IN THE SPOTLIGHT

Mechanisms of Resistance to PARP Inhibitors—Three and Counting

Tito Fojo and Susan Bates

Summary: Given the malleable nature of cancer cells, we should expect, and do see, the development of resistance to any new chemotherapeutic agent. Models that help us understand how this happens are a first step to better and more effective chemotherapeutics. *Cancer Discov; 3(1); 20–3. ©2012 AACR.*

See related article by Jaspers et al., p. 68 (1).

In this issue of *Cancer Discovery*, Jaspers and colleagues (1) report interesting and provocative results in an ongoing series of investigations aimed at dissecting mechanisms of drug resistance in BRCA-deficient breast cancer. They use a genetically engineered mouse model (GEMM) lacking both Brca1 and p53 (*K14cre;Brca1^{F/F};p53^{F/F}*), in which breast cancers arise spontaneously with a mean latency of approximately 213 days (2). They have previously shown marked differences in response to chemotherapy in the spontaneously arising tumors, in much the same way responses in tumors in patients vary. In subsequent studies, they transplanted tumors orthotopically into syngeneic wild-type mice, allowing comparisons of therapeutic efficacy with tumor sampling before and after treatment. Previous studies examined resistance to doxorubicin, paclitaxel, topotecan, cisplatin, and olaparib (AZD2281), with varying results and clinical implications (3, 4). The present study proves again the adage that cancer cells are very adaptable. And while many have criticized GEMMs as too narrow and thus too easily treated, the *K14cre;Brca1^{F/F};p53^{F/F}* model has elegantly identified several resistance mechanisms. In this issue of *Cancer Discovery*, we see how cancer cells adapt and develop resistance when first-order mechanisms of tolerance are eliminated. For the first time in an *in vivo* model, the authors report the development of resistance to inhibitors of PARP by partial restoration of homologous recombination (HR) due to somatic loss of 53BP1 (1).

The *K14cre;Brca1^{F/F};p53^{F/F}* model was engineered with a large deletion in the *BRCA* gene, and it is thus not surprising that resistance to PARP inhibitors (PARPi) did not involve secondary events that restore BRCA1 function, as reported in other clinical and preclinical studies (5). This may also have facilitated the emergence of the multidrug efflux transporter P-glycoprotein (Pgp) as the primary mechanism of resistance in the *K14cre;Brca1^{F/F};p53^{F/F}* model—a mechanism of drug tolerance first observed when doxorubicin and paclitaxel were used as the chemotherapeutics; and then subsequently with the PARPi olaparib. Although discouraging results in clinical trials attempting to reverse drug resistance mediated by Pgp have led many to discount drug efflux as a mechanism of resistance, we must remember that failure of a strategy to reverse resistance does not mean the resistance mechanism is not important clinically—it only speaks to the failure of the strategy (6). In the *K14cre;Brca1^{F/F};p53^{F/F}* model, murine tumors developed olaparib resistance mediated by Pgp, and the resistance could be reversed with the specific inhibitor tariquidar (3). In the study reported here, the investigators probed the model further, inactivating Pgp by breeding the *Mdr1a/b*-null alleles to homozygosity and isolating resistant tumors. In a subset of tumors, they found resistance mediated by reactivation of HR.

What have we learned? At a very simple level, the data remind us yet again what we have known for decades: the problem is not our drugs, the problem is the intrinsic resistance present at the outset or the acquired tolerance that emerges following drug exposure. Cisplatin, doxorubicin, paclitaxel, and even methotrexate are excellent drugs that can cure, but in a majority of cancers, intrinsic or acquired resistance precludes cure. While some had hoped "targeted therapies" would not suffer this malady, in fact, the data are now overwhelming that resistance is a major problem for these newer therapies. Furthermore, resistance is complex and refractory. For example, a number of mechanisms have been reported to confer resistance to the BRAF inhibitor vemurafenib in clinical samples (7). And as the current study shows, resistance is likely just as complex when it comes to PARPi. Previous studies have identified 2 mechanisms of resistance to PARPi in preclinical models and patients: secondary mutations that partially restore BRCA function and overexpression of Pgp (3, 5). To that, we can now add loss of 53BP1 with restoration of HR. Importantly, loss of 53BP1 occurred in only a fraction of the PARP-insensitive tumors, indicating the existence of as-yet additional uncharacterized resistance mechanisms.

