Beyond resistance to PARP inhibition: Mechanisms and effective treatment options

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Poly-ADP ribosylation polymerase (PARP) inhibition is a promising new strategy that specifically kills malignancies with mutation of BRCA genes. However, as in other agents, it is known that continuous treatment with PARP inhibitors generates acquired resistance in these tumors. In this review, we discuss about the potential mechanisms of acquired resistance to the PARP inhibition that is routed through up-regulation of the NF-κB signaling pathway and furthermore discuss about potential treatment options for malignancies that gain such resistance.

Keywords: PARP inhibitor; resistance; BRCA1; breast cancer; ovarian cancer; NF-κB and bortezomib


Introduction

Development of genomic sequencing techniques using next generation sequencers has revealed a multitude of genes are related to hereditary breast and ovarian cancer syndrome (HBOC) [1]. Among these genes, familial breast and ovarian cancer susceptible genes, BRCA1 and BRCA2, have been intensively studied because of their high penetrance [2]. Additionally, mutation of the BRCA1 is linked to basal-like breast cancer, a most aggressive subtype of breast cancer [3]. Breast cancer is divided into 5 subgroups based on gene expression pattern and the basal-like breast cancer does not display hormonal receptors and human epidermal growth factor receptor 2, HER2, resulting in limited therapeutic options using hormonal therapy and trastuzumab, an inhibitor for HER2 signaling [4, 5]. More importantly, basal-like breast cancer is linked to poorest clinical outcome of all the subtypes in breast cancer [5, 6]. Indeed, a meta-analysis across several reliable datasets has revealed that mutation of BRCA1 is linked to shorter survival in breast cancer [7]. Although impact of mutation in BRCA1 gene in ovarian cancer has not been as well studied as in the breast, this evidence clearly suggests that development of an effective therapy for BRCA1-deficient tumor is an urgent requirement.

Poly-ADP ribosylation polymerase (PARP) is involved in DNA repair pathway and inhibition of PARP enhances the effect of anti-cancer therapy, enhancing DNA damage [8]. Rucaparib, a class of PARP inhibitor, was developed as a drug that potentiates the effect of temozolamide, an alkylating agent, or radiotherapy, and it showed promising results in clinical trials [9, 10]. Olaprib is also the same class of PARP inhibitor and interestingly it was shown that single agent treatment with olaparib kills tumor cells with mutation in BRCA genes [11, 12]. These findings stimulated further study, as to whether other PARP inhibitors might result in the same effect, and indeed, three inhibitors such as rucaparib, olaprib and BMN 673 proved effective [9, 11-13]. Therefore these agents are currently underway in clinical trials [10, 14-17]. Also it was revealed that suppression of
protein product of BRCA gene (BRCAness) was sensitive to PARP inhibitor treatment \[18\]. Breast cancers with mutation of BRCA1 gene, such as the basal-like phenotype \[19\] displays similar clinical feature with tumors displaying BRCAness underlining the significance of this finding in the field of cancer treatment. Several recent reports have further revealed that disruption of genes that are involved in homologous recombination, the machinery involved in DNA double strand break repair, also resulted in increased sensitivity to PARP inhibition \[20\]. These evidence points to the underlying mechanism as to how PARP inhibitors preferentially kills BRCA-deficient tumor, however, the exact the complete mechanism awaits to be discovered. Nonetheless, olaparib showed promising results in clinical trials both in breast and ovarian cancer, and finally it has been approved by U.S. Food and Drug Administration (FDA) as a chemotherapy adjuvant in advanced ovarian cancer with mutation of BRCA genes \[15, 21\].

Acquired resistance of cancer cells to drugs is a common occurrence in cancer treatments. One of the promising anti-cancer drug pathway through PARP inhibition, also generates tumor resistance. Several reports have revealed underlying mechanisms of the resistance to PARP inhibition \[22-26\]. In this review, we not only discuss about the different mechanisms behind resistance to PARP treatment, but also discuss about candidate treatment options for malignancies which acquire resistance to PARP inhibition.

