Using functional genetic screens to understand and overcome PARP inhibitor resistance

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Chapter 4 Addendum

Filling in the gaps in PARP inhibitor-induced synthetic lethality

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ABSTRACT

Tumors with loss of BRCA1 are homologous recombination (HR) deficient and hypersensitive to poly(ADP-ribose) polymerase inhibitors (PARPi). However, these tumors may restore HR and acquire PARPi resistance via loss of end-protection of DNA double-strand breaks. We found that loss of nuclear DNA ligase III resensitizes HR-restored BRCA1-deficient cells to PARPi by exposing post-replicative single-stranded DNA (ssDNA) gaps. Our work, and that of others, identifies ssDNA gaps as a key determinant of PARPi response.

Developing targeted therapies is one of the main goals of current cancer research. The synthetic lethality of poly(ADP-ribose) (PAR) polymerase inhibitors (PARPi) in homologous recombination (HR)-deficient tumors provides an example of a targeted therapy that has been successfully translated into the clinic. Despite the success of this approach, drug resistance poses a major obstacle and combination treatment strategies are required to overcome PARPi resistance[1]. One of the best-studied mechanisms of PARPi resistance involves restoration of HR in cells with defects in *BRCA1* via loss of DNA double-strand break (DSB) end-protection factors (e.g., 53BP1, MAD2L2 or shieldin complex subunits 1-3)[1]. Recently, we have identified nuclear nuclear DNA ligase III (LIG3) as a critical mediator of PARPi resistance in BRCA1-deficient cells that have restored HR via loss of 53BP1[2]. Depletion of LIG3 resensitizes BRCA1/53BP1 double-deficient cells to PARPi *in vitro* and *in vivo*, rendering LIG3 as a synthetic dependency of BRCA1/53BP1 double-deficient cells and a potential therapeutic target. Importantly, loss of nuclear LIG3 does not revert HR restoration but exposes cells to MRE11 mediated post-replicative single-stranded DNA (ssDNA) gaps upon treatment with PARPi, leading to accumulation of chromosomal abnormalities and cell death[2].

The synthetic lethality observed between PARPi and *BRCA1/2* mutations has long been attributed to the requirement of BRCA1/2 for error-free repair of DSBs via HR and for replication fork protection (FP)[1]. Recent work from Cong and colleagues shows that BRCA1/2-deficient cells accumulate ssDNA gaps upon exposure to PARPi, which is reverted in cells that acquired resistance to PARPi[3]. Our work recapitulates these findings and shows that it is possible to revert PARPi resistance in BRCA1/53BP1 double-deficient cells by reinstating ssDNA gap exposure via depletion of LIG3. Together, these observations indicate that exposure to post-replicative ssDNA gaps is a key determinant of PARPi cytotoxicity, which is independent of HR restoration. Moreover, it was shown that depletion of BRCA1 interacting helicase 1 (BRIP1), which phenocopies loss of BRCA1 in regards to its HR and FP functions, does not result in exposure to PARPi-induced ssDNA gaps, which goes in line with the (unexpected) lack of response of BRIP1-deficient cells to PARPi[3]. Altogether, these findings identify ssDNA gap exposure as novel predictor of PARPi sensitivity, in addition to defects in HR and FP.

In the presence of 53BP1, loss of LIG3 enhances PARPi sensitivity and ssDNA gap exposure in BRCA1-deficient cells but not in BRCA1-proficient cells, indicating that the increase in PARPi toxicity resulting from LIG3 depletion is dependent on BRCA1 loss, but independent of 53BP1. Surprisingly, inhibition of MRE11 rescued the increase in PARPi-induced ssDNA gaps in LIG3-depleted BRCA1/53BP1 double-deficient cells but did not revert ssDNA gap exposure in BRCA1-deficient cells in the presence of LIG3[2]. This indicates that the PARPi-induced ssDNA gaps observed upon LIG3 loss are distinct from the gaps in BRCA1-deficient cells when LIG3 is present. Hence, the additional increase in ssDNA gaps and subsequent hypersensitivity to PARPi observed in LIG3-depleted BRCA1-deficient cells is most likely due to ablation of two independent gap-suppression (or gap-filling) pathways.

