PARP inhibitor resistance: the underlying mechanisms and clinical implications

He Li 1, Zhao-Yi Liu 1, Naiyuan Wu 1, Yong-Chang Chen 1, Quan Cheng 2 and Jing Wang 1,3*

Abstract

Due to the DNA repair defect, BRCA1/2 deficient tumor cells are more sensitive to PARP inhibitors (PARPi) through the mechanism of synthetic lethality. At present, several PARPi targeting poly (ADP-ribose) polymerase (PARP) have been approved for ovarian cancer and breast cancer indications. However, PARPi resistance is ubiquitous in clinic. More than 40% BRCA1/2-deficient patients fail to respond to PARPi. In addition, lots of patients acquire PARPi resistance with prolonged oral administration of PARPi. Homologous recombination repair deficient (HRD), as an essential prerequisite of synthetic lethality, plays a vital role in killing tumor cells. Therefore, Homologous recombination repair restoration (HRR) becomes the predominant reason of PARPi resistance. Recently, it was reported that DNA replication fork protection also contributed to PARPi resistance in BRCA1/2-deficient cells and patients. Moreover, various factors, such as reversion mutations, epigenetic modification, restoration of ADP-ribosylation (PARylation) and pharmacological alteration lead to PARPi resistance as well. In this review, we reviewed the underlying mechanisms of PARP inhibitor resistance in detail and summarized the potential strategies to overcome PARPi resistance and increase PARPi sensitivity.

Keywords: PARPi, Homologous recombination, Resistance, Synthetic lethality

Introduction

DNA damage response (DDR) is vital to maintaining genome stability [1]. When cells suffer from DNA damage, DDR is instigated and it can remove the damage by specified DNA repair pathways, including homologous recombination repair (HR), non-homologous end joining repair (NHEJ), single stranded break repair (SSBR) [2]. To cope with DNA single-strand breaks (SSB), base excision repair (BER) is activated in mammalian cells. Poly (ADP-ribose) polymerases (PARPs), especially PARP1, PARP2 and PARP3 are key to BER [3, 4]. As DNA damage sensors and signal transducers, they can bind damaged DNA at single strand DNA breaks sites, which result in the recruitment of DNA repair effectors to the sites of DNA breaks [4, 5]. NHEJ and HR are two mainly pathways to resolve the DNA double-strand breaks (DSB). NHEJ is an error prone pathway. In this mechanism, DSB sites are repaired by blunt end ligation with low fidelity [6]. While the use of NHEJ leads to accumulation of genetic aberrations, chromosomal instability, cell cycle arrest and apoptosis [7]. However, HR is a process of accurate restoration of the DSB with high fidelity [8]. BRCA1/2 proteins are crucial for the error-free repair of HR [9]. In the S/G2 phase, BRCA1 is recruited to the DSB sites, which counteracts 53BP1 and initiates ubiquitination of C-terminal binding protein interacting protein (CtIP) [10]. With the assistance of CtIP, the 5’ to 3’ resection occurs and generates 3’ overhangs. Afterwards, BRCA2 and PALB2 participate in the formation of the nucleoprotein filament and D-loop [11, 12] (Fig. 1). Given that DDR has the ability to overcome the cytotoxicity
induced by chemo- and radiotherapy treatment, it’s important to uncover the underlying mechanisms of DNA repair pathway and exploit new drugs.

Germline mutations in BRCA1/2 (gBRCAm) predispose to ovarian cancer and breast cancer. Besides, somatic mutations of BRCA1/2 (sBRCAm) have also been suggested in various cancer types. Especially, nearly 20% of patients (16% gBRCAm and 4% sBRCAm) occur in ovarian cancer [13]. More importantly, up to 50% high-grade serious ovarian cancer (HGSOC) patients present as HRD [14]. Therefore, inhibition of PARPs may cause both SSBR deficient and HRD in BRCA1/2 deficient patients, leading to cell death [15, 16]. This is the so-called “synthetic lethality”, which is a concept proposed a century ago to describe the condition whereby a defect of either one of two genes have no/little effect but the combination of both genes (BRCA and PARPs) lead to cell death [17].

PARPi are the first agents designed to exploit synthetic lethality and permitted to use in clinic. They have the ability to bind and trap PARPs on DNA, preventing the release of PARPs from DNA break sites and removing PARPs from their normal catalytic cycle [5]. Due to more benefits and less adverse effects, olaparib (lynparza), niraparib (ZEJULA) and rucaparib (RUBRACA) are indicated for the maintenance treatment of recurrent ovarian cancer patients, who are in a complete or partial response to platinum-based chemotherapy in United states [18–21]. Olaparib is also approved to treating gBRCAm advanced ovarian cancer as four lines of chemotherapy [18]. It can also be used to treat gBRCAm, HER2-negative metastatic breast cancer patients, who have been treated with chemotherapy in the neoadjuvant, adjuvant, or metastatic setting [22, 23]. Recently, it’s suggested that carriers with HRD but not gBRCAm or sBRCAm, which is termed as “BRCAness”, are also sensitive to PARPi [24]. However, BRCA1/2 mutations remain the strongest genetic predictor of sensitivity of PARPi [25].

Similarity with other chemotherapy agents, PARPi also faced the drug resistance. More than 40% of BRCAm ovarian cancer patients failed to benefit from PARPi [26, 27]. Considering the important roles of HR repair pathway and protection of stalled replication forks in the effect of

\[ \text{Fig. 1} \text{ Schematic describing the function the principle of synthetic lethality interaction between PARPs and BRCA1/2. When cells suffer from DNA response, single-strand breaks emerge. PARPs, especially PARP1, bind to the DNA break sites, which result in the PARylation of target proteins and recruitment of the DNA damage repair effectors. Then the auto-PARylation on PARPs leads to the dissociation of PARPs from DNA. Treatment HR-deficient tumor cells with PARPi, NHEJ is the only pathway to use to repair double-strand break, which lead to accumulation of genome instability and cell death for the low fidelity.} \]
PARPi, we described the effects of DNA repair response and protection of stalled replication forks on PARPi resistance in detail. Besides, we reviewed the association between PARPi resistance and other factors, such as reversion mutations, epigenetic modification, restoration of PARPylation and pharmacological alteration. Finally, we summarized the feasible strategies to overcome PARPi resistance and enhance PARPi sensitivity in clinic.

**Restoration of HR repair in PARPi resistance**

Due to HRD is the main premise of anticancer effects of PARPi, it is crucial to understand the HR repair pathway. When DSB happen in mammalian cells, the DDR is activated. Cooperatively, cells employ two typical mechanisms to repair DSB: HR and NHEJ. Normally, NHEJ is the mainly repair mechanism by ligating the broken DNA ends in a nonhomologous end-joining way and occurs throughout the cell cycle, especially in G0/G1 phase. However, HR predominates the S/G2 phase, due to the high DNA replication and available sister template [28]. In the process of HR, the DSB ends are firstly resected by Mre11-Rad50-Nbs1(MRN) complex together with CtIP and nucleases (EXO1, DNA2 and MUS8), leading to the formation of the single-strand DNA (ssDNA) and committing the cells to HR [29]. Afterwards, the resected DNA ends are coated by hyperphosphorylated single-strand DNA binding protein A (RPA) [30]. The variant H2AX (named γH2AX) is activated and phosphorylated by apical kinases, such as ataxia-telangiectasia mutated (ATM) and ATM- and Rad3-related (ATR). The spreading of γH2AX along the chromosome assists the recruitment and accumulation of additional DDR proteins, including p53-binding protein (53BP1) and BRCA1 to the DDR foci [31]. With the favor of PALB2, BRCA2 binds with BRCA1 and promotes the loading of recombinase RAD51 on the ssDNA [11]. The RAD51 mediates the invasion of the homologous sequence and formation of the nucleoprotein filament and D-loop by eliminating secondary structure formation and protecting DNA ends from degradation [32] (Fig. 2). Therefore, the restoration of HR pathway by inducing the process of DNA end resection and

![Fig. 2 Homologous recombination repair in S/G2 phase. The double-strand break ends are resected by MRE11-RAD50-NBS1(MRN) complex together with CtIP. ATM is recruited to DSBs through MRN and phosphorylates targets such as 53BP1 and MDC1. MDC1 phosphorylation recruits the E3 ubiquitin ligase RNF8, which, through recruitment of a second E3 ubiquitin ligase (RNF168), leads to histone H2A ubiquitylation. This modification, together with H4K20 methylation, allows for 53BP1 recruitment. 53BP1 phosphorylation allows its interaction with Rif1 and PTP1, which can be blocked by WIP1. 53BP1 blocks DNA resection by recruiting shieldin and presents cells to NHEJ. While, BRCA1 counteracts the protection function of 53BP1, leading to the resection of DNA ends. Afterwards, the resected DNA ends are coated by PRA. With the favor of PALB2, BRCA2 binds with BRCA1 and promotes the loading of RAD51. The RAD51 mediates the invasion of the homologous sequence and formation of the nucleoprotein filament and D-loop by eliminating secondary structure formation. EMI and DDB2 mediate the degradation of RAD51. TOPBP1 phosphorylates RAD51. BRD4 and HORMAD1 are key regulators of RAD51 accumulation on chromatin. P, phosphorylation; Ub, ubiquitylation; Me, methylation, SUMO, SUMOylation, red arrows, resection](image-url)
formation of nucleoprotein filament and D-loop may lead to PARPi resistance.