Materials and methods

Cell culture and cell viability assay

UWB1.289 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C.

Cell viability assay

Cells were plated into 96-well plates at a density of 3000 cells per well. Different doses of drugs were added, and the plates were incubated at 37°C for a week. Cell Titer-Blue reagent (Promega) was used to assay for cell viability according to the manufacturer’s guidelines. Briefly, the plates were incubated at 37°C for approximately 1-2 hours in a humidified 5% CO₂ atmosphere. The intensity of Titer-Blue signal, and thus fraction of viable cells / well was determined using the Plate Reader (Perkin Elmer/Packard) at A590 nm. Each experiment was done in quadruplicate.

Crosstalk of drug resistance among anti-cancer drugs

Several PARP inhibitors are currently under clinical trials \[10, 14-17\]. Among these PARP inhibitors, the closest in line to clinical use is olaparib \[14-17\]. Indeed, the drug olaparib was approved by FDA as an anti-cancer drug for advanced ovarian cancer with mutation in BRCA genes \[21\]. Rucaparib is one more PARP inhibitor that has been reported to be in clinical trials \[9, 10\]. Both olaparib and rucaprib have similar molecular effect, in that they inhibit both PARP1 and PARP2 with similar affinity \[13\]. Recently we generated PARP inhibitor-resistant cell lines by continuous exposure with olaparib, both in the ovarian cancer background (UWB1.289) and breast cancer background (HCC1937), both of which harbor homozygous mutations in BRCA1 gene, \[24\]. The PARP inhibitor-resistant lines both in UWB1.289 cells (UWB-R) and HCC1937 (HCC-R) displayed resistance in the micro-molar scale, even though they were treated with nano-molar dose of olaparib indicating that the drug resistance phenotype might be an outcome of long-term culture. Currently, only a few reports have investigated cross-sensitivity among PARP inhibitors \[27\]. Therefore we verified the cross-resistance to PARP inhibitors. The PARP
inhibitor-resistant lines, both the UWB-R and the HCC-R, displayed resistance to rucarabine as well [24]. Apart from crosstalk in resistance among PARP inhibitors, it is known that PARP inhibitor-resistant cells are also resistant to other anti-cancer drugs such as cross-linking agents, but not to microtubule poisons [22, 27]. We assessed whether our PARP inhibitor-resistant cells displayed similar phenotypes. The UWB-R cells display resistance to epirubicine, a topoisomerase II inhibitor (Figure 1a). Interestingly, the resistant UWB-R cells indeed are sensitive to taxol, a microtubule stabilizing agent as suggested previously (Figure 1b). Thus, PARP inhibitor-resistant cells are also resistant to other class of PARP inhibitors and other anti-cancer drugs causing DNA damage, but not to microtubule poisoning agents.

