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Targeting abnormal DNA double strand break repair in cancer

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Abstract A major challenge in cancer treatment is the development of therapies that target cancer cells with little or no toxicity to normal tissues and cells. Alterations in DNA double strand break (DSB) repair in cancer cells include both elevated and reduced levels of key repair proteins and changes in the relative contributions of the various DSB repair pathways. These differences can result in increased sensitivity to DSB-inducing agents and increased genomic instability. The development of agents that selectively inhibit the DSB repair pathways that cancer cells are more dependent upon will facilitate the design of therapeutic strategies that exploit the differences in DSB repair between normal and cancer cells. Here, we discuss the pathways of DSB repair, alterations in DSB repair in cancer, inhibitors of DSB repair and future directions for cancer therapies that target DSB repair.

Keywords Homologous recombination · Non-homologous end-joining

Introduction

Cells have evolved a complex network of pathways that function in response to DNA damage. Key components of this response include DNA repair pathways that remove various types of DNA lesions and DNA damage-activated signal transduction pathways that target fundamental cellular processes, including transcription and cell cycle progression. Here, we will focus on the cellular response induced by DNA double strand breaks (DSB)s, considered to be the most lethal form of DNA damage [1]. The cancer predispostion of autosomal recessive human syndromes, such as ataxia telangiectasia, that are characterized by hypersensitivity to DSB-inducing agents indicates the role of the DNA damage response in protecting against the genomic instability induced by DSBs that drives cancer formation and progression [2]. More recently, abnormalities in the DSB response, including defects in DSB repair, have been identified as the underlying cause of hereditary forms of breast cancer [3]. Since genomic instability is a common characteristic of both inherited and sporadic forms of cancer cells, it appears likely that abnormalities in the DNA damage response also contribute to the development and progression of sporadic cancers [1]. However, high-throughput sequencing studies have found that mutation of DNA repair genes occurs infrequently in sporadic cancers [4]. Instead, it has been suggested that genomic instability in sporadic cancers may be mainly due to oncogene-induced DNA replication stress and DNA damage [4]. In addition, oncogenes may also impact the repair and mutagenic consequences of DNA damage by altering the relative activities of DNA repair pathways that repair the same lesion, presumably by epigenetic mechanisms.

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DSB repair pathways

The cytotoxicity of DSBs presumably reflects the difficulty of repairing these lesions because, unlike almost all other types of DNA damage that have an intact undamaged

template strand to guide the repair, the integrity of both strands of the duplex is lost. Thus, cells that incur more than one DSB have the problem of distinguishing between the previously linked DNA ends and DNA ends from other molecules. The repair of DSBs occurs via two mechanistically distinct groups of pathways, homology-directed pathways and non-homologous end-joining (NHEJ) pathways.

Homology-directed repair

The predominant pathway that repairs replication-associated DSBs is characterized by the invasion of single strand DNA into a homologous duplex [1, 5]. This repair pathway, which is active in late S phase and in the G2 phase of the cell cycle, utilizes the undamaged sister chromatid as the template for repair and so is usually error-free (Fig. 1). Notably, many chemotherapeutic agents block DNA replication, leading to the stalling and/or collapse of replication forks and the generation of lesions that are repaired by homologous recombination (HR) [6]. If the HR pathway is inactivated, there are back-up pathways that can repair DSBs. These pathways, which are described in more detail below, are error-prone, generating deletions and chromosomal translocations (Fig. 1).

The first step in HR is resection of the DSB in a 5′–3′ manner that involves the human Mre11-Rad50-Nbs1 (MRN) complex and CtIP [7, 8]. The resultant 3′ single strands are bound by RPA, preventing degradation and providing the signal to activate the cell cycle checkpoint kinase ATR [9]. Next, hRad51 is recruited and assembled into a nucleoprotein filament, displacing RPA. This reaction involves several accessory proteins including hRad52, XRCC2, XRCC3 and BRCA2 [10–12]. The recruitment and assembly of hRad51 nucleoprotein filaments can be visualized in cell nuclei as foci formed after IR and in S phase at sites of replication-associated DSBs [13]. Strand invasion by the hRad51 nucleoprotein filament into the adjacent homologous sister chromatid results in formation of a D loop structure. DNA synthesis from the invading 3′ end extends the D loop, increasing its stability [14, 15]. The extension of the D loop also permits capture of the second DNA end, resulting in the formation of a Holliday junction. Resolution of the Holliday junction by a resolvase, such as Gen1 [16], completes the repair, generating two identical sister chromatids [17]. Alternatively, in break-induced replication, the entire sister chromatid is copied following formation of the D loop structure [18].