**DNA end resection in PARPi resistance**

Considering that DNA end resection is the key of different DNA repair pathways choices, it’s likely that DNA end resection dictates the different repair outcome and PARPi sensitivity. Recently, multiple reports have suggested that DNA end resection participated in the PARPi resistance (Fig. 2).

Cell cycle controls the choice of DSB repair pathways [33]. In the G1 phase, 53BP1 and RIF1 proteins localize to DSB sites, leading to the inhibition of BRCA1 recruitment, blocking DNA resection and promoting NHEJ repair pathway. Otherwise, DNA end resection is stimulated in the phase of S/G2 phase and promotes HR repair [34]. It is worth mentioning that DNA end resection is depended on cyclin-dependent kinases (CDKs) activity, which mediate phosphorylation of MRN complex and CtIP [35, 36]. It was reported that CDK5-silenced Hela cells were more sensitive to PARPi [36]. Besides, CDK12 was identified as a determinant of olaparib in the models of HGSOC by genome-wide synthetic lethal screen [37]. Loss-of functions (LOF) mutations in CDK12 disrupted HR repair and sensitized ovarian cancer cells to veliparib [38]. In triple-negative breast cancer (TNBC), deletion of CDK12 reversed both primary PARPi resistance and secondary PARPi resistance, no matter in BRCA wild-type and mutated models [39]. Besides, CDK18 facilitates ATR activation by interacting with ATR and regulating ATR-Rad9/ATR-ETAA1 interactions, promoting HR and PARPi resistance in glioblastoma stem-like cells [40]. Recently, a case report results indicated that PARPi combined with CDK4/6 inhibitor (palbociclib) revealed more excellent therapeutic effects than PARPi alone in the treating with BRCA-mutated, ER-positive breast cancer [41]. All these evidences suggested that CDKs blocked DNA end resection and lead to PARPi resistance and its inhibitors might overcome the PARPi resistance. Prospectively, the combination therapy of PARPi and CDKs inhibitors is applied in clinic.

In addition to Cell cycle and CDKs, accessory factors including 53BP1, REV7 and RIF1, contribute a lot to DNA end resection and PARPi resistance [42–44]. 53BP1, which is a chromatin-binding protein, blocks DNA resection by preventing the accession of CtIP to the DSB sites [45]. It has been suggested that the loss of 53BP1 induced DNA end resection and HR restoration, leading to PARPi resistance in various cancers, such as breast cancer [42], glioblastoma [46] and ovarian cancer [47]. Mainly, 53BP1 protects DNA ends from resection in two ways. One way is to strengthen the nucleosomal barrier to end-resection nucleases by recognizing and binding to the nucleosomes containing H4K20m2 and H2AK15ub [48]. The other way is to recruit effector complex proteins with end-protection activity [49]. Recently, it was demonstrated that shieldin, an effector complex composed by SHLD1, SHLD2, SHLD3 and REV7, were recruited by 53BP1 to the DSB sites in a 53BP1 and RIF1 depend manner [49]. Numerous evidences revealed that shieldin, as the key regulator of NHEJ repair and HR repair, was also associated with PARPi resistance [49–51]. REV7, as the component of shieldin, was also suggested to counteract DNA end resection and sensitize cells to PARPi [43]. Likewise, catalysed the inactivating conformational change of REV7 and dissociated REV7-Shieldin by TRIP13 ATPase promoted HR, leading to PARPi resistance [52]. The protection function of 53BP1 requires the interactions of PTIP and RIF1, which is depends on ATM [44, 53]. Hence, the interaction between 53BP1 and RIF1 plays pivotal roles in DNA end resection and PARPi resistance. As is known to us, only when 53BP1 is phosphorylated by ATM can it recruit RIF1 and PTIP [54]. It was demonstrated that ATM-deficient cancer cells was more sensitive to PARPi than ATM-proficient cells and the combination use of ATM inhibitors enhanced PARPi efficacy [55, 56]. Besides, multiple clinical trials results indicated that patients with low ATM proteins had a greater benefit from PARPi and more favorable prognosis [57–59]. Recently, it was disclosed that WIP1 dephosphorylated 53BP1 at Threonine 543 and attenuated its interaction with RIF1, leading to decreased sensitivity of cancer cells to PARPi [60], which confirmed the importance of the interaction between 5BP1 and RIF1 once more. Obviously, nucleases (i.e., MRE11 [61–63], DNA2 [64] and EXO1 [65, 66]), functioning as “DNA end clipping” in the process of DNA end resection, affected the sensitivity and resistance of PARPi.

**Formation of RAD51-ssDNA filament and D-loop in PARPi resistance**

The RAD51-ssDNA filament performs the central functions in homology search, DNA stand exchange and HR repair (Fig. 2). Especially, RAD51 foci is suggested to serve as a functional biomarker of HR repair and PARPi resistance beyond BRCA mutation [67–69]. In the issue, the balance between RAD51 filament formation and disruption seem particularly important. By using a genetic screen, EMI1 was identified to constitutively target RAD51 for degradation and function as a modulator of PARPi sensitivity. Downregulation of EMI1 enhanced the RAD51 accumulation, leading to restoring HR and developing PARPi resistance in BRCA1-deficient TNBC cells [70]. Similarly, DNA damage binding protein 2 (DDB2), a DNA damage recognition factor, was reported to participate in the regulation of RAD51 degradation by...
physical interaction in TNBC cells. The inhibition of DDB2 induced RAD51 polyubiquitination and proteasomal degradation, leading to defective HR and sensitivity to PARPi [71]. Topoisomerase IIβ-binding protein 1 (TOPBP1) was essential for RAD51 phosphorylation at serine 14, which was necessary for RAD51 recruitment on chromatin and formation of RAD51 foci. Absent of TOPBP1 abrogated the HR and increased sensitivity of ovarian cancer cells to olaparib [72]. Bromodomain protein 4 (BRD4) is a kind of key chromosomal regulator of genome stability. The inhibition of BRD4 recruited RAD51 accumulation without activation of ATM/ATR-dependent DNA damage response [73]. It was mentioned that BRD4 was amplified in various cancer [74]. Growing evidence suggested that BRD4 inhibitors (JQ1, INCB054329) sensitized to PARPi and expanded the utility of PARPi in clinic [74–77].

Table 1 Reversion mutations (variant allele fraction > 0.5%) conferred resistance to PARPi resistance