As regards our patients, clinical trial data and preclinical models such as the *K14cre;Brca1^{F/F};p53^{F/F}*;Mdr1a/b^{−/−} model make us confront reality. For women with breast or ovarian cancer in a background of BRCA deficiency, it seems likely that PARPi will provide a treatment alternative and some benefit, but for the overwhelming majority inherent and acquired resistance will emerge (8, 9). And we may find

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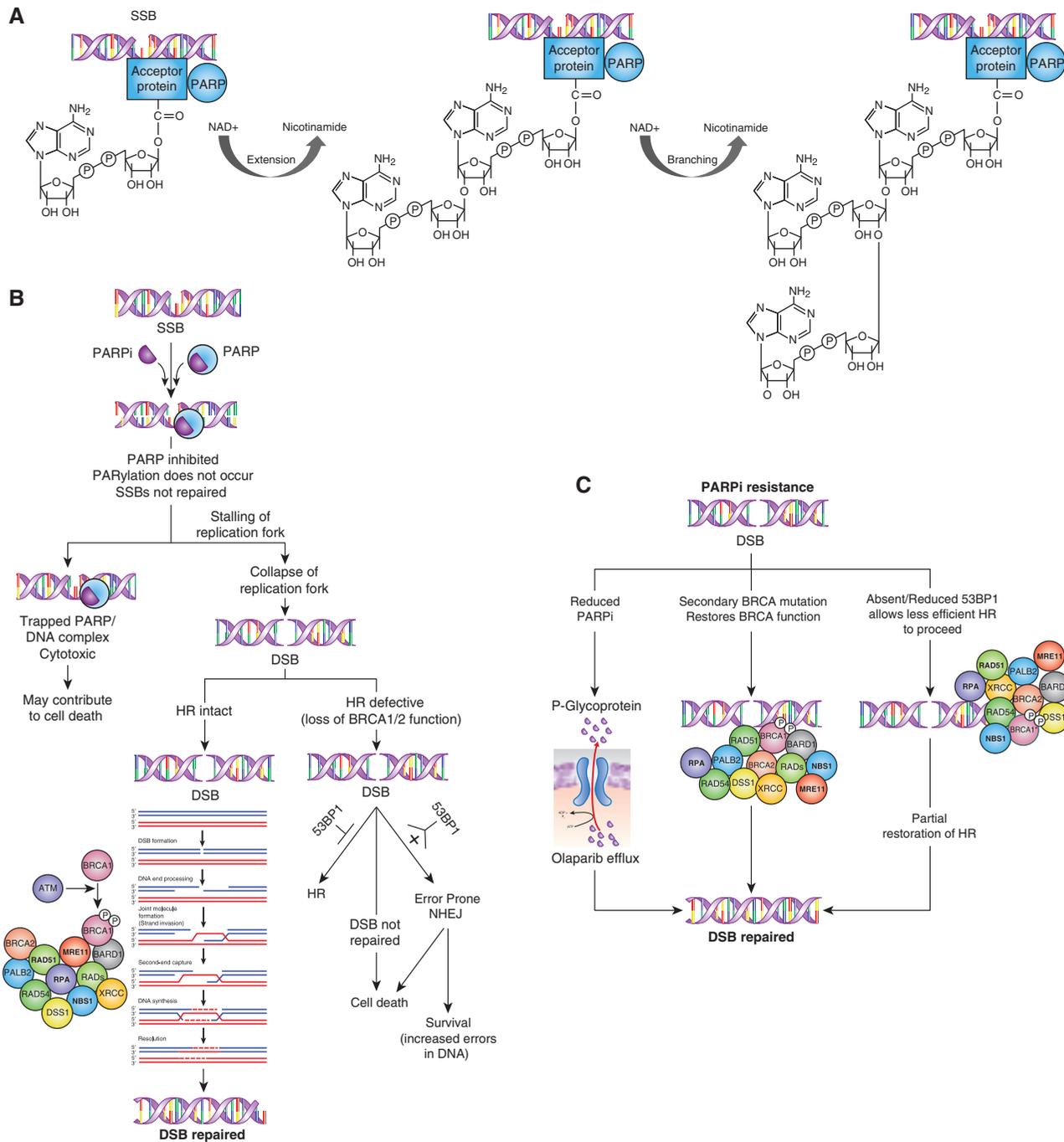
Figure 1. (**A**) **P**oly(**A**DP-**r**ibosyl**)ation** often referred to as PARylation is a post-translational protein modification catalyzed by poly(ADP-ribose) polymerases [PARPs]. PARylation involves the polymerization of ADP ribose units from donor nicotinamide adenine dinucleotide (NAD+) molecules and is catalytically activated by single strand breaks in DNA. PARylation forms heterogenous linear and branched chains of poly(ADP-ribose) or PAR. A chain consists of as many as 200 ADP-ribose units linked via glycosidic $1' \rightarrow 2'$ bonds, covalently bound to acceptor proteins, that include PARP1 itself (auto-PARylation), histones and other proteins involved in the recognition and repair of DNA strand breaks. Each residue in a PAR chain contains an adenine moiety capable of stacking and forming hydrogen bonds and two negatively charged phosphate groups. PAR recruits additional proteins such as p53, the base excision repair protein XRCC-1 and others, most of which contain a PAR binding motif (PBM) ≈20 amino acids in length. (**B**) PARP inhibitors (PARPi) in clinical development mimic the nicotinamide moiety of NAD+, and bind to the enzyme's catalytic domain. Inhibition of PARPs leads to persistence of DNA single strand breaks (SSBs) that collapse replication forks resulting in double strand breaks (DSBs) and in turn trigger homologous recombination (HR). Such DSBs are present in higher numbers when PARP has been inhibited. In cells with intact HR, DSBs are successfully repaired. However, in cells with defective HR such as those with dysfunctional BRCAs (asterisk, part **C**), DSB repair cannot occur efficiently leading to apoptosis or to the use of error prone non-homologous end joining (NHEJ), which may in turn lead to apoptosis. (**C**) Mechanisms of resistance to PARPi described to date include secondary BRCA mutations that restore BRCA function, increased drug efflux mediated by P-glycoprotein and reduced/absent 53BP1 expression resulting in partial restoration of HR.