Homology-directed repair (HR) does occur between homologous chromosomes in G1 cells albeit at a much lower frequency [19] (Fig. 2). These events may generate genetic alterations ranging from gene conversion to loss of heterozygosity (Fig. 2). The presence of repetitive sequences throughout the human genome presents a problem for homology-dependent DSB repair mechanisms. For example, strand invasion can occur with an inappropriate homologous sequence on the sister chromatid, homologous chromosome or even on a non-homologous chromosome, resulting in genomic rearrangements. Finally, a single strand annealing pathway that occurs between repeated

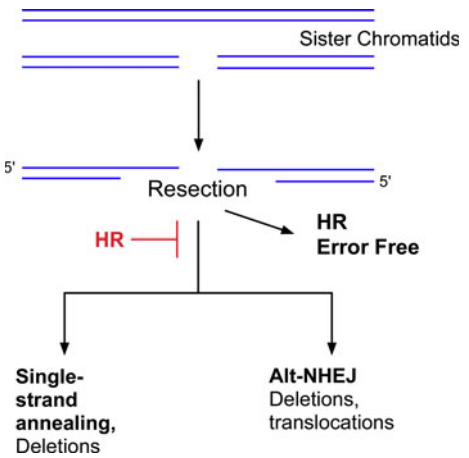

Fig. 1 DSB repair in the S and G2 phases of the cell cycle. In late S and the G2 phase of the cell cycle, DSBs can be repaired by homologous recombination (HR) using the undamaged sister chromatid. In the initial stage of HR, the ends of the DSBs are resected to generate 3′ single stranded regions. If the ends are resected but HR is inactivated, the DSBs can be joined by back-up pathways, single strand annealing and alternative non-homologous end joining. In contrast to the error-free homolgous recombination pathways, the back-up pathways generate genomic rearrangements

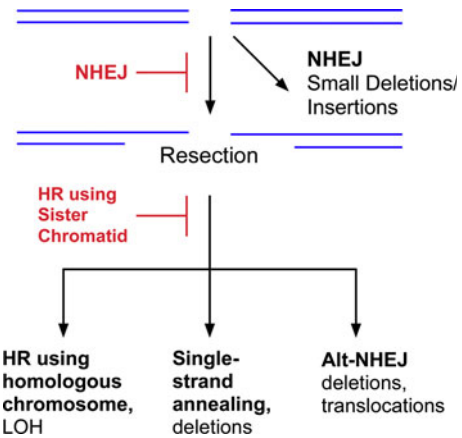

Fig. 2 DSB repair in the G1 phases of the cell cycle and in non-dividing cells. In the G1 phase of the cell cycle and non-dividing cells, the majority of DSBs are repaired by DNA-PK-dependent NHEJ. If this pathway is inactivated, the DSBs can be repaired by homologous recombination (HR) but, in the absence of the sister chromatid, the homologous chromsome will be used to guide the repair. Alternatively, the DSBs can be joined by back-up pathways, single strand annealing and alternative non-homologous end joining

sequences on the same chromosome generates intra-chromosomal deletions [20] (Figs. 1, 2).

Non-homologous end-joining

In the repair of DSBs by NHEJ, the DNA ends are brought together in a reaction that is independent of extensive DNA sequence homology and so is prone to introducing errors ranging from small insertions and deletions at the break site to the joining of previously unlinked DNA ends [1, 5, 21]. In addition to repairing DSBs caused by endogenous and exogenous DNA damaging agents, the NHEJ proteins also participate in immunoglobulin gene rearrangements [22]. While the repair of DSBs by NHEJ occurs throughout the cell cycle, NHEJ is the major DSB repair pathway in G0, G1 (Fig. 1) and early S phase [22, 23]. Most DSBs are rapidly repaired by NHEJ, but there is a slower phase that reflects the repair of a subset of DSBs that are either more complex DSB lesions or occur in condensed chromatin [24, 25].

As shown in Fig. 3, the NHEJ pathway is initiated by the Ku70/Ku86 heterodimer, a ring-shaped complex that binds to and encircles DNA ends [26]. This serves to protect the DNA ends from degradation and to recruit the catalytic subunit (cs) of the DNA-dependent protein kinase (DNA-PK) [27–29] to form the activated DNA-PK [30, 31]. The kinase activity of DNA-PK is critical for NHEJ with a key substrate being the DNA-PKcs itself [32]. In addition, DNA-PK phosphorylates Artemis, which binds to DNA-PKcs [33], and activates its endonuclease activity. The key step in NHEJ is the physical juxtaposition of DNA ends. This end-bridging occurs via interactions between DNA-bound DNA-PKcs molecules [34, 35].

