DNA repair defects in cancer and therapeutic opportunities

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DNA repair and DNA damage signaling pathways are critical for the maintenance of genomic stability. Defects of DNA repair and damage signaling contribute to tumorigenesis, but also render cancer cells vulnerable to DNA damage and reliant on remaining repair and signaling activities. Here, we review the major classes of DNA repair and damage signaling defects in cancer, the genomic instability that they give rise to, and therapeutic strategies to exploit the resulting vulnerabilities. Furthermore, we discuss the impacts of DNA repair defects on both targeted therapy and immunotherapy, and highlight emerging principles for targeting DNA repair defects in cancer therapy.

Human cells rely on a complex network of DNA repair and DNA damage signaling pathways, called the DNA damage response (DDR), to remove DNA lesions and aberrant structures from the genome and maintain genomic stability (Ciccia and Elledge 2010). The DDR pathways deal with not only the DNA damage generated by extrinsic insults, but also genomic problems arising from intrinsic cellular processes. In cancer cells, some of the DDR pathways are commonly compromised, allowing genomic instability to accumulate. On the one hand, genomic instability fuels tumorigenesis. On the other hand, the loss of DDR pathways in cancer cells renders them vulnerable to DNA damage and additional defects in the DDR network. Targeting specific DDR proteins in cancer cells harboring DDR defects or high levels of genomic instability can lead to synthetic lethality, providing a promising strategy for cancer therapy (O'Connor 2015). The DDR defects of cancer cells also affect the immune response to tumors, implicating the DDR as a modulator of immunotherapy (Pilger et al. 2021). In this review, we discuss the major classes of DDR defects in cancer, new developments in targeting the DDR pathways, and new strategies to exploit DDR defects in cancer therapy, providing an updated view of the implications of DDR defects in cancer and cancer treatment.

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Defects of DDR pathways in cancer

Mutations in a variety of genes encoding DNA repair and damage signaling proteins have been detected in human cancers. While many of these mutations are likely passenger events without a functional impact, some mutations impair the DDR and may contribute to tumorigenesis. The DDR defects resulting from the mutations can be divided into three classes, as discussed below.

Class I: defects in repair of DSBs and replication-associated DNA damage

Mutations in the tumor suppressor genes *BRCA1* and *BRCA2* are frequently found in several cancer types, including breast, ovarian, prostate, and pancreatic cancers (Venkitaraman 2002; Mersch et al. 2015). Both *BRCA1* and *BRCA2* genes encode proteins critical for DNA double-stranded break (DSB) repair through homologous recombination (HR) (Chen et al. 2018a). BRCA1 forms a complex with BARD1, which is rapidly recruited to sites of DNA damage after DSB formation (Fig. 1). At DSBs, BRCA1 regulates the formation of single-stranded DNA (ssDNA) and recruits the PALB2–BRCA2 complex. The PALB2–BRCA2 complex displaces the replication protein A (RPA) and assembles RAD51 filaments, a crucial HR intermediate, on ssDNA (Zhao et al. 2015). BRCA1/2-deficient cells cannot repair collapsed DNA replication forks properly. Both BRCA1 and BRCA2 restrict "long-tract" gene conversion, an error-prone repair process (Willis et al. 2014). BRCA1 also suppresses tandem duplications at stalled forks (Willis et al. 2017). Mutations in other HR genes, such as *PALB2*, *BRIP1*, *RAD51C*, and

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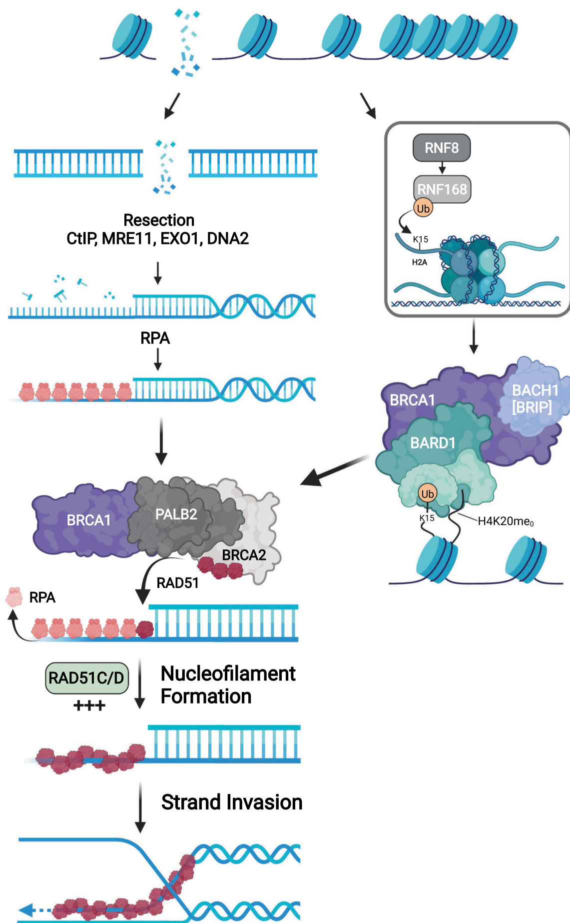

Figure 1. Functions of BRCA1/2 in homologous recombination. The BRCA1–BARD1 complex is recruited to the DSB-flanking chromatin marked by H2A K15ub and H4 K20me0 (Becker et al. 2021; Hu et al. 2021). The BRCA1 at DSBs recruits PALB2–BRCA2 to promote the assembly of RAD51 filaments, which enables D-loop formation following the resection by MRE11-CtIP and EXO1 or MRE11-CtIP and DNA2.

RAD51D, are also found in cancers (Antoniou et al. 2014; Suszynska et al. 2020). These findings suggest that the genomic instability in HR-deficient cancer cells may arise from defective repair of DSBs and collapsed replication forks.