that combinations of PARPi with chemotherapy are also not the answer. These combinations have proven toxic and difficult to administer, and there is, at present, no definite evidence that such combinations will prove more effective than serial use of the respective agents (10). Thus, we should not develop countless PARPi but rather focus on approaches to delay or avert the onset of resistance. Recognizing the role of Pgp in resistance to olaparib, the authors convincingly show that resistance was mitigated and response durability increased with the novel PARP inhibitor AZD2461, a poor Pgp substrate.

Normal metabolism and environmental insults create ongoing DNA damage. PARP1 and the less abundant PARP2 bind single-strand breaks (SSB) in DNA (Figure 1) (11). Binding of DNA activates the catalytic C-terminal domain to use the ADP-ribose monomers from NAD$^+$ to covalently modify proteins with large linear and branched poly(ADP-ribose) (PAR) chains as a signal for DNA repair enzymes and scaffolding proteins. After the repair is complete, the PAR chains are degraded. The rapid binding of PARP is critical for repairing DNA SSBs, and when PARP is inhibited, repair is impaired. When these unrepaired SSBs are encountered during DNA replication, the replication fork stalls, converting the SSBs into double-strand breaks (DSB) (Figure 1). DSB repair may occur via the more accurate and efficient HR pathway or by the error prone nonhomologous end joining (NHEJ) pathway. HR requires an orchestrated series of events that includes recognition signaled by γ-H2AX, chromatin remodeling, and DNA repair by BRCA1/2, RAD51, 53BP1, and numerous other proteins. This explains why cancer cells deficient in BRCA1 and BRCA2, two crucial components of the HR machinery, are hypersensitive to PARPi, a phenomenon sometimes referred to as synthetic lethality.

Although counterintuitive, loss of 53BP1 seemed to promote HR in the BRCA-deficient cells. Loss of 53BP1 was identified by immunohistochemistry in 3 of 11 olaparib-resistant tumors and in 3 of 12 AZD2461-resistant tumors. Expression loss was explained by truncating mutations, duplications, frame-shifts, and silencing. These observations agree with previous *in vitro* studies showing that loss of 53BP1 partially restores the HR defect that Brca-1 deletion confers (12, 13). How this occurs is not yet understood, but it may be explained by data generated using a dominant negative 53BP1 construct (14). These experiments showed that suppression of 53BP1 suppressed NHEJ and increased HR-mediated repair, suggesting that 53BP1 may help regulate the choice between NHEJ and HR-mediated repair. Targeting 53BP1 loss to restore sensitivity to a PARPi is theoretically possible, but may prove difficult.

We have envisioned the mechanism of action of a PARPi as a simple and straightforward inhibition of PARP activity. But we now know that PARPi, like all good drugs, have complex activity profiles, and this may be good. A recent study has convincingly shown that, like topoisomerase inhibitors, PARPi can act as poisons that trap the PARP1 and PARP2 enzymes on DNA, generating complexes that are more cytotoxic than the unrepaired SSBs caused by PARP inactivation (15). Furthermore, the potency in trapping PARP does not correlate with the ability of a PARPi to inhibit PARP catalytic activity and differs markedly among available inhibitors.

Although it is an interesting thesis, this needs to be proven, as resistance mechanisms that restore HR seem indifferent to the "trapped PARPs" that likely continue to form, suggesting such lesions may be tolerable. But if proven to be crucial, then as we understand which property is more important in cytotoxicity and which has greater effect on normal cells and possibly contributes to the poor tolerability of combinations, we may more rationally discriminate among existing PARPi.

As we look back on this decade of targeted therapies, we see therapies that have been thwarted by very adaptable cancer cells using alternate paths to the same end or exploiting redundancy (16). With imatinib, mutations that rendered BCR-ABL insensitive to drug allowed reactivation of a critical pathway. With agents targeting VEGF, the data indicate that alternate pathways emerge to drive angiogenesis. And in the case of BRAF inhibitors, multiple adaptations reconstitute signaling through the mitogen-activated protein kinase (MAPK) pathway. But in the case of PARP inhibition, the data, to date, show either exclusion of the drug by an efflux pump or reactivation of HR as mechanisms of tolerance. Surprisingly, a "work around" PARP has not been seen yet—the alternate pathway seems to be to work around BRCA. If this means cancer cells find it more difficult to adapt when PARP is inhibited, then we do well to continue pursuit of PARP as a target.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interests were disclosed.

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