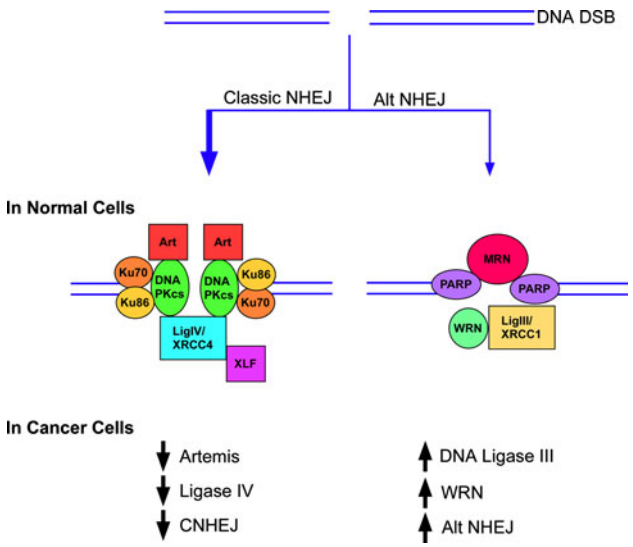

Fig. 3 In normal cells (*upper panel*), alternative NHEJ pathway (Alt NHEJ) is a minor DSB pathway repair pathway compared with DNA-PK-dependent NHEJ (Classic NHEJ). In cancer cells (*lower panel*), the steady state levels of key DNA-PK-dependent NHEJ proteins are reduced whereas the steady state levels of key alternative NHEJ are increased. This results in increased activity of the Alt NHEJ pathway and reduced activity of the DNA-PK-dependent NHEJ (CNHEJ) pathway

Approximately 10% of endogenous DSBs in mammalian cells have non-ligatable ends [36], whereas a significantly higher fraction of DSBs generated by ionizing radiation are not directly ligatable. When juxtaposed ends can be directly ligated, the repair reaction is completed by DNA ligase IV/XRCC4, which is recruited to DSBs by interactions with DNA-PK [37]. In contrast, there appear to be multiple factors involved in processing non-ligatable ends to generate a ligatable substrate. These include polynucleotide kinase [38], the nucleases FEN-1 [39] and Artemis [36], and the Pol X family members, Pol mu and lambda [40]. As a consequence of these processing reactions, the joining of DSBs by DNA-PK-dependent NHEJ often results in the loss or addition of a few nucleotides at the break site and the presence of short complementary sequences, microhomologies, at the break site that presumably contributed to the alignment of the DNA ends [41, 42]. Another NHEJ factor, XLF or Cernunnos, contributes to the joining of non-complementary DNA ends by interacting with DNA ligase IV/XRCC4 and stimulating the joining of mismatched DNA ends [43].

Although DNA-PK-dependent NHEJ frequently causes small alterations in DNA sequence around the break site, it usually does not join previously unlinked DNA ends [44]. There is, however, increasing evidence for an alternative (Alt) version of NHEJ that results in larger deletions and chromosomal translocations [44, 45] (Figs. 1 and 2). For example, chromosomal abnormalities, including c-myc/IgH translocations are observed in the absence of either Ku or DNA ligase IV/XRCC4 [46–48] and rare aberrant V(D)J coding joins are found in lymphocytes lacking either Ku or DNA-PKcs [49]. Cell-based assays measuring rearrangement of immunoglobulin genes have detected robust end joining by Alt NHEJ that is even detectable in cells with a functional DNA PK-dependent NHEJ pathway [50–52]. The hallmark features of the Alt NHEJ pathway are larger deletions and insertions, longer tracts of microhomology and a much higher frequency of chromosomal translocations compared with DNA PK-dependent NHEJ [44]. A number of DNA repair proteins, including PARP-1, MRN, WRN and DNA ligase IIIα/XRCC1 [53–60], have been implicated in alternative NHEJ, but the mechanisms and regulation of this repair pathway or pathways are poorly defined (Fig. 3).

Since the fidelity of DSB repair by the different pathways described above varies greatly (Figs. 1 and 2), the choice of DSB repair pathway will determine the effect that this type of DNA damage has on genome stability. There is

evidence for competition between the major HR and NHEJ pathways, in particular at the DSB binding stage. In addition, regulation of the end resection machinery by cyclin-dependent protein kinases plays a role in limiting HR to the S and G2 phases of the cell cycle [61]. Thus, there are many different potential alterations in DSB repair that could increase genome instability, thereby driving cancer progression. At the present time, our ability to define the DSB repair properties and capacities of tumor samples in biopsies is very limited, making it difficult to design therapeutic strategies that exploit the abnormal DNA repair in tumor cells.