In addition to HR, BRCA1 and BRCA2 are important for protecting stalled replication forks (Fig. 2; Schlacher et al. 2011, 2012). Replication forks undergo a remodeling process upon stress, giving rise to four-way DNA structures called reversed forks (Neelsen and Lopes 2015; Quinet et al. 2017). In cells lacking BRCA1/2, the nascent DNA at reversed forks is increasingly degraded by the MRE11 and EXO1 nucleases (Fig. 2; Lemaçon et al. 2017). The degradation of nascent DNA in BRCA2-deficient cells forces forks to recover through a MUS81- and POLD3-mediated pathway, which is associated with transient DSBs and fork instability (Fig. 2). Several groups reported that BRCA1/2-deficient cells display increased levels of ssDNA gaps in nascent DNA (Fig. 2; Cong et al. 2021; Kang et al. 2021; Paes Dias et al. 2021; Simoneau et al. 2021; Taglialatela et al. 2021). PrimPol, which reprimes for DNA synthesis ahead of stalled DNA polymerases, promotes the formation of ssDNA gaps in BRCA1/2-deficient cells (Quinet et al. 2020; Kang et al. 2021; Taglialatela et al. 2021). Both BRCA1 and BRCA2 are required for the repair of ssDNA gaps after replication (Tirman et al. 2021). The functions of BRCA1/2 at ssDNA gaps may be related to their roles in protecting stalled forks, as BRCA1/2 prevent MRE11-mediated DNA degradation in both contexts. The increase of ssDNA gaps in BRCA1/2-deficient cells elevates replication stress by sequestering RPA and causing fork collapse in a *trans* cell cycle manner (Cong et al. 2021; Simoneau et al. 2021). Thus, loss of the functions of BRCA1/2 in protecting stalled forks and ssDNA gaps may also contribute to the genomic instability in cancer cells.

BRCA1 and BRCA2 are also important for suppressing R-loops, a three-stranded polynucleotide structure formed by DNA–RNA hybridization during transcription (Crossley et al. 2019). R-loops are a source of genomic instability because they interfere with DNA replication forks and are cleaved by structure-specific nucleases. BRCA1 suppresses R-loops at transcription termination sites (TTSs) by recruiting the SETX helicase (Hatchi et al. 2015). An increase of R-loops is also detected near transcription start sites (TSSs) in BRCA1 mutation carriers (Chiang et al. 2019). Loss of COBRA1/NELFB, an inhibitor of transcription elongation, reduces R-loops in BRCA1-deficient cells and mitigates mammary tumorigenesis in mice (Zhang et al. 2017). In BRCA2-deficient cells, R-loops are also increased at TSSs due to impaired transcription elongation (Shivji et al. 2018). BRCA2 interacts with the TREX-2 complex, which is important for mRNA processing, to suppress R-loops (Bhatia et al. 2014). The functions of BRCA1/2 in suppressing R-loops may be important in tissues regulated by estrogen because estrogen induces R-loop-associated DNA damage (Stork et al. 2016). BRCA1 is important for maintaining the differentiation state of mammary epithelial cells (Wang et al. 2019c), suggesting a tissue- and cell type-specific function.

Genes in the Fanconi anemia (FA) pathway are also mutated in cancers, particularly in acute myeloid leukemia (AML) and squamous cell carcinoma (Niraj et al. 2019). To date, 22 FA genes have been identified, and some of them are also HR genes. The FA pathway is important for repairing DNA interstrand cross-links (ICLs). Upon collision of replication forks with ICLs, the FA core complex monoubiquitinates the FANCD2–FANCI (ID) complex, enabling it to bind DNA stably (Shakeel et al. 2019; Wang et al. 2021b). The ID complex recruits additional FA proteins to remove ICLs and allow completion of repair through HR. Furthermore, the FA pathway repairs DNA–protein cross-links (DPCs). Formaldehyde, a cellular metabolite generated by histone demethylases, is a source of DPCs. Loss of the aldehyde dehydrogenase ALDH2 and alcohol dehydrogenase ADH5 increases formaldehyde and DNA damage in mice (Dingler et al. 2020). Loss of ALDH2 induces spontaneous DNA

