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Review Article

Targeting DNA damage response in cancer therapy

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Cancer chemotherapy and radiotherapy are designed to kill cancer cells mostly by inducing DNA damage. DNA damage is normally recognized and repaired by the intrinsic DNA damage response machinery. If the damaged lesions are successfully repaired, the cells will survive. In order to specifically and effectively kill cancer cells by therapies that induce DNA damage, it is important to take advantage of specific abnormalities in the DNA damage response machinery that are present in cancer cells but not in normal cells. Such properties of cancer cells can provide biomarkers or targets for sensitization. For example, defects or upregulation of the specific pathways that recognize or repair specific types of DNA damage can serve as biomarkers of favorable or poor response to therapies that induce such types of DNA damage. Inhibition of a DNA damage response pathway may enhance the therapeutic effects in combination with the DNA-damaging agents. Moreover, it may also be useful as a monotherapy when it achieves synthetic lethality, in which inhibition of a complementary DNA damage response pathway selectively kills cancer cells that have a defect in a particular DNA repair pathway. The most striking application of this strategy is the treatment of cancers deficient in homologous recombination by poly(ADP-ribose) polymerase inhibitors. In this review, we describe the impact of targeting the cancer-specific aberrations in the DNA damage response by explaining how these treatment strategies are currently being evaluated in preclinical or clinical trials.

The genome DNA is constantly exposed to various genotoxic insults. Among the variety of types of DNA damage, the most deleterious is the DNA double-strand break (DSB).[1] Double-strand breaks can be generated by endogenous sources such as reactive oxygen species produced during cellular metabolic processes and replication-associated errors, as well as by exogenous sources including ionizing radiation and chemotherapeutic agents. Double-strand breaks are also generated in a programmed manner during meiosis and during the V(D)J recombination and class switch recombination required for the development of lymphocytes. If left unrepaired, DSBs can result in cell death. If accurately repaired, DSBs can result in survival of cells with no adverse effects. If insufficiently or inaccurately repaired, DSBs can result in survival of cells showing genomic alterations that may contribute to tumor development.[2] In order to maintain genomic integrity, cells have evolved a well coordinated network of signaling cascades, termed the DNA damage response, to sense and transmit the damage signals to effector proteins, and induce cellular responses including cell cycle arrest, activation of DNA repair pathways, and cell death (Fig. 1).[1]

Cancer chemotherapeutic agents and radiotherapy exert their cytotoxic effects by inducing DNA DSBs. As cancer cells often have specific abnormalities in the DNA damage response, therapeutic strategies based on such properties of cancer cells have been developed. Several inhibitors that block specific DNA damage responses or repair proteins have been tried not only as sensitizing agents in combination with DNA-damaging agents but also as single agents against cancers with defects in particular DNA repair pathways. The most prominent example of the latter is the killing effect of poly (ADP-ribose) polymerase (PARP) inhibitors on BRCA1- or BRCA2-defective tumors, which takes advantage of the defects in DNA repair in cancer cells.[3]

In this review, we will first outline the mechanism of the DNA damage response. Next, we will describe the aberrations in DNA damage responses in human cancers. Finally, we will explain how different DNA damage response pathways can be targeted for cancer therapy.

Mechanism of DNA Damage Response

DNA-damaging agents induce various types of DNA damage including modification of bases, intrastrand crosslinks, interstrand crosslinks (ICL), DNA–protein crosslinks, single-strand breaks (SSBs), and DSBs. Each type of DNA damage is recognized and processed by proteins involved in the DNA damage response (Fig. 1).

In response to DSBs, the MRE11–RAD50–NBS1 (MRN) complex senses and binds to DSB sites, and recruits and activates the ataxia telangiectasia mutated (ATM) kinase through its autophosphorylation.[4,5] Once activated, ATM

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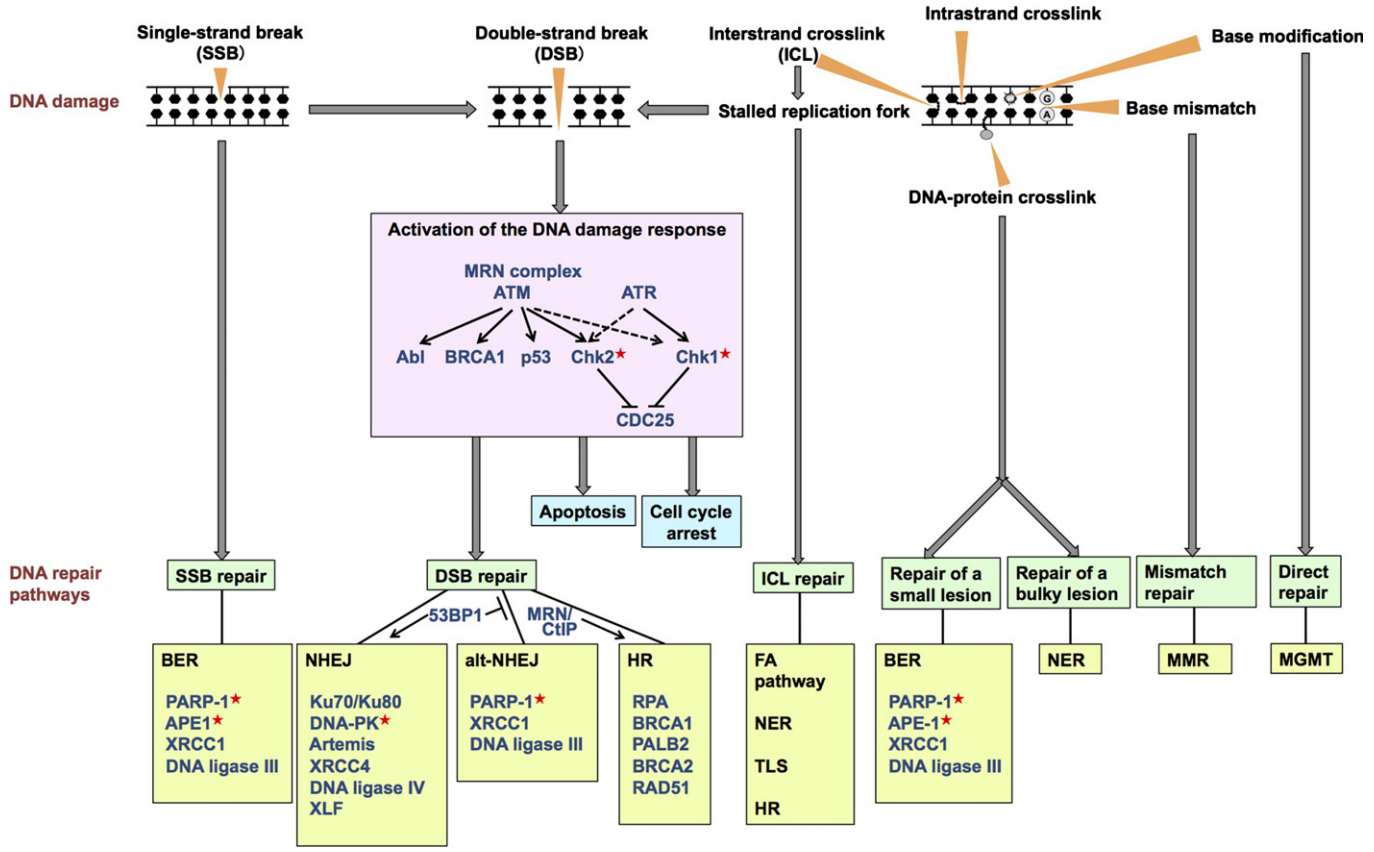

Fig. 1. Overview of the diverse spectrum of DNA damage and the DNA damage response. The major repair pathways and key proteins used to process each type of damage are shown. In non-homologous end-joining (NHEJ), the Ku70/Ku80 complex binds to the DNA double-strand break ends and recruits the other indicated components. In base-excision repair (BER), poly(ADP-ribose) polymerase-1 (PARP-1) detects and binds to single-strand breaks and ensures accumulation of other repair factors at the breaks. Single-strand breaks containing modified DNA ends are recognized by damage-specific proteins such as apurinic/apyrimidinic endonuclease (APE1), which subsequently recruits Polβ and XRCC1-DNA ligase IIIα to accomplish the repair. All the molecules indicated here are aberrated in sporadic cancers. The proteins targeted for cancer therapy in the present clinical trials are marked with red asterisks. alt-NHEJ, alternative NHEJ; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related; FA, Fanconi anemia; HR, homologous recombination; MGMT, O^6-methylguanine-DNA methyltransferase; MMR, mismatch repair; MRN, MRE11–RAD50–NBS1; NER, nucleotide excision repair; TLS, translesion synthesis.

