The DNA Damage Response: Implications for Tumor Responses to Radiation and Chemotherapy

Michael Goldstein and Michael B. Kastan

Department of Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, North Carolina 27710; email: michael.kastan@duke.edu, michael.goldstein@duke.edu

Annu. Rev. Med. 2015. 66:129–43

First published as a Review in Advance on November 24, 2014

The *Annual Review of Medicine* is online at med.annualreviews.org

This article's doi: 10.1146/annurev-med-081313-121208

Keywords

DNA repair, cancer resistance, radiosensitization, chemosensitization, synthetic lethality

Abstract

Cellular responses to DNA damage are important determinants of both cancer development and cancer outcome following radiation therapy and chemotherapy. Identification of molecular pathways governing DNA damage signaling and DNA repair in response to different types of DNA lesions allows for a better understanding of the effects of radiation and chemotherapy on normal and tumor cells. Although dysregulation of the DNA damage response (DDR) is associated with predisposition to cancer development, it can also result in hypersensitivity or resistance of tumors to therapy and can be exploited for improvement of cancer treatment. We highlight the DDR pathways that are activated after treatment with radiation and different classes of chemotherapeutic drugs and describe mechanisms determining tumor sensitivity and resistance to these agents. Further, we discuss approaches to enhance tumor sensitivity to radiation and chemotherapy by modulating the DDR with a goal of enhancing the effectiveness of cancer therapies.

THE DNA DAMAGE RESPONSE AND CANCER

DNA damage response (DDR): network of signaling pathways activated in cells in response to DNA damage

Nucleotide excision repair (NER): removes damaged nucleotides from DNA

Double-strand break (DSB): a DNA break affecting both strands of DNA

Homologous recombination (HR): repairs DNA double-strand breaks using a homologous chromatid as template

Poly(ADP-ribose) polymerase (PARP): a family of enzymes that synthesize poly(ADP-ribose) chains with PARP1 and PARP2 playing a role in DNA repair

Ataxia telangiectasia mutated (ATM): protein kinase that responds to DNA double-strand breaks

PI3K-like kinase (PIKK): family of phosphatidylinositol 3-kinase-like kinases including ATM, ATR, and DNA-PK that are involved in DNA damage response

Ataxia telangiectasia and Rad3-related (ATR): protein kinase that responds to single-stranded DNA regions

DNA-PK: protein kinase that initiates nonhomologous end joining

Cells are constantly dealing with damage to their DNA due to replication stress, telomere shortening, and a variety of exogenous and endogenous DNA targeting exposures, such as UV light, chemical toxins, and reactive oxygen species (ROS) that are generated during general metabolism and inflammation. An intricate network of signaling pathways and repair mechanisms has evolved to help mammalian cells cope with a variety of DNA lesions in order to protect genomic stability. Much of our understanding of the DNA damage response (DDR) stems from studying human diseases caused by hereditary defects in DNA damage signaling and repair pathways. In these patients, an inability to properly detect, repair, or respond to DNA lesions results in a cancer-prone phenotype. For example, xeroderma pigmentosum is caused by mutations in the *XP* family of genes and is characterized by a predisposition to skin cancer due to an inability to effectively repair UV-light-induced DNA lesions by nucleotide excision repair (NER). Inherited mutations in the *ATM* or *NBS1* genes, the products of which are involved in detecting and signaling DNA double-strand breaks (DSBs), result in the cancer-prone conditions ataxia telangiectasia and Nijmegen breakage syndrome, respectively, with a predisposition to lymphoid malignancies (1).

Mutations in the *Brca1* and *Brca2* genes result in defective repair of DSBs by homologous recombination (HR) and are associated with genomic instability and predisposition to breast and ovarian cancer (2). The hereditary nonpolyposis colon cancer (*HNPCC*) genes are involved in DNA mismatch repair, and their dysfunction predisposes individuals to colon and uterine tumors (3). Although such inherited mutations predispose to development of cancer, they can also create specific sensitivities of the consequent tumor cells to therapeutic agents, such as the extraordinary sensitivity of Brca1-null tumors to inhibitors of base excision repair (PARP inhibitors) and the hypersensitivity of ATM-null tumors to ionizing radiation and radiomimetic drugs. Further, clearly established links between certain environmental exposures to DNA damaging agents and cancer development also demonstrate the importance of the DDR in tumor development, even in the setting of nonhereditary malignancies (4).

The DDR has several distinct components that determine the outcome of the damaged cells, including (*a*) initial detection of the lesion resulting in activation of signaling networks and cell cycle checkpoint induction, (*b*) DNA repair, and (*c*) induction of cell death through activation of programmed cell death pathways. All parts of the DDR need to be properly coordinated to maintain genomic stability, and all can affect the outcome of cancer treatment. Thus, they are attractive targets for radio- and chemosensitization during cancer treatment. Knowing the DDR defects that are present in a tumor can also allow for selection of optimal treatments that can efficiently kill the tumor cells. This review explores the effects of dysregulation of DDR pathways on the response of tumors to radiation and chemotherapy and discusses strategies of targeting these pathways in order to sensitize tumors to genotoxic treatment (**Figure 1**).

THE DNA DAMAGE RESPONSE AND DNA REPAIR PATHWAYS

DNA Damage Signaling and Checkpoint Induction

Following induction of DNA breaks, initiation of signaling pathways is facilitated by members of the phosphatidylinositol 3-kinase (PI3K)-like kinase (PIKK) family, ATM, ATR (ataxia telangiectasia and Rad3-related protein), and DNA-PK (DNA-dependent kinase). ATM plays a critical role in DNA damage signaling originating at DSBs, whereas ATR responds to single-stranded DNA (ssDNA) regions. DNA-PK is directly involved in DNA repair by promoting DSB religation through nonhomologous end joining (NHEJ). An early event in DSB signaling is binding of the MRN complex to double-stranded DNA ends (5). MRN consists of the MRE11, RAD50,

Figure 1

DNA lesions induced by radiation and chemotherapy and their repair. The figure illustrates the specific DNA lesions induced by radiation and different classes of chemotherapeutic agents, the molecular pathways repairing these lesions, and the DNA damage response factors that can be targeted in order to improve the efficacy of the anticancer agents. Each color represents a repair machinery or multiple repair machineries involved in repair of a specific DNA lesion. Abbreviations: BER, base excision repair; HR, homologous recombination; DSB, DNA double-strand break; ICL, DNA interstrand cross-link; IR, ionizing radiation; MGMT, methyl-guanine methyl-transferase; MMR, mismatch repair; NER, nucleotide excision repair; NHEJ, nonhomologous end joining; SSB, DNA single-strand break.

and NBS1 proteins; MRE11 possesses endo- and exonuclease activities that facilitate resection of DNA ends at the DSB. The MRN complex plays a critical role in recruiting DDR proteins to the DSB, including ATM and other signaling molecules (5). Although activation of the ATM kinase appears to be initiated by steric changes in chromatin structures after DNA breakage (6), the MRN-dependent recruitment of activated ATM to the DSB appears to markedly amplify ATM activation (5, 7).

