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Previews

Capitalizing on Cancer Replication Stress by Preventing PAR Chain Turnover: A New Type of Synthetic Lethality

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Tumors resistant to PARP inhibitors frequently show signs of replication stress, with hyper-activated PARP. In this issue of *Cancer Cell*, Pillay et al. demonstrate that inhibiting PAR-chain turnover results in cell-cycle arrest, which is cytotoxic when combined with cell-cycle checkpoint inhibition and constitutes a novel cancer therapy.

Human cells survive DNA damage via complex networks of proteins that coordinate the repair of damage, regulate gene expression, and direct cell-cycle progression. Deficiencies in any of the DNA damage response pathways in tumor cells forces a greater dependency on the remaining pathways. This can be exploited with synthetic lethality strategies targeting the alternative pathways thereby reducing tumor cell survival. Spontaneously elevated levels of DNA damage in cancer are primarily thought to be a consequence of replication stress. A variation on synthetic lethality is to capitalize on the high levels of replication stress in many cancer cells, indicated by a high spontaneous level of γH2AX foci in S phase, and convert this stress into lethality by blocking the normal turnover of DNA damage stress responses. One such response is the hyperactivation of poly(ADP-ribose) polymerase (PARP) resulting in widespread PARylation of chromatin bound proteins. Inhibiting poly(ADP-ribose) glycohydrolase (PARG), the enzyme responsible for the normal turnover of poly(ADP-ribose) (PAR), results in the overaccumulation of PAR chains. In this issue of *Cancer Cell*, Pillay and colleagues are the first to show the potential therapeutic utility of this approach.

The regulation of PAR levels by PARP and PARG is a key component of the cellular response to the single-stranded DNA breaks observed in cells exhibiting replication stress. PARP1 and PARP2 are recruited to DNA damage sites where they synthesize large branched PAR chains on acceptor proteins. This results in the stabilization of the replication fork and allows for the recruitment of additional repair factors. In the absence of PARP activity, cells rely on the homologous recombination (HR) repair pathway to process DNA damage (Bryant et al., 2005; Farmer et al., 2005). Drugs that inhibit PARP1/2 have been shown to be effective in treating HR-deficient cancers, both as single agents and in combination with cytotoxic chemotherapy (Poggio et al., 2018). The success of PARP inhibitors in clinical applications has led to an increased focus on the development of additional therapies exploiting synthetic lethality.

PARG plays a prominent role in DNA damage repair by effecting the timely degradation of PAR chains at damage sites (Pascal and Ellenberger, 2015). Normal PAR turnover is important for successful damage processing and progression through the cell cycle (Pascal and Ellenberger, 2015) (Figure 1A). In the absence of PARG or its activity, PAR overaccumulation results in prolonged replication fork stalling and persistent ATR/CHK1 checkpoint activation (Min et al., 2013), blocking entry into mitosis. If the tumor cell is unable to process replication stress due to an underlying genetic alteration, the cell will undergo replication catastrophe, involving collapsed replication forks, extensive DNA damage, and cell death (Toledo et al., 2017). The importance of PARG in moderating DNA replication stress renders it a promising therapeutic target.

PDD00017273, a small molecule inhibitor of PARG (PARGi) developed by James et al., (2016), has been shown to stabilize PAR chains in cells and reduce clonogenic ability when combined with DNA damaging agents in sensitive cell lines. Pillay et al. (2019) investigate the mechanism of PARGi sensitivity and identify a subset of ovarian cancer models with underlying replication stress vulnerability that make them susceptible to PARG inhibition. In contrast to the cytotoxic effect seen with PARPi treatment of HR-deficient cells, the authors show that the inhibition of PARG generates a cytostatic effect, as demonstrated by an extended S-phase duration and activation of the G_2/M checkpoint in PARGi-sensitive cell lines. This phenotype is dependent on PARP activation, as co-treatment with a PARPi results in loss of efficacy. PARGi-sensitive cells exhibit characteristic markers of replication stress in response to prolonged exposure to the inhibitor, including prolonged ATM activation, pan nuclear γH2AX staining, increased RPA foci formation, and DNA replication fiber asymmetry. This phenotype is reproduced in untreated PARGi-sensitive cell lines by siRNA knockdown of RECQ1, a helicase involved in replication fork restart (Popuri et al., 2012). This effect is not shown in PARGi-resistant cells treated with siRECQ1. Together, these results suggest that the PARGi effect in sensitive cells is likely due to replication catastrophe induced by protracted replication stress.

