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Review Article

Resistance to poly ADP-ribose polymerase inhibitors and its clinical implications

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ABSTRACT

Homologous recombination repair-deficient tumor cells including altered breast cancer gene 1 (BRCA1) and breast cancer gene 2 (BRCA2) tumor cells are known to respond to poly ADP-ribose polymerase (PARP) inhibitor through the mechanisms of reaching critical levels of genomic instability followed by mitotic catastrophe and ultimately synthetic lethality. PARP inhibitors are oral targeted drugs that are well tolerated and have received FDA approval for the treatment of ovarian, breast, pancreatic, and prostate cancer. The common challenge one encounters in clinical usage is the PARP inhibitor resistance, which may be *de novo* or acquired. In this review, various mechanisms underlying PARP resistance will be highlighted and the rationale to overcome the resistance.

Keywords: DNA repair, Mutations, Molecular oncology, BRCA

INTRODUCTION

Genomic stability is maintained by various mechanisms involved in DNA damage response and DNA repair pathways that include homologous recombination repair (HR), non-homologous end-joining repair (NHEJ), and single-stranded break repair, and base excision repair. Poly (ADP-ribose) polymerases (PARPs), especially PARP1, PARP2, and PARP3, are key DNA damage sensors and signal transducers and are recruited at single-strand DNA break sites which result in the PARylation of target proteins and recruitment of the DNA damage repair effectors. Then, the auto-PARylation of PARPs leads to the dissociation of PARPs from DNA. On the other hand, NHEJ (error-prone) and HR (high fidelity) are two main pathways to resolve the DNA double-strand breaks (DSBs). BRCA1/2 proteins are essential for the error-free repair of HR, in the S/G2 phase, BRCA1 is recruited to the DSB sites, later, BRCA2 and Partner and localizer of BRCA2 (PALB2) participate in the formation of the nucleoprotein filament and D-loop, required for sister chromatid to work as a template for repair. Treatment with PARP inhibitors (PARPi) in HR-deficient tumor cells results in dependency on NHEJ (error-prone) pathway for low fidelity double-strand break repair that leads to accumulation of genome instability and cell death. PARPi in addition to synthetic lethality, can bind and trap PARPs on DNA, preventing the release of PARPs from DNA break sites and removing PARPs from their normal catalytic cycle.

Drug resistance is well reported for PARPi, very similar to chemotherapy. Up to 40% of BRCA-mutated ovarian cancer patients do not respond to PARPi. Various cellular mechanisms have been established for PARPi resistance.[1-3]

These mechanisms can be classified into four main categories:

a) Restoration of HR pathway
b) Cellular availability of the drug or PARPi
c) The activity and abundance of PAR chains
d) Replication fork protection

RESTORATION OF HR REPAIR PATHWAY

Normally, the DDR repair pathway is activated when DSB happens, employing two typical mechanisms to repair DSB: HR and NHEJ. Normally, NHEJ is the main repair mechanism for ligating the broken DNA ends in a NHEJ way occurring throughout the cell cycle, especially in G0/G1 phase. However, HR predominates in the S/G2 phase, where the DSB ends are firstly resected by the Mre11-Rad50-Nbs1(MRN) complex together with CtIP and nucleases (EXO1, DNA2, and MUS8), leading to the formation of the single-strand DNA (ssDNA) that is coated by hyperphosphorylated single-strand DNA binding protein A (RPA). The γH2AX is activated and phosphorylated by ataxia-telangiectasia mutated (ATM) and ATM and Rad3 related (ATR), which helps to recruit and accumulate DDR proteins such as p53-binding protein (53BP1) and BRCA1. Further, PALB2 with BRCA2 binds with BRCA1 and promotes the loading of recombinase RAD51 on the ssDNA. 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. Both BRCA1 and BRCA2 are crucial for HR. However, loss of BRCA1, but not of BRCA2, can be bypassed by concomitant loss of factors that regulate NHEJ, resulting in HR reactivation.

BRCA1 and *BRCA2* mutations are usually single nucleotide, or short insertions or deletions, leading to a frameshift mutation. Reactivation of BRCA1/2 function by secondary mutations, so-called reversion mutations, regains frame restoration and potentially BRAC1/2 protein activity, as depicted in [Table 1]. BRCA1 consists of N-terminal domains (BRCT), an N-terminal RING domain, and a coiled-coil domain. Reversion mutations that restored the functions of BRCT and N-terminal RING domain are responsible for PAPRi resistance. Further, the deletion of exon 11 of BRCA1 was associated with partial PARPi resistance. BRCA2 contains a DNA-binding domain and eight BRC repeats that bind to RAD51. BRCA2 mutations lacking BRC 6–8 lead to PARPi resistance. In addition to reversion mutations in BRCA1/2, secondary somatic mutations restoring Rad51C and Rad51D were also associated with acquired resistance to the PARPi. Another way of restoring BRCA1 levels and activity is the demethylation of the hypermethylated promoter of *BRCA1*. Thus, reversion mutations and restoration of BRCA expression can lead to PARPi resistance as well as resistance to other DNA-damaging drugs.[3]