Once the activated ATM kinase is at the DSB, it phosphorylates a variety of targets facilitating cellular responses to the damage. One important ATM target within chromatin is the histone protein H2AX. The phosphorylated form of H2AX, referred to as γH2AX, serves as a platform for recruitment of additional DDR factors and enhancement of signaling pathways. γH2AX recruits MDC1, which then recruits additional MRN and ATM molecules to regions surrounding DSBs, resulting in amplification of ATM signaling (5). Furthermore, MDC1 recruits RNF8, an E3 ubiquitin ligase that initiates conjugation of ubiquitin chains to histone H2A. Brca1 is then recruited to the DSB site by binding ubiquitin chains via a protein complex containing Rap80 and Abraxas (5). Activated ATM also phosphorylates substrates such as NBS1, Chk2, Brca1, and p53, which are involved in activation of cell cycle checkpoints (5). ATM-dependent phosphorylation stabilizes p53, which upregulates p21 expression, and induces the G_1 arrest, whereas Chk2 and Brca1 appear to be involved in S and G_2/M checkpoints (8).

In contrast to ATM, which responds to DSBs, ATR kinase is activated by single-strand–double-strand junctions that arise as intermediates of DNA repair pathways, at resected DSBs, or as a result of replication fork collapse. The latter can occur naturally during DNA replication or as a consequence of DNA interstrand cross-links (ICLs) due to uncoupling of minichromosome maintenance (MCM) helicase, which is essential for DNA replication, and DNA polymerase (9). Because of its role in resolution of stalled replication forks, ATR is essential for survival under basal conditions (10, 11). Notably, although ATM and ATR are activated by different stimuli, their substrates significantly overlap (8). An important substrate of ATR is the Chk1 kinase, which induces cycle arrest in the S and G_2/M phases in response to DNA damage (5, 8).

Nonhomologous end joining (NHEJ): directly religates DNA double-strand breaks

MRN: complex of MRE11, RAD50, and NBS1 proteins, which detects DNA double-strand breaks

Interstrand cross-link (ICL): a chemical adduct that covalently cross-links both DNA strands

Minichromosome maintenance (MCM) helicase: DNA helicase that unwinds DNA and separates annealed DNA strands

Ionizing radiation (IR): radiation that releases electrons from molecules and atoms, thereby ionizing them

Single-strand break (SSB): DNA break affecting a single strand of DNA

DNA Double-Strand Break Repair

Double-strand DNA breaks are among the most toxic DNA lesions. Therefore, agents that induce DSBs, such as ionizing radiation (IR) and topoisomerase inhibitors, are widely used for cancer treatment. DSB formation can arise directly or indirectly from other DNA lesions, for instance, when a replication fork collides with a single-strand break (SSB) or an ICL during replication. This mechanism plays an important role in cancer therapy because toxicity of many chemotherapeutic agents depends on the conversion of primary DNA lesions induced by these drugs into DSBs. The two major DSB repair pathways are NHEJ and homologous recombination (HR). NHEJ is an error-prone repair pathway that can occur throughout all cell cycle phases, whereas HR is an error-free pathway that can be used only in the late S and G_2 phases of the cell cycle as it requires a homologous sequence located on the sister chromatid. HR is the major pathway repairing DSBs in lower eukaryotes, and NHEJ predominates in mammalian cells (12). However, despite its minor role in repair of directly induced DSBs in human cells, HR is specifically required for repair of DSBs that are formed at collapsed replication forks in the S phase of the cell cycle. Further, HR is involved in repair of ICLs, where it collaborates with NER.

The initial step in NHEJ is the binding of the Ku70/Ku80 complex to the DSB, followed by recruitment and activation of the catalytic subunit DNA-PKcs (5). Active DNA-PK phosphorylates a variety of targets including XRCC4 (13). Finally, DNA ends are religated by the XRCC4-ligase

IV complex (14). This process requires a modulation of chromatin structure by nucleolin, which allows XRCC4 to access the break site (15).

During HR, a homologous sequence located on the sister chromatid serves as a template to replace the region flanking the DSB on the damaged DNA strand. It requires nucleolytic processing of the DNA ends at the DSB that is initiated by CtIP in an MRN-, Brca1-, and ATM-dependent manner (2) and extended by exonuclease 1 (Exo1) (16). The resulting ssDNA regions are covered by the RPA complex, which keeps ssDNA unwound, followed by Rad51 recruitment (5). Rad51 nucleofilaments promote the invasion of the damaged DNA strand into the undamaged DNA duplex of the sister chromatid and the pairing of the complementary strands. Thereafter, strand exchange occurs and the resulting Holliday junction is resolved (14).

Replication protein A (RPA) complex: covers and stabilizes single-stranded DNA regions

BER: base excision repair

Apurinic/apyrimidinic endonuclease 1 (APE1): performs the incision of DNA during base excision repair

Apurinic/apyrimidinic (AP) site: a DNA site where the base was removed from the nucleotide

Proliferating cell nuclear antigen (PCNA): facilitates DNA ligation during long-patch base excision repair

Mismatch repair (MMR): removes base mismatches from DNA

Base Excision Repair

Base excision repair (BER) is responsible for removal of damaged bases such as N-alkylated purines and 8-oxo-7,8-dihydroguanine (8-OxoG). A damaged base is recognized and removed by specific glycosylases, resulting in an apurinic/apyrimidinic (AP) site, followed by a cleavage of the AP site by either the glycosylase itself or AP endonuclease 1 (APE1). This DNA "nick," along with directly induced SSBs, is a substrate for further steps of BER. In the next step, a displacement of either a single nucleotide (short-patch BER) or up to 13 nucleotides (long-patch BER) occurs at the cleaved DNA strand, followed by DNA resynthesis. Ligation is then performed by ligase III, XRCC1, and PARP1 in the case of short-patch BER or by ligase I and proliferating cell nuclear antigen (PCNA) in long-patch BER (14).

Nucleotide Excision Repair

Nucleotide excision repair (NER) serves to repair bulky DNA adducts such as UV-light-induced lesions and intrastrand cross-links. Also, it performs the first step of ICL repair, which is then completed by HR. NER is divided into transcription-coupled repair that removes DNA lesions blocking RNA polymerase progression along the DNA strand and global genomic repair that is independent of transcription. The NER pathway consists of recognition of the DNA lesion by members of the XP protein family, DNA unwinding, excision of the lesion by XPG and ERCC1-XPF, filling of the DNA gap, and sealing of DNA by ligase I (14).

Mismatch Repair

Mismatch repair (MMR) removes base mismatches caused by replication errors and by spontaneous or induced base modifications such as methylation and oxidation. The mismatch is recognized by the MutSα complex, which consists of MSH2 and MSH6 proteins that bind to the lesion and associate with the MutLα complex composed of MLH1 and PMS2. Then DNA excision at the daughter strand containing the incorrect base is performed by Exo1, and the gap is filled by Polδ (14).