Mechanism of resistance to PARP inhibition

Secondary mutation in BRCA genes

It was reported that continuous treatment with PARP inhibitor to patients with breast or ovarian cancer resulted in resistance to the drug [22]. A finding that has been reproduced both in vivo as well as in vitro [22, 28]. First report of the resistance to PARP inhibitor was the study using pancreatic cancer cell line with c.6174delT frame shift mutation in BRCA2 [22]. The c.6174delT mutation produces truncated form of BRCA2 protein, which lacks two BRC repeats, the DNA-binding/DSS1 interacting domain and a C-terminus domain that binds to Rad51, a recombinase required for homologous recombination repair for DNA double strand breaks. In terms of function, this mutation prevents the accumulation of Rad51 at the sites of DNA damage and this results in deficiency in homologous recombination [29]. Cells with deficiency in homologous recombination display increased sensitivity to PARP inhibitors, therefore loss of the accumulation of Rad51 at the sites of DNA damage in the cell line is one possible reason for the increased sensitivity to PARP inhibitor treatment [20]. This report revealed that the PARP inhibitor-resistant cells had the original BRCA2 gene with the original mutation as well as a new BRCA2-like gene that had an intact open reading frame (ORF), resulting from an intragenic deletion of the non-sense mutation, leading to restoration of the ORF, BRC repeats and the DNA binding domain. This novel BRCA2-like gene compensates for Rad51 accumulation at sites of DNA damage. Another study identified revertant mutants in BRCA2 gene that restored gene function in three cell lines that were established from the same patient with ovarian carcinoma with a preexisting loss of function mutation in BRCA2 gene [28]. Two of the three cell lines were derived from collection of ascites after tumor relapsing with acquiring resistance to cisplatin, an intra-strand cross-linking agent and also resistant to PARP inhibitor. Surprisingly, the cell lines that were resistant to both cisplatin and PARP inhibitor reverted the original mutation to regain wild type BRCA2 gene status, but the cell line that was sensitive to these drugs conserved the original mutation in BRCA2 gene. Thus both these studies exemplify restoration of mutation in BRCA2 gene to wild type status as a possible mechanism of acquired resistance to PARP inhibitors [28]. Apart from PARP inhibitor, it is also reported that reversion of the original mutation in BRCA genes occurs after acquiring resistance. In BRCA1, for instance, the restoration of the original mutation is seen more frequently in tumors with acquired resistance to cisplatin than in tumors without the resistance [30]. Although direct evidence suggesting restoration of mutation in BRCA1 gene after acquiring resistance to PARP inhibitor is not available to date, accumulated evidence suggest that PARP inhibition could be the major route for reversion of the original mutations.

Increased drug export

P-glycoproteins, identified in multidrug resistant cancer cells, located at cell membrane, regulate removal of cellular toxins such as drugs [31]. The protein family contains ATP-binding domain, which is required for ATP-binding cassette (ABC) transporters, amongst them the best-known ABC transporter is ABCB1, a protein product of ABCB1 (MDR1). In normal human tissues, ABCB1 is expressed in brain and gastrointestinal tract to remove harmful chemicals from these tissues. In cancer cells, expression of ABCB1 is increased when the cells acquire resistance to anti-cancer drugs such as anthracyclines, vinca alkaloids, colchicines, epipodophyllotoxins, and paclitaxel. Other family members such as ABCC1 are also involved in drug resistance [31] through the same mechanism of action. An elegant study analyzed the impact of the efflux pump on PARP inhibitor-resistance using an in vivo model to mimic BRCA1-deficient breast cancer as accurately as possible. By using BRCA1-deficient tumor from conditional BRCA1 knockout mouse as xenografts in wild type mice, and continuous intra-peritoneal administration of PARP inhibitor allowed a subset of the transplanted tumor to develop resistance to the drug [25]. The degree of function of the efflux pump is simply reflected by the expression level of transcripts of the transporter genes [31] and therefore resection of the tumors, and expression levels of transcripts of the efflux transporter genes in these xenografts revealed that mRNA expressions of Abcb1a or Abcb1b were elevated. Furthermore, they found that tariquidar, a specific inhibitor of P-glycoprotein, reversed the resistance to PARP inhibitor [25]. Furthermore, a recent report shows that knockout of Abcb1 in a background of BRCA1 knockout delays acquired resistance following PARP inhibition [23]. However, as
discussed in the following section, regardless of effective treatments, all the tumors with knockout of the Abcb1 obtain resistance eventually [23]. These evidence suggest that over-expression of efflux pumps is a possible mechanism of resistance to PARP inhibitors but this mechanism may be involved only in an early stage of acquiring drug resistance.