DNA damage-activated cell cycle checkpoints

The potential mutagenic and cytotoxic consequences of unrepaired DNA damage are mitigated by cell cycle checkpoints that prevent the replication and/or segregation of damaged genomes. The Ataxia Telangiactasia Mutated (ATM) protein that is defective in the cancer-prone radiation-sensitive human disease, ataxia telangiectasia, is a central player in the activation of cell cycle checkpoints by DSBs. ATM is recruited to DSBs by the MRN complex [62]. This results in ATM autophosphorylation and its conversion from an inactive dimer to an active monomer [63]. Once activated, ATM phosphorylates MRN and downstream effector proteins to initiate cell cycle checkpoints at the G1/S, intra-S, and G2/M boundaries [2]. The activation of these checkpoints allows increased time for the repair of DNA damage before it is replicated and/or passed onto daughter cells thereby increasing cell survival and preserving genomic integrity. In addition to its role in DSB-activated signal transduction pathways, there is emerging evidence that ATM is involved in the repair of a subset of DSBs in both G0 and G2 cells [24, 64].

Alterations in DSB repair in cancer

Homology-directed repair

Inherited mutations in *BRCA1* and *BRCA2* predispose these individuals to breast, ovarian and other cancers [65]. Following the classic paradigm of tumor suppressor genes [66], the inheritance of one defective copy of *BRCA1* or *BRCA2* in the germline is enough to cause cancer predisposition, because it increases the probability of losing the remaining wild-type allele, an event that is consistently observed in tumor cells from these individuals. Cancer susceptibility genes fall into two general classes, "gatekeeper" genes whose altered expression relieves normal controls on cell division, death, or lifespan, promoting the out-growth of cancer cells, and "caretaker genes" whose disruption causes genome instability [67]. Several lines of evidence suggest that *BRCA1* and *BRCA2* act as caretakers and that loss of these genes lead to spontaneous chromosomal abnormalities. Mouse BRCA2-deficient cells sustain spontaneous chromosomal aberrations that accumulate during cell proliferation [68]. Microscopically, the abnormalities are not restricted to broken chromosomes and chromatids but also include triradial and quadriradial structures, markers of defective mitotic recombination that are hallmarks of the inherited cancer-prone human diseases, Bloom's syndrome (BS) and Fanconi's anemia (FA) [69, 70].

Analysis of the genes involved in FA, which is characterized by cellular hypersensitivity to DNA cross-linking agents, revealed that *FANCD2* and *BRCA2* are in fact the same gene. Furthermore, while homozygous mutation of *FANCD1*, *FANCN*, or *FANCJ* results in FA, heterozygous mutations in these same genes have been linked to familial breast and ovarian cancer predisposition, highlighting the role of both the *BRCA* and *FA* genes as tumor suppressors in the same tissues [71]. In response to DNA damage or replication fork stalling during S phase, the FA core complex is activated and monoubiquitylates FANCD2 and FANCI, leading to their retention in chromatin foci, which colocalize with downstream components of the repair pathway, including FANCD1 (BRCA2), FANCN (PALB2), and FANCJ (BRIP) [72]. BLM is a member of the RecQ helicase family that is involved in both regulating homologous recombinational repair and replication fork regression [73]. Recently, it has been shown that, after treatment of cells with agents that introduce DNA interstrand cross-links, a complex containing BLM associates with the FA core complex to form a 1.5- to 2-MDa supercomplex named BRAFT [74], suggesting that the genomic instability observed in FA, BS and inherited breast cancers may be due to a failure in BRAFT assembly that in turn results in a defect in homologous recombination at stalled replication forks.

Although evidence is emerging that the gross chromosomal alterations observed in BRCA-deficient cells result from inappropriate DSB repair, the exact mechanisms that generate these abnormalities are still not understood. Recent work from several groups shows that, while BRCA1- or BRCA2-deficient rodent cells or human tumors are specifically deficient in HR, NHEJ (and sometimes SSA) remains intact [75]. This suggests that spontaneous or induced DSBs in BRCA-deficient cells are rerouted for repair by error-prone mechanisms, because the preferred mode of (error-free) processing by HR is unavailable (Fig. 1). In accord with this hypothesis, it has been shown that error-prone DSB repair mechanisms predominate in murine BRCA2-deficient cells [76–78], and possibly in BRCA1-deficient cells [79].