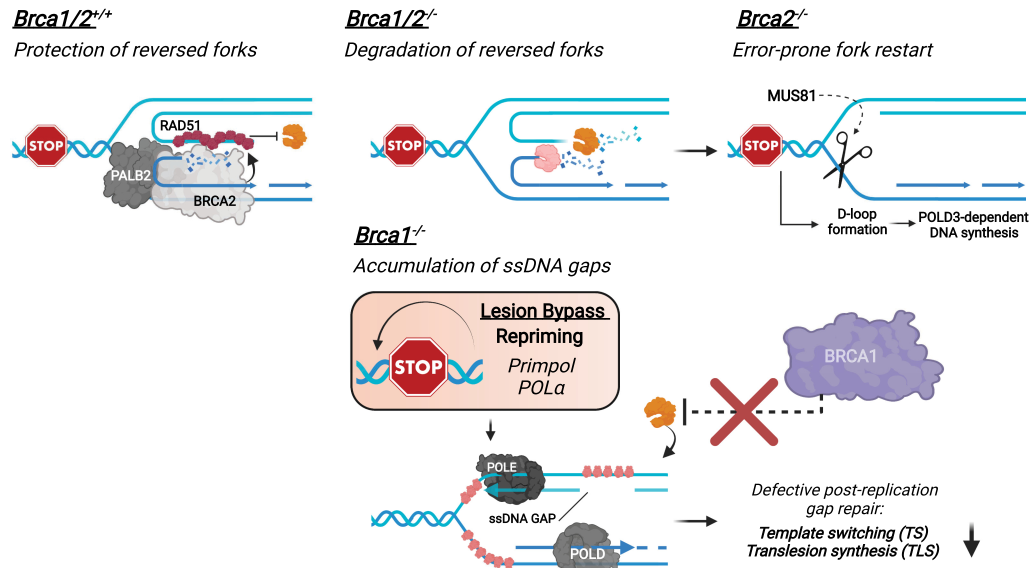

Figure 2. Functions of BRCA1/2 in protecting replication forks. In BRCA1/2-proficient cells, reversed replication forks are protected from nucleolytic degradation by RAD51 in a BRCA1/2-dependent manner. In BRCA1/2-deficient cells, nascent DNA at reversed forks is increasingly degraded. Degradation of nascent DNA in BRCA2-deficient cells leads to MUS81- and POLD3-dependent, error-prone fork restart. Furthermore, ssDNA gaps accumulate during replication in BRCA1/2-deficient cells. BRCA1-deficient cells are defective for protecting ssDNA gaps against the MRE11 nuclease, reducing TS- and TLS-mediated gap repair.

damage in *FANCD2*$^{-/-}$ mice, suggesting that the FA pathway protects cells against DPCs (Langevin et al. 2011). FA proteins are also implicated in HR (Adamson et al. 2012), replication stress response (Gari et al. 2008), R-loop suppression (García-Rubio et al. 2015; Schwab et al. 2015), and maintenance of the epithelial state (Wang et al. 2019c). Collectively, the cancer-associated mutations in HR and FA pathways may compromise the ability of cells to cope with intrinsic DNA damage, affecting their genomic integrity, RNA biogenesis, and differentiation states.

Class II: defects in DNA damage signaling and checkpoints

Mutations in DNA damage checkpoint genes *ATM*, *ATR*, *CHK1*, and *CHK2* are also found in cancers. For example, ATM mutations are detected in a wide range of cancers, including colorectal, uterine, prostate, and lung cancers (Jette et al. 2020). ATM and its downstream kinase CHK2 are important for signaling DSBs (Shiloh and Ziv 2013). In response to DSBs, ATM is required for the checkpoint responses in G1, S, and G2/M phases of the cell cycle. NBS1, MRE11, and RAD50, which are involved in ATM activation at DSBs, are also mutated in cancers (McPherson et al. 2020). ATM and CHK2 phosphorylate p53 after DNA damage, and CHK2 is a regulator of apoptosis (Hirao et al. 2002). Upon DSBs, ATM phosphorylates NEMO and promotes activation of the NF-κB pathway (Wu et al. 2006). ATM is also activated by reactive oxygen species (ROS), protecting cells from oxidative DNA damage (Lee and Paull 2021). Thus, loss of ATM in cancer cells may lead to multiple defects in checkpoints and apoptosis, allowing cancer cells to continue proliferation and accumulate chromosomal alterations.

Compared with the ATM–CHK2 pathway, the ATR–CHK1 pathway plays a more important role in the response to DNA replication stress (Marechal and Zou 2013; Saldivar et al. 2017). In S phase, ATR and CHK1 stabilize DNA replication forks and prevent excessive firing of replication origins. ATR also alleviates replication stress by promoting dNTP synthesis (Buisson et al. 2015). Even during the unperturbed cell cycle, ATR ensures proper S–G2 and G2–M transitions and accurate chromosome segregation in mitosis (Kabeche et al. 2018; Lemmens et al. 2018; Saldivar et al. 2018). In response to high replication stress, ATR and CHK1 mediate S-phase and G2/M checkpoint responses. Loss of ATR leads to a range of defects in the cell cycle, including high replication stress, premature entry to mitosis, and mitotic aberrations. Although ATR is a haploid-insufficient tumor suppressor in mice (Fang et al. 2004), ATR mutations are less common than ATM mutations in human cancers because ATR is an essential gene and cancer cells with high levels of genomic instability are especially dependent on ATR signaling for survival.

Class III: elevation of mutation burden

Mutations in mismatch repair (MMR) genes *MSH2*, *MSH6*, *MLH1*, and *PMS2* are found in a range of cancers, particularly in colorectal cancer (Lynch and de la Chapelle 2003). While heterozygous mutations in MMR genes cause Lynch syndrome and adult-onset of colorectal cancer, biallelic MMR deficiency (bMMRD) is an autosomal recessive disorder characterized by early onset of cancers.

Because MMR plays a critical role in repairing DNA mismatches after DNA replication, loss of MMR results in a drastic increase of mutations in the genome (Jiricny 2013). In addition, MMR prevents DNA replication errors in microsatellite repeats, and loss of MMR leads to microsatellite instability (MSI). Notably, cancer cells with MSI are dependent on the WRN helicase for survival (Chan et al. 2019). WRN suppresses DNA secondary structures at expanded microsatellite repeats, preventing cleavage by the MUS81 nuclease and formation of DSBs (van Wietmarschen et al. 2020). Defects in other DNA repair pathways also contribute to mutations in cancer cells. For example, loss of nucleotide excision repair (NER) compromises repair of UV damage and increases the risk of skin cancer, which is high in UV signature mutations. Loss of MUTHY, a base excision repair (BER) protein, increases single-base substitutions in tumors.

The proofreading activities of DNA polymerases are also critical for suppressing mutations. Mutations in the proofreading domains of DNA polymerases ε and δ (*POLE* and *POLD1*) are found in a number of cancers, including colorectal and endometrial cancers (Mur et al. 2020). *POLE* and *POLD1* mutations drastically increase mutation burden in tumors (Campbell et al. 2017). In mouse models, *Pole* and *Pold1* mutants promote tumorigenesis (Albertson et al. 2009; Li et al. 2018). Interestingly, modeling a cancer mutation of *POLE* in yeast generates a hyperactive polymerase that bypasses DNA mismatches and hairpins, suggesting that *POLE/POLD1* mutations may affect replication fidelity in multiple ways (Xing et al. 2019).