THE DNA DAMAGE RESPONSE IN TUMOR THERAPY

Ionizing Radiation

Ionizing radiation (IR), a common modality used to treat malignancies, particularly solid tumors, generates ROS that cause a variety of lesions in DNA. Most IR-induced DNA lesions are base

modifications such as 8-OxoG (17). Additionally, IR causes DNA breaks; 1 Gy IR induces approximately 1,000 SSBs and 35 DSBs per cell (18, 19). Although DSBs constitute a minority of DNA lesions induced by IR, they play a central role in IR-induced cytotoxicity. Therefore, pathways involved in signaling and repair of DSBs are critical for the outcome of radiation therapy.

Alterations of DSB repair pathways that occur in tumors due to genetic or epigenetic changes affect the sensitivity of cancer cells to IR and can be exploited for an effective radiation therapy. Willems et al. identified several single-nucleotide polymorphisms (SNPs) within the *Ku70* and *Ku80* genes that are associated with an increased sensitivity of tumors to radiation (20). Furthermore, decreased Brca1 expression due to hypermethylation of the *Brca1* promoter is a common event in multiple nonhereditary cancer types (21, 22), and a loss of functional Brca1 was reported to cause radiosensitization in tumors (23, 24), suggesting that the methylation status of the *Brca1* promoter can potentially be used as a marker to identify tumors that are responsive to radiation.

As described above, the ATM kinase is activated following DSB induction, and ATM signaling contributes to DSB repair via HR by promoting DNA end-processing and Brca1 recruitment (2, 25). A protective role of ATM after exposure to IR is mirrored by the profound radiosensitivity observed in ataxia telangiectasia patients (1), suggesting that ATM is a suitable target for radiosensitization. Accordingly, small-molecule inhibitors of ATM were demonstrated to sensitize cancer cells to IR in preclinical models (26–28). It is worth noting that ATM loss results in radiosensitization independent of the p53 status (29), and chemical ATM inhibition efficiently sensitizes p53-mutant glioblastoma cells to IR (27). The ability of ATM inhibitors to sensitize p53-deficient cancer cells to radiation is highly important in light of the tumor resistance to therapy associated with p53 mutations and the high frequency of p53 mutations in malignant tumors.

Another cornerstone of radiosensitization is the inhibition of NHEJ, which plays a major role in repair of DSBs in human cells. Indeed, DNA-PK inhibitors exhibit a strong radiosensitizing effect in cancer cell lines (30). Because a partial redundancy between NHEJ and HR exists in repair of IR-induced DSBs (31), a simultaneous inhibition of DNA-PK and ATM appears to be an optimal approach to radiosensitization. Interestingly, the small-molecule inhibitor NVP-BEZ235, initially developed as an mTOR inhibitor, was found to inhibit both PIKKs, ATM and DNA-PK, and proved to be a potent radiosensitizer in preclinical experiments (32, 33). However, a potential drawback of NVP-BEZ235 is its ability to inhibit ATR (34). Because ATR is crucial in resolving stalled replication forks, it is generally required for survival, even in the absence of exposure to genotoxic stress. Consequently, disruption of ATR function results in lack of viability at both cellular and organism levels (10, 11). These findings suggest that an optimally designed radiosensitizing PIKK inhibitor might target both ATM and DNA-PK, but not affect ATR activity, in order to avoid systemic toxicity.

Another approach to radiosensitization is the inhibition of checkpoint kinase signaling. Specific inhibitors of Chk1 were shown to potentiate the toxicity of DSB-inducing agents, including IR (35, 36), whereas a loss of Chk2 appears to have an opposite effect, causing radioresistance in vivo (37). Thus, most research has focused on the development of Chk1 inhibitors. It is not clear, however, whether the abrogation of the cell cycle arrest is, indeed, responsible for radiosensitization by Chk1 inhibitors. In addition to checkpoint activation, Chk1 is involved in HR (5), and a recent study suggested that radiosensitization caused by a Chk1 inhibitor is mediated through its inhibitory effect on HR activity rather than checkpoint signaling (38).

Importantly, IR-induced lesions such as 8-OxoG and SSBs that have a low cytotoxic potential per se can indirectly induce toxic DSBs by blocking replication forks. Both 8-OxoG and SSBs are dealt with by BER. Consistently, a high expression level of the BER factor APE1 is associated with radioresistance in cervical cancer (39) and germ cell tumors (40). These findings suggest that inhibition of BER may be a suitable approach to radiosensitization. So far, methoxyamine has

been identified as an inhibitor of APE1 and was shown to potentiate radiosensitization caused by 5-Iodo-20-deoxyuridine (IdUrd) in colon cancer cells but did not increase sensitivity to IR on its own (41). PARP inhibitors provide another possibility for blocking BER. Small-molecule inhibitors of PARP1 sensitized cancer cells to IR in preclinical settings (42, 43) and are currently undergoing clinical trials in combination with radiotherapy (44).

Methylguanine methyl-transferase (MGMT): directly repairs DNA alkylations at O6-guanine

DNA Methylating Agents

The chemical simplicity of methyl adducts in DNA is in contrast to a surprisingly complicated mechanism of cytotoxicity caused by these DNA lesions. The main products of methylating agents such as temozolomide (TMZ) and procarbazine are N^7- and N^3-methylguanine (N^7MeG, N^3MeG) and N^3-methyladenine (N^3MeA). O^6-methylguanine (O^6MeG) is a minor product, but it possesses a high potential to induce cytotoxicity. O^6MeG does not induce cell death directly but rather by converting to a DSB in an MMR- (45) and replication- (46) dependent manner. Mechanistically, it mispairs with cytosine or thymine during DNA synthesis in the S phase, and repetitive, futile attempts to remove the mismatch by MMR result in formation of DSBs that induce cell death (46, 47). Thus, efficient MMR is required for tumor sensitivity to TMZ (48), and loss of expression of MLH1, a member of the MMR pathway, is associated with resistance of gliomas (49) and medulloblastomas (50) to TMZ.

The O^6MeG lesion is reversed in a stoichiometric manner by methylguanine methyltransferase (MGMT) (51). Therefore, MGMT expression is an important determinant of cellular responses to methylating agents, and high MGMT expression levels in cancer were shown to correlate with resistance to TMZ (52, 53). However, a silencing of MGMT expression due to *MGMT* promoter hypermethylation was found in several cancer types (54). This epigenetic mark correlates with hypersensitivity of gliomas to TMZ (55), suggesting that *MGMT* promoter methylation can be used as a biomarker predictive of tumor response to methylating agents. It should be noted that in addition to removing methyl residues, MGMT can reverse other DNA alkylations, such as chlorethyl adducts induced by nitrosourea drugs, e.g., bis(2-chloroethyl)nitrosourea (BCNU), that are later converted into ICLs. Therefore, MGMT expression levels affect the tumor response to this class of agents as well.