| Gene     | Primary mutations               | Reversion mutations                        | Variant allele fraction | Cancer type |
|----------|---------------------------------|--------------------------------------------|-------------------------|-------------|
| BRCA1    | Q1756fs*74 (c.5266dupC)         | Q1756_D1757 > PG (c.5263_5272 > TCCCCAGGAC) | 3.2%                    | HGPSC*      |
| BRCA1    | I1479delAG (c.1360_1361del)     | s454_i467del (c.1361_1402del)              | 57%                     | TNBCb       |
| BRCA2    | K2162fs*5 (c.6486_6489delACAA)  | K2150fs*17 (c.6448_6473del26)              | 33%                     | Breast cancer |
| BRCA2    | V1283fs*2 (c.3847_3848delGT)    | D1280_N1288del (c.3838_3864del27)         | 57%                     | mPCc        |
| BRCA2    | V1804Kfs (c.5410_5411del)       | Y1480_A1896del (c.4434_5686delinsTT)      | 0.60%                   | Breast cancer |
| BRCA2    | V1804Kfs (c.5410_5411del)       | I1633_L2269del (c.4897_6807del)            | 0.40%                   | mPCc        |
| BRCA2    | Q2960X (c.9106C > T)            | Q2960E (c.9106C > G)                       | 67%                     | Breast cancer |
| BRCA2    | E1493Vfs*9 (c.4705_4708delGAAA) | I1490_E1493del (c.4608_4709delAAATACTGAAA) | 55–56%                  | HGPSC*      |
| BRCA2    | S1982fs (c.5946delTT)           | S1982_A1996del (c.5946_5990del45)          | 1%                      | Prostate cancer |
| BRCA2    | S1982fs (c.5946delTT)           | S1982fs (c.5946_5952dupAAA)               | 0.5%                    | Prostate cancer |
| BRCA2    | N1910fs*2 (5727_5728insG)       | A1843_S1984del (5528_5956del420)           | 0.53%                   | Prostate cancer |
| BRCA2    | N1910fs*2 (5727_5728insG)       | A1891_M1936del (5671_5808del138)           | 0.54%                   | Prostate cancer |
| BRCA2    | N1910fs*2 (5727_5728insG)       | D1909_D1911 > EDY (5727_5731TAATG > AGACT) | 0.63%                   | Prostate cancer |
| BRCA2    | N1910fs*2 (5727_5728insG)       | L1908_S1917del (5721_5750del30)            | 1.8%                    | Prostate cancer |
| BRCA2    | N1910fs*2 (5727_5728insG)       | N1766_Q2009del (5202_6025 > CA)            | 1.3%                    | Prostate cancer |
| BRCA2    | N1910fs*2 (5727_5728insG)       | N1910_D1911del (5278_5733delAATGAT)        | 3.3%                    | Prostate cancer |
| BRCA2    | N1910fs*2 (5727_5728insG)       | S1788_P2114 > DTT (5362_6340 > GATACCA)    | 1.2%                    | Prostate cancer |
| BRCA2    | N1910fs*2 (5727_5728insG)       | NA (splice site 5333_6841 + 197del1706)    | 4.8%                    | Prostate cancer |

*HGPSC: High-grade papillary serous carcinoma; 
*TNBC: triple-negative breast cancer; 
*mPC: metastatic pancreatic cancer; 
*NA: unknown

Reversion mutations in PARPi resistance

In 2008, the influence of reversion mutations on PARPi resistance was independently discovered by two groups. Ashworth et al derived PARPi-resistant clones by deleting the BRCA2 c.6174delT frameshift mutation of human CAPAN1 pancreatic cancer cell line, a BRCA2-deficient cell line. Consequently, the reconstituted BRCA2-deficient cells acquired PARPi resistance [82]. Meanwhile, Sakai et al demonstrated that secondary mutations restored the wild-type BRCA2 reading frame was a major clinical mediator of acquired resistance to platinum and PARPi [83]. By using liquid biopsy or circulating cell-free DNA (cfDNA), lots of BRCA reversion mutations have been discovered to restore the open reading frame (ORF) of BRCA1/2 and confer resistance to PARPi-based therapy in various cancers [84–90] (Table 1). Full length BRCA1 consists of N-terminal domains (BRCT), N-terminal RING domain and coiled-coil
domain. BRCT is responsible for binding phosphorylated proteins such as CtIP. N-terminal RING domain can stabilize BRCA1 and ensure the E3 ligase domain can stabilize BRCA1 and ensure the E3 ligase domain 

Multiple evidence suggested that reversion mutations, which restored the functions of BRCT and N-terminal RING domain, played essential roles in PARPi resistance [92–94]. In addition, cancer cells lacking the exon 11 of BRCA1 promoted partial PARPi resistance [95].

BRCA2 contains a DNA-binding domain and eight BRC repeats that bind to RAD51 and mediates the recruitments of RAD51 and strand exchange in HR [91]. It was suggested that each BRC repeat was divided into two categories and only BRC 1–4 bound to RAD51 with high affinity and enhanced DNA strand exchange while BRC 5–8 bound to RAD51 with low affinity and did not affect DNA strand exchange [96]. However, an in vitro study indicated that BRCA2 mutations lacking BRC 6–8 also lead to PARPi resistance [82]. Recently, two reversion mutations (c.4434_5686delinsTT and c.4897_6807del) produced truncated BRCA2 protein were thought to be competent in conferring PARPi resistance [89]. In addition to reversion mutations in BRCA1/2, Secondary somatic mutations restoring Rad51C and Rad51D were also demonstrated to be associated with acquired resistance to the PARPi [84]. With the development of gene editing, CRISPR-Cas9 screens were recently used to identify point mutations in PARPi conferring PARPi resistance. Several mutations in PARPi1 including p.R591C and p.848delY, were identified to cause PARPi resistance. More importantly, the CRISPR-Cas9 “tad-mutagenesis” mutagenesis screens approach could be employed in the analysis of other gene mutations [97].

Taken together with the growing body of data identifying reversion mutations in PARPi resistance, it seems to be the most well-validated mechanism of PARPi resistance in BRCAm cancer patients. However, we must notice that whether the reversion mutations are induced by PARPi itself or other anticancer drugs or even spontaneous is unclear. After all, cancer cells harboring BRCA mutations prefer to NHEJ repair, which lead to accumulation of genetic aberrations and increased risk of reversion mutations. Moreover, before or even during treating with PARPi-based therapy, other anticancer drugs, such as platinum, were also administered to patients, which invisibly make the study more difficult to investigate the influence of PARPi-based therapy on secondary mutation in clinic.

Furthermore, the frequency of reversion mutations occurred among patient population is still known. Recently, the prevalence of BRCA reversion mutations in metastatic castration-resistant prostate cancer (mCRPC) was estimated. By using a large genomic database, 24 gBRCAm carriers were selected from 1534 patients with mCRPC underwent ctDNA testing. At the time of the blood draw, 5 of these 24 patients were given either a PARPi inhibitor or platinum-based chemotherapy. Two patients, one receiving olaparib and one carboplatin, had BRCA2 reversion mutations. Therefore, in this germline mutation–positive, platinum- or PARPi-exposed cohort, the frequency of BRCA2 reversion mutations was 40% [98]. However, another clinical trial result showed that 8 of 97 HGSOC patients with gBRCAm or sBRCAm (8.2%) were identified to have BRCA reversion mutations before treating with rucaparib. After treating with rucaparib, only 8 of 78 postprogression patients had BRCA reversion mutations and the occurrence rate of reversion mutations was only 10.3% [99]. All these results reflected that the BRCA reversion mutations might be different in various cancers. Due to the small sample size, additional studies with more patients and various cancers are needed to carry out.

Protection of DNA replication fork in PARPi resistance

In addition to DNA repair, PARPi and BRCA1/2 participate in DNA replication. PARPi1 has a key role in mediating the accumulation of regressed forks and avoiding an untimely restart of reversed forks, leading to DSB formation [100]. Both BRCA1 and BRCA2 protect nascent DNA at stalled replication forks from MRE11/DNA2-dependent degradation [101, 102]. When PARPi inhibitors trap PARPi on DNA to block DNA replication, cells will rely on BRCA1/2 to stabilize their stalled replication forks and prevent them from being extensively degraded by nucleases (i.e., MRE11, DNA2, MUS81). As BRCA1/2 is defective, the absence of DNA replication forks protection leads to genome instability and cell death [103] (Fig. 3). Recently, more and more evidence suggested that DNA replication fork protection but not HRR caused PARPi resistance in BRCAm cells and patients, which challenged the HR dominance in synthetic lethality (Fig. 3). Rondinelli et al. showed that low EZH2 levels reduced H3K27 methylation, prevented MUS81 recruitment at stalled forks and caused fork stabilization, which promoted PARPi resistance in BRCA2-deficient cells but not in BRCA1-deficient cells [104]. Besides, Ray et al. demonstrated that PTIP, MELL3/4 and CHD4 deficiency did not restore HR activity at DSB. Instead, their absence inhibited the recruitment of the MRE11 nuclease to stalled replication forks and protected nascent DNA strands from extensive degradation, which in turn lead to acquisition of PARPi resistance in BRCA2-deficient cells [105]. FANCDD2 suppresses MRE11-mediated fork degradation in a manner dependent on nucleoprotein filaments and plays an important role in the stabilization of stalled replication forks [106]. It’s reported that FANCDD2 was highly expressed in BRCA1/2-mutated breast cancer, ovarian cancers and uterine cancers. FANCDD2 overexpression conferred resistance to PARPi...
Due to the DNA translocase activity, SMARCAL1, a member of SNF2 family, could reverse the nascent DNA degradation induced by FANCD2 deficiency in BRCA1/2-mutated breast cancer cells. It promoted the formation of ssDNA gaps at replication forks and reversed forks catalyzed by SMARCAL1 was prone to be degraded by MRE11. More importantly, its deletion promoted PARPi and cisplatin resistance [109]. In addition to SMARCAL1, the SNF2-family DNA translocases ZRANB3 and HLTF exhibited fork-remodeling activities similar to SMARCAL1, indicating that they might be associated with PARPi resistance as well [110]. RADX deletion restored fork protection but not HR by regulating RAD51 at replication forks and conferred PARPi resistance in BRCA2-mutated cancer cell lines [111]. These collective results refocus our PARPi resistance spotlight onto fork protection, which might make significant contributions to PARPi resistance [112]. Consequently, it might provide us a novel strategy in considering the future cancer therapy.