It should be noted that DNA repair can be altered in cancer cells by mechanisms other than mutations, such as gene expression, RNA processing, protein modifications, and protein degradation. Furthermore, DNA repair can be affected by other pathways, such as the cell cycle, DNA replication, nucleotide metabolism, chromatin modulation, transcription, and RNA biogenesis. The DDR defects in cancer cells caused by indirect mechanisms can also contribute to genomic instability and affect the therapeutic response.

Targeting the DDR in cancer therapy

Ionizing radiation and genotoxic chemotherapies have been long used to exploit the DNA repair defects in tumors. For example, cisplatin is effective in ovarian cancer probably because HR deficiency is common in this cancer type. Compared with traditional radiotherapy and chemotherapy, targeting the DDR proteins indispensable in cancer cells provides a potentially more selective and less toxic therapeutic approach.

PARP inhibitors

One of the best characterized examples of synthetic lethality in cancer cells is the selective killing of HR-deficient cancer cells by PARP inhibitor (PARPi) (Bryant et al. 2005; Farmer et al. 2005; Fong et al. 2009). To date, several PARPis are approved or under trials for the treatment of breast, ovarian, prostate, and pancreatic cancers (Table 1). Inhibition of PARP1/2 by PARPi impairs BER and increases DNA single-stranded breaks (SSBs) in the genome, which are converted to DSBs during DNA replication. Cells with HR defects are unable to repair replication-associated DSBs and therefore are sensitive to PARPi. The efficacy of PARPi is associated with the trapping of PARP1/2 on chromatin (Murai et al. 2012). Loss of the chromatin remodeler ALC1/CHD1L leads to persistent BER intermediates, increases PARP1 trapping, and renders HR-deficient cancer cells more sensitive to PARPi (Blessing et al. 2020; Juhász et al. 2020; Hewitt et al. 2021; Verma et al. 2021). Loss of RNaseH2, which is required for ribonucleotide excision repair (RER), leads to topoisomerase 1 (TOP1)-mediated cleavage of misincorporated ribonucleotides and trapping of PARP1, rendering cells hypersensitive to PARPi (Zimmermann et al. 2018). Inhibition of DNPH1, which suppresses genomic incorporation of 5-hydroxymethyl deoxyuridine (hmdU), increases BER intermediates and PARP1 trapping and further sensitizes BRCA-deficient cancer cells to PARPi (Fugger et al. 2021).

PARP1 is also important at replication forks. PARP1 is required for the proper maturation of Okazaki fragments on the lagging strand (Hanzlikova et al. 2018). PARP1-mediated PARylation inhibits RECQ1, which resolves reversed forks (Berti et al. 2013). Inhibition of PARP1/2 reduces fork reversal and increases repriming by PrimPol, generating ssDNA gaps on the leading strand (Genois et al. 2021). The induction of ssDNA gaps by PARPi may be critical for the killing of BRCA1/2-deficient cells. One study suggests that PARPi generates more ssDNA gaps in BRCA1/2-deficient cells, driving RPA exhaustion and replication catastrophe (Fig. 3A; Cong et al. 2021). In contrast, another study shows that PARPi induces ssDNA gaps similarly in BRCA1-deficient and -proficient cells (Fig. 3B; Simoneau et al. 2021). However, the trapping of PARP1 by PARPi prevents the completion of gap repair and allows the gaps to persist. During the ensuing mitosis, more chromosome aberrations are observed in BRCA1/2-deficient cells (Schoonen et al. 2017). During the subsequent S phase, a new round of DNA replication leads to collisions of replication forks with ssDNA gaps and a surge of DSBs. BRCA1/2-deficient cells fail to activate ATR to suppress replication origin firing and to repair DSBs at collapsed forks, providing a plausible explanation of how PARPis selectively kill *BRCA1/2* mutant cells over multiple cell cycles (Simoneau et al. 2021).

ATR, CHK1, and WEE1 inhibitors

Inhibitors of ATR, CHK1, and WEE1 have shown efficacy in cancer cells with specific DDR defects or high genomic instability (Table 1). The ATR–CHK1 pathway is critical for S-phase and G2/M checkpoints. Even in the absence of extrinsic DNA damage, inhibition of ATR or CHK1 in cancer cells elevates CDK1/2 activities, increases firing of replication origins, and reduces replication fork stability (Petermann et al. 2006; Buisson et al. 2015). Similarly, WEE1 inhibitor (WEE1i) also elevates CDK1/2 activities,

Table 1. *DDR inhibitors in clinical use or trials*

Target	Inhibitor	Manufacturer	Reference
PARP1 or PARP1/2	Olaparib	AstraZeneca	Fong et al. 2009
	Rucaparib	Clovis Oncology	Coleman et al. 2017
	Niraparib	Tesaro	González-Martín et al. 2019
	Talazoparib	Medivation	Litton et al. 2018
	Veliparib	AbbVie	Coleman et al. 2019
	AZD5305	AstraZeneca	Johannes et al. 2021
ATR	Berzosertib (M6620, VX-970)	Merck KGaA	Yap et al. 2020
	Ceralasertib (AZD6738)	AstraZeneca	Yap et al. 2021a
	BAY 1895344	Bayer	Yap et al. 2021b
	RP-3500	Repare	Roulston et al. 2022
	ART0380	Artios	Unpublished
	ATRN-119	Artin	Unpublished
CHK1	Prexasertib (LY2606368)	Eli Lily	Lee et al. 2018
	SRA737	Sareum	Rogers et al. 2020
WEE1	Adavosertib (AZD1775)	AstraZeneca	Leijen et al. 2016
ATM	AZD1390	AstraZeneca	Jin and Oh 2019
	AZD0156	AstraZeneca	Jin and Oh 2019
DNA-PK	AZD7648	AstraZeneca	Fok et al. 2019
	Peposertib (M3814)	Merck KGaA	van Bussel et al. 2021
	BAY-8400	Bayer	Berger et al. 2021
ATM/DNA-PK	XRD-0394	XRad	Unpublished
DNA-PK/mTOR	CC-115	Celgene	Munster et al. 2019
POLθ	ART4215	Atrios	Zatreanu et al. 2021
	Novobiocin (VP-006)	Varsity Pharma	Zhou et al. 2021
RAD51	CYT-0851	Cyteir	Ratiu et al. 2017
USP1	KSQ-4279	KSQ	Unpublished
PKMYT1	RP-6306	Repare	Gallo et al. 2021
PRMT5	GSK3326595	GSK	Gerhart et al. 2018
	JNJ-64619178	Janssen	Brehmer et al. 2021
	TNG908	Tango	Unpublished
	PRT 811	Prelude	Unpublished