phosphorylates a large number of downstream proteins.[6] Phosphorylation of Chk2 induces phosphorylation of the protein phosphatase CDC25A, leading to cell cycle arrest. Phosphorylation of BRCA1 leads to DSB repair as well as cell cycle arrest in the S phase, whereas activation of p53 triggers cell cycle arrest in the G_1 phase or cell death. In the initiation of the response to SSBs or DNA replication fork collapse, the ataxia telangiectasia and Rad3-related (ATR) kinase is activated and recruited to the sites of DNA damage.[7] ATR phosphorylates and activates Chk1,[8] which plays a role in the S and G_2/M cell checkpoints by regulating the stability of the CDC25 phosphatases. Activation of the 53BP1 protein, a mediator of the DNA damage response, contributes to the choice of the DSB repair pathways by promoting non-homologous end joining (NHEJ).[9]

The DNA repair pathways can either work independently or coordinately to repair different types of DNA damage (Fig. 1). Double-strand breaks are predominantly repaired by either NHEJ or homologous recombination (HR).[10] Non-homologous end joining is an error-prone repair pathway that is mediated by the direct joining of the two broken ends.[10] Factors involved in NHEJ include the Ku70/Ku80 complex, DNA-PK catalytic subunit (DNA-PKcs), the Artemis nuclease, XLF, XRCC4, and DNA ligase IV. Homologous recombination is an error-free repair pathway that requires a non-damaged sister chromatid to serve as a template for repair (Fig. 2).[10] Factors involved in HR include the MRN complex, CtIP, replication protein A (RPA), BRCA1, PALB2, BRCA2, and RAD51. In addition to NHEJ and HR, an alternative form of NHEJ, namely, alt-NHEJ, is also involved in DSB repair.[11] It exhibits a slower process than the classical NHEJ and can catalyze the joining of unrelated DNA molecules, leading to the formation of translocations as well as large deletions and other sequence alterations at the junction. Factors involved in this pathway include PARP-1, XRCC1, DNA ligase IIIα, polynucleotide kinase, and Flap endonuclease 1.

Single-strand breaks and subtle changes to DNAs are repaired using base-excision repair (BER) proteins,[12] which include PARP-1, XRCC1, DNA ligase IIIα, and apurinic /apyrimidinic endonuclease (APE1). Bulky DNA lesions such as pyrimidine dimers caused by UV irradiation are processed by the nucleotide excision repair (NER) pathway,[13] which requires the excision repair cross-complementing protein 1 (ERCC1). Base mismatches arising as a result of replication errors can be repaired by the mismatch repair pathway.[14]

In the repair of ICL, ubiquitin-mediated activation of the Fanconi anemia (FA) pathway plays a key role.[15] The FA pathway is constituted by at least 15 FA gene products, whose germline defects result in FA, a cancer predisposition syndrome.

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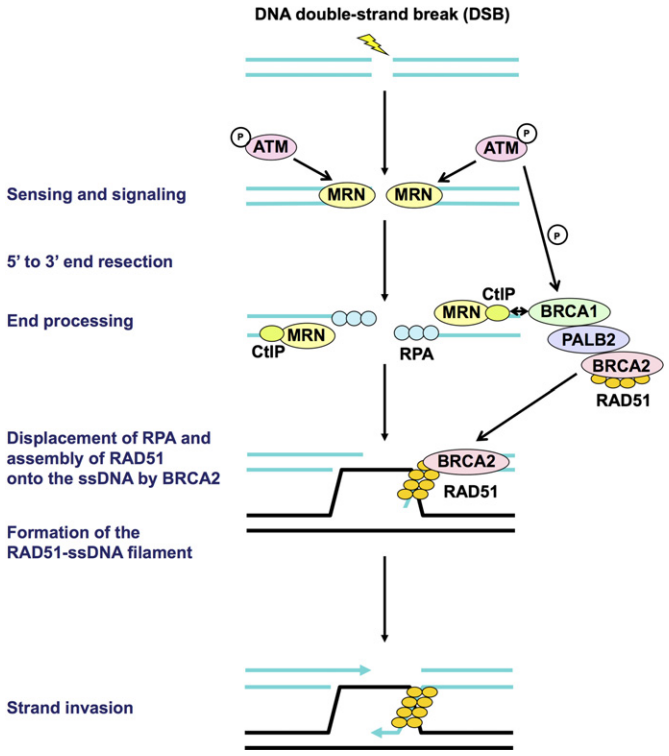

Fig. 2. Early steps of homologous recombination. First, the DNA double-strand break is sensed by the MRE11–RAD50–NBS1 (MRN) complex, which subsequently recruits and activates the ataxia telangiectasia mutated (ATM) kinase. Then, the DNA ends are resected by the MRN complex and CtIP, resulting in generation of 3′ single-stranded DNA (ssDNA) overhangs on both sides of the break. These overhangs are coated and stabilized by replication protein A (RPA). Next, BRCA2, which forms the BRCA1–PALB2–BRCA2 complex, directly binds RAD51 and recruits it to the double-stranded DNA–ssDNA junction, and promotes the loading of RAD51 onto ssDNA. This step is followed by displacement of RPA from ssDNA ends and assembly of the RAD51–ssDNA filament, which is mediated by BRCA2, leading to strand invasion into an undamaged homologous DNA template. All the molecules indicated here are aberrated in sporadic cancers. None of the proteins indicated here are targeted for cancer therapy in the present clinical trials. P, phosphorylation.

Activation of the FA core complex, which is comprised of eight FA proteins (FANCA/B/C/E/F/G/L/M) and associated proteins, leads to monoubiquitination of FANCD2 and FANCDI, which subsequently coordinates three critical DNA repair processes, including nucleolytic incision by XPF-ERCC1 and SLX4 endonucleases, translesion DNA synthesis, and HR.

Aberrations in DNA Damage Responses in Human Cancers

In sporadic cancers, both activation and inactivation of the DNA damage response are found in various cancers,[16–62] as summarized in Table 1.

Regarding activation of the DNA damage response proteins, increased autophosphorylation of ATM and ATM-dependent phosphorylation of Chk2 are reported in early-stage tumors, suggesting that the DNA damage response may serve as a barrier to the malignant progression of tumors.[16,17] In contrast, a recent study reports that ATM is hyperactive in late-stage breast tumor tissues, suggesting that the ATM-mediated DNA damage response also plays a role in tumor progression and metastasis.[18] Increased expression of NBS1, RAD50, Chk1, Chk2, CDC25A, CDC25B, and CDC25C are also reported.[19–28] DNA-PK catalytic subunit is reported to be upregulated in radiation-resistant tumors or in tumors with poor survival.[29,30] Overexpression of RAD51, BRCA1, ERCC1, APE1, and PARP1 is also observed in various cancers and is associated with resistance to chemotherapy.[31–46]

However, inactivation of DNA damage response proteins is also observed in various cancers. The *p53* gene is one of the most frequently mutated genes in human sporadic cancers. Although the reported frequencies of *p53* mutation vary among the types of cancer, it is estimated that more than half of cancers might have inactivated p53 due to mutations, deletion, loss of heterozygosity of the gene, or decreased expression.[47,48] Although inactivating mutations in *ATM*, *BRCA1,* or *BRCA2* are less frequent than those in the *p53* gene,[49–53] decreased expression of ATM, the MRN complex, Chk2, RAD51, BRCA1, BRCA2, and ERCC1 is frequently observed, suggesting that aberration of the DNA damage response is common in sporadic cancers.[19,22,23,26,54–62] Promoter hypermethylation of the *BRCA1* gene is frequently observed and may be one of the predominant mechanisms for deregulation of the *BRCA1* gene.[62] Furthermore, our group reported the functional inactivation of BRCA2 in cancer cells aberrantly expressing SYCP3, a cancer-testis antigen.[63] Disruption of the FA pathway resulting from mutations or decreases or loss of expression due to promoter hypermethylation has been also described in various cancers.[64,65]

As described above, both activation and inactivation of the DNA damage response are observed in cancers, and are expected to determine important properties of the DNA damage response machinery present in each cancer. The status of BRCA has been adopted as an important condition factor in current clinical trials, however, the status of other DNA damage response proteins have not yet been translated into clinical trials. In the next section, we will introduce various approaches for taking advantage of these cancer-specific properties of the DNA damage response in cancer therapy.