ssDNA regions can originate upon DNA damage induced by e.g., ultraviolet radiation[4,5], and have been recently associated with chemotherapy response. Panzarino et al. suggested

that exposure to ssDNA gaps upon induction of replication stress underlies “BRCAness” and dictates response to cisplatin[6]. Further support for the existence of two independent gap suppression pathways is provided by Tirman et al., who showed two temporally distinct post-replicative repair mechanisms to fill primase and DNA directed polymerase (PRIMPOL) dependent ssDNA gaps generated in cisplatin-treated (BRCA1/2-proficient) cells with defective replication fork reversal due to PARP inhibition or loss of SMARCAL1 (SWI/SNF related, matrix-associated, actin-dependent regulator of chromatin, subfamily A-like 1)[7]. In line with our findings that one gap suppression mechanism is mediated by HR while another is dependent on LIG3 but HR-independent, they show that one pathway requires the RAD51 recombinase, whereas the other does not rely on recombination-mediated repair[7]. Moreover, they found that BRCA1/2 promotes gap filling by limiting MRE11 activity upon simultaneous cisplatin treatment and suppression of fork reversal. Together with our finding that MRE11 inhibition fails to rescue PARPi-induced ssDNA gaps in BRCA1-deficient cells, these results suggest that the role of BRCA1/2 in gap filling is context-dependent.

An increase in ssDNA gaps as a result of PRIMPOL-mediated repriming has also been shown to arise during unperturbed replication in HR-deficient tumors, which were shown to rely on error-prone translesion synthesis (TLS) for their repair, rendering TLS a synthetic dependency of HR-deficient cells[8]. Moreover, HR-restoration via 53BP1 depletion rescued the synthetic lethality between BRCA1-deficiency and loss of TLS repair, indicating that, in unperturbed conditions, BRCA1/53BP1 double-deficient cells do not rely on TLS for ssDNA gap repair[8]. While loss of nuclear LIG3 does not increase ssDNA gap exposure in unchallenged conditions, it remains to be tested if loss of LIG3 restores the need for TLS in BRCA1/53BP1 double-deficient cells. It also remains to be determined if repriming activities (e.g., by PRIMPOL) are responsible for the ssDNA gaps observed upon PARPi treatment. Of note, depletion of PRIMPOL did not rescue PARPi-induced ssDNA gaps in LIG3-depleted BRCA1/53BP1 double-deficient cells to the same extent as inhibition of MRE11 or depletion of CHD4 ([2] and unpublished data).

While the ssDNA gap model remains to be validated in the clinic, the identification of ssDNA gap exposure as a vulnerability in BRCA1/2-deficient cells and as a predictor of PARPi sensitivity creates new opportunities for designing combination therapies to improve response to PARPi. Novel therapeutic strategies to increase exposure to ssDNA gaps or block gap suppression might effectively counteract PARPi resistance and thereby improve progression-free survival of patients.

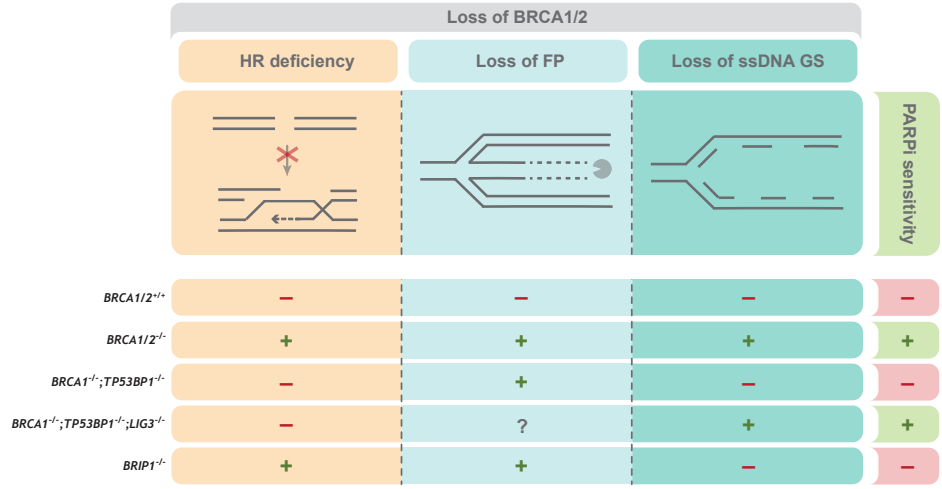

Figure 1 | Predictors of PARPi sensitivity in BRCA1/2-deficient cells. A | To date, three key determinants of response to poly(ADP-ribose) polymerase inhibitors (PARPi) have been identified: homologous recombination (HR) deficiency, loss of replication fork protection (FP) and loss of single-stranded DNA (ssDNA) gap suppression (GS). Cells with loss of BRCA1/2 display all three hallmarks, rendering these cells highly sensitive to PARP inhibition. Loss of 53BP1 in BRCA1-deficient cells restores HR and GS, hindering response to PARPi. Additional loss of nuclear DNA ligase III (LIG3) in BRCA1/53BP1 double-deficient cells restores ssDNA gap exposure and re-sensitizes these cells to PARPi. BRIP1-deficient cells show HR deficiency and loss of FP but maintain GS, which renders these cells resistant to PARPi.

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