Another mechanism for the restoration of the HR pathway is by inducing the process of DNA end resection and the formation of nucleoprotein filament and D-loop may lead to PARPi resistance. DNA end resection is dependent on cyclin-dependent kinases (CDKs) activity that phosphorylates of MRN complex and CtIP. Among the CDKs, CDK12 plays an important role. CDK12 loss of functions inhibits HR repair and sensitizes ovarian cancer cells to PARPi and reverses both primary and secondary PARPi resistances in Triple-negative breast cancer (TNBC).[4] This evidence suggested that CDK inhibitors might overcome the PARPi resistance.

In addition, accessory factors 53BP1, RIF1, and REV7 contribute significantly to DNA end resection. 53BP1 is involved in chromatin binding, blocks DNA resection by preventing the access to CtIP to the DSB sites and its loss induces HR restoration, leading to PARPi resistance in various cancers, such as breast cancer, glioblastoma, and ovarian cancer. The loss of 53BP1 and downstream factors RIF1 and REV7 that are involved in NHEJ could reverse the HR defect caused by BRCA1 deficiency but not BRCA2-deficient cells. In addition, the Shieldin (SHLD) protein complex comprising REV7, SHLD1, SHLD2, and SHLD3 acts as an active inhibitor of resection.[2,4] Thus, loss of Shieldin factors may result in PARP resistance.

Similarly, the loss of two other proteins DYNLL1 and its transcriptional activator ATMIN, which are known as an interactor of 53BP1, can lead to PARP resistance in BRCA1-deficient tumor cells.[2]

CELLULAR AVAILABILITY OF THE PARPi

In the majority of breast and ovary tumor cell line models, overexpression of drug efflux transporter genes (*Abcb1a* and *Abcb1b* encoding for MDR1/P-gp and *Abcg2*) displayed resistance to PARPi.[2] Consequently, coadministration of the MDR1 inhibitor tariquidar, elacridar, and verapamil resensitizes the tumors to the PARPi. Furthermore, *ABCB1* expression is often upregulated in chemotherapy-treated ovarian and breast cancers.

THE ACTIVITY AND ABUNDANCE OF PAR CHAINS

PARylation is a process catalyzed by PARP proteins that are covalently adding PAR chains transiently and reversibly. PARP1 is the main protein responsible for the bulk of cellular PARylation, up to 90%, on DNA damage.[2] PARPi inhibits the catalytic activity of PARP and traps PARP proteins on damaged chromatin. These two mechanisms are responsible for PARPi activity and enable cytotoxicity. PARP1 mutations that diminish the trapping of the protein on DNA induce PARPi resistance even in HR-deficient cells. Further, PARP1 mutation ablating the PARP1 expression can result in PARPi resistance.

Table 1: Reversion mutations in *BRCA* Genes

Gene	Primary mutation	Reverse mutation	Tumor type
BRCA1	Q1756fs*74 (c. 5266dupC)	Q1756_D1757>PG (c. 5263_5272>TCCCCAGGAC)	High-grade papillary serous carcinoma
BRCA1	1479delAG (c. 1360_1361del)	s454_l467del (c. 1361_1402del)	TNBC
BRCA2	K2162fs*5 (c. 6486_6489delACAA)	K2150fsa17 (c. 6448_6473del26)	Pancreatic cancer
BRCA2	V1283fs*2 (c. 3847_3848delGT)	D1280_N1288del (c. 3838_3864del27)	Breast cancer
BRCA2	V1804Kfs (c. 5410_5411del)	Y1480_A1896del (c. 4434_5686delinsTT)	Pancreatic cancer
BRCA2	V1804Kfs (c. 5410_5411del)	I1633_I2269del (c. 4897_6807del)	Pancreatic cancer
BRCA2	Q2960X (c. 9106C>T)	Q2960E (c. 9106C>G)	Breast cancer
BRCA2	E1493Vfs*9 (c. 4705_4708delGAAA)	I1490_E1493del (c. 4698-4709delAAATACTGAAAG)	High-grade papillary serous carcinoma
BRCA2	S1982fs (c. 5946delT)	S1982_ A1996del (c. 5946_5990del45)	Prostate
BRCA2	S1982fs (c. 5946delT)	S1985fs (c. 5949_5952dupAAAA)	Prostate
BRCA2	N1910fs*2 (5727_5728insG)	A1843_S1985del (5528_5956del429)	Prostate
BRCA2	N1910fs*2 (5727_5728insG)	A1891_M1936del (5671_5808del138)	Prostate
BRCA2	N1910fs*2 (5727_5728insG)	D1909_D1911>EDY (5727_5731TAATG>AGACT)	Prostate
BRCA2	N1910fs*2 (5727_5728insG)	L1908_S1917del (5721_5750del30)	Prostate
BRCA2	N1910fs*2 (5727_5728insG)	N1766_Q2009del (5292_6025>CA)	Prostate
BRCA2	N1910fs*2 (5727_5728insG)	N1910_D1911del (5728_5733delAATGAT)	Prostate
BRCA2	N1910fs*2 (5727_5728insG)	S1788_P2114>DTT (5362_6340>GATACCA)	Prostate

The activity of PAR glycohydrolase (PARG) reverses PARylation resulting in the degradation of PAR chains. Thus, PARG works similarly to PARPi by preventing PAR accumulation. Genetic alterations cause loss of PARG as a cause for PARPi resistance.