**Resuming homologous recombination**

DNA double strand breaks are repaired by homologous recombination or non-homologous end joining [24]. The BRCA genes are involved in homologous recombination and loss of function of these genes results in homologous recombination defect [32]. On the other hand, p53-binding protein (53BP1) is a positive regulator of non-homologous end joining [33], 53BP1 accumulates at the sites of DNA damage through binding of ubiquitination-dependent recruitment (UDR) motif to lysine 20-dimethylated histone H4 (H4K20me2) and lysine 15-ubiquitinated histone H2A (H2AK15-Ub) catalyzed by RNF168 [33]. BRCA1-deficient cells tend to lean towards non-homologous end joining for DNA repair, preferentially because of the homologous recombination-defect, however interestingly, forced inactivation of 53BP1 in BRCA1-deficient cells drives homologous recombination. Interestingly, partial knockdown of 53BP1 in BRCA1-deficient cells causes resistance to PARP inhibitor [34]. Therefore whether 53BP1-deficiency could influence secondary resistance to PARP inhibitor-resistant cells was investigated. As discussed above, hyper-action of the efflux pump is one possible mechanism of resistance to PARP inhibitor (especially in early step of acquiring the resistance) [25]. Consistently, genetic inactivation of Abcb1 results in longer latency to acquire resistance to PARP inhibitor in BRCA1 deficient cancer, however all the tumors eventually obtain the resistance to PARP inhibitor [23]. Interestingly, about 30 percent of the PARP inhibitor-resistant tumor showed complete absence of 53BP1 protein expression, suggesting protein loss by suppression of expression was crucial to mediating this resistance. Interestingly, genetic alteration in 53BP1 gene due to mutations was found to be inadequate in mediating resistance [23]. This evidence suggests that inactivation of 53BP1 may indeed be a critical resistance mechanism to overcome PARP inhibition through re-activation of homologous recombination mediated repair.

**Increased phosphorylation of ribosomal protein S6**

Phosphoinositide 3-kinase (PI3K) activates Akt, a serine-threonine kinase, and the PI3K/Akt pathway is implicated in cancer growth and survival [35]. Among many types of cancers, including breast and ovarian cancer, the PI3K/Akt pathway is often found up-regulated [35]. PI3K/Akt pathway is linked to BRCA1 mutation, as it is known that BRCA1-deficiency causes up-regulation of Akt pathway [36]. The actuated PI3K/Akt pathway activates mTORC1 (mTOR complex1), a direct mediator of the pathway, and this complex activates p70 S6 kinase that promotes cell growth and angiogenesis [37]. A recent report revealed that phosphorylation of ribosomal protein S6 was increased after prolonged exposure to PARP inhibitor in BRCA1-deficient cells, but not in BRCA1-proficient cells. Inversely, knock-in mutation of ribosomal S6 in which phosphorylatable serine residues are no longer available in BRCA1-deficient cells did not develop resistance to PARP inhibitor [26]. The report also showed that co-treatment of PARP inhibitor with rapamycin, a selective inhibitor of mTORC1, reversed the resistance to PARP inhibitor both in vitro and in vivo [28], suggesting phosphorylation of ribosomal protein S6 through activation of BRCA1-PI3K/Akt/mTOR axis may play a pivotal role in acquiring resistance to PARP inhibitor. However, implication of the phosphorylation of the serine residues in ribosomal protein S6 in DNA damage response is not well known. Recent work revealed that the knock-in mutation that prevents phosphorylation of ribosomal protein S6 in mice generates precancerous lesion in pancreas with increased foci formation of γH2AX as well as 53BP1 [38]. This suggests that phosphorylation of ribosomal protein S6 is involved in DNA damage response and abrogation of the phosphorylation causes defect in the DNA damage repair. Taken together, hyperphosphorylation of ribosomal protein S6 mediated by BRCA1-PI3K/Akt/mTOR axis can also be a mechanism of acquired resistance to PARP inhibition, through alteration of the cellular response to DNA damage.