The major role of BRCA2 in DSB repair is through control of the hRad51 recombinase, while BRCA1 performs a distinct and more general function as a link between the sensing/signaling and effector components involved in the response to DNA damage, helping to ensure that the response is appropriate for the initiating lesion [80]. Overexpression of hRad51 in a chicken DT40 BRCA1 null mutant rescues defects in proliferation, DNA damage survival, and HR [81]. Furthermore, retrospective analyses of microarray expression data in BRCA1-deficient breast tumors revealed elevated expression of hRad51 and two of its late-acting cofactors, RAD54 and RAD51AP1. Together, these results suggest that upregulation of hRad51 in cells lacking BRCA1 function circumvents the normal requirement for BRCA1 in subnuclear assembly of hRad51 foci [81], Interestingly, while mutations in BRCA genes rarely occur in sporadic breast cancer, hRad51 is frequently upregulated, resulting in increased HR in these cells [82]. It is possible that this dysregulated HR may also lead to inappropriate repair of DSBs.

It is now widely accepted that a variety of tumor cell lines display elevated steady state levels of hRad51 and increased numbers of hRad51 nuclear foci compared with nonmalignant control cell lines [83]. The elevated steady state levels of hRad51 are not caused by gene amplification or changes in protein stability but instead are the result of transcriptional up-regulation [83]. In contrast, decreased levels of Rad51 were observed in multiple cancer cell types under hypoxic conditions but were not associated with the cell cycle distribution or expression of hypoxia-inducible factor [84]. With the accumulating evidence that abnormalities in HR occur frequently in cancer and that these abnormalities are potential therapeutic targets, there is growing interest in the identification of biomarkers that are diagnostic of HR abnormalities. Since alterations in expression of key recombination proteins have been observed, the use of focused microarrays to determine the expression of HR proteins may lead to the identification of diagnostic gene expression patterns for different HR abnormalities. A problem with this approach is that it does not directly measure HR. Recently, Powell and colleagues have developed an ex vivo assay based on formation of BRCA1, hRad51 and FANCD2 foci to detect FA/BRCA pathway defects in breast cancer biopsies [85].

Non-homologous end-joining

The genetic instability caused by deletion of any one of the key components of the main DNA-PK dependent non-homologous end-joining (NHEJ) pathway is characterized by chromosomal translocations [44, 86]. In addition, NHEJ deficiencies animals result in increased rates of neoplastic transformation. For example, DNA-PKcs and Ku70 mutant mice have a high incidence of T-cell lymphomas and Ku70$^{-/-}$ mice have increased rates of fibroblast transformation [87, 88]. In contrast, while fibroblasts from Ku80-null mice show chromosomal instability associated with chromosome aberrations, including breakage, translocations and aneuploidy, the animals have only a slightly earlier onset of cancer compared with wild-type animals. However, p53 inactivation synergizes with Ku80 to promote tumorigenesis such that all the double mutant mice succumb to pro-B-cell lymphoma at an early age [47]. These tumors display a specific set of chromosomal translocations and gene amplifications involving the immunoglobulin heavy chain IgH/Myc locus, reminiscent of Burkitt lymphoma [89]. Similar translocations are also seen in pro-B cell tumors that result from XRCC4- or DNA ligase IV-deficiency in a Trp53-null animals [90]. Since almost all the malignancies observed in NHEJ-deficient mice occur in lymphoid-derived cells, it is possible that they arise as a consequence of the role of NHEJ in immunoglobulin gene rearrangements. However, studies by the Alt laboratory have shown that the cancer predisposition resulting from NHEJ-deficiency occurs in other tissues and cell types [91].

In humans, there is emerging circumstantial evidence that defects in DNA-PK-dependent NHEJ result in cancer predisposition. One of the five individuals identified with mutant *LIG4* alleles [92, 93] had leukemia. A more recent study provided evidence that *LIG4* polymorphisms might influence the risk of acute lymphoblastic leukemia in children [94]. Finally, there is also evidence showing that the steady state levels of key factors in DNA-PK-dependent NHEJ are frequently reduced in cancer cell lines [55, 91] (Fig. 3). Specifically, it has been shown that DNA ligase IV is reduced in colon, cervical and breast cancer cell lines [95] and both DNA ligase IV and Artemis are reduced in chronic myeloid leukemia (CML) cell lines [55]. Notably, primary CML cells and cell lines established from CML patients expressing BCR-ABL1 have elevated levels of ROS and increased endogenous DSBs [96, 97]. Furthermore, repair of DSBs by NHEJ in CML cells is characterized by large deletions around the break-point junction and joining of DNA ends at regions of DNA sequence microhomology [98]. This abnormal and error-prone DSB repair is not only due to the reduced activity of the DNA-PK-dependent NHEJ pathway [55] but also the increased activity of the Alt NHEJ pathway, resulting from elevated steady state levels of WRN and DNA ligase IIIα [55] (Fig. 3). Together these studies suggest that Alt NHEJ is upregulated in a variety of cancers and is likely to contribute to the deletions and translocations that drive cancer progression. Importantly, knockdown of DNA ligase IIIα reduces DSB repair by NHEJ in CML but not normal myeloid cells, indicating that the upregulated Alt

NHEJ pathway in cancer cells is a potential therapeutic target [55].