increases origin firing, and overrides the G2/M checkpoint (Moiseeva et al. 2019). ATR, CHK1, and WEE1 inhibitors exacerbate the replication stress in cancer cells and impair checkpoint responses. In addition, both ATR and CHK1 are important for HR (Sorensen et al. 2005; Buisson et al. 2017b). Thus, ATR and CHK1 inhibitors not only induce replication-associated DSBs, but also prevent DSB repair. ATR inhibitor (ATRi) induces mitotic catastrophe if cells enter mitosis with high levels of DSBs. In cancer cells under high replication stress, ATRi induces excessive ssDNA, leading to RPA exhaustion and replication catastrophe (Toledo et al. 2013). All these effects of ATR, CHK1, and WEE1 inhibitors may contribute to the synthetic lethality in cancer cells.

ATRi has shown efficacy in ATM-deficient tumors (Villaruz et al. 2016; Rafiei et al. 2020). Both ATM and ATR contribute to the S-phase and G2/M checkpoints. Furthermore, ATM and ATR function in concert in DSB signaling (Shiotani and Zou 2009). The loss of ATM in cancer cells may render them increasingly reliant on ATR for damage signaling and checkpoint responses. Notably, ATM-deficient cancer cells are not highly sensitive to PARPi, highlighting the difference between ATRi and PARPi (Rafiei et al. 2020). The functional redundancy between ATM and ATR, rather than an indispensable role of ATM in HR, likely underlies the sensitivity of ATM-deficient cells to ATRi. BRCA1/2-deficient cancer cells are sensitive to ATRi (Kim et al. 2017; Yazinski et al. 2017). In this context, ATRi may suppress residual HR activity, induce replication-associated DSBs, and prevent repair of ssDNA gaps and DSBs, leading to selective killing of cancer cells. In nonsmall cell lung cancer (NSCLC), low expression of ERCC1, which regulates the XPF nuclease in NER and FA pathways, is associated with a better response to cisplatin (Lord et al. 2002). Loss of ERCC1 is synthetically lethal with ATR inhibition (Mohni et al. 2014), suggesting that cancer cells with defects in HR and FA pathways may be generally sensitive to ATRi.

ATRi also selectively kills cancer cells under high replication stress. Cancer cells overexpressing Cyclin E harbor high replication stress and are sensitive to ATRi and CHK1i (Toledo et al. 2011). Loss of ARID1A or SMARCA4/BRG1, two components of SWI/SNF chromatin remodeling complexes, alters chromatin compaction, elevates replication stress, and confers ATRi sensitivity (Williamson et al. 2016; Gupta et al. 2020; Kurashima et al. 2020). Cancer cells reliant on the alternative lengthening of telomeres (ALT) pathway display high replication stress at telomeres and are sensitive to ATRi (Flynn et al. 2015). In cancer cells expressing the APOBEC3A/B cytidine

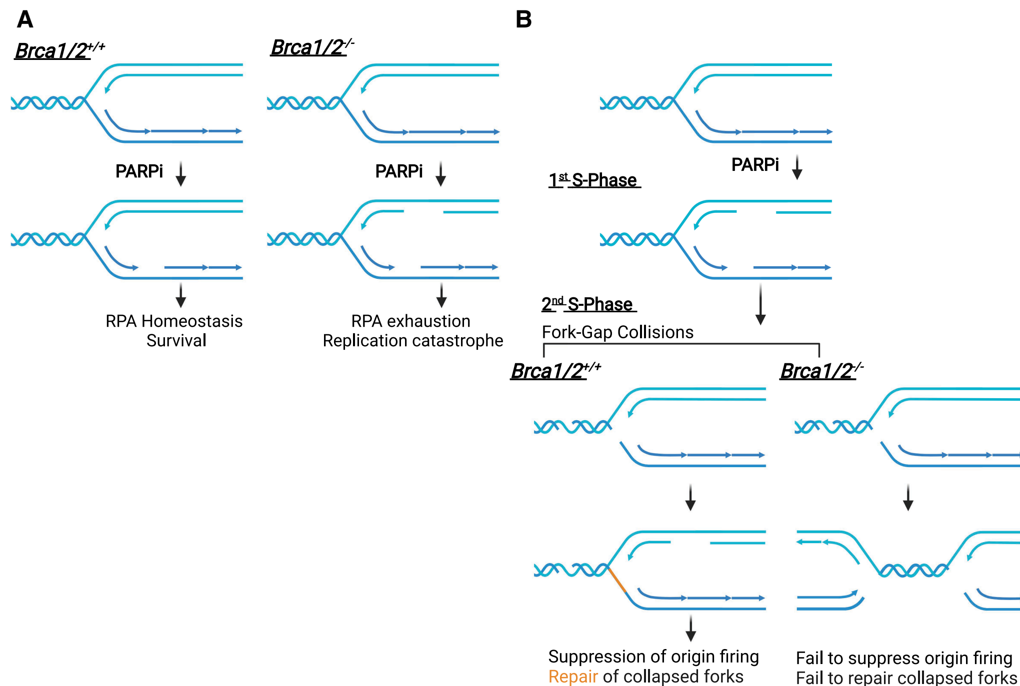

Figure 3. Models for the selective killing of BRCA1/2-deficient cells by PARPi. (*A*) PARPi induces more ssDNA gaps in BRCA1/2-deficient cells, promoting RPA exhaustion and replication catastrophe. (*B*) PARPi induces ssDNA gaps during replication and prevents complete gap repair, allowing gaps to persist into the next cell cycle and generate DSBs upon collisions with replication forks. BRCA1/2-deficient cells fail to repair collapsed forks and mount a checkpoint response, leading to progressive accumulation of DSBs over multiple cell cycles and cell death.