Stalled replication forks are a major source of genome instability in proliferating cells, which need to be stabilized or restarted to promote cell survival. Through decades’ efforts, multitude of mechanisms were found to protect stalled replication forks to preserve genome stability under replication stress. Except for the pathways mentioned above, RecQ helicases and pathways involved in ATR/CHK1-dependent checkpoint activation also play essential roles in replication fork protection and genome stability maintenance [103]. Therefore, they might function as part of mechanisms of PARPi resistance. However, there is no relevant preclinical and clinical studies up to now, which are expected to be taken into consideration in the future.
Epigenetic modification, restoration of PARylation and pharmacological alteration of PARPi resistance

Epigenetic modification may affect PARPi sensitivity and lead to PARPi resistance. Multiple lines of treatment prior PARPi lead to loss of BRCA1 promoter methylation, which rescued the expression of BRCA1 and conferred resistance of PARPi [113]. MiR-622 and miR-493-5p induced PARPi resistance by suppressing NHEJ reparation and impacting multiple pathways pertinent to genome stability, respectively [114, 115]. Deubiquitination of BARD1 BRCT domain by USP15 assisted BRCA1 retention at DSBs and causes PARPi resistance [116]. Moreover, similar to deletion of 53BP1, acetylation of 53 bp1 inhibited NHEJ and promoted HR by negatively regulating 53 bp1 recruitment to DSBs, which made BRCA1-deficient cells acquire resistance to PARPi [117]. The role of N6-methyladenosine (m6A) modification in PARPi resistance was recently explored. Even though that there was no difference in total m6A-modified mRNA between parental and PARPi-resistant ovarian cancer PEO1 cells, the increased expression and N6-methylation modification of FZD10 were confirmed in resistant PEO1 cells. FZD10 contributed to PARPi resistance by upregulating the Wnt/β-catenin pathway [118].

As is known to us, PARPi kill tumor cells via PARPs activity inhibition and PARP trapping. PARPs activity increase and restoration of PARylation are responsible to PARPi resistance. Phosphorylation of PARP1 at Tyr907, mediated by c-Met, increased PARP1 enzymatic activity and reduced its binding to PARPi, thereby rendering cancer cells resistant to PARPi [119]. By combing genetic screens with multi-omics analysis of matched PARP-sensitive and -resistance BRCA2-mutated mouse mammary tumors, PAR glycohydrolase (PARG) was found, the loss of which resulted in restoring PARylation formation and PARPi resistance [120]. Furthermore, the expression of PARPi was significantly associated with PARPi toxicity. It has been revealed that both cells with low expression of PARPi and cells harboring PARPi LOF mutations were more resistant to PARPi [121, 122].

Pharmacological alteration also modulates PARPi inhibitor response. PARPi are substrates of multidrug resistance protein (MDR1, P-gp), encoded by ABCB1 gene [123]. Both in vivo and in vitro studies indicated the enhanced P-gp-mediated drug efflux contributed to the acquired resistance to PARPi [124, 125]. What’s more, the resistance could be reversed by coadministration of the P-gp inhibitors or genetic inactivation of P-gp [42, 123–125]. The overexpression of ABCB1 might be induced by long-term treating with PARPi but the mechanisms are still unclear. Compared to other factors, the weight of contribution in pharmacological changes to PARPi resistance in clinic is uncertain. More and more researches are needed to uncover the underlying mechanisms.

Clinical implications towards PARPi resistance

To enhance PARPi sensitivity and overcome PARPi resistance, several feasible strategies should be considered and implemented in the future (Table 2): 1) PARPi-DRM combinatorial therapy; 2) PARPi-ionizing radiation (IR) combination; Nuclear localization is required for BRCA1 to participate in HR-mediated DNA repair [128]. IR can initiate the export of BRCA1 from the nucleus to the cytoplasm, leading to increased sensitivity of PARPi in wild-type BRCA1 and HR-proficient tumor cells [129, 130]. However, because of the synthetic lethality of the combination therapy is p53-depend, it can only be used in wild-type p53 patients [131]. Meanwhile, PARPi induce radiosensitization in vitro and in vivo models [132]. What’s even more refreshing is HR restoration by 53BP1 pathway inactivation further increased radiosensitivity in preclinical model systems. It was showed that BRCA1-mutated tumors, which acquired drug resistance due to BRCA1-independent HR restoration, could be sensitized to radiotherapy [133]. In addition to the preclinical results, clinical studies were also attempted to exploit the efficacy of PARPi-IR combination. A phase 1, open-label dose escalation study (NCT00649207) evaluating veliparib in combination with whole brain radiation therapy (WBRT) in patients with brain metastases were originated with Mehta and his colleagues [134]. The preliminary efficacy results were better than predicted outcome based on the graded prognostic factors in the published nomogram. Based on encouraging safety and preliminary efficacy results, a randomized, controlled phase 2b study is ongoing. Other two phase 1 trials (NCT01264432,
Table 2 The feasible combination therapy to enhance PARPi sensitivity and overcome PARPi resistance

| Combination therapy | Trials | NCT          | Phase | Treatment                                                                 | Status            | Study population                                                                 |
|---------------------|--------|--------------|-------|---------------------------------------------------------------------------|-------------------|---------------------------------------------------------------------------------|
| PARPi-oHSVs combination | No     |              |       |                                                                           |                   |                                                                                  |
| PARPi-IR combination | Yes    | NCT00649207  | I     | Veliparib + WBRT*                                                         | Completed         | Solid tumors with brain metastases                                               |
| PARPi-IR combination | Yes    | NCT01264432  | I     | Veliparib + IR                                                            | Completed         | Peritoneal carcinomatosis; fallopian tube, ovarian and primary peritoneal cancers |
| PARPi-IR combination | Yes    | NCT01589419  | I     | Veliparib + capecitabine + IR                                            | Completed         | Locally advanced rectal cancer                                                   |
| PARPi-IR combination | Yes    | NCT02412371  | I/II  | Veliparib + Paclitaxel/ Carboplatin + IR                                  | Completed         | Stage III NSCLC                                                                  |
| PARPi-IR combination | Yes    | NCT01386385  | I/II  | Veliparib + Paclitaxel/ Carboplatin + IR                                  | Active, not recruiting | Stage III NSCLC                                                                  |
| PARPi-IR combination | Yes    | NCT01618357  | I     | Veliparib + IR                                                            | Recruiting        | Breast cancer                                                                    |
| PARPi-CDKi combination | No     |              |       |                                                                           |                   |                                                                                  |
| PARPi-immunotherapy | Yes    | NCT02734004  | I/II  | Olaparib + MED14736                                                       | Active, not recruiting | Ovarian, breast, SCLC and gastric cancers                                      |
| PARPi-immunotherapy | Yes    | NCT03824704  | II    | Rucaparib + Nivolumab                                                     | Active, not recruiting | Epithelia ovarian cancer, fallopian tube cancer, primary peritoneal cancer, HGSC and endometrioid adenocarcinoma |
| PARPi-immunotherapy | Yes    | NCT02849496  | II    | Olaparib + Atezolizumab                                                   | Recruiting        | Locally advanced unresectable; metastatic non-HER2-positive breast cancer        |
| PARPi-epigenetic drugs | No     |              |       |                                                                           |                   |                                                                                  |
| PARPi-HSP90 inhibitors | No     |              |       |                                                                           |                   |                                                                                  |
| PARPi-WEE1 inhibitors | Yes    | NCT03579316  | II    | Olaparib + AZD1775                                                       | Recruiting        | Recurrent fallopian tube, ovarian and primary peritoneal cancers                 |
| PARPi-WEE1 inhibitors | Yes    | NCT04197713  | I     | Olaparib + AZD1775                                                       | Not yet recruiting | Advanced solid tumors with selected mutations and PARP Resistance               |
| PARPi-WEE1 inhibitors | Yes    | NCT02576444  | II    | Olaparib + AZD1775                                                       | Active, not recruiting | Tumors harboring either TP53 or KRAS mutations or mutations in KRAS and TP53   |
| PARPi-WEE1 inhibitors | Yes    | NCT02511795  | I     | Olaparib + AZD1775                                                       | Completed         | Refractory solid tumors; Relapsed SCLC                                          |
| PARPi-ATR inhibitors | Yes    | NCT02576444  | II    | Olaparib + AZD6738                                                       | Active, not recruiting | Tumors harboring mutations leading to dysregulation of the PI3K/AKT pathway     |
| PARPi-ATR inhibitors | Yes    | NCT04062569  | II    | Olaparib + AZD6738                                                       | Recruiting        | Gynaecological cancers                                                           |
| PARPi-ATR inhibitors | Yes    | NCT03787680  | II    | Olaparib + AZD6738                                                       | Recruiting        | Prostate cancer                                                                  |
| PARPi-ATR inhibitors | Yes    | NCT03330847  | II    | Olaparib + AZD6738/ AZD1775                                              | Recruiting        | Metastatic triple negative breast cancer                                          |
| PARPi-ATR inhibitors | Yes    | NCT03878095  | II    | Olaparib + AZD6738                                                       | Recruiting        | IDH1 and IDH2 mutant tumors                                                      |
| PARPi-ATR inhibitors | Yes    | NCT03462342  | II    | Olaparib + AZD6738                                                       | Recruiting        | HGSC                                                                            |
| PARPi-ATR inhibitors | Yes    | NCT03428607  | II    | Olaparib + AZD6738                                                       | Active, not recruiting | SCLC                                                                            |
| PARPi-ATR inhibitors | Yes    | NCT03682289  | II    | Olaparib + AZD6738                                                       | Recruiting        | Clear cell renal cell cancer; Metastatic renal cell cancer; Metastatic urothelial cancer; Metastatic pancreatic cancer; Locally advanced |
Table 2 The feasible combination therapy to enhance PARPi sensitivity and overcome PARPi resistance (Continued)