Use of DNA repair inhibitors in cancers with DSB repair defects

Cancer therapy has until recently been focused on creating cytotoxicity through DNA damaging agents, such as ionizing radiation, cis platinum and temozolomide [99]. Although differences in the DNA damage response between normal and cancer cells presumably underlie the ability of these agents to preferentially kill cancer cells, their use is often limited by normal tissue toxicity. Since abnormalities in the DNA damage response of cancer cells are becoming more clearly defined, there is growing interest in the development of small molecules that will selectively target the abnormal DNA repair in cancer cells with the hope that these compounds either alone or in combination with DNA damaging agents will effectively kill cancer cells, while minimizing damage to normal cells.

DSB-activated cell cycle checkpoints

Since ATM is the predominant kinase responsible for the activation of multiple cell cycle checkpoints following DSB induction and ATM-deficient cells are exquisitely sensitive to ionizing radiation [2], inhibitors of ATM should potentiate the cytotoxicity of ionizing radiation and chemotherapeutic drugs that cause DSBs. Initial studies with caffeine and LY294002 that inhibit a broad range of protein kinases, including ATM, provided support for this idea [100, 101] and prompted the identification of a more specific ATM inhibitor, KU-55933, from a library of LY294002 derivatives [101]. As expected, KU-55933 efficiently sensitizes tumor cells to ionizing radiation and DSB-inducing chemotherapeutic agents, such as camptothecin and etoposide [101]. A potential problem with using ATM inhibitors as cancer therapeutics is that they may also sensitize normal tissues to DNA damage. In this scenario, the inhibitor of the DNA damage response will not preferentially enhance killing of the cancer cell and so there will be no therapeutic gain. Since cancer cells are presumed to have abnormalities in the DNA damage response, a subset of cancers with a particular DNA repair abnormality may be uniquely sensitive to ATM inhibition. In support of this idea, ATM was identified in a high-throughput siRNA screen for gene products whose knockdown is lethal to cells with a defective FA pathway [102]. Since the FA pathway is known to be disrupted in several types of sporadic cancers [102], inhibition of ATM may be an effective therapeutic strategy in this subset of cancers.

HR

As mentioned above, the key strand exchange protein in HR, hRad51, is overexpressed in a variety of tumors, and elevated hRad51 expression is correlated with a poor prognosis [103]. Although the DSB repair function of hRad51 protects normal cells from acquiring genetic changes that drive tumor development, overexpressed hRad51 in tumors contributes to their resistance to chemotherapy agents such as cisplatin [103–105], indicating that hRad51 is a potential target for antitumor drugs. Hine and colleagues exploited the overexpression of Rad51 in cancer cells to design a hRad51 promoter-based anticancer therapy. They cloned 2,931 bp of upstream regulatory sequences, the first noncoding exon of the Rad51, and the sequence encoding the first 12 amino acids of the hRad51 open reading frame (ORF) into a luciferase plasmid construct. The resultant plasmid was transfected into normal and cancer cells and luciferase activity was analyzed by flow cytometry. They found that the difference in promoter activity between normal and cancer cells increases to an average of 840-fold with a maximum difference of 12,500-fold. Based on this dramatic difference in promoter activity between normal and cancer cells, they designed a therapeutic strategy in which the hRad51 promoter was fused to a sequence encoding diphtheria toxin A (DTA), and the resultant plasmid was transfected into a variety of cancer cell types, including fibrosarcoma, breast and cervical cancer cells, and normal breast epithelial cells and fibroblasts. Notably, the cancer but not normal cells were killed by the plasmid, presumably as a consequence of increased expression of DTA. While the strategy described above has the problem of specifically introducing a nucleic acid into the cancer cells, these results suggest that therapies based on the hRad51 promoter could be highly tumor specific and may open new avenues for targeting a broad range of cancers [103].