deaminases, APOBEC3A/B generate replication stress by increasing abasic sites, conferring ATRi sensitivity (Green et al. 2017; Buisson et al. 2017a). Hotspot mutations in RNA splicing factors such as SF3B1, U2AF1, and SRSF2 are prevalent in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Some of these splicing factor mutants induce aberrant R-loop accumulation, rendering cells sensitive to ATRi (Nguyen et al. 2018; Chen et al. 2018b; Singh et al. 2020). Cancer cells with reduced RNaseH2 are also sensitive to ATRi, presumably because misincorporated ribonucleotides induce SSBs and interfere with replication (Wang et al. 2019a). A mutation signature in cancer cells was recently linked to low RNaseH2 expression and TOP1-mediated RER (Reijns et al. 2022), providing a potential biomarker for the use of ATRi and PARPi in therapy.

Notably, ATRi and PARPi display a strong synergy in BRCA1/2-deficient cancer cells (Kim et al. 2017, 2020; Yazinski et al. 2017; Shah et al. 2021; Roulston et al. 2022). Because both ATRi and PARPi induce ssDNA and both ATRi and PARPi sensitivities associate with high ssDNA levels (Buisson et al. 2015; Cong et al. 2021), the synergy between ATRi and PARPi may arise from ssDNA accumulation. Alternatively, ATRi may increase replication origin firing and the collision of replication forks with PARPi-induced ssDNA gaps, enhancing the effects of PARPi (Simoneau et al. 2021). ATRi may also reduce the stability of replication forks in response to PARPi and repair of PARPi-induced ssDNA gaps and DSBs.

Other DDR inhibitors and opportunities to exploit genomic instability

ATM inhibitor (ATMi) and DNA-PKcs inhibitor (DNA-PKi) sensitize cancer cells to radiation and chemotherapy (Table 1; Durant et al. 2018; Fok et al. 2019). ATMi and DNA-PKi display a synergy in inhibiting cell proliferation, suggesting that loss of both ATM and DNA-PK is synthetic lethal (Wang et al. 2021a). DNA-PKi prevents the reappearance of CHK1 phosphorylation after ATRi treatment (Buisson et al. 2015), suggesting that DNA-PKi may enhance the effects of ATRi in cancer cells.

POLθ is required for DSB repair through microhomology-mediated end joining (MMEJ), an error-prone repair pathway (Yu and McVey 2010; Mateos-Gomez et al. 2015). HR-deficient cells are hypersensitive to POLθ loss because DSB repair is increasingly dependent on MMEJ in the absence of HR (Ceccaldi et al. 2015). Inhibitors of the ATPase or polymerase activity of POLθ selectively kill BRCA1/2-deficient cancer cells and enhance the effects of PARPi (Table 1; Fig. 4; Zatreanu et al. 2021; Zhou et al. 2021). REV1 forms a complex with the translesion synthesis (TLS) DNA polymerase POLζ and enables its localization to DNA lesions. A REV1 inhibitor (REV1i) that disrupts the interaction of REV1 with POLζ (Chatterjee et al. 2020) enhances the effects of cisplatin in cancer cells (Yoon et al. 2021). REV1i also inhibits the repair of ssDNA gaps in BRCA1/2-deficient cells, leading to cytotoxicity (Fig. 4; Taglialatela et al. 2021).

Additional inhibitors have been developed to target the HR pathway or BRCA-deficient tumors. A RAD51 inhibitor displays efficacy in chronic lymphoblastic leukemia (CLL) expressing the AID cytidine deaminase (Lamont et al. 2013), presumably because it attenuates the repair of AID-induced DSBs (Table 1). An inhibitor of TRDMT1, an RNA methyltransferase involved in the transcription-coupled HR, sensitizes cancer cells to PARPi and ATRi (Zhu et al. 2021). Furthermore, a RAD52 inhibitor induces synthetic lethality in BRCA2-deficient cells (Yang et al. 2021), which is likely attributed to the indispensable HR function of RAD52 in the absence of BRCA2 (Feng et al. 2011). The deubiquitinase USP1 is required for the deubiquitination of PCNA and FANCD2, which are involved in TLS, template switching (TS), FA, and HR pathways (Huang et al. 2006; Oestergaard et al. 2007). The protection of replication forks in the absence of BRCA1 requires USP1, and a USP1 inhibitor selectively kills BRCA1-deficient cells (Table 1; Lim et al. 2018). In TNBC, an inhibitor of CDK12/13, suppressors of intronic polyadenylation, reduces expression of HR genes and enhances the effects of PARPi and chemotherapy (Quereda et al. 2019). In leukemia cells overexpressing the protein arginine methyltransferase PRMT5, an inhibitor of PRMT5 impairs HR by altering the splicing of repair regulators and synergizes with PARPi to induce cell death (Table 1; Hamard et al. 2018). As discussed above, an inhibitor of DNPH1 synergizes with PARPi in BRCA-deficient cells by increasing BER-generated SSBs and PARP trapping (Fugger et al. 2021). Loss of ALC1 also promotes PARP trapping and enhances the effects of PARPi in BRCA-deficient cells (Blessing et al. 2020; Juhász et al. 2020; Hewitt et al. 2021; Verma et al. 2021), making ALC1 inhibitors potential therapeutics for BRCA-deficient tumors (Abbott et al. 2020).

