PARP inhibitors – trapped in a toxic love affair

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Abstract

It is often the case that when an investigational cancer drug first enters clinical development, its precise mechanism of action is unclear. This was the case for PARP inhibitors (PARPi) used to treat homologous recombination (HR) defective cancers. In 2012, nearly a decade after the first PARPi entered clinical development, work from Murai and colleagues demonstrated that clinical PARPi not only inhibit the catalytic activity of PARP1, PARylation, but also "trap" PARP1 on DNA, this latter effect being responsible for much of the tumour cell cytotoxicity caused by these drugs. We discuss how this work not only changed our understanding about how PARPi work, but also stimulated subsequent dissection of how PARP1 carries out its normal function in the absence of inhibitor.

Main text

The primary target of clinically used PARPi is PARP1, a chromatin-associated poly-ART (ADP-riboslytransferase) enzyme which is activated by binding to damaged DNA. Once bound to DNA, PARP1 uses NAD^+ to synthesize multiple ADP-ribose units on adjacent substrate proteins. The resultant ADP-ribose homopolymer chains, now known as Poly(ADP-ribose) (PAR), facilitate DNA repair by driving local changes in chromatin structure and also the recruitment of DNA repair effector proteins. PARP1's role in these processes began to be uncovered in earnest from the late 1970s onwards, when alkylating agents and ionizing radiation were both shown to increase PARP1's catalytic activity, reducing cellular levels of NAD^+ whilst increasing PAR (1). Relatively non-specific, low potency "toolbox" PARP1 inhibitors, such as 3-aminobenzamide (3-AB), reversed these NAD^+/PAR phenotypes and sensitized cells to alkylating agents, largely by impairing the repair of DNA damage. Early studies also demonstrated that PARP1 has a "shuttle mechanism" where its catalytic activity is linked to its binding to- and dissociation from DNA (1). We now know (Figure 1) that PARP1 binds DNA via N-terminal zinc fingers (ZnF). PARP1-DNA binding initiates an allosteric signalling cascade and conformational change in the protein that ultimately leads to the release of an autoinhibitory interaction

between the helical domain (HD) and the catalytic domain (Cat). The change in the HD/Cat interaction allows NAD^+ to access to the catalytic site, an event which drives PARylation and the subsequent recruitment of substrate proteins and the repair of the double helix. PARP1 eventually PARylates itself (autoPARylation), an act which drives the dissociation of PARP1 from DNA. In keeping with this PAR-dependent shuttle mechanism, reducing the level of PAR on PARP1 via PAR glycohydrolase (PARG) activity results in PARP1 being retained on DNA. The connection between autoPARylation and the residence of PARP1 on DNA also led to the suggestion that catalytic inhibitors of PARP1 might impair the fitness of cells by preventing the release of PARP1 from damaged DNA (1).

Small molecule inhibitors of PARP1's catalytic activity (which also inhibit PARP1 paralogs, such as PARP2) were originally developed as potentiators of DNA damaging chemo- or radiotherapy; the first-in-human trial using a PARPi (initiated in 2003) assessed a PARPi combined with the alkylating agent temozolomide. However, once drug-like potent and selective PARP1 inhibitors were discovered, their potential as single-agent drugs was uncovered. Pre-clinical work demonstrated the ability of PARPi to kill tumour cells with HR defects, including those with *BRCA1* or *BRCA2* mutations, leaving non-mutant cells relatively unharmed (2,3). This led to clinical trials demonstrating the efficacy of PARPi in treating HR defective cancers and the eventual regulatory approval of five PARPi (talazoparib, rucaparib, niraparib, olaparib and pamiparib), with others, such as veliparib, still undergoing clinical development.