**Up-regulation of NF-κB signaling**

Yet until now, comprehensive screening to investigate the mechanism of acquired resistance to PARP inhibitor has not been reported. Towards this end we compared gene expression pattern in parental UWB1.289 (UWB) and UWB-R using RNA sequencing, and this was the first report to investigate the mechanism of PARP inhibitor-resistance exclusively through genome scale expression changes [24]. From these analyses almost all the downstream pathways of NF-κB signaling were preferentially up-regulated in UWB-R compared to the parental cells. NF-κB is a transcription factor and it is involved in immunity and inflammatory response [39]. NF-κB is often up-regulated in many types of malignancies resulting in activation of cancer cell proliferation, apoptotic resistance and cell migration [39, 40]. Also it is known that NF-κB effect on drug resistance supercedes its influence as an anti-apoptotic effector [41]. There are two pathways that activate NF-κB signaling, either the canonical or the non-canonical pathway. In canonical pathway, stimuli such as microbial and viral infection
activate IκB kinase (IKK) complex (IKKα, IKKβ and NEMO), mainly IKKβ, and the activated IKKβ phosphorylates IκBα at Ser-32 and Ser-36. This phosphorylation event triggers ubiquitination of IκBα by SCFβTrCP E3 ubiquitin ligase followed by degradation by 26S proteasome [39, 42]. When IκBα is degraded, p65/p50 heterodimer, a NF-κB complex, is targeted into nucleus to promote transcription of their responsive gene. In non-canonical pathway, stimuli such as B cell-activating factor activate IKKα and the activated IKKα phosphorylates p100 [43]. The phosphorylated p100 undergoes an ubiquitin dependent partial degradation to generate p52. After processing of p100, the p52-REL-B heterodimer is targeted to nucleus for transcription [39]. Given this perspective, up-regulation of NF-κB signaling can be confirmed with 1. Quantitative RTPCR, to evaluate whether transcripts of downstream genes in NF-κB signaling are up-regulated, 2. Nuclear localization of p65, 3. Luciferase reporter gene assay. All three assays showed that NF-κB signaling were indeed up-regulated in UWB-R compared to the parental UWB cells [24]. Apart from UWB cell line that was used for screening, HCC-R also displayed up-regulation of NF-κB signaling. Furthermore, knock down of p65, a core component of NF-κB signaling, reversed the resistance to PARP inhibitor treatment [24]. These observations indicate that up-regulation of NF-κB signaling as a new mechanism of acquired resistance to PARP inhibitor treatment. As mentioned above, up-regulation of NF-κB signaling is only one of the several mechanisms in resistance to anti-cancer therapy. However interestingly, up-regulation of genes that are involved in anti-apoptosis was not seen both in UWB-R and in HCC-R [24, 41] indicating that NF-κB signaling is involved in drug resistance distinct from its known anti-apoptotic effect.

**Therapeutic implication for PARP inhibitor-resistance**

**Crosstalk in drug resistance**

PARP inhibitor-resistant cells also display resistance to other anti-cancer agents including PARP inhibitors, but not to microtubule poisoning drugs such as taxane (Figure 1b) [22], suggesting that there is a distinct drug toxicity synergy in action. This suggests that microtubule poisoning might be a therapeutic option for cancers acquiring resistance to PARP inhibitor treatment, since currently microtubule poisons are widely in use for cancer treatment, much knowledge about their safety and efficacy is already available.