How Can Different DNA Damage Response Pathways be Targeted for Cancer Therapy?

Because the efficacy of cancer chemotherapy and radiotherapy relies on generation of DNA damage that will be recognized and repaired by intrinsic DNA repair pathways, aberrant expression of a particular DNA damage response protein should be a biomarker of resistance or favorable response to therapies that induce the corresponding types of DNA damage.[66] For example, patients with surgically treated non-small-cell lung cancer whose tumors lacked expression of ERCC1 were shown to benefit from cisplatin-based adjuvant chemotherapy in a clinical study.[38] Another example is the case of RAD51, whose expression can serve as a marker of cisplatin resistance in non-small-cell lung cancer, which is consistent with the role of HR in the repair of ICL.[31]

In contrast, many inhibitors of the DNA damage response have been developed and some of them have been tested for their potential to enhance DNA damage-induced tumor cell killing in preclinical studies and clinical trials (Tables 2 and 3).

Inhibitors of ATM/ATR and the MRN complex. As ATM and the MRN complex play central roles as sensors or mediators in the DNA damage response, these molecules have been considered to be promising targets for radiosensitization or chemosensitization.[67] Several promising ATM inhibitors have been developed (Table 2). KU55933, the first specific inhibitor of ATM, inhibits radiation-induced ATM-dependent phosphorylation events and sensitizes cancer cells to radiation and

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Table 1. Examples of aberrations in DNA damage responses in human sporadic cancers

Molecule	Activation or inactivation	Type of aberrations	Type(s) of cancer	Frequency	Phenotypes	Reference(s)
ATM	Activation	Increased autophosphorylation	Bladder, breast cancers	30–68%	Cancer barrier function	(16,18)
		Increased copy number	Prostate cancers	~2%		(51)
	Inactivation	Mutation	Pancreatic, lung, colon, endometrial, prostate, skin, kidney, breast, central nervous system, ovarian cancers	1–7%		(49,50)
			Hematopoietic and lymphoid malignancies	~11%		(49)
		Loss of heterozygosity, loss	Pancreatic cancers	~5%		(50)
		Decreased copy number	Prostate cancers	~5%		(51)
		Decreased expression	Breast, head and neck cancers	25–75%		(54,55)
MRE11	Inactivation	Decreased expression	Breast cancers	7–31%		(19,54,56)
			Colorectal, gastric, pancreatic cancers with microsatellite instability	67–100%		(19)
RAD50	Activation	Increased expression	Colorectal cancers	~24%		(21)
	Inactivation	Decreased expression	Breast cancers	3–28%		(19,54,56)
			Colorectal, gastric cancers with microsatellite instability	28–71%		(19)
NBS1	Activation	Increased expression	Esophageal, head and neck, non-small-cell lung cancers, hepatomas	40–52%	Poor prognosis	(19,20)
	Inactivation	Decreased expression	Breast cancers	10–46%		(19,54,56)
Chk1	Activation	Increased phosphorylation	Cervical cancers	~25%		(27)
		Increased expression	Lung, liver, breast, colorectal, ovarian, cervical cancers	46–100%	Resistance to chemotherapy, poor prognosis	(22–27)
	Inactivation	Decreased expression	Lung, ovarian cancers, hetapocellular carcinomas	9–32%		(22,23,26)
Chk2	Activation	Increased phosphorylation	Bladder, colon, lung cancers, melanomas	30–50%	Cancer barrier function	(16,17)
		Increased expression	Ovarian cancers	~37%		(26)
	Inactivation	Decreased expression	Breast, non-small cell lung cancers	28–47%		(57,58)
p53	Inactivation	Mutation	Solid tumors	~50%		(47)
			Hematopoietic malignancies	~10%		(47)
		Decreased expression	Solid and hematopoietic tumors	~50%	Resistance to chemotherapy, poor prognosis	(48)
CDC25A	Activation	Increased expression	Thyroid, breast, ovarian, liver, colorectal, laryngeal, esophageal cancers, non-Hodgkin's lymphomas	17–70%		(28)
CDC25B	Activation	Increased expression	Thyroid, breast, ovarian, liver, gastric, colorectal, laryngeal, esophageal, endometrial, prostate cancers, gliomas, non-Hodgkin's lymphomas	20–79%		(28)
CDC25C	Activation	Increased expression	Colorectal, endometrial cancers, non-Hodgkin's lymphomas	13–27%		(28)
DNA-PKcs	Activation	Increased expression	Glioblastoma, prostate cancers	~49%	Poor survival	(29,30)

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Table 1. (continued)

Molecule	Activation or inactivation	Type of aberrations	Type(s) of cancer	Frequency	Phenotypes	Reference(s)
RAD51	Activation	Increased expression	Breast, head and neck, non-small-cell lung cell, pancreatic cancers, soft tissue sarcomas	24–66%	Resistance to platinum agents, poor outcome	(31–35)
	Inactivation	Decreased expression	Breast, colorectal cancers	~30%		(59,60)
BRCA1	Activation	Increased expression	Lung cancers	~22%	Resistance to chemotherapy	(36)
	Inactivation	Mutation	Breast, ovarian cancers	<10%		(52,53)
		Decreased expression	Breast, ovarian, lung cancers	9–30%		(60–62)
BRCA2	Inactivation	Mutation	Breast, ovarian cancers	<10%		(52,53)
		Decreased expression	Ovarian cancers	13%		(61)
ERCC1	Activation	Increased expression	Colorectal, ovarian, gastric, head and neck, non-small-cell lung cancers	14–70%	Resistance to platinum agents	(31,37–43)
	Inactivation	Decreased expression	Colorectal, gastric, non-small-cell lung cancers	30–77%		(37,38,42,43)
APE1	Activation	Increased expression	Bladder, breast, cervical, head and neck, liver, non-small-cell lung cancers, ovarian cancers, medulloblastomas, gliomas, osteosarcomas, germ cell tumors	19–99%	Resistance to chemotherapy and/or radiation	(44)
PARP	Activation	Increased expression	Breast cancers, germ cell tumors	5–47%		(45,46)
FANCA	Inactivation	Decreased expression /loss of expression	Acute myelogenous leukemias	4–40%		(64,65)
		Mutation	Acute myelogenous leukemias	~7.6%		(64)
FANCC	Inactivation	Mutation, loss of heterozygosity	Pancreatic cancers	~9%		(64)
FANCF	Inactivation	Decreased expression /loss of expression	Breast, cervical, head and neck, non-small-cell lung, ovarian cancers, acute myelogenous leukemias, germ cell tumors	6.7~30%		(64,65)
FANCG	Inactivation	Loss of expression	Acute myelogenous leukemias	27%		(65)

Expression has been confirmed at mRNA and/or protein levels. Studies using cultured cancer cells are excluded.

topoisomerase inhibitors.[67] KU60019, an improved analog of KU55933, inhibits the DNA damage response and effectively radiosensitizes human glioma cells.[68] Mirin is an inhibitor of the MRN complex, which prevents MRN-dependent activation of ATM without affecting ATM protein kinase activity and inhibits MRE11-associated exonuclease activity.[67] Telomelysin is another inhibitor that inhibits the MRN complex through the adenoviral E1B-55 kDa protein.[67] The therapeutic outcomes of these agents remain to be tested in clinical trials. Although the long search for selective inhibitors of ATR has not yet paid off, schisandrin B was recently identified as a moderate selective ATR inhibitor, although it will also affect ATM at high concentrations.[69] Recently, two novel ATR inhibitors, NU6027 and VE-821, were also shown to sensitize cells to a variety of DNA-damaging agents in preclinical studies.[70,71]

Inhibitors of Chk1/Chk2 and CDC25. As the triggering of cell cycle checkpoints is crucial in the DNA damage response, these checkpoints have also been widely investigated as a potential target for cancer therapy (Table 3).[72] Among the inhibitors for Chk1 and/or Chk2, UCN-01 was the first to enter clinical trials, but it was discontinued due to toxicities such as symptomatic hypotension and neutropenia and a lack of convincing efficacy after phase II trials.[72] Other Chk1/Chk2 inhibitors with improved specificities, including XL844 and AZD7762, also entered clinical trials but failed to achieve a good response.[72] The selective Chk1 inhibitor SCH900776 has been used in phase I trials for acute leukemia in combination with cytarabine and for solid tumors in combination with gemcitabine, and showed some partial responses and stable disease.[72] The Chk1 inhibitor LY2603618 and the dual Chk1/Chk2 inhibitor LY2606368 are also currently being tested in early clinical trials. CDC25 phosphatases, the key factors in cyclin-dependent kinase activation crucial for cell cycle regulation, are also considered to represent promising novel targets in cancer therapy. CDC25 inhibitors have also been developed, and some have entered into clinical trials, although the clinical data is limited.[73]