A small molecule inhibitor of the MRN complex, Mirin, has been identified by high throughput screening of a small molecule library using a *X. laevis* extract assay. As expected, Mirin inhibits MRN-dependent activation of ATM and homology-dependent repair of DSBs [106]. Since HR factors such as MRN and hRad51 are required for cell viability, there is a concern that small molecule inhibitors of these essential proteins will be cytotoxic for normal as well as cancer cells and so will not have utility as anti-cancer agents. Factors such as hRad52, XRCC2 and XRCC3 that contribute to hRad51 strand exchange, but are not essential, may be alternative therapeutic targets [5]. It has been suggested that, while there appears to some functional redundancy among these factors, abnormalities in HR may make cancer cells more dependent upon one or more of these factors [107]. While these accessory factors

do not have enzymatic activities, their activity appears to be mediated by protein–protein interactions, so it may be possible to design high throughput screens for small molecules that block protein–protein interactions. Although this is a relatively unexplored approach compared with targeting enzyme active sites, it has been used successfully to identify a small molecule that prevents an inhibitory protein binding to wild-type p53, thereby restoring the tumor suppressor function of p53 [108].

PARP inhibitors

The abundant nuclear protein Poly(ADP-ribose) polymerase (PARP-1) binds avidly to DNA single strand breaks, an event that activates PARP-1 polymerase activity [109]. Activated PARP-1 utilizes NAD to synthesize poly (ADP-ribose) polymers that are attached to PARP-1 itself and to other nuclear proteins. Poly (ADP-ribosylated) PARP-1 serves as a recruitment factor for DNA ligase IIIα/XRCC1 and other factors involved in the repair of DNA single strand breaks [110]. Although there are other PARP family members, PARP-1 is the predominant enzyme that synthesizes poly (ADP-ribose) in response to DNA damage [111]. The replication of DNA containing single strand breaks cause DSBs, and so preventing the repair of DNA single strand breaks by inhibiting PARP-1 results in an increase in DSBs. Since these replication-associated DSBs would normally be repaired by HR, cells that are defective in HR are hypersensitive to PARP inhibitors. Based on this rationale, potent and specific inhibitors of PARP were developed as therapeutic agents for inherited forms of breast and ovarian cancer as the PARP inhibitors should be cytotoxic for *brca* mutant tumors but not normal tissues with a functional *BRCA* allele [112, 113].

As expected, PARP inhibitors increased the cytotoxicity of a range of anti-cancer agents including temozolomide and ionizing radiation that cause DNA single strand breaks [114, 115], and both *brca1*- and *brca2*-mutant cell lines were hypersensitive to PARP inhibitors in cell culture and mouse xenograft assays [116]. These results formed the basis for a phase I clinical trial, which demonstrated that the PARP inhibitor AZD2281 (Olaparib) exhibited antitumor activity in patients with ovarian and breast tumors resulting from either *BRCA1* or *BRCA2* mutations [117]. This phase I study was conducted in patients with advanced solid tumors ($n = 60$). The patient population was enriched for *BRCA1* or *BRCA2* mutation carriers ($n = 23$), including three breast cancer patients with inherited *BRCA2* mutations, in order to assess an objective antitumor effect of the PARP inhibitor in BRCA-deficient tumors. Within this group, treatment with Olaparib resulted in a confirmed partial response rate of 39% (9/23) with a sustained response in one patient for more than 76 weeks. As expected, no responses were observed in the tumors of non-BRCA mutation carriers. Olaparib treatment was evaluated further in two separate phase II studies for BRCA-associated breast and ovarian cancer [118, 119]. These studies recruited *BRCA1* or *BRCA2* mutation carriers with advanced breast cancer who had progressed following at least one previous cycle of chemotherapy. Twenty-seven patients were treated in each cohort and the activity of Olaparib as a single agent was confirmed. In the 400-mg group, an objective response rate of 41% (11/27) was seen with a progression-free survival of 5.7 months. Response was lower in the 100-mg group suggesting that the extent of PARP inhibition may be important. These proof of concept studies were the first to report single agent activity for a PARP inhibitor in BRCA-related breast cancer. Although a potentially exciting breakthrough in breast cancer treatment, the results of these small nonrandomized trials will require confirmation in larger phase III trials. A phase II trial investigating the single agent activity of the PARP inhibitor AG014699 (Pfizer GRD, La Jolla, California, USA) and a phase I trial investigating the PARP inhibitor ABT-888 (Abbott, North Chicago, Illinois, USA) in BRCA-associated breast and ovarian cancers are ongoing. Since these trials employ different modes of administration, scheduling, specificity and potency of PARP inhibition, it will be interesting to see if the responses differ from those observed with Olaparib. The promising results obtained with PARP inhibitors as a single agent in the treatment of hereditary breast cancer have prompted the design of trials to determine whether combining PARP inhibitors with other cancer therapeutics improves the outcome in other forms of cancer.