Because of its critical role in resistance to alkylating drugs, MGMT was considered a promising target for inhibition. In fact, chemical inhibitors of MGMT highly potentiate the toxicity of TMZ and other alkylating agents in cancer cells (56) and entered clinical trials more than a decade ago (57). Unfortunately, clinical benefits of MGMT inhibition were limited owing to enhanced adverse side effects, mainly myelotoxicity, when administered in combination with TMZ and BCNU (58), which required a reduction of the chemotherapy dosage. This effect was attributed to MGMT inhibition in healthy tissues as the inhibitors lack specificity for tumor cells. Therefore, coupling MGMT inhibitors to molecules that can be used for targeted delivery, such as DNA or RNA aptamers (59), could be used in the future to avoid systemic side effects.

Unlike O^6MeG, N^3MeA and N^3MeG cannot be repaired by MGMT but instead are substrates of BER. Notably, the toxicity of the N-methylations is dependent on the formation of BER intermediates such as AP sites and SSBs that can block replication forks during the S phase, resulting in replication fork collapse and DSB formation. Therefore, changes in protein activities that participate in early and late steps of BER generating and repairing these intermediates, respectively, have opposite effects on the cytotoxicity induced by methylating agents. For instance, an overexpression of N-methylpurine DNA glycosylase (MPG), which facilitates the initial step of BER, correlates with an increased formation of AP sites, resulting in hypersensitivity of cancer cells to TMZ (60). Importantly, increased expression levels of MPG were found in breast cancer

(61) and astrocytoma (62), suggesting a potential benefit of methylating agents in treatment of these tumors.

Topoisomerases (TOPs): relax supercoiled DNA structures by cleaving and religating DNA

In contrast, an overexpression of proteins that are involved in late steps of BER can cause resistance to methylating agents due to an enhanced repair of toxic BER intermediates. High levels of APE1 and PARP1, which are involved in processing of AP sites and ligation of SSBs, respectively, were found to be a common trait in cancer and can result in resistance to chemotherapy (63–65). Accordingly, high nuclear APE1 expression correlates with a shorter overall survival in patients with ovarian and gastroesophageal cancer treated with alkylating drugs (64). On the other hand, a defect in SSB religation due to low expression of XRCC1 and ligase III results in accumulation of DNA breaks and hypersensitivity to TMZ (66).

On the basis of these findings, PARP1 and APE1 appear to be attractive targets for inhibition, which could overcome the resistance of cancer cells to methylating agents caused by an overexpression of these repair factors. In fact, inhibition of PARP1 (67) and APE1 (68) was shown to sensitize cancer cell lines to TMZ in preclinical settings. Currently, APE1 inhibitors (69, 70) and PARP1 inhibitors (71) are undergoing clinical trials in combination with TMZ.

Topoisomerase Inhibitors

Topoisomerases (TOPs) are proteins that relax supercoiled DNA structures by cleaving either one (TOP I) or both (TOP II) DNA strands followed by unwinding of the supercoils and religation of DNA by TOPs. TOP I inhibitors, such as camptothecin and topotecan, and TOP II inhibitors, such as etoposide and anthracyclines, block the final religation step, resulting in accumulation of SSBs and DSBs, respectively. DNA breaks induced by TOP inhibitors differ from chemically induced DNA breaks because the inactive TOP remains covalently bound to the DNA at the break site and has to be removed during break repair. SSBs induced by TOP I inhibitors are repaired by a complex consisting of tyrosyl-DNA-phosphodiesterase 1 (TdP1), which hydrolyzes the bond between TOP I and the 3′ DNA end at the break, and BER machinery (72). Consequently, PARP inhibitors, which blunt BER, sensitize cells to TOP I inhibitors (73). A dual PARP1/2 inhibitor has entered a clinical trial in combination with topotecan (74).

Whereas TOP II inhibitors induce DSBs independent of proliferation (75), TOP I inhibitors induce SSBs that can cause stalled replication forks during the S phase, resulting in their conversion into DSBs (76). Thus, the cytotoxicity induced by TOP I poisons is proliferation dependent (75). As discussed above, HR is involved in repair of DSBs that are generated from SSBs in the S phase. Therefore, alterations in HR affect sensitivity to TOP I poisons. For instance, Brca1 loss results in hypersensitivity to camptothecin (77), which could be exploited for treatment of Brca1-deficient tumors. Further, inhibition of ATM may sensitize cells to camptothecin and etoposide (28), suggesting a potential benefit of ATM inhibitors in combination with TOP I and TOP II inhibitors. Ultimately, because TOP I–induced cytotoxicity is mainly mediated by DSBs, targeting the NHEJ pathway by DNA-PK inhibitors is another approach to sensitize cells to TOP I poisons and was shown to be effective in combination with irinotecan (78).

Inhibition of the checkpoint kinase Chk1 also potentiates the toxicity induced by TOP poisons in preclinical settings (36). However, a phase II clinical trial investigating the efficacy of the Chk1 inhibitor UCN-01 in combination with irinotecan reported aggravation of chemotherapy side effects and showed little benefit of UCN-01 (79).

In contrast to TOP I poisons, DSBs are the primary lesion induced by TOP II inhibitors. Consequently, cells can be sensitized to etoposide by inhibitors of DNA-PK (80) and ATM (28). A recent study showed that ATM regulates TOP II expression, and ATM loss results in increased TOP II levels and enhances sensitivity to TOP II inhibition (81). This finding is in accordance

with the concept that TOP expression levels correlate with the number of DNA breaks induced by TOP inhibitors. Thus, ATM inhibitors may cause sensitization to TOP II inhibitors not only by affecting the cellular response to DSBs but also by enhancing TOP II expression and elevating the level of the breaks induced by TOP II poisons.

DNA Cross-Linking Agents

DNA cross-linking agents were among the first drugs used for cancer treatment. Cyclophosphamide was introduced into the clinic in 1958. This agent belongs to the group of nitrogen mustard (NM) compounds, which are bifunctional alkylating agents and preferentially target N^7G on both DNA strands, thus inducing DNA ICLs. NM-induced toxicity is dependent on replication and has been mainly attributed to replication perturbations during the S phase and inhibition of transcriptional activity (82). Another group of agents that induce ICLs are nitrosourea drugs; BCNU, for example, conjugates a chlorethyl residue to O^6 of guanine, which is then converted into an N^1-guanine-N^3-cytosine ICL (83). Finally, cisplatin, which covalently binds to guanine and adenine, generates DNA intra- and interstrand cross-links (84).