Inhibition of NHEJ by DNA-PK inhibitors. Regarding NHEJ, inhibitors of DNA-PK, including NU7026 and NU7441, were found to induce extreme sensitivity to ionizing radiation as

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Table 2. Examples of DNA damage response inhibitors in preclinical studies

Pathway	Target(s)	Name(s)	Preclinical evidence
DNA damage sensors and mediators	MRE11	Mirin, telomelysin	Sensitization to ionizing radiation
	ATM	KU55933, KU60019, CP466722	Sensitization to ionizing radiation and topoisomerase inhibitors
	ATR	Schisandrin B	Sensitization to UV treatment
		NU6027, VE-821	Sensitization to ionizing radiation and a variety of chemotherapy
Cell cycle checkpoints	Chk1	SAR-020106	Sensitization to irinotecan and gemcitabine
	Chk2	VRX0466617	Sensitization to ionizing radiation
Non-homologous end joining	DNA-PK	NU7026, NU7441	Sensitization to ionizing radiation and topoisomerase II inhibitors
	DNA-PK and PI3K	KU-0060648	Sensitization to etoposide and doxorubicin
	DNA ligase IV	SCR7	Sensitization to ionizing radiation and etoposide
Alternative non-homologous end joining	DNA ligases I and IIIα	L67	Sensitization to ionizing radiation and methyl methanesulfonate
Homologous recombination (HR)	RAD51	B02, A03, A10	Identified by high-throughput screenings of RAD51 inhibitors

well as DNA-damaging agents in preclinical studies (Table 2).[74] However, the therapeutic efficacy of DNA-PK inhibitors depends on the expression levels of DNA-PK in cancer cells versus normal cells, and their clinical application is currently restricted because of their toxicity to normal cells. The dual mTOR and DNA-PKcs inhibitor CC-115 is undergoing early clinical evaluation (Table 3). KU-0060648 is a potent dual inhibitor of DNA-PK and PI-3K, which has recently been reported to enhance etoposide and doxorubicin cytotoxicity (Table 2).[75]

Inhibition of NHEJ or alt-NHEJ by DNA ligase inhibitors. DNA ligases are required for both NHEJ and alt-NHEJ pathways as well as other DNA repair pathways such as BER and NER. Small molecule inhibitors of human DNA ligases have been identified and shown to be cytotoxic and also to enhance the cytotoxicity of DNA-damaging agents. SCR7 is an inhibitor of DNA ligase IV, which is involved in the NHEJ pathway. SCR7 reduces cell proliferation in a DNA ligase IV-dependent manner and increases the tumor-inhibitory effects of agents that cause DSBs.[76] L67 is an inhibitor of DNA ligases I and IIIα, which are involved in the alt-NHEJ pathway as well as BER and NER. The levels of the alt-NHEJ proteins such as DNA ligase IIIα and WRN are reported to be elevated in BCR-ABL-positive CML cell lines,[77] so inhibition of alt-NHEJ factors may be an additional therapeutic approach in BCR-ABL-positive CML, which is usually treated by tyrosine kinase inhibitors. Indeed, CML cell lines with increased alt-NHEJ were shown to be hypersensitive to the combination of L67 and PARP inhibitor.[78]

Inhibitors of RAD51 and tyrosine kinases regulating HR. With respect to HR, there are currently few inhibitors that directly target HR proteins. Along with the RAD51 inhibitors that were recently identified (Table 2),[79] the molecules that indirectly regulate HR may also be candidate targets for inhibiting HR. For example, the non-receptor tyrosine kinase c-Abl is activated by ATM in response to DNA damage, and subsequently phosphorylates RAD51.[80] Oncogenic fusion tyrosine kinases, such as BCR-ABL, TEL-ABL, TEL-JAK2, TEL-PDGFβR, and NPM-ALK, enhance the expression levels and/or tyrosine phosphorylation of RAD51.[81,82] From these findings, inhibitors of oncogenic tyrosine kinases are expected to sensitize cancer cells to DNA-damaging agents. Consistent with this hypothesis, treatments with the tyrosine inhibitor imatinib have been shown to enhance sensitivity to DNA crosslinking agents and ionizing radiation in cancer cells.[81] Furthermore, targeting RAD51 was shown to overcome imatinib resistance in CML cells.[83]

Inhibitors of histone deacetylases, heat shock protein 90, and DSB repair. Histone deacetylases (HDACs) are powerful regulators of the stability of the genome, and many HDAC inhibitors are shown to downregulate multiple components of the DNA damage response and repair, including HR, NHEJ, the MRN complex, and ATM.[84] Thus, the use of HDAC inhibitors in combination with DNA-damaging agents may be an area of great interest with potential clinical utility. The HDAC inhibitor PCI-24781 caused increased apoptosis by inhibiting RAD51-mediated HR when used in combination with the PARP inhibitor PJ34 in a preclinical study.[85] The inhibitor of heat shock protein 90, 17-allylamino-17-demethoxygeldanamycin, radiosensitizes human tumor cell lines by inhibiting RAD51-mediated HR.[86] Curcumin is a natural product that has been tested for its chemosensitizing potential, and sensitizes cancer cells to PARP inhibitors by inhibiting NHEJ, HR, and the DNA damage checkpoint.[87]

Inhibitors of PARP and APE1 in combination with DNA-damaging agents. Inhibitors of PARP, which inhibit the BER and SSB repair pathways, are the most advanced and promising drugs that target DNA repair.[88] A number of clinical trials using PARP inhibitors are currently underway (Table 3). Inhibitors of PARP were first tried in combination with DNA-damaging agents. Some clinical responses were observed in the phase I and II trials of the PARP inhibitor rucaparib in combination with temozolomide.[89,90] Further clinical trials of PARP inhibitors have been carried out in combination with various DNA-damaging agents and/or ionizing radiation (Table 3). Inhibitors of another BER protein APE1 are also being tested in combination with DNA-damaging agents in clinical trials (Table 3).

Using PARP inhibitors as single agents in BRCA-deficient cancers based on the principle of synthetic lethality. In 2005, PARP inhibitors were shown to selectively inhibit the growth of cells with defects in either the *BRCA1* or *BRCA2* genes, suggesting a new use of PARP inhibitors as single agents.[91,92] A possible explanation for this lethality is as follows. The cancer cells with defects in the *BRCA* gene are defective in HR, as the wild-type *BRCA* allele is absolutely lost. However, HR is intact in normal cells of the same patients who carry one wild-type *BRCA* allele and one mutant *BRCA* allele. Inhibition of PARP1 results in the accumulation of SSBs, which are converted to lethal DSBs that require HR for their repair.

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Table 3. Examples of DNA damage response inhibitors in clinical trials

