated with PARP inhibitor, unligated Okazaki fragments persist, potentially leading to replication conflicts in the subsequent S phase (Figure 1).

Maya-Mendoza et al. (2018) showed that PARP inhibition, but also depletion of FEN1 or LIG1, increases the speed of replication forks. These observations indicate that the increased fork speed in the absence of exogenous DNA damage might be a result of unligated Okazaki fragments behind the fork. How could an increase in fork speed be explained by unligated Okazaki fragments behind the fork? It has previously been reported that PARP inhibition blocks reversal of stalled forks, which is required for efficient fork restart (Ray Chaudhuri et al., 2012). Perhaps fork reversal also occurs when unligated Okazaki fragments are detected behind the fork, allowing them to be processed properly. PARP inhibition would both prevent fork reversal and also block SSB repair from functioning as a backup pathway to resolve unligated Okazaki fragments. This would lead to increased fork spead but persistent SSBs, which would eventually be resolved by the HR machinery in the subsequent S phase. Maya-Mendoza et al. (2018) also report that treatment with a high dose of olaparib activates the DNA damage response, and show that damage occurs mainly at sites of DNA replication. This is indicative of DNA breaks that would normally be repaired via HR, but would remain unrepaired and become toxic in HR-deficient cells.

Although PARylation is one of the most rapid responses to DNA damage, it is also highly transient, making it difficult to decipher its targets. It was previously believed that PARP was activated stochastically as a result of damage from reactive oxygen species. Hanzlikova et al. (2018) and Maya-Mendoza et al. (2018) indicate that PARP mainly acts at the replication fork, promoting repair of unligated Okazaki fragments (Figure 1). How many of the 30–50 million Okazaki fragments synthesized during each cycle require the “backup” SSB repair pathway remains unclear. Nevertheless, these findings indicate that lagging strand DNA synthesis can be targeted in cancer treatment. For example, Hanzlikova et al. (2018) show that *XRCC1*$^{-/-}$, *PARP1*$^{-/-}$, and *PARP1*$^{-/-}$*PARP2*$^{-/-}$ cells are highly sensitive to FEN1 inhibition. This indicates that FEN1 could be targeted in the treatment of tumors with defects in SSB repair. Interestingly, it has been reported that HR-deficient cells are also sensitive to FEN1 inhibition (Ward et al., 2017), consistent with the results from Hanzlikova

et al. (2018) and Maya-Mendoza et al. (2018). Conversely, cancers with defects in Okazaki fragment processing (Forbes et al., 2017) would be predicted to be sensitive to PARP inhibitors. Thus, by filling gaps in our knowledge about endogenous mutagens, the current studies suggest new ways to exploit the rewiring of DNA repair pathways in cancer.

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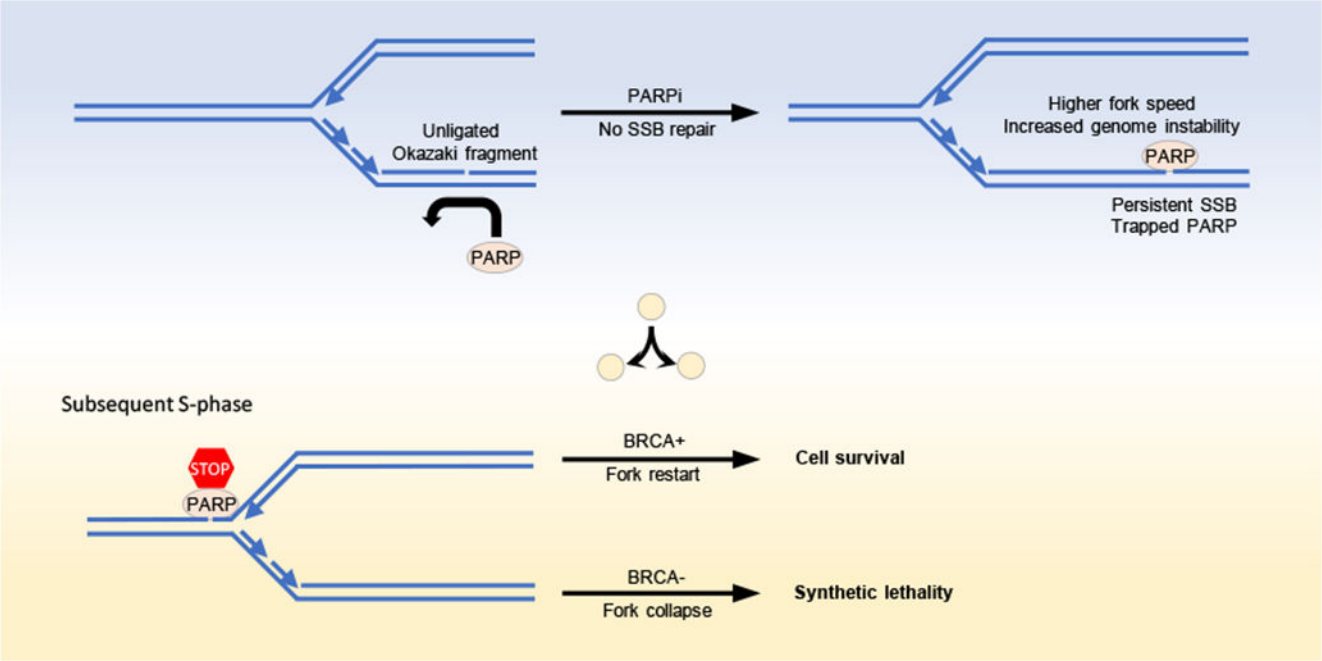

Figure 1. Role for PARP during Lagging Strand DNA Synthesis

Top: during unperturbed DNA replication, a fraction of Okazaki fragments is not ligated properly, leading to SSB formation. These lesions are recognized by PARP, triggering the backup SSB repair pathway. In cells treated with PARP inhibitor, PARP is trapped at unligated Okazaki fragments and cells will undergo mitosis with persistent SSBs. PARP inhibition also appears to increase replication fork speed and induce elevated DNA damage through an as yet unknown mechanism. Bottom: trapped PARP at persistent SSB forms a replication barrier during subsequent S phase, leading to fork stalling. BRCA-mediated HR repair is required for fork restart. In the absence of functional BRCA, DNA damage persists, leading to cell death.