Whereas DNA intrastrand cross-links are repaired by NER alone, ICL repair involves parts of NER, HR, and translesion synthesis. Consequently, NER defects due to mutations of ERCC1 and xeroderma pigmentosum (XP) family proteins result in hypersensitivity to cisplatin in cultured cells (85). Consistent with these observations, low ERCC1 expression was found to cause sensitivity of testicular tumors to cisplatin (86), potentially explaining the high responsiveness of testicular cancer to cisplatin-based chemotherapy (87). Although no specific NER inhibitors are currently available, several drugs initially designed for other purposes, such as cyclosporine A (88), cetuximab (89), and spironolactone (90), were shown to inhibit NER by downregulating expression of NER factors and to increase sensitivity to platinum agents.

An important example of sensitivity to cross-linking agents due to HR deficiency is the loss of functional Brca1 in breast cancer, which results in high responsiveness to cisplatin (91, 92). Because the Fanconi anemia (FANC) pathway plays an important role in promoting HR during ICL repair, a loss of members of this pathway increases sensitivity to agents that induce ICLs (93). Interestingly, an inactivation of the FANC pathway due to epigenetic modification of various *FANC* genes was shown to be a common event in ovarian cancer (94), head and neck squamous cell carcinoma, and non–small cell lung cancer (95). This finding suggests that screening tumors for genetic and epigenetic alterations affecting either Brca1 or the FANC pathway may be useful in predicting tumor response to ICL-inducing agents.

SYNTHETIC LETHALITY

The concept of synthetic lethality has gained increasing popularity in the field of oncology. Synthetic lethality describes a condition where a disruption of two redundant DDR pathways results in accumulation of DNA damage leading to cell death, whereas abrogation of only one pathway is tolerated by the cell. This can be exploited therapeutically if cancer cells lack one of the pathways due to a genetic defect and another pathway is inhibited by a targeted drug. Ideally, this approach will kill cancer cells while sparing healthy tissues, in which both pathways are functional. A proof of principle was demonstrated using cancer cell xenograft models that were deficient in HR due to *Brca1* or *Brca2* mutations. These cancer cells are specifically sensitive to PARP inhibitors at concentrations that are nontoxic to healthy tissues (96, 97). The suggested mechanism behind this phenomenon is based on the accumulation of naturally occurring SSBs due to BER inhibition by PARP inhibitors resulting in conversion of these SSBs into DSBs at stalled replication forks

during the S phase. In noncancerous cells these DSBs can be repaired by HR, but cancer cells lacking HR due to *Brca1/2* mutations accumulate DSBs that induce cell death.

However, the idea of treating nonhereditary forms of Brca1/2-deficient tumors with a PARP inhibitor alone bears some caveats. These tumors appear to develop resistance to PARP inhibitors owing to a restoration of Brca1 and Brca2 functions by genetic reversion (92, 98). Another mechanism of resistance is pathway rewiring due to the loss of 53BP1 or DNA-PK, which restores HR activity by blunting NHEJ (99, 100). These findings suggest that treatment of Brca1/2-deficient tumors with a PARP inhibitor monotherapy may not be the optimal strategy. Notably, loss of functional Brca1 results in tumor sensitivity to IR (23) and to chemotherapeutic drugs such as TOP I inhibitors (77) and cisplatin (91, 92). Given that PARP inhibition also increases sensitivity to IR and multiple chemotherapeutic agents, as described above, a combination of IR and/or chemotherapy with PARP inhibitors in patients with Brca1/2-deficient tumors appears to be a superior strategy. By exploiting the tumor sensitivity caused by Brca1/2 deficiency in multiple ways, it would be more efficient than PARP inhibitors alone and could potentially impede resistance development.

CONCLUDING REMARKS

Since the introduction of radiation and chemotherapy, oncologic treatment regimens have been largely developed by trial and error and validated through clinical trials. Detailed characterization of DDR pathways over the past two decades has revealed many details about the cellular response to radiation and chemotherapeutic agents, and this knowledge is ready to be exploited for cancer treatment. Analysis of biomarkers such as *MGMT* promoter methylation and *Brca1* mutations has potential to become an important tool for predicting tumor response to specific types of chemotherapeutic drugs and assigning an appropriate therapy regimen. Designing an individual tumor therapy based on the molecular phenotype of the cancer rather than on its histologic appearance will increase the efficacy of treatment and reduce systemic toxicity by avoiding drugs that the tumor is resistant to.

Alteration of DDR pathways by small-molecule inhibitors is another promising approach to improve the efficacy of radiation and chemotherapy. However, the use of these drugs as chemosensitizers requires caution because a systemic application of DDR inhibitors and chemotherapeutic agents not only potentiates the killing of cancer cells but also results in an increased toxicity of DNA-damaging agents in healthy tissues. During initial clinical trials including DDR inhibitors, an aggravation of side effects frequently forced a decrease in the dosage of chemotherapy, thus limiting the benefits of DDR modulators. The use of specific delivery methods such as nanoparticles or nucleic acid aptamers coupled to DDR inhibitors could improve their specificity for cancer cells and reduce the side effects when combined with chemotherapy. Importantly, inhibition of DDR in noncancerous tissues by small-molecule inhibitors is a lesser issue during radiation therapy. Unlike chemotherapy, the radiation beam is delivered specifically to the tumor, so systemic toxicity should be avoided if chemical DDR inhibitors are combined with radiation therapy. Therefore, the currently available DDR modulating agents appear to have better clinical prospects as radiosensitizers than as chemosensitizers.

An ideal cancer therapy would specifically target cancer cells while avoiding toxicities to normal tissues. The concept of synthetic lethality promised such a possibility. However, the enthusiasm about synthetic lethality aiming to specifically kill cancer cells by nontoxic doses of a DDR inhibitor has been challenged by reports showing an acquired resistance of tumors to synthetically lethal therapeutics due to genetic reversion of DDR defects and rewiring of DDR pathways. Nonetheless, exploiting a defective DDR pathway in cancer by targeting the redundant repair