**Inhibition of efflux transporter**

Increased action of efflux pump is a key resistance mechanism to PARP inhibition, it proposes that inhibition of drug transporter may reverse the resistance to PARP inhibitor. Co-treatment with an inhibitor of P-glycoprotein, indeed restores the sensitivity to PARP inhibitor [25]. Several inhibitors of efflux pump have been developed and are widely in use such as the first generation inhibitors like cyclosporine, amiodarone and tamoxifen. Sadly, these failed to restore sensitivity to the multidrug resistance in clinical trials because of lack of specificity and potency. Second generation inhibitors such as PSC 833 are more potent and selective, however it often requires reduction in dose of anti-cancer agents (i.e., 25% reduction for etoposide and 66% reduction for paclitaxel). This dosing reduction might affect and could have eventually resulted in failure of sensitizing tumors to chemotherapy in clinical trials [31, 44]. Tariquidar is a third generation efflux pump inhibitor, the most potent and specific inhibitor for P-glycoprotein. A recent study reveals that tariquidar also inhibits breast cancer resistance protein (BCRP), another efflux transporter at a certain concentration [45]. Nevertheless, inhibition of the efflux transporters may provide benefit for the PARP inhibitor-resistance in the clinic. However, the effects of non-specific binding of tariquidar to efflux pump in normal tissues such as blood-brain barrier is still under investigation [46]. A phase II clinical trial to evaluate the effect of tariquidar in restoration of resistance to anthracycline or taxane in breast cancer has been reported, although the cohort is not large, however it showed limited efficacy [44]. As described above, efflux transporters obviously play an essential role in eliminating toxins from human body, therefore, inhibition of the efflux transporters may cause unexpected adverse effects. Furthermore, genetic inactivation of Abcb1, a responsive gene for P-glycoprotein, causes prolonged latency to obtain resistance to PARP inhibition, but eventually the tumor acquire resistance [23]. These evidences indicate that inhibitors of efflux pump may have limited effect in restoration of resistance not only to PARP inhibitor but also to other agents in practical clinical use.

**Targeting PI3K/Akt/mTOR**

In breast cancer, PI3K/Akt/mTOR pathway is involved in resistance to hormonal therapy. A phase II clinical trial of co-treatment of everolimus, an mTOR inhibitor, with letrozole, an aromatase inhibitor, to estrogen receptor-positive breast cancer significantly increases the efficacy of the hormonal therapy [47]. Similarly, as discussed above, phosphorylation of ribosomal protein S6, a downstream effector of PI3K/Akt/mTOR, is up-regulated in PARP inhibitor-resistant cells, therefore rapamycin, an inhibitor of mTORC1, reverses resistance to PARP inhibitor [26]. In past, the fact that rapamycin or its analogs called rapalogs displayed limited anti-tumor activity, engendered the development of new generation of inhibitors of PI3K/Akt/mTOR such as dual PI3K-mTOR inhibitor [48].
Indeed, preclinical and clinical experiments showed that the dual inhibitors displayed inhibitory effects on growth in many types of tumors including breast, hepatic, ovarian, prostate and pancreatic cancers [47, 49]. This new generation of PI3K-mTOR inhibitors or even rapalogs are well tolerated and do not show severe side effects in clinical trials [49].

Therefore, inhibitors of PI3K/Akt/mTOR signaling including rapamycin are candidate drugs that could restore resistance to PARP inhibition. Especially the new generation of inhibitor for PI3K/Akt/mTOR signaling has an anti-tumor effect, suggesting even single agent treatment with the inhibitor may be beneficial for a subset of PARP inhibitor-resistant tumors. Thus, targeting PI3K/Akt/mTOR may be an effective treatment. However, it is noteworthy that BEZ235, a dual PI3K-mTOR inhibitor, also causes acquired resistance in the tumor [49]. Furthermore it is known that inhibition of mTORC1 results in activation of mitogen activated protein kinase (MAPK) via PI3K feedback loop [50]. The MAPK is a core member of Raf-MAPK/extracellular signal-regulated kinase (ERK) (Raf-MAPK-ERK) signaling pathway that promotes cell proliferation, cell survival and metastasis and the pathway is often up-regulated in certain types of cancers [51]. Thus, treatment with inhibitor for the PI3K/Akt/mTOR signaling in turn may promote tumor growth or metastasis.