Pathway	Target(s)	Name	Combination	Type of cancer	Clinical trial number	Stage	Trial periods
Cell cycle checkpoints	Chk1	UCN-01	Combination therapy				
			Carboplatin	Advanced solid tumor	NCT00036777	Phase I	Completed
			Irinotecan	Metastatic or unresectable solid tumor, triple negative breast cancer	NCT00031681	Phase I	Completed
			Cytarabine	Refractory or relapsed acute myelogenous leukemia, myelodysplastic syndrome	NCT00004263	Phase I	Completed
			Perifosine	Relapsed or refractory acute leukemia, chronic myelogenous leukemia, high risk myelodysplastic syndrome	NCT00301938	Phase I	Completed
			Gemcitabine	Unresectable or metastatic pancreatic cancer	NCT00039403	Phase I	Completed
			Topotecan	Relapsed or progressed small-cell lung cancer	NCT00098956	Phase II	Completed
			Cisplatin	Advanced malignant solid tumor	NCT00012194	Phase I	Terminated
			Fluorouracil	Metastatic pancreatic cancer	NCT00045747	Phase II	Completed
			Prednisone	Refractory solid tumor, lymphoma	NCT00045500	Phase I	Completed
			Irinotecan	Advanced solid tumor	NCT00047242	Phase I	Completed
			Fluorouracil, leucovorin	Metastatic or unresectable solid tumor	NCT00042861	Phase I	Completed
			Topotecan	Advanced ovarian epithelial, primary peritoneal, fallopian tube cancer	NCT00072267	Phase II	Completed
			Fludarabine	Recurrent or refractory lymphoma or leukemia	NCT00019838	Phase I	Completed
			Fluorouracil	Advanced or refractory solid tumor	NCT00004059	Phase I	Completed
			Cisplatin	Advanced or metastatic solid tumor	NCT00006464	Phase I	Completed
			Topotecan	Recurrent ovarian epithelial cancer, fallopian tube cancer, primary peritoneal cavity cancer	NCT00045175	Phase I	Completed
			Fludarabine	Chronic lymphocytic leukemia or lymphocytic lymphoma	NCT00045513	Phase I, II	Active, not recruiting
			Monotherapy				
				Relapsed or refractory T-cell lymphoma	NCT00082017	Phase II	Completed
				Metastatic melanoma	NCT00072189	Phase II	Completed
				Breast cancer, lymphoma, prostatic neoplasm	NCT00001444	Phase I	Completed
				Leukemia/lymphoma /unspecified adult solid tumor	NCT00003289	Phase I	Completed
				Advanced or metastatic kidney cancer	NCT00030888	Phase II	Active, not recruiting
		SCH900776	Combination therapy				
			Cytarabine	Relapsed acute myeloid leukemia	NCT01870596	Phase II	Until January, 2016
			Cytarabine	Acute myelogenous leukemia/acute lymphocytic leukemia	NCT00907517	Phase I	Terminated
			Gemcitabine	Solid tumor/lymphoma	NCT00779584	Phase I	Completed
			Hydroxyurea	Advanced solid tumors	NCT01521299	Phase I	Withdrawn

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Table 3. (continued)

Pathway	Target(s)	Name	Combination	Type of cancer	Clinical trial number	Stage	Trial periods
		LY2603618	Combination therapy				
			Desipramine, pemetrexed, gemcitabine	Cancer	NCT01358968	Phase I	Completed
			Pemetrexed, gemcitabine	Advanced or metastatic solid tumor	NCT01296568	Phase I	Completed
			Pemetrexed, cisplatin	NSCLC	NCT01139775	Phase I, II	Until March, 2014
			Gemcitabine	Pancreatic cancer	NCT00839332	Phase I, II	Completed
			Gemcitabine	Solid tumor	NCT01341457	Phase I	Until December, 2014
			Pemetrexed	Cancer	NCT00415636	Phase I	Completed
			Pemetrexed	NSCLC	NCT00988858	Phase II	Until April, 2014
	Chk1 and Chk2	XL844	Combination therapy				
			Gemcitabine	Advanced cancer, lymphoma	NCT00475917	Phase I	Terminated
			Monotherapy				
				Advanced cancer, lymphoma	NCT00475917	Phase I	Terminated
				Chronic lymphocytic leukemia	NCT00234481	Phase I	Terminated
		AZD7762	Combination therapy				
			Gemcitabine	Solid tumor	NCT00413686	Phase I	Completed
			Gemcitabine	Solid tumor	NCT00937664	Phase I	Terminated
			Irinotecan	Solid tumor	NCT00473616	Phase I	Terminated
		PF-00477736	Combination therapy				
			Gemcitabine	Advanced solid tumor	NCT00437203	Phase I	Terminated
Non-homologous end joining	DNA-PK and mTOR	CC-115	Monotherapy				
				Multiple myeloma, non-Hodgkin's lymphoma, glioblastoma, squamous cell carcinoma of head and neck, prostate cancer, Ewing's osteosarcoma, chronic lymphocytic leukemia	NCT01353625	Phase I	Until April, 2015
Base excision repair	APE1	TRC102	Combination therapy				
			Pemetrexed	Neoplasm	NCT00692159	Phase I	Completed
			Temozolomide	Lymphoma, solid tumor	NCT01851369	Phase I	Until February, 2015
			Fludarabine	Relapsed or refractory hematologic malignancy	NCT01658319	Phase I	Until January, 2015
		Lucanthone					
			Combination therapy				
			Radiotherapy	Brain metastases from NSCLC	NCT02014545	Phase II	Until Decemcer, 2017
			Temozolomide and radiation	Glioblastoma multiforme	NCT01587144	Phase II	Terminated
	PARP	Rucaparib (AG014688)	Combination therapy				
			Cisplatin	Triple negative breast cancer or ER/PR+, HER2− breast cancer with known BRCA1/2 mutations	NCT01074970	Phase II	Until May, 2014
			Carboplatin	Advanced solid tumor	NCT01009190	Phase I	Until Dec, 2013
			Monotherapy				
				Platinum-sensitive, relapsed, high-grade epithelial ovarian, fallopian tube, or	NCT01891344	Phase II	Until December, 2015

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Table 3. (continued)

Pathway	Target(s)	Name	Combination	Type of cancer	Clinical trial number	Stage	Trial periods
				primary peritoneal cancer			
				Solid tumor (Phase I), ovarian cancer with germline BRCA mutations (Phase II)	NCT01482715	Phase I, II	Until March, 2014
				Platinum-sensitive, high-grade serous or endometrioid epithelial ovarian, primary peritoneal or fallopian tube cancer	NCT01968213	Phase III	Until November, 2016
				BRCA-mutated locally advanced or metastatic breast cancer or advanced ovarian cancer	NCT00664781	Phase II	Until September, 2014
		Olaparib (AZD2281)	Combination therapy				
			Cediranib	Recurrent ovarian, fallopian tube, peritoneal cancer or recurrent triple-negative breast cancer	NCT01116648	Phase I, II	Until May, 2014
			Abiraterone, prednisone, or prednisolone	Metastatic castration-resistant prostate cancer	NCT01972217	Phase II	Until July, 2018
			Bkm120	Recurrent triple-negative breast cancer or recurrent high-grade serous ovarian cancer	NCT01623349	Phase I	Until Dec, 2014
			Radiotherapy	Esophageal cancer	NCT01460888	Phase I	Until August, 2018
			Paclitaxel	Recurrent or metastatic gastric cancer	NCT01063517	Phase II	Completed
			Radiotherapy with or without cisplatin	Locally advanced NSCLC	NCT01562210	Phase I	Until March, 2015
			Irinotecan, cisplatin, mitomycin C	Advanced pancreatic cancer	NCT01296763	Phase I, II	Until January, 2016
			Temozolomide	Relapsed glioblastoma	NCT01390571	Phase I	Until September, 2015
			Paclitaxel	Advanced gastric cancer	NCT01924533	Phase III	Until December, 2017
			Carboplatin and paclitaxel	Stage III, stage IV relapsed ovarian cancer or uterine cancer	NCT01650376	Phase I, II	Until February, 2015
			Radiation therapy and cetuximab	Advanced squamous cell carcinoma of the head /neck with heavy smoking histories	NCT01758731	Phase I	Until July, 2016
			Gefitinib	EGFR mutation-positive advanced NSCLC	NCT01513174	Phase I, II	Until June, 2015
			Temozolomide	Advanced Ewing's sarcoma	NCT01858168	Phase I	Until July, 2017
			Carboplatin	Mixed muellerian cancer, cervical cancer, ovarian cancer, breast cancer, primary peritoneal cancer, fallopian tube cancer, endometrial cancer, carcinosarcoma	NCT01237067	Phase I	Until September, 2014
			Carboplatin and paclitaxel	Advanced ovarian cancer	NCT01081951	Phase II	Until June, 2013
			Cisplatin-based chemoradiotherapy	Locally advanced squamous cell caricinoma of the head and neck	NCT01491139	Phase I	Withdrawn

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Table 3. (continued)