A number of processes could have conceivably explained the *BRCA1/2* synthetic lethal effects. Phenotypically, PARPi exposure in both *BRCA1/2* mutant and wild type cells elicits a DNA damage response. In wild type cells, this response co-occurs with activation of HR, but in *BRCA1/2* mutant cells the inability to carry out HR and effectively repair the damage caused by PARPi results in gross chromosomal aberrations and cell death (2,3). Importantly, only prolonged exposure to PARPi caused the maximal synthetic lethal effect in *in vitro* and *in vivo* models of *BRCA1/2* mutant cancer (2) suggesting that an accumulation of DNA damage over a series of cell cycles is required. Based on

these observations and the known function of PARP1, it was originally proposed that the inhibition of PARylation by PARPi caused synthetic lethality by impairing the recruitment and activity of DNA repair proteins, phenocopying the effects of deleting the *PARP1* gene; the persistence of unrepaired DNA lesions would ultimately cause the structural collapse of replication forks. Alternatively, PARPi could potentially "trap" PARP1 on DNA either by preventing the autoPARylation required for PARP1 release and/or by altering the conformation of PARP1 such that its release from DNA was prevented and/or delayed; this trapped PARP1 could conceivably cause a steric barrier to the normal progression of the replication fork, again causing a greater reliance upon HR (1). Indeed, in experiments where cells were exposed to the combination of the DNA alkylating agent methyl methanesulfonate (MMS) and the toolbox PARPi (4-AN), the amount of chromatin-associated PARP1 was increased (4). Deletion of the *PARP1* gene caused resistance to the 4-AN/MMS combination, suggesting that the trapping of the PARP1 protein by a PARPi could indeed cause cytotoxicity (4).

Whether the trapping hypothesis bore any relevance to clinical PARPi only became clear with work from Murai and colleagues published in *Cancer Research* in 2012 (5). This work established that chicken DT40 cells with a deletion in the *Parp1* gene or human tumour cells with *PARP1* gene silencing were profoundly resistant to the clinical PARPi olaparib. When exposed to olaparib, *Parp1*$^{-/-}$ DT40 cells also did not display an enhanced DNA damage response, suggesting that the presence of Parp1 was an absolute requirement for the DNA damage normally caused by PARPi. Olaparib exposure also increased the amount of chromatin-associated PARP1 and PARP2 in cells co-exposed to MMS, suggesting that olaparib stabilises a PARP1/DNA complex. Interestingly, veliparib, as potent inhibitor of PARylation as olaparib and niraparib, but a poor PARP1 "trapper", did not elicit same level of *BRCA* synthetic lethality in DT40 cells (5). Subsequent "head-to-head" studies in isogenic human *BRCA1/2* wild type and null cells showed that *BRCA1/2* synthetic lethal effects are better elicited by PARPi that effectively trap PARP1, and less correlated with the ability of a PARPi to inhibit PARylation. Consistent with this, unbiased mutagenesis screens showed that *PARP1* deletion or

PARP1 mutations that prevent trapping are drivers of resistance to those PARPi that trap PARP1 on DNA (6).

In totality, this work made the critical link between PARP1 trapping and the cytotoxic effects of clinical PARPi and led Murai and colleagues to propose that many clinical PARPi induce “reverse allostery”, where PARPi binding in the catalytic site alters the PARP1 allosteric signalling required for PARP1 release from DNA and/or fixes the conformation of PARP1 in such way that the inhibitor-bound state has a higher affinity for DNA (5). Previous work had also shown that gene silencing of *PARP1* elicited *BRCA1/2* synthetic lethality, as did PARPi later shown to trap PARP1 (2,3). Thus, the work of Murai and colleagues suggested that two parallel (and potentially non-exclusive) mechanisms might operate: genetic inactivation or catalytic inhibition of PARP1 in the absence of trapping might cause the persistence of DNA lesions normally repaired by PARP1, such as single strand DNA breaks (SSBs). Upon encountering replication forks, these SSBs might stall and collapse replication forks, thus activating HR in *BRCA1/2* wild type cells but causing synthetic lethality in *BRCA1/2* mutant cells; alternatively, the PARP1-DNA complex formed by trapping PARPi could itself be a DNA lesion which similarly requires *BRCA1/2* function to mediate DNA repair and prevent cell death (5). The precise balance of each mechanism in cells is unknown, but several lines of evidence suggest trapping might be more important for tumour cell cytotoxicity. For example, PARPi that are effective at trapping *and* catalytic inhibition (e.g. talazoparib, rucaparib, olaparib) elicit greater *BRCA1/2* synthetic lethal effects in *in vitro* cell-based models of *BRCA1/2* mutation than PARPi which are less effective in PARP1 trapping (e.g. veliparib) but have similar effects on catalytic activity (5). However, clinical trials that compare different PARPi in a head-to-head fashion have not, as yet, been performed, so it is not completely clear whether PARPi like veliparib have different therapeutic effects than PARPi with greater trapping capabilities.