pathway has clinical potential. It is conceivable that it is easier for cancer cells to adapt to a relatively low level of DNA damage that is indirectly induced by synthetically lethal DDR inhibitors and accumulates over a period of time than to high levels of DNA damage that are rapidly induced by chemotherapy and radiation. Therefore, a combination of a synthetically lethal drug with radiation and chemotherapeutic agents that the cancer cells are specifically sensitive to due to the loss of a DDR pathway appears to be a superior approach, as it would more broadly exploit the DDR dysregulation and might hinder the development of resistance.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Shiloh Y. 1997. Ataxia-telangiectasia and the Nijmegen breakage syndrome: related disorders but genes apart. *Annu. Rev. Genet.* 31:635–62
2. Huen MS, Sy SM, Chen J. 2010. BRCA1 and its toolbox for the maintenance of genome integrity. *Nat. Rev. Mol. Cell Biol.* 11:138–48
3. Modrich P, Lahue R. 1996. Mismatch repair in replication fidelity, genetic recombination, and cancer biology. *Annu. Rev. Biochem.* 65:101–33
4. Luch A. 2005. Nature and nurture—lessons from chemical carcinogenesis. *Nat. Rev. Cancer* 5:113–25
5. Ciccia A, Elledge SJ. 2010. The DNA damage response: making it safe to play with knives. *Mol. Cell* 40:179–204
6. Bakkenist CJ, Kastan MB. 2003. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* 421:499–506
7. Kitagawa R, Bakkenist CJ, McKinnon PJ, Kastan MB. 2004. Phosphorylation of SMC1 is a critical downstream event in the ATM-NBS1-BRCA1 pathway. *Genes Dev.* 18:1423–38
8. Kastan MB, Bartek J. 2004. Cell-cycle checkpoints and cancer. *Nature* 432:316–23
9. Byun TS, Pacek M, Yee MC, et al. 2005. Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint. *Genes Dev.* 19:1040–52
10. Brown EJ, Baltimore D. 2000. ATR disruption leads to chromosomal fragmentation and early embryonic lethality. *Genes Dev.* 14:397–402
11. Brown EJ, Baltimore D. 2003. Essential and dispensable roles of ATR in cell cycle arrest and genome maintenance. *Genes Dev.* 17:615–28
12. Cromie GA, Connelly JC, Leach DR. 2001. Recombination at double-strand breaks and DNA ends: conserved mechanisms from phage to humans. *Mol. Cell* 8:1163–74
13. Leber R, Wise TW, Mizuta R, Meek K. 1998. The XRCC4 gene product is a target for and interacts with the DNA-dependent protein kinase. *J. Biol. Chem.* 273:1794–801
14. Christmann M, Tomicic MT, Roos WP, Kaina B. 2003. Mechanisms of human DNA repair: an update. *Toxicology* 193:3–34
15. Goldstein M, Derheimer FA, Tait-Mulder J, Kastan MB. 2013. Nucleolin mediates nucleosome disruption critical for DNA double-strand break repair. *Proc. Natl. Acad. Sci. USA* 110:16874–79
16. Mimitou EP, Symington LS. 2008. Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature* 455:770–74
17. Cadet J, Delatour T, Douki T, et al. 1999. Hydroxyl radicals and DNA base damage. *Mutat. Res.* 424:9–21
18. Nikjoo H, Uehara S, Wilson WE, et al. 1998. Track structure in radiation biology: theory and applications. *Int. J. Radiat. Biol.* 73:355–64
19. Rothkamm K, Lobrich M. 2003. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. *Proc. Natl. Acad. Sci. USA* 100:5057–62

20. Willems P, Claes K, Baeyens A, et al. 2008. Polymorphisms in nonhomologous end-joining genes associated with breast cancer risk and chromosomal radiosensitivity. *Genes Chromosomes Cancer* 47:137–48
21. Lee MN, Tseng RC, Hsu HS, et al. 2007. Epigenetic inactivation of the chromosomal stability control genes BRCA1, BRCA2, and XRCC5 in non-small cell lung cancer. *Clin. Cancer Res.* 13:832–38
22. Esteller M, Silva JM, Dominguez G, et al. 2000. Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. *J. Natl. Cancer Inst.* 92:564–69
23. Ernestos B, Nikolaos P, Koulis G, et al. 2010. Increased chromosomal radiosensitivity in women carrying BRCA1/BRCA2 mutations assessed with the G2 assay. *Int. J. Radiat. Oncol. Biol. Phys.* 76:1199–205
24. Fourquet A, Stoppa-Lyonnet D, Kirova YM, et al. 2009. Familial breast cancer: clinical response to induction chemotherapy or radiotherapy related to BRCA1/2 mutations status. *Am. J. Clin. Oncol.* 32:127–31
25. Jazayeri A, Falck J, Lukas C, et al. 2006. ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. *Nat. Cell Biol.* 8:37–45
26. Rainey MD, Charlton ME, Stanton RV, Kastan MB. 2008. Transient inhibition of ATM kinase is sufficient to enhance cellular sensitivity to ionizing radiation. *Cancer Res.* 68:7466–74
27. Biddlestone-Thorpe L, Sajjad M, Rosenberg E, et al. 2013. ATM kinase inhibition preferentially sensitizes p53-mutant glioma to ionizing radiation. *Clin. Cancer Res.* 19:3189–200
28. Hickson I, Zhao Y, Richardson CJ, et al. 2004. Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. *Cancer Res.* 64:9152–59
29. Westphal CH, Hoyes KP, Canman CE, et al. 1998. Loss of ATM radiosensitizes multiple p53 null tissues. *Cancer Res.* 58:5637–39
30. Veuger SJ, Curtin NJ, Richardson CJ, et al. 2003. Radiosensitization and DNA repair inhibition by the combined use of novel inhibitors of DNA-dependent protein kinase and poly(ADP-ribose) polymerase-1. *Cancer Res.* 63:6008–15
31. Takata M, Sasaki MS, Sonoda E, et al. 1998. Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. *EMBO J.* 17:5497–508
32. Konstantinidou G, Bey EA, Rabellino A, et al. 2009. Dual phosphoinositide 3-kinase/mammalian target of rapamycin blockade is an effective radiosensitizing strategy for the treatment of non-small cell lung cancer harboring K-RAS mutations. *Cancer Res.* 69:7644–52
33. Mukherjee B, Tomimatsu N, Amancherla K, et al. 2012. The dual PI3K/mTOR inhibitor NVP-BEZ235 is a potent inhibitor of ATM- and DNA-PKCs-mediated DNA damage responses. *Neoplasia* 14:34–43
34. Toledo LI, Murga M, Zur R, et al. 2011. A cell-based screen identifies ATR inhibitors with synthetic lethal properties for cancer-associated mutations. *Nat. Struct. Mol. Biol.* 18:721–27
35. Tao Y, Leteur C, Yang C, et al. 2009. Radiosensitization by Chir-124, a selective CHK1 inhibitor: effects of p53 and cell cycle checkpoints. *Cell Cycle* 8:1196–205
36. Shao RG, Cao CX, Shimizu T, et al. 1997. Abrogation of an S-phase checkpoint and potentiation of camptothecin cytotoxicity by 7-hydroxystaurosporine (UCN-01) in human cancer cell lines, possibly influenced by p53 function. *Cancer Res.* 57:4029–35
37. Takai H, Naka K, Okada Y, et al. 2002. Chk2-deficient mice exhibit radioresistance and defective p53-mediated transcription. *EMBO J.* 21:5195–205
38. Engelke CG, Parsels LA, Qian Y, et al. 2013. Sensitization of pancreatic cancer to chemoradiation by the Chk1 inhibitor MK8776. *Clin. Cancer Res.* 19:4412–21
39. Herring CJ, West CM, Wilks DP, et al. 1998. Levels of the DNA repair enzyme human apurinic/apyrimidinic endonuclease (APE1, APEX, Ref-1) are associated with the intrinsic radiosensitivity of cervical cancers. *Br. J. Cancer* 78:1128–33
40. Robertson KA, Bullock HA, Xu Y, et al. 2001. Altered expression of Ape1/ref-1 in germ cell tumors and overexpression in NT2 cells confers resistance to bleomycin and radiation. *Cancer Res.* 61:2220–25
41. Taverna P, Hwang HS, Schupp JE, et al. 2003. Inhibition of base excision repair potentiates iododeoxyuridine-induced cytotoxicity and radiosensitization. *Cancer Res.* 63:838–46
42. Liu SK, Coackley C, Krause M, et al. 2008. A novel poly(ADP-ribose) polymerase inhibitor, ABT-888, radiosensitizes malignant human cell lines under hypoxia. *Radiother. Oncol.* 88:258–68