| Combination therapy | Trials | NCT | Phase | Treatment | Status | Study population |
|---------------------|--------|-----|-------|-----------|--------|-----------------|
| NCT01589419) indicated that the PARPi-IR combination treatment was well-tolerated and show good responses as well [135, 136]. Undoubtedly, further evaluation of PARPi-IR combination treatments is currently underway in multiple phase 2 clinical trials in patients with NSCLC and breast cancer (NCT02412371, NCT01386385, NCT01618357). 3) PARPi-CDKs inhibitors; DNA end resection is depended on cyclin-dependent kinases (CDKs) activity. A number of studies indicated that CDKs played important roles in PARPi resistance [36–41]. CDK inhibitor dinaciclib resensitized TBNC cells, which had acquired resistance to niraparib. In addition to TBNC cells, synthetic lethal strategy combining dinaciclib with niraparib was also highly efficacious in ovarian, prostate, pancreatic, colon, and lung cancer cells [137]. Currently, CDK12 attracted more attentions in PARPi resistance, due to its inactivating somatic alterations were recurrently observed in various cancers. Numerous evidences proved that CDK12 mutation or deficiency lead to cancer cells sensitivity to PARPi [37]. Furthermore, CDK12 inhibitors reversed de novo and acquired PARPi resistance in BRCA1-mutant breast cancer cells [39]. 4) PARPi-immunotherapy; Jiao et al and her colleagues revealed that PARPi upregulated PD-L1 expression in breast cancer cell lines via inactivating GSK3β, which in return leading to attenuate anticancer immunity. Moreover, the combination of PARPi and anti-PD-L1 therapy showed better therapeutic efficacy than each alone [138]. PARPi-mediated modulation of the immune response contributes to their therapeutic effects independently of BRCA1/2 mutations. Recently results suggested that PARPi promoted accumulation of cytosolic DNA fragments because of unresolved DNA lesions, which in turn activated the DNA-sensing cGAS-STING pathway and stimulated production of type I interferons to induce antitumor immunity independent of BRCAness [139]. At present, several clinical trials (NCT02734004, NCT03824704 and NCT02849496) are ongoing. In this term, all these trials may be informative. 5) PARPi-epigenetic drugs; As previously mentioned, epigenetic modification was associated with PARPi sensitivity [113, 117, 118]. Acetylation and deacetylation of histones is one of the most important mechanisms of posttranslational regulation of gene expression [140]. So far, numerous studies have declared that treating with histone deacetylation inhibitors (HDACi) and PARPi exhibited synergy effects due to the induction of HDACi on HRD, which as a result sensitized cancer cells to PARPi [141–144]. Several mechanisms have been observed. Firstly, it was reported that HDACi decreased the expression of DNA repair genes such as RAD51, CHK1, BRCA1 and RAD21 mediated through transcription factor E2F1 [145]. Secondly, HDACi blocked the deacetylation and expression of HSP90, resulting in the degradation of its substrates BRCA1, Rad52, ATR and CHK1 [146]. Finally, recent studies showed that acetylation blocked DNA damage-induced chromatin PARylation and HDACi treatment significantly increased the trapping of PARPi at DSB sites in chromatin [147, 148]. Additionally, low doses of DNA methyltransferase inhibitor (DNMTi) induced BRCAness phenotype through downregulating expression of key HR genes [149]. The combination DNMTi and PARPi enhanced the cytotoxic effect by increasing the PARP “trapping” on DSB sites independent on BRCA mutations [150, 151]. However, there is no clinical trial to evaluate its effect until now. 6) PARPi-other drugs; In addition to the above mentioned, PARPi was also suggested to combine with HSP90 inhibitors, ATR/CHK1 inhibitors and WEE1 inhibitors [152, 153]. BRCA1 function is reliant on HSP90. HSP90 inhibitor, 17-AAG, could induce HRD and increase Olaparib sensitivity of HR-proficient ovarian cancer cell lines [154]. Treating PARPi-resistant cells with 7-dimethylaminoethylamino-17-demethoxygeldanamycin, a HSP90 inhibitor, reversed the resistance state by decreasing the quantity of BRCA1 protein [92]. ATR/CHK1 and WEE1 have emerged as putative BRCAness factors that function in both checkpoint activation and in replication fork stability. ATR/CHK1 inhibitors and WEE1 inhibitors treatment were recently shown to reverse PARPi resistance in cancer cells [152]. Currently, several trials to the safety and efficacy of these combination treatments in sporadic cancers are in progress (NCT03579316, NCT04197713, NCT02576444, NCT02511795, NCT04065269, NCT03787680, NCT03330847, NCT03878095, NCT03462342, NCT03428607, NCT03330847, NCT03878095, NCT03462342, NCT03428607, NCT03682289). In a word, the combination therapy to overcome PARPi resistance and enhance PARPi sensitivity is still in its infancy and has a long way to go. More and more studies are needed to investigate the feasibility in clinic.**

Conclusions and perspectives

In the past few decades, PARPi was successfully developed in treating BRCA mutation patients, which provided proof-of concept that synthetic lethal interactions could be translated into cancer therapy. However, the
preclinical and clinical investigation of PARPi is far from complete. In terms of PARPi resistance, multiple potential resistance mechanisms, such as HR restoration and protection of DNA replication fork have been identified. Nonetheless, the contribution weight of them to PARPi resistance is incomprehensible. Recently, the PRIMA trial results suggested that among patients with newly diagnosed advanced ovarian cancer who had a response to platinum-based chemotherapy, those who received niraparib had significantly longer progression-free survival (PFS) than those who received placebo, regardless of the presence or absence of HRD [155]. Based on it, we assumed that PARPi might kill cancer cells in ways other than DNA repair. The association between PARPi resistance and protection of DNA replication fork confirmed this conjecture. Therefore, we should comprehensively understand how PARPi functions, especially, how do the roles of PARPi in processes unrelated to DNA repair influence the anti-cancer activity of PARPi, which would be conductive to understand the development of resistance. Also, to overcome PARPi resistance and increase PARPi sensitivity, the optimal combination of PARPi and other treatment regimens are urgently needed to identify.

In addition to PARPi resistance, a serious of unanswered questions that could guide the optimal use of PARPi in the future, are not addressed. For example, what other proteins beyond BRCA1 and BRCA2 contribute to the efficacy of PARPi? Currently, PTEN has received a lot of attention as a promising biomarker to predicting the sensitivity of PARPi. PTEN is one of the tumor suppressor genes most frequently inactivated in human cancers [156]. It is reported that loss of PTEN lead to HRD, increased genomic instability and replication fork collapse [157–159]. At present, there is a growing body of preclinical evidence that tumors with loss of PTEN function are defective in HR and may, therefore, be hypersensitive to PARPi [159–161]. Likewise, there are lots of conflicting results that PTEN deficiency has no effect on PARPi sensitivity [162–164]. In a word, vulnerabilities of PTEN-deficient sporadic cancers to PARPi inhibition remain controversial.