Pathway	Target(s)	Name	Combination	Type of cancer	Clinical trial number	Stage	Trial periods
			Carboplatin and/or paclitaxel	Triple-negative metastatic breast cancer, advanced ovarian cancer	NCT00516724	Phase I	Until December, 2014
			Irinotecan	Locally advanced or metastatic colorectal cancer	NCT00535353	Phase I	Until December, 2013
			Dacarbazine	Advanced melanoma	NCT00516802	Phase I	Completed
			Paclitaxel	Metastatic triple negative breast cancer	NCT00707707	Phase I	Until December, 2012
			Liposomal doxorubicin	Advanced solid tumor	NCT00819221	Phase I	Until August, 2013
			Topotecan	Advanced solid tumor	NCT00516438	Phase I	Completed
			Gemcitabine	Pancreatic cancer	NCT00515866	Phase I	Completed
			Bevacizumab	Advanced solid tumor	NCT00710268	Phase I	Completed
			Cisplatin	Advanced solid tumor	NCT00782574	Phase I	Until December, 2014
			Carboplatin	Breast and ovarian cancer with BRCA mutations or family histories	NCT01445418	Phase I	Recruiting
			Monotherapy				
				Advanced solid tumor	NCT01900028	Phase I	Until February, 2015
				Advanced solid tumor	NCT01921140	Phase I	Until March, 2015
				Advanced solid tumor	NCT01929603	Phase I	Until May, 2015
				Advanced solid tumor	NCT01851265	Phase I	Until July, 2014
				Advanced solid tumor with normal or impaired liver function	NCT01894243	Phase I	Until December, 2015
				Advanced solid tumor normal or impaired kidney function	NCT01894256	Phase I	Until December, 2015
				Metastatic breast cancer with germline BRCA1/2 mutations	NCT02000622	Phase III	Until February, 2021
				Advanced castration-resistant prostate cancer	NCT01682772	Phase II	Until July, 2015
				Advanced solid tumor	NCT01813474	Phase I	Until November, 2014
				BRCA-mutated ovarian cancer after a complete or partial response following platinum-based chemotherapy	NCT01874353	Phase III	Until June, 2020
				BRCA-mutated advanced cancer	NCT01078662	Phase II	Until December, 2013
				BRCA-mutated advanced ovarian cancer following first line platinum based chemotherapy	NCT01844986	Phase III	Until January, 2022
				Advanced Ewing's sarcoma	NCT01583543	Phase II	Until April, 2015
				Stage IV colorectal cancer with microsatellite instability	NCT00912743	Phase II	Completed
				BRCA-deficient ovarian, peritoneal, fallopian tube cancer	NCT01661868	Phase II	Withdrawn
				Advanced NSCLC	NCT01788332	Phase II	Until May, 2015
				BRCA-positive advanced breast cancer	NCT00494234	Phase II	Until December, 2013
				BRCA-positive advanced ovarian cancer	NCT00494442	Phase II	Until December, 2013
				Platinum-sensitive relapsed serous ovarian cancer	NCT00753545	Phase II	Completed
				Advanced solid tumor	NCT00572364	Phase I	Completed

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Table 3. (continued)

Pathway	Target(s)	Name	Combination	Type of cancer	Clinical trial number	Stage	Trial periods
				Advanced or metastatic solid tumor	NCT00633269	Phase I	Completed
				Ovarian cancer	NCT00516373	Phase I	Until December, 2014
				Advanced solid tumor	NCT00777582	Phase I	Until March, 2014
				High grade ovarian cancer, triple-negative breast cancer, BRCA-mutated breast cancer or ovarian cancer	NCT00679783	Phase II	Until December, 2012
				BRCA-positive advanced ovarian cancer	NCT00628251	Phase II	Until December, 2013
		Veliparib (ABT-888)	Combination therapy				
			Gemcitabine, cisplatin	Locally advanced or metastatic pancreatic cancer with BRCA or PALB2 mutations	NCT01585805	Phase II	Until July, 2017
			Temozolomide or combination with carboplatin and paclitaxel	Locally recurrent or metastatic breast cancer with BRCA mutations	NCT01506609	Phase II	Until May, 2015
			Radiotherapy and temozolomide	Newly diagnosed childhood diffuse pontine glioma	NCT01514201	Phase I, II	Until August, 2019
			Radiotherapy	Advanced solid malignancies with peritoneal carcinomatosis	NCT01264432	Phase I	Until April, 2014
			Bendamustine, rituximab	Advanced lymphoma, multiple myeloma, or solid tumors	NCT01326702	Phase I, II	Until November, 2015
			Topotecan	Relapsed epithelial ovarian, primary fallopian tube, or primary peritoneal cancer with negative or unknown BRCA status	NCT01690598	Phase I, II	Until April, 2015
			Gemcitabine and radiotherapy	Locally advanced, unresectable pancreatic cancer	NCT01908478	Phase I	Until July, 2019
			Dinaciclib with or without carboplatin	Advanced solid tumors with BRCA mutations	NCT01434316	Phase I	Until January, 2016
			Radiotherapy, carboplatin, paclitaxel	Stage III NSCLC that cannot be removed by surgery	NCT01386385	Phase I, II	Until December, 2016
			Doxorubicin, carboplatin, bevacizumab	Recurrent ovarian cancer, primary peritoneal cancer, or fallopian tube cancer	NCT01459380	Phase I	Until August, 2015
			Cisplatin, gemcitabine	Advanced biliary, pancreatic, urothelial, NSCLC	NCT01282333	Phase I	Terminated
			Cisplatin, vinorelbine	Recurrent and/or metastatic breast cancer with BRCA mutations, triple-negative breast cancer	NCT01104259	Phase I	Until September, 2014
			Mitomycin C	Metastatic, unresectable, or recurrent solid tumor	NCT01017640	Phase I	Until June, 2014
			Capecitabine, radiotherapy	Locally advanced rectal cancer	NCT01589419	Phase I	Until June, 2015
			Cyclophosphamide	Locally advanced or metastatic HER2-negative breast cancer	NCT01351909	Phase I, II	Until May, 2015

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Table 3. (continued)

Pathway	Target(s)	Name	Combination	Type of cancer	Clinical trial number	Stage	Trial periods
			Docetaxel, cisplatin, fluorouracil, radiotherapy, hydroxyurea, paclitaxel	Stage IV head and neck cancer	NCT01711541	Phase I, II	Until October, 2014
			Temozolomide	Solid tumor	NCT01193140	Phase II	Completed
			Cisplatin, etoposide	Extensive stage small-cell lung cancer, metastatic large cell neuroendocrine NSCLC, small-cell carcinoma of unknown primary or extrapulmonary origin	NCT01642251	Phase I, II	Until January, 2018
			Paclitaxel, carboplatin	Metastatic, unresectable solid tumor with liver or kidney dysfunction	NCT01366144	Phase I	Until July, 2015
			Oxaliplatin, capecitabine	BRCA-related malignancy, metastatic colorectal cancer, metastatic ovarian cancer, metastatic gastrointestinal malignancies in which oxaliplatin has shown some activity	NCT01233505	Phase I	Until July, 2014
			Carboplatin	Stage III or stage IV breast cancer with BRCA mutations	NCT01149083	Phase II	Until June, 2014
			Temozolomide	Acute leukemia	NCT01139970	Phase I	Until October, 2013
			Carboplatin, paclitaxel	Solid tumor	NCT01617928	Phase I	Completed
			Topotecan	Recurrent ovarian epithelial cancer, primary peritoneal cavity cancer, unspecified solid tumor	NCT01012817	Phase I, II	Until June, 2018
			Carboplatin, paclitaxel	Advanced NSCLC	NCT01560104	Phase II	Until September, 2014
			Carboplatin	HER2-negative metastatic or locally advanced breast cancer	NCT01251874	Phase I	Until September, 2013
			Paclitaxel, cisplatin	Advanced, persistent, or recurrent cervical cancer	NCT01281852	Phase I, II	Until March, 2020
			Topotecan with or without carboplatin	Relapsed or refractory acute leukemia, high-risk myelodysplasia, or aggressive myeloproliferative disorders	NCT00588991	Phase I	Until December, 2012
			Abiraterone, prednisone	Metastatic hormone-resistant prostate cancer	NCT01576172	Phase II	Until February, 2014
			Topotecan and filgrastim or pegfilgrastim	Persistent or recurrent cervical cancer	NCT01266447	Phase II	Until November, 2016
			Gemcitabine	Solid tumor	NCT01154426	Phase I	Until October, 2013
			Modified FOLFOX6	Metastatic pancreatic cancer	NCT01489865	Phase I, II	Until December, 2014
			FOLFIRI	Advanced gastric cancer	NCT01123876	Phase I	Until December, 2014
			Temozolomide	Recurrent or refractory childhood central nervous system tumor	NCT00946335	Phase I	Until October, 2011
			Temozolomide	Hepatocellular carcinoma	NCT01205828	Phase II	Until December, 2013
			Carboplatin, paclitaxel	Advanced solid tumor	NCT01281150	Phase I	Until December, 2013
			Carboplatin, paclitaxel, doxorubicin, cyclophosphamide	Stage IIb-IIIc triple-negative breast cancer	NCT01818063	Phase II	Until April, 2018

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Table 3. (continued)