Several other inhibitors are generated to exploit the replication stress in cancer cells. An RPA inhibitor increases replication stress and synergizes with chemotherapy to suppress tumor growth in mice (Glanzer et al. 2014), possibly by enhancing replication catastrophe. Similar to WEE1, the PKMYT1 kinase restricts CDK1 activity, and a PKMYT1 inhibitor induces synthetic lethality in cancer cells harboring Cyclin E amplification by causing unscheduled CDK1 activation (Table 1; Gallo et al. 2021). Moreover, loss of the WRN helicase increases replication stress at expended microsatellite repeats in MMR-deficient cancer cells, leading to synthetic lethality (van Wietmarschen et al. 2020). Inhibitors of WRN are potentially useful for the treatment of MMR-deficient tumors (Sommers et al. 2019).

A few other potential therapeutic targets have emerged in various contexts of DNA repair defects or genomic instability, although specific inhibitors of these targets are not yet available. APEX2 and CIP2A were identified as synthetic lethal hits in BRCA1/2-deficient cells through CRISPR–Cas9 screens (Mengwasser et al. 2019; Álvarez-Quilón et al. 2020; Adam et al. 2021). Loss of FANCM induces cell death in ALT$^+$ cancer cells (Pan et al. 2019). Loss of HMCES reduces the survival of cancer cells overexpressing the APOBEC3A cytidine deaminase (Mehta et al. 2020; Biayna et al. 2021).

Overcoming resistance to DDR targeted drugs

Although PARPis show efficacy in cancer patients carrying *BRCA1/2* mutations, patients inevitably acquire resistance to these drugs over time. A number of PARPi resistance mechanisms have been observed in cell lines, mouse tumor models, and patient samples, including reversion mutations of *BRCA1/2* genes (Sakai et al. 2008; Weigelt et al. 2017), up-regulation of drug efflux pumps (Rottenberg et al. 2008), stabilization of truncated BRCA1 protein (Johnson et al. 2013), and loss of PARP1 (Pettitt et al. 2013). Loss of the 53BP1–RIF1–Shieldin pathway increases DNA end resection in BRCA1-deficient cells, conferring resistance to PARPi (Bunting et al. 2010; Jaspers et al. 2013; Xu et al. 2015; Dev et al. 2018; Ghezraoui et al. 2018; Gupta et al. 2018; Mirman et al. 2018; Noordermeer et al. 2018). Loss of the CTC1–STN1–TEN1 (CST) complex, which is recruited by Shieldin, also leads to PARPi resistance (Barazas et al. 2018). Furthermore, loss of other inhibitors of DNA end resection, such as HELB, DYNLL1, and ZPET, also increase PARPi resistance (Tkáč et al. 2016; He et al. 2018; Moquin et al. 2019). When BRCA2-deficient cells regain the ability to protect stalled replication forks, they become resistant to PARPi (Ray Chaudhuri et al. 2016). Finally, loss of SLFN11 confers PARPi resistance (Lok et al. 2017), possibly due to the lack of SLFN11-mediated replication inhibition.

Using cell lines and patient-derived xenograft models, it was shown that ATRi can effectively overcome the PARPi resistance of BRCA1/2-deficient cancer cells (Kim et al. 2017, 2020; Yazinski et al. 2017). Notably, multiple PARPi resistance mechanisms are observed in single-cell-derived cell lines, suggesting that different mechanisms can operate in the same cells to confer resistance (Yazinski et al. 2017). ATRi inhibits the restored RAD51 loading and fork protection in resistant cells (Yazinski et al. 2017; Parmar et al. 2019). ATRi may also override the regained checkpoint of resistant cells, leading to more collisions between replication forks and ssDNA gaps (Simoneau et al. 2021). Finally, ATRi overcomes the PARPi resistance of SLFN11-deficient cells (Murai et al. 2016). Besides ATRi, POLθi overcomes the PARPi resistance in BRCA1/2-deficient cells by blocking MMEJ (Zatreanu et al. 2021; Zhou et al. 2021). REV1i overcomes the PARPi resistance in BRCA1-deficient cells by inhibiting ssDNA gap repair (Taglialatela et al. 2021). Inhibitors of DNPH1 and USP1 overcome the PARPi resistance in BRCA-deficient cells by increasing SSBs and destabilizing replication forks, respectively (Lim et al. 2018; Fugger et al. 2021). Furthermore, CDK12 inhibition overcomes the PARPi resistance in TNBC by reducing the expression of HR genes (Johnson et al. 2016).

The resistance to ATRi is less understood. Loss of CDC25A, which reduces CDK1/2 activities, confers ATRi resistance (Ruiz et al. 2016). Loss of the mediator protein MED12 renders cells resistant to ATRi by stabilizing replication forks (Schleicher et al. 2020). Finally, loss of Cyclin C and CDK8, two components of the mediator