**NF-κB inhibition**

The other mechanism of resistance to PARP inhibitor is up-regulation of NF-κB signaling [24]. This suggests that inhibition of NF-κB signaling should restore the sensitivity to PARP inhibition. NF-κB is a well-established deregulated signaling cascade in several malignancies; therefore NF-κB signaling is an attractive target for the development of anti-cancer agents. However, inhibitors of NF-κB signaling have not been yet approved for clinical use. In preclinical experiments, several inhibitors of IKK indeed suppress growth of multiple myeloma cells through inhibition of NF-κB signaling. BAY 11-7082, for instance, inhibits phosphorylation of IκBα, in turn, suppresses NF-κB signaling [52]. The first instance of utilizing an inhibitor of NF-κB signaling such as BAY 11-7082 in PARP inhibitor-resistant cells was based on the premise that mis-regulated NF-κB signaling resulted in resistance to PARP inhibitor. However, interestingly, PARP inhibitor-resistant cells display increased sensitivity to monotherapy with BAY 11-7082 [24]. This suggests that mis-regulated NF-κB signaling leads to a signaling addiction in these cells and as a secondary feature they might acquire resistance to PARP inhibition. These observations are seen both in ovarian cancer cells and breast cancer cells [24]. Therefore NF-κB signaling is an attractive target in PARP inhibitor-resistant breast and ovarian cancers, however again lack of NF-κB inhibitors approved for clinical use limits this intervention strategy.

Bortezomib, a reversible 26S proteosome inhibitor, is approved by FDA as an anti-cancer agent for multiple myeloma (MM), an example of malignancy with up-regulation of NF-κB signaling [53-55]. The exact mechanism how bortezomib exerts anti-tumor activity in multiple myeloma is not known. However, one explanation for the mechanism is that bortezomib protects IκB proteins, an inhibitory factor in NF-κB signaling, from ubiquitin-dependent proteasomal degradation as it is an inhibitor of 26S proteasome, resulting in inhibition of NF-κB signaling [53-55]. However, it is still controversial whether bortezomib indeed suppresses NF-κB signaling, because following reports suggest that bortezomib promotes, rather than inhibits, the NF-κB signaling [56, 57]. Nevertheless, it is clear that bortezomib inhibits growth of MM cells harboring up-regulation of NF-κB signaling. Our latest report showed that bortezomib inhibits NF-κB signaling at a certain dose and co-treatment with PARP inhibitor preferentially inhibits PARP inhibitor-resistant cells [24]. Furthermore, similar to BAY 11-7082, PARP inhibitor-resistant cells are sensitive to bortezomib [24]. These evidences suggest that bortezomib or inhibitors for NF-κB signaling may be candidate drugs for a subset of PARP inhibitor-resistant tumors with up-regulation of NF-κB signaling.

**Discussion**

Although PARP inhibitor has a potential to generate acquired resistance in malignancies, it is still attractive anti-cancer drug. This demands a study of the underlying mechanism, of how cancer cells acquire resistance. Several reports have appeared in biomedical literature, arguing this issue, but none of the mechanisms suggested to date are overlapping. This prompted us to perform comprehensive gene expression analysis for identifying the underlying mechanism. We systematically ruled out other potential drug resistance mechanisms that have been suggested to play a role in PARP inhibitor resistance. The novel mechanism we discovered identifies a key role played by NF-κB signaling in acquired resistance to PARP inhibitor. This suggests that cancer cells have myriad options to survive and the advantage of utilizing PARP inhibitor is based on the principle of synthetic lethality that selectively exploits an acquired deficiency for DNA repair in cancer cells, but not in normal cells. In order to take an advantage of this agent, it is vital to understand the exact mechanism of resistance and predict the best drug option that can be used to override the resistance. Though novel mechanisms may well play a causal role in PARP inhibitor resistance, it is vital to study effective therapeutic opportunities such resistance mechanisms invariably reveal, and this is possible only by detailed study
of the resistance mechanisms.

**Conflicting interests**

The authors declare no conflict of interest.

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**Author contribution**

ASS and KS carried out the experiments and all authors wrote the manuscript.

**Abbreviation**

PARP: Poly-ADP ribosylation polymerase.

**References**