43. Dungey FA, Loser DA, Chalmers AJ. 2008. Replication-dependent radiosensitization of human glioma cells by inhibition of poly(ADP-Ribose) polymerase: mechanisms and therapeutic potential. *Int. J. Radiat. Oncol. Biol. Phys.* 72:1188–97
44. Powell C, Mikropoulos C, Kaye SB, et al. 2010. Pre-clinical and clinical evaluation of PARP inhibitors as tumour-specific radiosensitisers. *Cancer Treat. Rev.* 36:566–75
45. Hickman MJ, Samson LD. 1999. Role of DNA mismatch repair and p53 in signaling induction of apoptosis by alkylating agents. *Proc. Natl. Acad. Sci. USA* 96:10764–69
46. Roos W, Baumgartner M, Kaina B. 2004. Apoptosis triggered by DNA damage O6-methylguanine in human lymphocytes requires DNA replication and is mediated by p53 and Fas/CD95/Apo-1. *Oncogene* 23:359–67
47. Karran P, Stephenson C. 1990. Mismatch binding proteins and tolerance to alkylating agents in human cells. *Mutat. Res.* 236:269–75
48. Pepponi R, Marra G, Fuggetta MP, et al. 2003. The effect of O6-alkylguanine-DNA alkyltransferase and mismatch repair activities on the sensitivity of human melanoma cells to temozolomide, 1,3-bis(2-chloroethyl)1-nitrosourea, and cisplatin. *J. Pharmacol. Exp. Ther.* 304:661–68
49. Shinsato Y, Furukawa T, Yunoue S, et al. 2013. Reduction of MLH1 and PMS2 confers temozolomide resistance and is associated with recurrence of glioblastoma. *Oncotarget* 4:2261–70
50. von Bueren AO, Bacolod MD, Hagel C, et al. 2012. Mismatch repair deficiency: a temozolomide resistance factor in medulloblastoma cell lines that is uncommon in primary medulloblastoma tumours. *Br. J. Cancer* 107:1399–408
51. Pegg AE, Dolan ME, Moschel RC. 1995. Structure, function, and inhibition of O6-alkylguanine-DNA alkyltransferase. *Prog. Nucleic Acid. Res. Mol. Biol.* 51:167–223
52. Pegg AE. 1990. Mammalian O6-alkylguanine-DNA alkyltransferase: regulation and importance in response to alkylating carcinogenic and therapeutic agents. *Cancer Res.* 50:6119–29
53. Zaidi NH, Liu L, Gerson SL. 1996. Quantitative immunohistochemical estimates of O6-alkylguanine-DNA alkyltransferase expression in normal and malignant human colon. *Clin. Cancer Res.* 2:577–84
54. Esteller M, Hamilton SR, Burger PC, et al. 1999. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. *Cancer Res.* 59:793–97
55. Hegi ME, Diserens AC, Gorlia T, et al. 2005. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N. Engl. J. Med.* 352:997–1003
56. Kaina B, Margison GP, Christmann M. 2010. Targeting O^6-methylguanine-DNA methyltransferase with specific inhibitors as a strategy in cancer therapy. *Cell. Mol. Life Sci.* 67:3663–81
57. Friedman HS, Kokkinakis DM, Pluda J, et al. 1998. Phase I trial of O6-benzylguanine for patients undergoing surgery for malignant glioma. *J. Clin. Oncol.* 16:3570–75
58. Rabik CA, Njoku MC, Dolan ME. 2006. Inactivation of O6-alkylguanine DNA alkyltransferase as a means to enhance chemotherapy. *Cancer Treat. Rev.* 32:261–76
59. Cerchia L, de Franciscis V. 2010. Targeting cancer cells with nucleic acid aptamers. *Trends Biotechnol.* 28:517–25
60. Trivedi RN, Wang XH, Jelezcova E, et al. 2008. Human methyl purine DNA glycosylase and DNA polymerase beta expression collectively predict sensitivity to temozolomide. *Mol. Pharmacol.* 74:505–16
61. Cerda SR, Turk PW, Thor AD, Weitzman SA. 1998. Altered expression of the DNA repair protein, N-methylpurine-DNA glycosylase (MPG) in breast cancer. *FEBS Lett.* 431:12–18
62. Kim NK, Ahn JY, Song J, et al. 2003. Expression of the DNA repair enzyme, N-methylpurine-DNA glycosylase (MPG) in astrocytic tumors. *Anticancer Res.* 23:1417–23
63. Abbotts R, Madhusudan S. 2010. Human AP endonuclease 1 (APE1): from mechanistic insights to druggable target in cancer. *Cancer Treat. Rev.* 36:425–35
64. Al-Attar A, Gossage L, Fareed KR, et al. 2010. Human apurinic/apyrimidinic endonuclease (APE1) is a prognostic factor in ovarian, gastro-oesophageal and pancreatico-biliary cancers. *Br. J. Cancer* 102:704–9
65. Zaremba T, Ketzer P, Cole M, et al. 2009. Poly(ADP-ribose) polymerase-1 polymorphisms, expression and activity in selected human tumour cell lines. *Br. J. Cancer* 101:256–62