Besides, due to additional biological process beyond HR related to sensitivity of PARPi, we need to redefine the concert of concept of “BRCAness” and exploit new techniques of companion diagnostics to predict the response of patients to PARPi [24, 152]. Current BRCAnalysis assay could not effectively identify BRCAness. For example, genomic scars of BRCAness, as they are currently measured, probably reflect the alteration of the genome in the absence of HR over the entire lifetime of a tumor, they might not provide an accurate estimation of whether HR is still defective in tumor cells at the time that treatment is delivered. Other proposed approaches such as the use of mRNA expression signatures and the individual analysis of genetic alterations in HR-related genes are both lack of specificity. RAD51 accumulation and the formation of RAD51-ssDNA play key roles in both HR and protection of stalled DNA replication fork, therefore, RAD51 assay may be feasible in identifying PARPi-sensitive cancer patients and broadening the population who may be response to PARPi-based therapy.

In conclusion, if all these issues can be figured out, we firmly believe that a substantial subset of cancer patients could benefit from PARPi.

**Abbreviations**

PARP: Poly (ADP-ribose) polymerase; PARPi: PARP inhibitor; HR: Homologous recombination; HRD: Homologous recombination repair deficient; PARylation: ADP-riboseylation; DDR: DNA damage response; NHEJ: Non-homologous end joining repair; SSBR: Single stranded break repair; SSB: Single-strand breaks; BER: Base excision repair; gBRCAm: Germline mutations in BRCA1/2; sBRCAm: Somatic mutations of BRCA1/2; HGSOC: High-grade serious ovarian cancer; BRCAm: Mutation of BRCA1/2; MRN: Mre11-Rad50-Nbs1; ssDNA: Single-strand DNA; CDKs: Cyclin-dependent kinases; LOF: Loss-of functions; TNBC: Triple-negative breast cancer; LUAD: Lung adenocarcinoma; cfDNA: Circulating cell-free DNA; ORF: Open reading frame; BRCT: N-terminal domains of BRCA1; mCRPC: Metastatic castration-resistant prostate cancer; m²A: N2-methyladenosine; PAR: PAR glycohydrolase; MDR1: Multidrug resistance protein; oHSV: Oncolytic herpes simplex viruses; GSCs: Glioblastoma stem cells; IR: Ionizing radiation; WBRT: Whole brain radiation therapy; HDAC: Histone deacetylation inhibitors; DNMTi: DNA methyltransferase inhibitor; PFS: Progression-free survival

**Acknowledgements**

Not applicable.

**Authors’ contributions**

He Li and Jing Wang conceived the structure of manuscript and revised the manuscript. Zhao-Yi Liu and He Li made the figures and table. All authors revised the manuscript and approved the final manuscript.

**Funding**

This research was supported by the National Natural Science Foundation of China (No.81972836) and National Key R&D Program (2016YFC1303703).

**Availability of data and materials**

All the data obtained and/or analyzed during the current study were available from the corresponding authors on reasonable request.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

All authors give consent for the publication of manuscript in Molecular Cancer.

**Competing interests**

The authors declare that there is no potential competing interest.

**Author details**

1Hunan Clinical Research Center in Gynecologic Cancer, Hunan Cancer Hospital and The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, 283, Tongzipo Road, Changsha 410013, Hunan, P. R. China. 2Department of Neurosurgery, Xiangya Hospital, Central South University, Changsha 410103, Hunan, People’s Republic of China. 3Department of Neurosurgery, Xiangya Hospital, Central South University, Changsha 410103, Hunan, People’s Republic of China. 4Department of Gynecologic Cancer, Hunan Cancer Hospital and The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, 283, Tongzipo Road, Changsha 410013, Hunan, People’s Republic of China.
1. Jeggo PA, Pearl LH, Carr AM. DNA repair, genome stability and cancer: a historical perspective. Nat Rev Cancer. 2016;16:35–42.

2. Chatterjee N, Walker GC. Mechanisms of DNA damage, repair, and mutagenesis. Environ Mol Mutagen. 2017;58:235–63.

3. Laneinger MF, Riccio AA, Pascal JM. PARP-2 and PARP-3 are selectively activated by 5’ phosphorylated DNA breaks through an alositc regulatory mechanism shared with PARP-1. Nucleic Acids Res. 2014;42:7762–75.

4. Eustermann S, Wu W-F, Laneinger MF, Yang J-C, Easton LE, Riccio AA, Pascal JM, Neuhaus D. Structural basis of detection and signaling of DNA single-Strand breaks by human PARP-1. Mol Cell. 2015;60:742–54.

5. Laneinger MF, Planck JL, Roy S, Pascal JM. Structural basis for DNA damage-dependent poly(ADP-ribosyl)ation by human PARP-1. Science (New York, NY). 2012;336:728–32.

6. Bétermier M, Bertrand P, Lopez BS. Is non-homologous end-joining really an inherently error-prone process? PLoS Genet. 2014;10:e1004086.

7. Jiang X, Li X, Li W, Bai H, Zhang Z. PARP inhibitors in ovarian cancer: sensitivity prediction and resistance mechanisms. J Cell Mol Med. 2019;23: 2303–10.

8. Hoyer W-D, Ehmsen KT, Liu J. Regulation of homologous recombination in eukaryotes. Annu Rev Genet. 2010;44:113–39.

9. Gudmundsdottir K, Ashworth A. The roles of BRCA1 and BRCA2 and associated proteins in the maintenance of genomic stability. Oncogene. 2006;25:5854–74.

10. Isono M, Niimi A, Oike T, Hagiwara Y, Sato H, Seikine R, Yoshida Y, Isobe S-Y, Obuse C, Nishi R, et al. BRCA1 directs the repair pathway to homologous recombination by promoting S3BP1 Diphosphorylation. Cell Rep. 2017;18: 520–32.

11. Sy SJH, Huen MSY, Chen J. PALB2 is an integral component of the BRCA complex required for homologous recombination repair. Proc Natl Acad Sci U S A. 2009;106:7155–60.

12. Hanenberg H, Andreasen PR. PALB2 (partner and localizer of BRCA2). Atlas Genet Cytogenet Oncol Haematol. 2018;22:484–90.

13. Nielsen FC, van Overeem HT, Sørensen CS. Hereditary breast and ovarian cancer: new genes in confined pathways. Nat Rev Cancer. 2016;16:599–612.

14. Cancer Genome Atlas Research N. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474:609–15.

15. Farmer H, McCabe N, Lord CJ, Tutt ANJ, Johnson DA, Richardson TB, Stratford I, Birbeck G, Marais R, et al. GEM in ovarian cancer correlates with platinum-free interval. J Clin Oncol. 2010;28:2512–9.

16. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Anderson C, Smalley SR, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005;434:913–17.

17. Meuth M, Curtin NJ, Helleday T. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005;434:917–20.

18. Lord CJ, Ashworth A. BRCA2 revisited. Nat Rev Cancer. 2016;16:110–24.
nonhomologous end joining and suppression of DNA double-strand break resection. Mol Cell. 2013;49:858–71.

45. Escrivan-Díaz C, Orthez A, Fradet-Turcotte A, Xing M, Young JTF, Tkâj J, Cook MA, Rosebrook AP, Munro M, Canny MD, et al. A cell cycle-dependent regulatory circuit controlled by complex 353BP1-R1F1 and BRCA1-1CIP controls DNA repair pathway choice. Mol Cell. 2013;49:872–83.

46. Wang Y-T, Yuan B, Chen H-D, Xu L, Tian Y-N, Zhang A, He J-X, Miao Z-H. Acquired resistance of phosphatase and tensin homolog-deficient cells to poly(ADP-ribose) polymerase inhibitor and Ara-C mediated by 353BP1 loss and SAMHD1 overexpression. Cancer Sci. 2018;109:21–31.

47. Hurley RM, Wahner Hendrickson AE, Wisser DW, Ansell P, Harrell MI. (2020) 19:107. resection in BRCA1-deficient cells. Nature. 2018;563:522–3.