Although this clinical issue remains to be resolved, the work of Murai and colleagues was hugely influential and stimulated a series of other discoveries that may be informative in terms of understanding how PARPi are best used

clinically. Subsequent high-density CRISPR-Cas9 mutagenesis of the *PARP1* gene established that in addition to mutations in the DNA-facing zinc finger domains, mutations in regions of PARP1 not predicted to directly bind DNA also impair trapping and cause PARPi resistance (6). These include a PARP1 p.R591C (WGR-domain) mutation also seen in a case of clinical *de novo* PARPi-resistance (6). Mutations at p.R591 could conceivably reduce PARP1 trapping by interfering with the PARP1-nucleosome interaction, by facilitating PARP1 reverse allostery in the presence of a PARPi (6), or by altering the conformation of PARP1 when it is bound to a PARPi (7). Interestingly, hydrogen-deuterium exchange mass spectrometry analysis of PARP1 suggests that PARPi with different trapping properties induce different allosteric changes in PARP1, adding weight to the suggestion that these conformational changes might be more important to the PARP1 trapping phenotype than the lack of autoPARylation (7). Maximal PARP1-trapping has also been shown to require an interaction between PARPi, which largely sit within the catalytic site, and the p.D766/D770 residues in the PARP1 helical domain that sit opposite to the catalytic site; this information not only reinforces how the helical domain/catalytic domain interaction is critical to whether the catalytic domain is active or not, but also indicated how veliparib, a PARPi with limited trapping properties, could be modified to a structural derivative that interacts with p.D766/D770 and elicits greater PARPi trapping and cytotoxicity (7). These discoveries, which in part were stimulated by Murai and colleagues' 2012 work, thus illustrate how dissecting the PARP1 trapping phenotype has been informative both in terms of understanding how the inter-domain interactions of PARP1 control its normal function and how PARPi could be improved to target these features.

Whilst alterations in PARP1 that impair PARP1 trapping cause PARPi resistance, PARPi sensitivity can be enhanced by increasing the number of DNA lesions that PARP1 normally binds to. For example, inactivation of RNA ribonuclease H2 proteins increases the amount of genomic uracil, which is normally processed into a PARP1-binding substrate. Thus, RNA ribonuclease H2 inactivation, in concert with PARPi, leads to more trapped PARP1 and more PARPi sensitivity (8). Similarly, maximal activation of PARPi-induced cGAS-

STING signalling (a potential driver of PARPi-mediated immune activation in cancer) is reversed by *PARP1* deletion, thus implicating PARP1 trapping in this phenotype as well (9). It is also possible that one of the deleterious side effects of PARPi, myelosuppression, might be driven by PARP1 trapping. Finally, the synergistic effects of PARPi when used in combination with chemotherapy (at least in pre-clinical models) also appear to be determined by trapping: PARP1 trapping and catalytic inhibition are critical for PARPi/temozolomide synergy but not for PARPi/topoisomerase I inhibitor synergy, where only catalytic inhibition of PARP1 is required (10). This suggests, once again, that a consideration of trapping and the original 2012 *Cancer Research* work, might be important in determining how these drugs are best used clinically.

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Figure 1. Effect of PARP1 trapping by PARP inhibitors. In trapping-free conditions (left), PARP1 binds a DNA lesion, which in turn activates its catalytic activity, PARylation. PARylation facilitates the recruitment of DNA repair factors such as XRCC1. Accumulated autoPARylation causes release of PARP1 from DNA. PAR chains are degraded by PAR glycosylase, PARG, which permits PARP1 re-activation. In the presence of a trapping PARP inhibitor (right), as well as the catalytic activity of PARP1 being inhibited, PARP1 is trapped at the DNA lesion for an extended duration. Trapped PARP1 becomes an obstacle for replication forks and *BRCA1/2* are required to protect the replication fork from collapse and to repair collapsed forks. In the absence of HR and *BRCA1/2*, the DNA lesions caused by PARPi are repaired by more error-prone methods, which over several cell cycles, leads to the accumulation of an increasingly disordered genome and loss of fitness.