66. Bauer M, Goldstein M, Heylmann D, Kaina B. 2012. Human monocytes undergo excessive apoptosis following temozolomide activating the ATM/ATR pathway while dendritic cells and macrophages are resistant. *PLOS ONE* 7:e39956
67. Tentori L, Ricci-Vitiani L, Muzi A, et al. 2014. Pharmacological inhibition of poly(ADP-ribose) polymerase-1 modulates resistance of human glioblastoma stem cells to temozolomide. *BMC Cancer* 14:151
68. Taverna P, Liu L, Hwang HS, et al. 2001. Methoxyamine potentiates DNA single strand breaks and double strand breaks induced by temozolomide in colon cancer cells. *Mutat. Res.* 485:269–81
69. Gordon MS, Rosen LS, Mendelson D, et al. 2013. A phase 1 study of TRC102, an inhibitor of base excision repair, and pemetrexed in patients with advanced solid tumors. *Invest. New Drugs* 31:714–23
70. Del Rowe JD, Bello J, Mitnick R, et al. 1999. Accelerated regression of brain metastases in patients receiving whole brain radiation and the topoisomerase II inhibitor, lucanthone. *Int. J. Radiat. Oncol. Biol. Phys.* 43:89–93
71. Plummer R, Jones C, Middleton M, et al. 2008. Phase I study of the poly(ADP-ribose) polymerase inhibitor, AG014699, in combination with temozolomide in patients with advanced solid tumors. *Clin. Cancer Res.* 14:7917–23
72. Plo I, Liao ZY, Barcelo JM, et al. 2003. Association of XRCC1 and tyrosyl DNA phosphodiesterase (Tdp1) for the repair of topoisomerase I-mediated DNA lesions. *DNA Repair* 2:1087–100
73. Smith LM, Willmore E, Austin CA, Curtin NJ. 2005. The novel poly(ADP-ribose) polymerase inhibitor, AG14361, sensitizes cells to topoisomerase I poisons by increasing the persistence of DNA strand breaks. *Clin. Cancer Res.* 11:8449–57
74. Kummar S, Chen A, Ji J, et al. 2011. Phase I study of PARP inhibitor ABT-888 in combination with topotecan in adults with refractory solid tumors and lymphomas. *Cancer Res.* 71:5626–34
75. Holm C, Covey JM, Kerrigan D, Pommier Y. 1989. Differential requirement of DNA replication for the cytotoxicity of DNA topoisomerase I and II inhibitors in Chinese hamster DC3F cells. *Cancer Res.* 49:6365–68
76. Strumberg D, Pilon AA, Smith M, et al. 2000. Conversion of topoisomerase I cleavage complexes on the leading strand of ribosomal DNA into 5′-phosphorylated DNA double-strand breaks by replication runoff. *Mol. Cell. Biol.* 20:3977–87
77. Fedier A, Steiner RA, Schwarz VA, et al. 2003. The effect of loss of Brca1 on the sensitivity to anticancer agents in p53-deficient cells. *Int. J. Oncol.* 22:1169–73
78. Davidson D, Coulombe Y, Martinez-Marignac VL, et al. 2012. Irinotecan and DNA-PKcs inhibitors synergize in killing of colon cancer cells. *Invest. New Drugs* 30:1248–56
79. Ma CX, Ellis MJ, Petroni GR, et al. 2013. A phase II study of UCN-01 in combination with irinotecan in patients with metastatic triple negative breast cancer. *Breast Cancer Res. Treat.* 137:483–92
80. Willmore E, de Caux S, Sunter NJ, et al. 2004. A novel DNA-dependent protein kinase inhibitor, NU7026, potentiates the cytotoxicity of topoisomerase II poisons used in the treatment of leukemia. *Blood* 103:4659–65
81. Tamaichi H, Sato M, Porter AC, et al. 2013. Ataxia telangiectasia mutated-dependent regulation of topoisomerase II alpha expression and sensitivity to topoisomerase II inhibitor. *Cancer Sci.* 104:178–84
82. Goldstein M, Roos WP, Kaina B. 2008. Apoptotic death induced by the cyclophosphamide analogue mafosfamide in human lymphoblastoid cells: contribution of DNA replication, transcription inhibition and Chk/p53 signaling. *Toxicol. Appl. Pharmacol.* 229:20–32
83. Tong WP, Kirk MC, Ludlum DB. 1982. Formation of the cross-link 1-[N^3-deoxycytidyl],2-[N^1-deoxyguanosinyl]-ethane in DNA treated with *N,N′*-bis(2-chloroethyl)-*N*-nitrosourea. *Cancer Res.* 42:3102–5
84. Fichtinger-Schepman AM, van der Veer JL, den Hartog JH, et al. 1985. Adducts of the antitumor drug cis-diamminedichloroplatinum(II) with DNA: formation, identification, and quantitation. *Biochemistry* 24:707–13
85. Damia G, Imperatori L, Stefanini M, D'Incalci M. 1996. Sensitivity of CHO mutant cell lines with specific defects in nucleotide excision repair to different anti-cancer agents. *Int. J. Cancer* 66:779–83
86. Usanova S, Piee-Staffa A, Sied U, et al. 2010. Cisplatin sensitivity of testis tumour cells is due to deficiency in interstrand-crosslink repair and low ERCC1-XPF expression. *Mol. Cancer* 9:248

87. Horwich A, Shipley J, Huddart R. 2006. Testicular germ-cell cancer. *Lancet* 367:754–65
88. Kuschal C, Thoms KM, Boeckmann L, et al. 2011. Cyclosporin A inhibits nucleotide excision repair via downregulation of the xeroderma pigmentosum group A and G proteins, which is mediated by calcineurin inhibition. *Exp. Dermatol.* 20:795–99
89. Prewett M, Deevi DS, Bassi R, et al. 2007. Tumors established with cell lines selected for oxaliplatin resistance respond to oxaliplatin if combined with cetuximab. *Clin. Cancer Res.* 13:7432–40
90. Alekseev S, Ayadi M, Brino L, et al. 2014. A small molecule screen identifies an inhibitor of DNA repair inducing the degradation of TFIIH and the chemosensitization of tumor cells to platinum. *Chem. Biol.* 21(3):398–407
91. Vollebergh MA, Lips EH, Nederlof PM, et al. 2011. An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients. *Ann. Oncol.* 22:1561–70
92. Swisher EM, Sakai W, Karlan BY, et al. 2008. Secondary BRCA1 mutations in BRCA1-mutated ovarian carcinomas with platinum resistance. *Cancer Res.* 68:2581–86
93. Kim H, D'Andrea AD. 2012. Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. *Genes Dev.* 26:1393–408
94. Taniguchi T, Tischkowitz M, Ameziane N, et al. 2003. Disruption of the Fanconi anemia-BRCA pathway in cisplatin-sensitive ovarian tumors. *Nat. Med.* 9:568–74
95. Marsit CJ, Liu M, Nelson HH, et al. 2004. Inactivation of the Fanconi anemia/BRCA pathway in lung and oral cancers: implications for treatment and survival. *Oncogene* 23:1000–4
96. Farmer H, McCabe N, Lord CJ, et al. 2005. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 434:917–21
97. Bryant HE, Schultz N, Thomas HD, et al. 2005. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 434:913–17
98. Edwards SL, Brough R, Lord CJ, et al. 2008. Resistance to therapy caused by intragenic deletion in BRCA2. *Nature* 451:1111–15
99. Bouwman P, Aly A, Escandell JM, et al. 2010. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. *Nat. Struct. Mol. Biol.* 17:688–95
100. Patel AG, Sarkaria JN, Kaufmann SH. 2011. Nonhomologous end joining drives poly(ADP-ribose) polymerase (PARP) inhibitor lethality in homologous recombination-deficient cells. *Proc. Natl. Acad. Sci. USA* 108:3406–11

Annual Review of Medicine

Volume 66, 2015

Contents

Indexes

Errata

An online log of corrections to *Annual Review of Medicine* articles may be found at http://www.annualreviews.org/errata/med