REPLICATION FORK PROTECTION

PARP1 and BRCA1/2 also participate in DNA replication. PARP1 mediates the accumulation of regressed forks and stops the restart of reversed forks essential for DSB formation. BRCA1/2 protects nascent DNA at stalled replication forks from nucleases (i.e., MRE11, DNA2, and MUS81).[2,4] In BRCA 2 deficient, low EZH2 levels reduced H3K27 methylation, prevented MUS81 recruitment at stalled forks, and caused fork stabilization, which promoted PARPi resistance but not in BRCA1-deficient cells. Further, the absence of PTIP, MELL3/4, and CHD4 deficiency inhibited the recruitment of the MRE11 nuclease and protected nascent DNA strands from extensive degradation, leading to PARPi resistance in BRCA2-deficient cells. FANCD2 suppresses MRE11-mediated fork degradation and plays an important role in the stabilization of stalled replication forks and its overexpression confers resistance to PARPi. SMARCAL1, a member of the SNF2 family, reverses the nascent DNA degradation induced by FANCD2 deficiency and its deletion promoted PARPi resistance.[2,4]

CLINICAL IMPLICATIONS TOWARD PARPI RESISTANCE

New strategies to enhance PARPi sensitivity and overcome PARPi resistance are evaluated at various stages of clinical trials. PARPi-oncolytic herpes simplex viruses (oHSVs) combination; recently, MG18L, a oHSV, was reported to proteasomally degrade RAD51 and sensitize glioblastoma stem cells to PARPi killing in a synthetic lethal-like fashion. PARPi-ionizing radiation (IR) combination – IR helps 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 but can only be used in wild-type p53 patients.[1] HR restoration by loss of 53BP1 pathway increases the radiosensitivity, thus supporting the rationale of PARPi-IR combination. PARPi-CDKs inhibitors combination – DNA end resection is dependent on CDKs activity. A synthetic lethal strategy combining dinaciclib with niraparib was also effective in TNBC, ovarian, prostate, pancreatic, colon, and lung cancer cells.[1,4] Numerous studies proved that CDK12 mutation or deficiency leads to cancer cell's sensitivity to PARPi, thus, CDK12 inhibitors may reverse *de novo* and acquired PARPi resistance. PARPi-immunotherapy combination – PARPi upregulated PD-L1 expression in breast cancer cell lines through inactivating GSK3β causing attenuation of anticancer immunity. Thus, the combination of PARPi and anti-PD-L1 therapy shows better therapeutic efficacy than each alone. PARPi leads to the accumulation of cytosolic DNA fragments activating the DNA-sensing cGAS-STING pathway and stimulating the production of type I interferons to induce antitumor immunity independent of BRCAness.[4] PARPi-epigenetic drugs – epigenetic modification is associated with PARPi sensitivity. Mechanistically, HDACi decreases the expression of DNA repair genes such as RAD51, CHK1, BRCA1, and RAD21 mediated through

transcription factor E2F1 and blocked the deacetylation and expression of HSP90, resulting in the degradation of its substrates BRCA1, Rad52, ATR, and CHK1. Further, HDACi treatment significantly increased the trapping of PARP1 at DSB sites in chromatin. In addition, low doses of DNA methyltransferase inhibitor (DNMTi)-induced BRCAness phenotype by downregulating the expression of key HR genes. The combination DNMTi and PARPi enhanced the cytotoxic effect by increasing the PARP "trapping" on DSB sites independent of BRCA mutations. PARPi – other drug combinations such as HSP90 inhibitors, ATR/CHK1 inhibitors, and WEE1 inhibitors could induce homologous recombination repair-deficient and increase PARP sensitivity.

CONCLUSION

In recent times, PARPi has been successful in treating BRCA mutation patients based on the synthetic lethal interactions. PARPi kills cancer cells beyond DNA repair, especially by a PARP trapping mechanism. However, PARPi resistance their multiple potential resistance mechanisms, such as HR restoration and protection of DNA replication fork, has been identified, posing a major clinical challenge. To overcome PARPi resistance and increase PARPi sensitivity, the optimal combination of PARPi and other drugs is required.

Declaration of patient consent

Patient consent is not required as there are no patients in this study.

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Conflicts of interest

Author Amit Verma is one of the Editor of the journal.

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