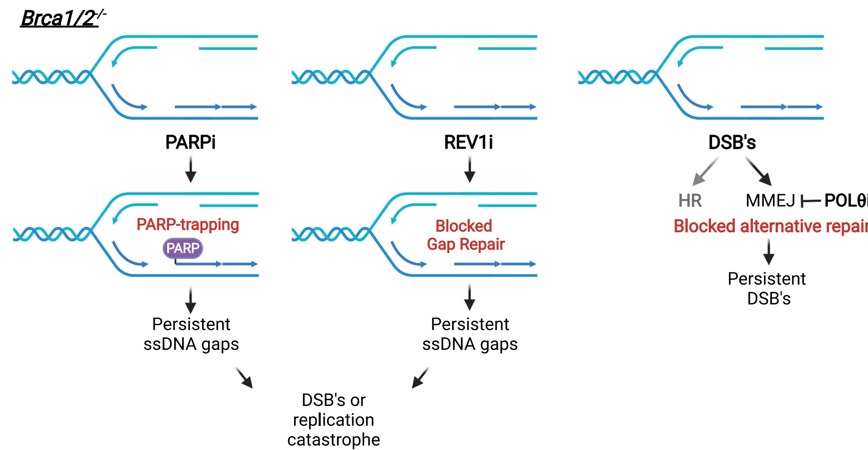

Figure 4. Selective killing of BRCA1/2-deficient cells by different DDR targeted drugs. Both PARPi and REV1i render the ssDNA gaps in BRCA1/2-deficient cells persistent, promoting DSBs or replication catastrophe. POLθi blocks MMEJ and prevents alternative repair of DSBs in HR-defective cells, rendering DSBs persistent in BRCA1/2-deficient cells.

complex, reduces R-loops and leads to ATRi resistance (Lloyd et al. 2021).

Targeting DDR defects with immunotherapy

The production of neoantigens by cancer cells is a key determinant for the antitumor immunity. Cancer cells defective in MMR accumulate high levels of mutations, increasing tumor mutation burden (TMB) (Ballhausen et al. 2020). Tumors with MMR deficiency (MMR-D) or high MSI (MSI-H) are more responsive to immune checkpoint inhibitors (ICIs) (Le et al. 2015). Pembrolizumab, an anti-PD-1 monoclonal antibody, has been approved by the FDA for the treatment of advanced colorectal cancer with MMR-D or MSI-H. Defects in MLH1, which regulates the EXO1 nuclease during MMR, lead to unrestricted EXO1 activity at DSBs and activation of the cGAS–STING pathway (Guan et al. 2021). The cGAS–STING-mediated interferon response enhances the response of MLH1-deficient tumors to immunotherapy independently of changes in neoantigens (Lu et al. 2021). A recent study on MMR-D colorectal tumors revealed multiple spatially organized hubs of interacting malignant and immune cells, providing insights into how MMR-D malignant cells elicit the immune response (Pelka et al. 2021). *POLE* and *POLD1* mutations in tumors are also associated with responses to immunotherapy (Wang et al. 2019b; Li et al. 2020). Thus, a subset of class III repair defects in cancer cells can lead to high TMB and an interferon response, providing an opportunity for immunotherapy.

ICIs have been tested in BRCA1/2-deficient tumors. A recent study suggested that the combination of BRCA2 mutations and TMB is a potential predictor of immunotherapy response (Zhou and Li 2021). Another study showed that BRCA2-deficient tumors are enriched for adaptative and innate immunity (Samstein et al. 2021). In mouse models of *BRCA1*-deficent ovarian cancer and TNBC, PARPi enhanced the effects of ICIs by eliciting the cGAS–STING pathway (Ding et al. 2018; Pantelidou et al. 2019). PARPi enhances infiltration and activation of $CD8^+$ T cells through the cGAS–STING pathway in tumor cells and paracrine activation of dendritic cells. Combinations of PARPi and ICIs have produced promising results in BRCA1/2-deficient tumors in trials (Vikas et al. 2020). The combinations of PARPi and ICIs are effective in some patients without *BRCA1/2* mutations, suggesting that this strategy may exploit the genomic instability from different sources.

In response to DSBs, PD-L1 expression is up-regulated in an ATR-dependent manner (Sato et al. 2017). ATRi reduces the expression of PD-L1 in cancer cells, sensitizing them to T-cell-mediated cell killing (Sun et al. 2018; Vendetti et al. 2018). ATRi also enhances the radiation-induced inflammatory tumor microenvironment (Dillon et al. 2019; Sheng et al. 2020). When treated with ATRi, irradiated cancer cells undergo mitosis in the presence of DSBs, increasing micronuclei and activation of the cGAS–STING pathway (Chen et al. 2020). The RNA-induced and RIG-I-mediated interferon response is also activated in irradiated cells upon ATR inhibition (Chen et al. 2020; Feng et al. 2020). Collapse of replication forks may give rise to AT-rich DNA fragments, which are transcribed into RNA to trigger the RIG-I pathway (Feng et al. 2020). Collectively, ATRi may potentiate the tumor-immune microenvironment in multiple ways, enhancing the efficacy of combined radioimmunotherapy.

Perspectives

Although a number of DNA repair and damage signaling pathways have been extensively studied, we still lack a complete understanding of how these pathways function in various oncogenic contexts. Why are certain DDR pathways lost in specific cancer types? Are the consequences of DDR defects distinct in different tissues and cell types? Our understanding of the interplays between oncogenic stress and the DDR is still limited. Does the oncogenic stress in cancer cells determine which DDR pathways to lose? Does the loss of DDR pathways in cancer cells determine which oncogenic events to acquire? Understanding the different sources of genomic instability in cancer cells and the effects of DDR defects in different oncogenic contexts is critical for developing specific strategies to induce

synthetic lethality. It is also important to note that the wiring of DDR pathways can be distinct in various tissues and cell types and under different selective pressures. The loss of specific DDR pathways in cancer cells or the selective pressure during tumor evolution and cancer therapy could reshape the wiring of the DDR network. A better understanding of the rewiring of the DDR network in cancer cells is important for targeting DDR pathways and overcoming the resistance to DDR targeted drugs. Finally, it is important to investigate how the DDR defects in cancer cells affect tumor microenvironments, and how tumor microenvironments impact the DDR in cancer cells. It is conceivable that future studies using patient samples, in vivo models, and single-cell analyses will profoundly improve our understanding of the genomic instability in tumors and the efficacy of DDR targeted therapy.

Competing interest statement

The authors declare no competing interests.

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