Pathway	Target(s)	Name	Combination	Type of cancer	Clinical trial number	Stage	Trial periods
			Floxuridine	Metastatic epithelial ovarian, primary peritoneal cavity, or fallopian tube cancer	NCT01749397	Phase I	Until March, 2016
			Liposomal doxorubicin	Recurrent ovarian cancer, fallopian tube cancer, or primary peritoneal cancer or metastatic triple-negative breast cancer	NCT01145430	Phase I	Until March, 2014
			Bortezomib, dexamethasone	Relapsed refractory multiple myeloma	NCT01495351	Phase I	Until October, 2013
			Temozolomide	Recurrent small-cell lung cancer	NCT01638546	Phase II	Until June, 2017
			Cyclophosphamide, doxorubicin	Metastatic or unresectable solid tumor, non-Hodgkin's lymphoma	NCT00740805	Phase I	Until December, 2013
			Whole brain radiation	Brain metastases from NSCLC	NCT01657799	Phase II	Until November, 2014
			Temozolomide	Recurrent high grade serous ovarian, fallopian tube, or primary peritoneal cancer	NCT01113957	Phase II	Completed
			Temozolomide	Metastatic or locally advanced breast cancer and BRCA1/2-associated breast cancer	NCT01009788	Phase II	Until December, 2014
			Carboplatin, paclitaxel	Advanced cancer with liver or kidney problems	NCT01419548	Phase I	Withdrawn
			Whole brain radiation	Cancer with brain metastases	NCT00649207	Phase I	Completed
			Radiotherapy	Inflammatory or loco-regionally recurrent breast cancer	NCT01477489	Phase I	Until December, 2016
			Carboplatin, paclitaxel, bevacizumab	Newly diagnosed ovarian epithelial cancer, fallopian tube cancer, or primary peritoneal cancer	NCT00989651	Phase I	Until July, 2014
			Carboplatin, paclitaxel	Advanced solid tumor or BRCA1/2-associated advanced solid tumor	NCT00535119	Phase I	Until October, 2012
			Temozolomide	Colorectal cancer	NCT01051596	Phase II	Until December, 2013
			Cyclophosphamide	Refractory BRCA-positive ovarian, primary peritoneal or ovarian high-grade serous carcinoma, fallopian tube cancer, triple-negative breast cancer, and low-grade non-Hodgkin's lymphoma	NCT01306032	Phase II	Until November, 2014
			Irinotecan	Metastatic or unresectable solid tumor, lymphoma	NCT00576654	Phase I	Until December, 2013
			Temozolomide	Recurrent or refractory childhood central nervous system tumor	NCT00994071	Phase I	Completed
			Cyclophosphamide	Refractory solid tumor or lymphoma	NCT01445522	Phase I	Completed
			Temozolomide	Recurrent high-grade glioma	NCT01026493	Phase I, II	Until February, 2014

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Table 3. (continued)

Pathway	Target(s)	Name	Combination	Type of cancer	Clinical trial number	Stage	Trial periods
			Cyclophosphamide	Solid tumor or lymphoma that did not respond to previous therapy	NCT00810966	Phase I	Active, not recruiting
			Radiotherapy, temozolomide	Grade IV astrocytoma	NCT00770471	Phase I, II	Completed
			Temozolomide	Metastatic prostate cancer	NCT01085422	Phase I	Completed
			Temozolomide	Advanced non-hematologic tumor	NCT00526617	Phase I	Completed
			Topotecan	Refractory solid tumor or lymphoma	NCT00553189	Phase I	Completed
			Temozolomide	Metastatic melanoma	NCT00804908	Phase II	Until March, 2014
			Carboplatin, gemcitabine	Advanced solid tumor	NCT01063816	Phase I	Until September, 2014
			Radiotherapy	Breast cancer	NCT01618357	Phase I	Until April, 2016
			Monotherapy				
				Solid tumor	NCT01199224	Phase I	Completed
				Locally advanced or metastatic pancreatic cancer	NCT01585805	Phase II	Until July, 2017
				Metastatic, unresectable, or recurrent solid tumors	NCT01017640	Phase I	Until June, 2014
				Stage III or Stage IV breast cancer with BRCA mutations	NCT01149083	Phase II	Until June, 2014
				BRCA-mutated metastatic or unresectable malignancy, high grade serous ovarian, fallopian tube, or peritoneal cancer	NCT01853306	Phase I	Until January, 2015
				BRCA-mutated epithelial ovarian, fallopian tube, or primary peritoneal cancer	NCT01540565	Phase II	Until April, 2014
				Advanced solid tumor	NCT02009631	Phase I	Until December, 2014
				BRCA-related malignancy, platinum-refractory ovarian, fallopian tube, or primary peritoneal cancer or basal-like breast cancer, advanced solid tumor	NCT00892736	Phase I	Until December, 2013
				Relapsed epithelial ovarian, primary fallopian or primary peritoneal cancer with BRCA mutations	NCT01472783	Phase I, II	Until December, 2015
				Chronic lymphocytic leukemia, follicular lymphoma, unspecified solid tumor	NCT00387608	Phase I	Completed
				Invasive breast cancer	NCT01042379	Phase II	Until November, 2014
				Advanced solid tumor	NCT01827384	Phase II	Until March, 2017
		INO-1001	Combination therapy				
			Temozolomide	Unresectable melanoma	NCT00272415	Phase I	Terminated
		MK4827	Combination therapy				
			Liposomal doxorubicin	Advanced solid tumor, platinum-resistant high grade serous ovarian cancer	NCT01227941	Phase I	Terminated
			Temozolomide	Advanced solid tumor, glioblastoma multiforme, melanoma	NCT01294735	Phase I	Completed

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Table 3. (continued)

Pathway	Target(s)	Name	Combination	Type of cancer	Clinical trial number	Stage	Trial periods
			Carboplatin, paclitaxel, liposomal doxorubicin	Advanced solid tumor	NCT01110603	Phase I	Terminated
			Monotherapy				
				Advanced solid tumor	NCT01226901	Phase I	Terminated
				Mantle cell lymphoma	NCT01244009	Phase II	Withdrawn
				Advanced solid tumors, chronic lymphocytic leukemia, T-cell-prolymphocytic leukemia	NCT00749502	Phase I	Completed
				Advanced HER2-negative, germline BRCA mutation-positive breast cancer	NCT01905592	Phase III	Until October, 2015
		CEP-9722	Combination therapy				
			Gemcitabine, cisplatin	Advanced solid tumor or mantle cell lymphoma	NCT01345357	Phase I	Completed
			Temozolomide	Advanced solid tumor	NCT00920595	Phase I	Completed
			Monotherapy				
				Advanced solid tumor	NCT01311713	Phase I, II	Terminated
				Advanced solid tumor	NCT00920595	Phase I	Completed
		E7016	Combination therapy				
			Temozolomide	Advanced solid tumor	NCT01127178	Phase I	Completed
			Temozolomide	Wild-type BRAF stage IV melanoma, unresectable stage III melanoma	NCT01605162	Phase II	Until March, 2014
		BMN673	Monotherapy				
				Acute myeloid leukemia, myelodysplastic syndrome, chronic lymphocytic leukemia, mantle cell lymphoma	NCT01399840	Phase I	Until June, 2013
				Advanced or recurrent solid tumor	NCT01286987	Phase I	Until June, 2013
				Advanced solid tumor with deleterious BRCA mutations	NCT01989546	Phase I, II	Until August, 2016
				Advanced breast cancer with BRCA mutations	NCT01945775	Phase III	Until June, 2016

For current status and information of clinical trials, refer to http://clinicaltrials.gov/, a service of the US National Institutes of Health. NSCLC, non-small-cell lung cancer.

Although such lesions would be repaired by HR in normal cells, they are not repaired in BRCA1- or BRCA2-deficient cancer cells because these cells are defective in HR repair, and thus the tumor cells are led to death. This concept is termed synthetic lethality, namely, the process by which defects in two different genes or pathways together result in cell death while defects in one of the two different genes or pathways do not affect viability (Fig. 3).[3] This attractive new therapeutic strategy based on the principle of synthetic lethality relies on the frequent defects in the DNA damage response observed in cancer as summarized in the previous chapter and Table 1, in which alternative DNA damage response pathways may be activated to allow cancer cells to survive in the presence of genotoxic stress. Because this strategy targets the cancer-specific aberrations in the DNA damage response, it will cause few or no toxicities on normal cells. The first report of a clinical trial of a PARP inhibitor as a single agent in patients with *BRCA* mutations was the phase I study of the oral PARP inhibitor olaparib.[93] It established the safety of olaparib as a single agent, and good responses were observed in patients with *BRCA*-mutated breast, ovarian, or prostate tumors. In subsequent phase II studies, approximately one-third of the patients with breast or ovarian cancer with germline *BRCA* mutations showed a favorable response to the drug with no severe toxicities.[94] Several other PARP inhibitors are currently being investigated in patients with germline *BRCA* mutations as single agents (Table 3). It is likely that PARP inhibitors have significant benefit to at least a subpopulation of cancer patients with defects in BRCA-mediated HR pathways.