48. DYNLL1 binds to MRE11 to limit DNA end double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol. 2017;18:1258.

49. Pedrajas D, Robinson D, Omlin A, Tunariu N, et al. DNA-repair defects and Olaparib plus paclitaxel in patients with sensitivity to PARP1- and ATR inhibitors in lung adenocarcinoma. Cancer Res. 2017;77:3040–7.

50. Gupta R, Somranyi K, Narita T, Maskey E, Stanlie A, Kremer M, Typs D, Lammens M, Mailand N, Nussenzweig A, et al. DNA Repair Network Analysis Reveals Shieldin as a Key Regulator of NHEJ and PARP Inhibitor Sensitivity. Cell. 2018;173:972–82.

51. Dev H, Chang T-YW, Lescalle C, de Kruijer I, Martin AG, Pilger D, Coates J, Fradet-Turcotte A, Canny MD, orthography error. Ansell P, Harrell MI. (2020) 19:107. resection in BRCA1-deficient cells. Nature. 2018;563:522–3.

52. Adelmant G, Chowdhury D, Marto JA, D’Andrea AD. TRIP13 regulates DNA repair pathway choice through REV7 conformational change. Nat Cell Biol. 2018;20:2287–96.

53. SChina-Clift M, Wei W, Ostermaier M, et al. Shieldin complex mediates 53BP1-dependent DNA repair. Nature. 2018;560:117–21.

54. Rodriguez D, Robinson D, Omlin A, Tunariu N, et al. DNA-repair defects and Olaparib plus paclitaxel in patients with sensitivity to PARP1- and ATR inhibitors in lung adenocarcinoma. Cancer Res. 2017;77:3040–7.

55. Sczaniecka-Clift M, Wei W, Ostermaier M, et al. Shieldin complex mediates 53BP1-dependent DNA repair. Nature. 2018;560:117–21.

56. Wang Y-T, Yuan B, Chen H-D, Xu L, Tian Y-N, Zhang A, He J-X, Miao Z-H. Acquired resistance of phosphatase and tensin homolog-deficient cells to poly(ADP-ribose) polymerase inhibitor and Ara-C mediated by 353BP1 loss and SAMHD1 overexpression. Cancer Sci. 2018;109:21–31.

57. Hurley RM, Wahner Hendrickson AE, Wisser DW, Ansell P, Harrell MI. (2020) 19:107. resection in BRCA1-deficient cells. Nature. 2018;563:522–3.

58. DYNLL1 binds to MRE11 to limit DNA end double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol. 2017;18:1258.

59. Pedrajas D, Robinson D, Omlin A, Tunariu N, et al. DNA-repair defects and Olaparib plus paclitaxel in patients with sensitivity to PARP1- and ATR inhibitors in lung adenocarcinoma. Cancer Res. 2017;77:3040–7.

60. Gupta R, Somranyi K, Narita T, Maskey E, Stanlie A, Kremer M, Typs D, Lammens M, Mailand N, Nussenzweig A, et al. DNA Repair Network Analysis Reveals Shieldin as a Key Regulator of NHEJ and PARP Inhibitor Sensitivity. Cell. 2018;173:972–82.

61. Dev H, Chang T-YW, Lescalle C, de Kruijer I, Martin AG, Pilger D, Coates J, Fradet-Turcotte A, Canny MD, orthography error. Ansell P, Harrell MI. (2020) 19:107. resection in BRCA1-deficient cells. Nature. 2018;563:522–3.

62. Adelmant G, Chowdhury D, Marto JA, D’Andrea AD. TRIP13 regulates DNA repair pathway choice through REV7 conformational change. Nat Cell Biol. 2018;20:2287–96.

63. SChina-Clift M, Wei W, Ostermaier M, et al. Shieldin complex mediates 53BP1-dependent DNA repair. Nature. 2018;560:117–21.
mechanism of cisplatin resistance in BRCA2-mutated cancers. Nature. 2008;451:1116–20.

45. Kondrashova O, Nguyen M, Shield-Artin K, Tinker AV, Teng NNH, Harrell MI, Kuiper MU, Ho BY, Barker H, Jasim M, et al. Secondary somatic mutations restoring RAD51C and RAD51D associated with acquired resistance to the PARP inhibitor Rucaparib in high-grade ovarian carcinoma. Cancer Discov. 2017;7:984–98.

46. Simmons AD, Nguyen M, Pintus E. Polyclonal BRCA2 mutations following carboplatin treatment confer resistance to the PARP inhibitor rucaparib in a patient with mCRPC: a case report. BMC Cancer. 2020;20:215.

47. Plishayai MI, Blankin AV, Bailey P, Chang DK, Laheru D, Wolfgang CL, Brody JR. BRCA2 secondary mutation-mediated resistance to platinum and PARP inhibitor-based therapy in pancreatic cancer. Br J Cancer. 2017;116:1021–6.

48. Gorinstein EL, Sandofe SD, Chung JH, Gay LM, Holmes O, Erlich RL, Somar, Martin LR, Rose AV, Stephens PJ, et al. BRCA2 reversion mutation associated with acquired resistance to Olaparib in estrogen receptor-positive breast cancer detected by genomic profiling of tissue and liquid biopsy. Clin Breast Cancer. 2018;18:184–8.

49. Mayer F, Gay LM, Lele S, Elvin JA. BRCA1 reversion mutation acquired after treatment identified by liquid biopsy. Gynecol Oncol Rep. 2017;21:57–62.

50. Tao H, Liu S, Huang D, Han X, Wu X, Shao YW, Hu Y. Acquired multiple secondary BRCA2 mutations upon PARP resistance in a metastatic pancreatic cancer patient harboring a BRCA2 germline mutation. Am J Transl Res. 2020;12:612–7.

51. Barber LJ, Sandhu S, Chen L, Campbell J, Kozarewa I, Fenwick K, Assiotis I, Barber LJ, Sandhu S, Chen L, Campbell J, et al. A genetic screen using the PiggyBac transposon in haploid cells identifies Parp1 as a mediator of olaparib toxicity. PLoS One. 2013;8:e61520.

52. Peng Y, Liao Q, Tan W, Peng C, Hu Z, Chen Y, Li Z, Li J, Zhen B, Zhu W, et al. The deubiquitylating enzyme USP15 regulates homologous recombination. Nucleic Acids Res. 2015;43:893–903.

53. Kais Z, Rondinelli B, Holmes A, O’Leary C, Kozono D, D’Andrea AD, Ceccaldi R. FANC2 maintains fork stability in BRCA1/2-deficient tumors and promotes alternative end-joining DNA repair. Cell Rep. 2016;15:2488–99.

54. Michl J, Zimmer J, Buffa FM, McDermott U, Tarsounas M. FANC2 limits replication stress and genome instability in cells lacking BRCA2. Nat Struct Mol Biol. 2016;23:755–7.

55. Tagalilatela A, Alvarez S, Leuzig V, Sannino V, Ranjha L, Huang JW, Madubata C, Anand R, Levy B, Rabadan R, et al. Restoration of Replication Fork Stability in BRCA1- and BRCA2-Deficient Cells by Inactivation of SNF2-Family Fork Remodelers. Mol Cell. 2017;68:414–30.e418.

56. Bérous T, Couch FB, Mason AC, Eichman BF, Manosas M, Cortez D. Substrate-selective repair and restart of replication forks by DNA topoisomerase I inhibition. Nat Struct Mol Biol. 2017;24:1589–600.

57. Kondrashova O, Topp M, Neric K, Lieschke E, Ho HY, Harrell MI, Zapparoli GV, Hadley A, Hollar R, Boehm E, et al. Methyltransferase of all BRCA1 copies predicts response to the PARP inhibitor rucaparib in ovarian carcinoma. Nat Commun. 2018;9:3970.

58. Choi YE, Meghani K, Brait M, Leclerc L, He YJ, Day TA, Elias KM, Drapkin R, Weinstock DM, Dao F, et al. Platinum and PARP inhibitor resistance due to overexpression of MicroRNA-622 in BRCA1-mutant ovarian Cancer. Cell Rep. 2016;14:429–39.

59. Meghani K, Fuchs W, Detappe A, Drané P, Gogola E, Rottenberg S, Sathe AA, Zhao R, Cortez D. RAD51 Promotes Genome Stability and Modulates Chemosensitivity by Regulating RAD51 at Replication Forks. Mol Cell. 2017;67:374–86.e375.

60. Schlaicher K. PARPs focus the spotlight on replication fork protection in cancer. Nat Cell Biol. 2017;19:1309–10.

61. Kondrashova O, Topp M, Neric K, Lieschke E, Ho HY, Harrell MI, Zapparoli GV, Hadley A, Hollar R, Boehm E, et al. The debiquitylating enzyme USP15 regulates homologous recombination repair and cancer cell response to PARP inhibitors. Nat Commun. 2019;10:1224.