NHEJ

In addition to DNA damage-activated cell cycle checkpoints and HR, there is evidence that the NHEJ pathway may be a valid target for the development of more effective cancer treatments. Cells deficient in Ku70/80 or the catalytic subunit of DNA-PK (DNA-PKcs) are sensitive to DSBs induced by IR or chemotherapeutic agents [120]. In addition, DNA-PK is upregulated in some cancers, suggesting that it may be an important factor for tumor growth and survival. Indeed, upregulation of DNA-PK activity has been shown to impair apoptosis in B-cell chronic lymphocytic leukemia [121, 122]. In initial studies, the broad-spectrum phosphoinositide-3-kinase-related protein kinase (PIKK) inhibitors, wortmannin and LY294002 [123], that inhibit DNA-PK and other PIKKs such as ATM and ATR, were found to sensitize tumor cells to chemotherapeutic agents, prompting the development of more specific PIKK inhibitors. Treatment with a flavone-based DNA-PK inhibitor IC87361 led to tumor regression [124]. Similarly,

a highly potent and selective DNA-PK inhibitor NU7441 with an IC_{50} of 13 nM caused sensitization of tumor cells to radiation and chemotherapeutic agents both in culture and in mouse xenograft models [125].

DNA ligase inhibitors

DNA joining events are required for the completion of almost all DNA repair pathways and for DNA replication [126]. In human cells, there are three genes that encode DNA ligases with different but partially overlapping cellular functions [126]. Thus, inhibitors of DNA ligases are predicted to be cytotoxic and to sensitize cells to a variety of DNA damaging agents depending upon the specificity of the inhibitor for the three human DNA ligases. Using computer-aided drug design based on the structure of human DNA ligase I complexed with nicked DNA, a series of small molecule inhibitors of human DNA ligases, which exhibit different specificities for the three human DNA ligases in vitro, have been identified [95, 127]. In cell culture assays, a subset of these compounds were cytotoxic, killing both normal and cancer cells. Interestingly, the cytotoxic ligase inhibitors inhibit both DNA ligases I and III whereas a DNA ligase I-specific inhibitor was cytostatic [95]. It is possible that DNA ligase III substitutes for DNA ligase I, the replicative DNA ligase, and so cytotoxicity is observed only when both enzymes are inhibited. Since expression of phosphorylation site mutants of human DNA ligase I induces cell senescence [128], it is possible that the cytostatic effect of the DNA ligase I-specific inhibitor is due to induction of cellular senescence. Alternatively, the cytotoxic effects of the DNA ligase inhibitors may be a consequence of inhibition of DNA repair and/or DNA ligase III-dependent mitochondrial DNA metabolism [129].

Notably, subtoxic concentrations of the ligase inhibitors preferentially sensitized cancer cells to DNA alkylating agents and ionizing radiation [95]. The molecular mechanisms that underlie the ability of the DNA ligase inhibitors to enhance the killing of cancer cells by DNA damaging agents are not known but presumably reflect differences in the DNA damage response between cancer and normal cells. In theory, DNA ligase inhibitors constitute an extremely versatile group of agents that, depending on their specificity for the three human DNA ligases, can be used to target a variety of DNA repair pathways that would be chosen based on the DNA damaging agent. For example, a DNA ligase IV-specific inhibitor is predicted to sensitize cells with a functional DNA-PK-dependent NHEJ pathway to ionizing radiation. The situation with inhibitors of DNA ligases I and III is more complex because these enzymes participate in multiple nuclear DNA repair pathways, and DNA ligase III also functions in mitochondrial DNA metabolism [126].

Future directions in cancer therapeutics

A wide variety of DNA damaging agents are used as cancer therapeutics. It is generally assumed that differences in the DNA damage response between normal and cancer cells underlies the utility of DNA damaging agents in cancer treatment. However, abnormalities in the DNA damage response in cancer cells are poorly defined. An understanding of the alterations in the DNA damage response of cancer cells would permit the design of novel therapeutic strategies involving the use of inhibitors of the DNA damage response alone or in combination with DNA damaging agents that selectively target the altered DNA damage response in cancer cells. The development of PARP inhibitors that selectively target the defect in DSB repair in breast tumors in women with herditary breast cancer is the first example of this therapeutic strategy [116]. This has prompted interest in defining and then targeting DNA repair abnormalities in sporadic cancers. Although there are a growing number of publications that describe DNA repair abnormalities in cancer cell lines, there is a need for the development of assays and identification of biomarkers that can be used to identify DNA repair abnormalities in clinical samples. In addition, the indentification and characterization of a wider repertoire of small molecules that target DSB repair proteins will not only increase our ability to probe DSB repair abnormalities in cancer cells but also to develop different combinatorial therapeutic strategies that selectively target the DSB repair abnormality in cancer cells.

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