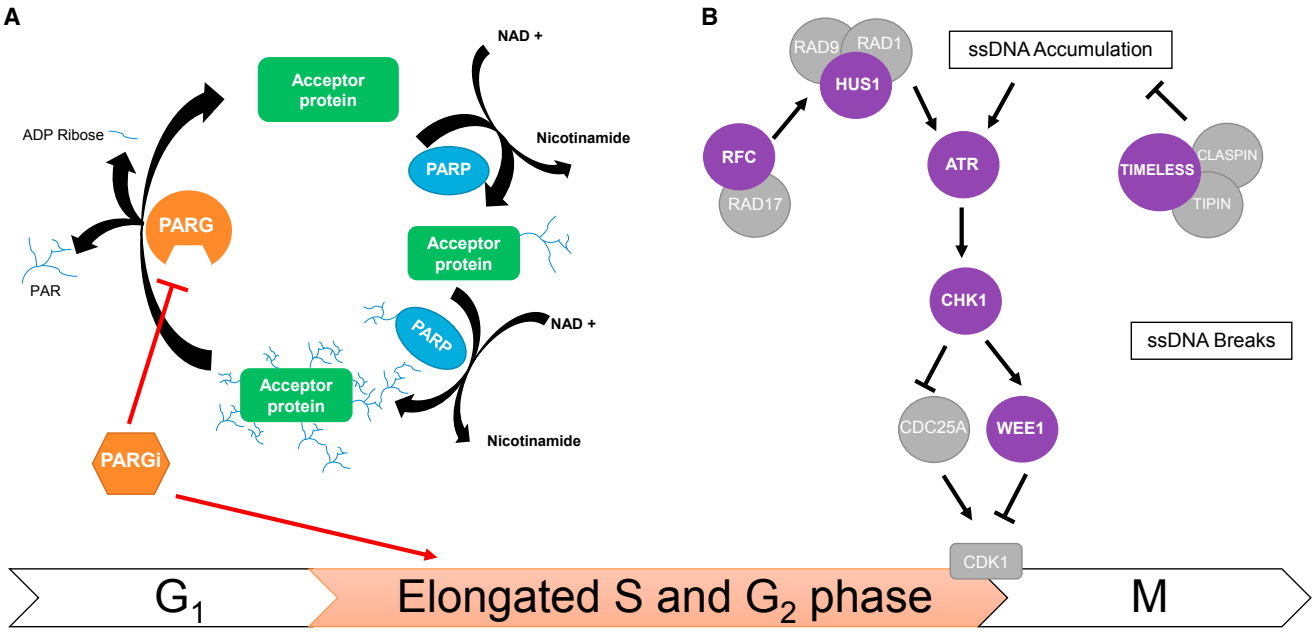

Figure 1. DNA Replication Stress Sensitizes Ovarian Cancer Cells to PARG Inhibition

(A) PARP and PARG regulate PAR levels in response to DNA damage. During DNA replication, PARP is recruited to sites of single-stranded DNA breaks and synthesizes PAR chains on acceptor proteins. This stabilizes the replication fork and signals the recruitment of additional repair factors. PARG then degrades these PAR chains to promote fork restart and cell-cycle progression. PARG inhibition results in overaccumulation of PAR chains, prolonged replication fork stall, and elongated S phase.

(B) PARG inhibition is synthetically lethal with DNA replication factors. siRNA-mediated depletion of proteins involved in replication fork stability and cell-cycle checkpoint signaling sensitized cells to PARGi. These include TIMELESS, HUS1, RFC2, CHK1, ATR, and WEE1 (purple).

To further elucidate the underlying mechanism of PARGi sensitivity, Pillay et al. (2019) developed an siRNA screen for synthetic lethal targets in PARGi-resistant cells using pan nuclear γH2AX as the endpoint marker. The top hits were TIMELESS, HUS1, RFC2, and CHK1, all of which are involved in checkpoint signaling at stalled replication forks (Forment and O'Connor, 2018). siRNA-mediated knockdown of these factors individually results in sensitization of the initially resistant OVCAR3 cell line to PARGi (Figure 1B). These results suggest that the pattern of DDR gene expression has the potential to predict cellular response to PARG inhibitors. In this study, interrogation of the expression profiles for 40 candidate genes in 47 ovarian cancer cell lines resulted in the identification of six cell lines with downregulation of DNA replication genes, of which three were found to be sensitive to PARGi.

As a result of these observations, Pillay et al. propose that pharmacological inhibition of certain DDR proteins could sensitize non-responsive cell lines to PARG inhibition, hence broadening the potential applications of this therapeutic strategy. Interestingly, PARGi in combination with chemotherapy-induced replication stress showed only a modest reduction in clonogenic ability; however, pharmacological inhibition of CHK1 in combination with PARGi resulted in decreased proliferation and increased apoptosis. The discovery of this new type of synthetic lethal relationship presents the opportunity for exploitation in the clinic, not only as a monotherapy in tumors with an intrinsic replication vulnerability but also in combination with the inhibition of proteins involved in the G_2/M checkpoint such as WEE1 and ATR (Forment and O'Connor, 2018). Additionally, the apparent mutually exclusive nature of the PARPi and PARGi relationship indicates a potential for utilization of PARGi in PARPi-resistant tumors. It is important to note that the PARGi response appears to be context dependent. Therefore, going forward, it will be interesting to see whether PARG inhibitors show efficacy in other cancer types and whether manipulation of other pathways in combination with PARGi will result in synthetic lethality, as Pillay and colleagues have shown for the ovarian cancer cell lines in this study.

DECLARATION OF INTERESTS

S.N.P. is on the advisory board of Varian Medical Systems and on the scientific advisory board of Artios Therapeutics.

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Update

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Correction

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In the originally published version of this preview article, the name of the compound was mislabeled. The correct name is PDD00017273. The compound name has now been corrected in the online version of the preview article. The authors apologize for any confusion this error may have caused.