62. Guo X, Bai Y, Zhao M, Zhou M, Shen Q, Yun CH, Zhang H, Zhu WG, Wang J. Acetylation of 53BP1 dictates the DNA double strand break repair pathway. Nucleic Acids Res. 2018;46:869–73.

63. Fukumoto T, Zhu H, Nacarilli T, Karasheva S, Fatkhutdinov N, Wu S, Liu P, Kosenkov AV, Showe LC, Jean S, et al. N(6)-methyladenosine of FZD10 mRNA contributes to PARP inhibitor resistance. Cancer Res. 2019;79:2812–20.

64. Du Y, Yamaguchi H, Wei Y, Hu JL, Wang HL, Hu YH, Lin WC, Yu WH, Leonard PG, Lee GR, et al. Blocking c-met-mediated PARP1 phosphorylation enhances anti-tumor effects of PARP inhibitors. Nat Med. 2016;22:194–201.

65. Gogola E, Duarte AA, de Ruiter JR, Wiegent WW, Schmid JA, de Bruijn R, James DJ, Guerrero Llobet S, Vis DJ, Annunzio S, et al. Selective Loss of PARP Restores PARPylation and Counteracts PARP Inhibitor- Mediated Synthetic Lethality. Cancer Cell. 2018;33:1078–93.e1012.

66. Pettitt SJ, Rehman FL, Bajrami I, Brough R, Wallberg F, Kozarewa I, Fenwick K, Assiotis I, Chen L, Campbell J, et al. A genetic screen using the PiggyBac transposon in haploid cells identifies Parp1 as a mediator of olaparib toxicity. PLoS One. 2013;8:e61520.
132. Liu X, Han SK, Anderson M, Shi Y, Semizarov D, Wang G, McGonigal T, Roberts L, Lasko P, Palma J, et al. Acquired resistance to combination treatment with temozolomide and ABT-888 is mediated by both base excision repair and homologous recombination DNA repair pathways. Mol Cancer Res. 2009;7:1686–92.

133. Christie EL, Pattnaik S, Beach J, Copeland A, Rashoo N, Fereday S, Hendley J, Alsop K, Brady SL, Lamb G, et al. Multiple ABCB1 transcriptional fusions in drug resistant high-grade serous ovarian and breast cancer. Nat Commun. 2019;10:1295.

134. Mehta MP, Wang D, Wang F, Kleinberg L, Brade A, Robins HI, Turaka A, Czito BG, Deming DA, Jameson GS, Mulcahy MF, Vaghefi H, Dudley MW, Rottenberg S, Jaspers JE, Kersbergen A, van der Burg E, Nygren AO, Zander SA, Derksen PW, de Bruin M, Zevenhoven J, Lau A, et al. High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. Proc Natl Acad Sci U S A. 2008;105:17079–84.

135. Oplustilova I, Wolanin K, Mistrk M, Korinkova G, Simkova D, Bouchal J, Lenobbel B, Barkova J, Lau A, O'Connor MJ, et al. Evaluation of candidate biomarkers to predict cancer cell sensitivity or resistance to PARP-1 inhibitor treatment. Cell Cycle. 2012;11:3857–60.

136. Kohlhapp FJ, Kaufman HL. Molecular pathways: mechanism of action for PARPi triggers the STING-dependent immune response and enhances the therapeutic efficacy of immune checkpoint blockade independent of PARP inhibitor olaparib in triple-negative breast cancer. Cancer Res. 2015;75:33.

137. Kachhap SK, Rosmus N, Collis SJ, Korstenhuis MS, Wissing MD, Hedayati M, Shabbeer S, Mondonca J, Deangelis J, Marchimoniali L, et al. Downregulation of homologous recombination pathway DNA repair enzymes by HDAC inhibition in prostate cancer is mediated through the E2F1 transcription factor. PLoS One. 2015;10:e011208.

138. Kim Y, Kim A, Sharp A, Sharip A, Jiang J, Yang Y, Xie Y. Reverse the resistance to PARP inhibitors. Int J Biol Sci. 2017;13:198–208.

139. Liszczak G, Diehl K, Dann GP, Muir TW. Acetylation blocks DNA damage-induced chromatin ADP-ribosylation. Nat Chem Biol. 2018;14:4837–40.

140. Robert C, Nagaria PK, Pawar N, Adevuyi A, Gopi J, Meyers DJ, Cole PA, Rassoul FV. Histone deacetylase inhibitors decrease NHEJ both by acetylation of repair factors and trapping of PARP1 at DNA double-strand breaks in chromatin. Leuk Res. 2016;45:14–23.

141. Abbotts R, Topper MJ, Biondi C, Fontaine D, Goswami R, Stojanovic L, Choi EY, McLaughlin L, Kogan AA, Xia L, et al. DNA methyltransferase inhibitors induce a BRCA1analogous phenotype that sensitizes NSCLC to PARP inhibitor and ionizing radiation. Proc Natl Acad Sci U S A. 2019;116:26689–88.

142. Baldan F, Mio C, Allegri L, Puppin C, Russo D, Filetti S, Damante G, D’Arrigo S, Ferrari E, Mariani R, et al. Combination therapy with temozolomide and PARP inhibitor PJ34 and histone deacetylase inhibitor vorinostat on leukemia cell lines. Anticancer Res. 2014;34:1849–56.

143. Baldan F, Mio C, Allegri L, Puppin C, Russo D, Filetti S, Damante G. Synergy between HDAC and PARP inhibitors on proliferation of a human anaplastic thyroid Cancer-derived cell line. Int J Endocrinol. 2015;2015:978371.

144. Ha K, Fiskus W, Choi DS, Bhaskara S, Cerchietti L, Devaraj SG, Shah B, Sharma S, Chang JC, Melnick AM, et al. Histone deacetylase inhibitor treatment induces ‘BRCAles’ and synergistic lethality with PARP inhibitor and cisplatin against atypical triple-negative breast cancer cancers. Oncotarget. 2014;5:6357–50.

145. Min A, Im SA, Kim DK, Song SH, Kim HJ, Lee KH, Kim TY, Han SW, Oh DY, Kim TY, et al. Histone deacetylase inhibitor, suberylanilide hydroxamic acid (SAHA), enhances anti-tumor effects of the poly (ADP-ribose) polymerase (PARP) inhibitor olaparib in triple-negative breast cancer cancers. Breast Cancer Res. 2015;17:33.

146. Christie EL, Pattnaik S, Beach J, Copeland A, Rashoo N, Fereday S, Hendley J, Alsop K, Brady SL, Lamb G, et al. Multiple ABCB1 transcriptional fusions in drug resistant high-grade serous ovarian and breast cancer. Nat Commun. 2019;10:1295.

147. Audia JE, Campbell RM. Histone modifications and Cancer. Cold Spring Harb Perspect Biol. 2016;8:a019521.
endometrioid endometrial adenocarcinomas predicts sensitivity to PARP inhibitors. Sci Transl Med. 2010;2:53ra75.

161. Forster MD, Dedes KJ, Sandhu S, Frentzas S, Kristeleit R, Ashworth A, Poole CJ, Weigelt B, Kaye SB, Molife LR. Treatment with olaparib in a patient with PTEN-deficient endometrioid endometrial cancer. Nat Rev Clin Oncol. 2011;8:302–6.

162. Fraser M, Zhao H, Luoto KR, Lundin C, Coackley C, Chan N, Joshua AM, Bismar TA, Evans A, Helleday T, Bristow RG. PTEN deletion in prostate cancer cells does not associate with loss of RAD51 function: implications for radiotherapy and chemotherapy. Clin Cancer Res. 2012;18:1015–27.

163. Sandhu SK, Schelman WR, Wilding G, Moreno V, Baird RD, Miranda S, Hylands L, Risnes R, Forster M, Omlin A, et al. The poly(ADP-ribose) polymerase inhibitor niraparib (MK4827) in BRCA mutation carriers and patients with sporadic cancer: a phase 1 dose-escalation trial. Lancet Oncol. 2013;14:882–92.

164. Bian X, Gao J, Luo F, Rui C, Zheng T, Wang D, Wang Y, Roberts TM, Liu P, Zhao JJ, Cheng H. PTEN deficiency sensitizes endometrioid endometrial cancer to compound PARP-Pi3K inhibition but not PARP inhibition as monotherapy. Oncogene. 2018;37:341–51.

**Publisher’s Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.