Using PARP inhibitors as single agents in cancers with no *BRCA* mutations. The potential for PARP inhibitors as single agents has also been tested in clinical trials of cancers with no germline *BRCA* mutations, such as high-grade serous ovarian cancers and triple-negative breast cancers.[95] Inhibitors of PARP were also effective in a subset of cancers with no germline *BRCA* mutations, suggesting that there may be a subset of sporadic cancers that show features of "BRCAness," which may show good response to PARP inhibitors.[96] Indeed, cancer cells expressing the cancer-testis antigen SYCP3, in which BRCA2 is functionally inactivated, as described above, show extreme hypersensitivity to a PARP inhibitor.[63] Defects in other HR-related proteins such as RAD51, RAD54, and RPA also confer selective sensitivity to PARP inhibition.[97] Moreover, defects in the DNA damage response proteins, such as NBS1, MRE11, ATR, ATM,

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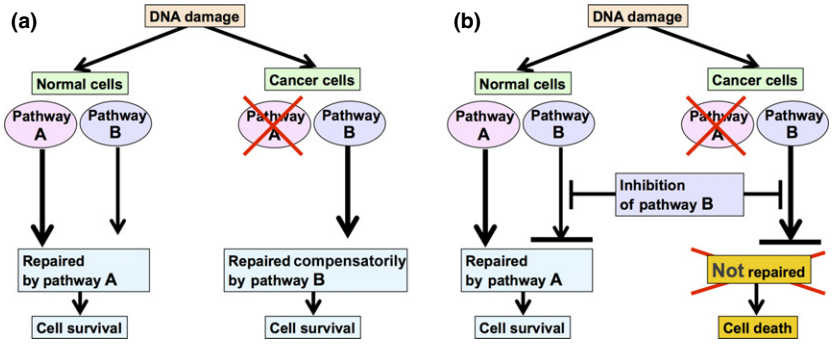

Fig. 3. Principle of synthetic lethality. DNA damage is often processed by multiple DNA repair pathways. In the example shown here, pathways A and B are both intact in normal cells, whereas pathway A is defective in cancer cells. (a) In the absence of the pathway B inhibitor, cancer cells can survive, because the defect in pathway A is compensated by the alternative pathway B. (b) When the cells are treated with the pathway B inhibitor, both pathways will be blocked in cancer cells, which will result in cell death. However, normal cells will not be affected, because inhibition of pathway B will be compensated by pathway A.

FANCD2, FANCA, FANCC, Chk1, Chk2, and ERCC1, also confer selective sensitivity to PARP inhibition.[97,98]

Exploitation of other synthetic lethalities by DNA damage response. Taking advantage of the dysregulated DNA damage response in cancer using the synthetic lethality approach may be one of the most promising prospects for the future of cancer treatment. From this point of view, many efforts have been made to identify defects of two different DNA damage response genes or pathways that are synthetically lethal when combined. For example, ATM inhibition is shown to be synthetically lethal with FA pathway deficiency.[99] The suggested explanation for this lethality is as follows. The FA pathway-deficient cancer cells are defective in the repair of DNA replication fork stalling, which is normally repaired by ATR and the FA pathway. In FA pathway-deficient conditions, the stalled fork will collapse and form a DSB that will alternatively activate an ATM-dependent DNA damage response. Inhibition of ATM in such FA pathway-deficient cells will leave no alternative mechanism for repair, leading to cell death. The FA pathway-deficient cells are also hypersensitive to Chk1 silencing,[100] which may be explained by the hyperdependence of the FA pathway-deficient cells on G_2/M checkpoint activation mediated by Chk1 for viability. Because defects in the FA pathway are frequently observed in a number of different types of cancer (Table 1),[64,65] the use of ATM inhibitors or Chk1 inhibitors in FA pathway-deficient tumors will be a promising approach that should be evaluated in clinical trials in the future. In another example, RAD54B deficiency is shown to be synthetically lethal in cells with reduced Flap endonuclease 1 expression, but the mechanisms of this lethality remain to be elucidated.[101] Recently, inhibition of APE1 was shown to be synthetically lethal in BRCA- and ATM-deficient cells, presenting a novel model for APE inhibition as a synthetic lethal strategy in cells deficient in DSB repair.[102] Briefly, APE1 inhibition leads to AP site accumulation and results in indirect generation of SSBs that are eventually converted to toxic DSBs, which cannot be repaired in cells deficient in DSB repair. The APE1 inhibitors are being tested in combination with DNA-damaging agents in current clinical trials, and they may be evaluated further as a synthetic lethal strategy. More recently, inactivation of the HR protein RAD52 was shown to be synthetically lethal with deficiencies in BRCA2, BRCA1, and PALB2.[103,104] This lethal effect may be due to the loss of RAD51-dependent HR function mediated by the BRCA1–PALB2–BRCA2 complex, because human RAD52 is suggested to function in an independent and alternative repair pathway of RAD51-dependent HR when deficiencies exist in BRCA1, PALB2, or BRCA2. As no inactivating mutations of RAD52 have been documented in human sporadic cancers, inhibition of RAD52 could be an attractive strategy for improving cancer therapy in the BRCA- or PALB2-defective subgroup of cancers. Although no inhibitors of RAD52 have been developed yet, it would be of great interest to assess the effects of inhibition of RAD52 on cancer-specific killing of the cancers with "BRCAness" profiles and compare them with those of PARP inhibitors in future clinical trials. There might be additional synthetic lethalities to be discovered and exploited in future.

Current Limitations and Future Perspectives

Although the data from clinical trials of the inhibitors of DNA damage response, including PARP inhibitors, seem encouraging, we should note that the use of PARP inhibitors also faces significant limitations.

The first limitation is the evolution of resistance. In the case of using PARP inhibitors in cancer cells carrying mutations in *BRCA1* or *BRCA2*, the drug resistance can be caused by secondary mutations in the *BRCA1* or *BRCA2* gene that restore the open reading frame of the gene and enable the generation of functional BRCA proteins possessing the ability to repair DNA damage caused by PARP inhibitors.[105–107] Other suggested mechanisms underlying the resistance to PARP inhibitors include the loss of 53BP1 expression in BRCA-deficient cells and the upregulation of genes that encode P-glycoprotein efflux pumps,[108–111] although the importance of these factors in clinical resistance to PARP inhibitors has not been elucidated. In future clinical trials, it would be desirable to periodically monitor the sequences of *BRCA1* and *BRCA2* and the expression levels of the key proteins such as 53BP1 or P-glycoprotein efflux pumps.

The second limitation is the lack of reliable biomarkers of response or resistance to the inhibitors. There is a pressing need to identify biomarkers to predict the response to the inhibitors. Regarding the sensitivities to PARP inhibitors, elevated levels of PARP and CDK12 deficiency are suggested to be possible biomarkers for favorable responses.[45,112] We should also keep in mind that many factors might affect the DNA damage response and take into account the complexity of the networks regulating DNA repair. For instance, most cancer cells grow under hypoxia, a condition that activates hypoxia inducible factor-1 (HIF-1). Because HIF-1 contributes to therapy resistance, it is considered an attractive target molecule for cancer therapy. Diverse functional interactions

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between HIF-1 and the DNA damage response have also been described,[113] so the efficacy of the combination of HIF-1 inhibitors and inhibitors of the DNA damage response proteins should be examined in the future.

Conclusions

Defects or upregulation of the proteins involved in DNA damage response and repair are common in cancers, and may be induced by both genetic and epigenetic causes. Inhibition of the DNA damage response proteins can be used to enhance chemotherapy and radiotherapy, and also to selectively kill cancer cells showing deficiencies in particular DNA repair pathway(s) based on the principle of synthetic lethality. Inhibition of PARP in BRCA-defective cancers seemed effective in early clinical trials. Better understanding of the basic biology underlying the DNA damage response and the mechanisms responsible for its dysregulation in cancer will provide exciting opportunities for new and efficient cancer therapy targeting the DNA damage response.

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Disclosure Statement

The authors have no conflict of interest.

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