An Emerging Regulatory Role for the Tumor Microenvironment in the DNA Damage Response to Double-Strand Breaks

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ABSTRACT

Radiation, alkylating agents, and platinum-based chemotherapy treatments eliminate cancer cells through the induction of excessive DNA damage. The resultant DNA damage challenges the cancer cell's DNA repair capacity. Among the different types of DNA damage induced in cells, double-strand breaks (DSB) are the most lethal if left unrepaired. Unrepaired DSBs in tumor cells exacerbate existing gene deletions, chromosome losses and rearrangements, and aberrant features that characteristically enable tumor progression, metastasis, and drug resistance. Tumor microenvironmental factors like hypoxia, inflammation, cellular metabolism, and the immune system profoundly influence DSB repair mechanisms. Here, we put into context the role of the microenvironment in governing DSB repair mechanisms.

Introduction

DNA repair during carcinogenesis

Genomic stability is essential to guarantee the inheritance of correct genetic information. Nevertheless, endogenous or exogenous environmental factors can act as DNA-damaging agents leading to genetic alterations (1). Endogenous factors include byproducts of cell metabolism, while exogenous agents encompass ionizing radiation, UV light, and chemotherapeutic drugs (1). Integrity of DNA is vitally important as mutations can lead to carcinogenesis (2). Because the resolution of DNA lesions is critical to cell survival, repair processes are in place to protect DNA integrity (2). These repair processes involve the activation of the cell-cycle checkpoints (CHK1, CHK2, p53, and p21) to stall the cell cycle, removal of mutagenic lesions in DNA, and cell death through apoptosis or senescence if repair fails (1). The goal is to avoid erroneous information being passed to the progeny and to potentially interrupt neoplastic transformations.

Genomic instability is a characteristic of most cancers. This is supported by the observation that tumor cells often have unchecked proliferation, chromosomal translocations, and aneuploidy as a consequence of mutagenic lesions in DNA (2). Furthermore, mutations in DNA repair genes form the basis of many hereditary cancers underscoring their importance in oncogenesis (3–5). Xeroderma pigmentosum is a hereditary disease caused by a defect in nucleotide excision repair (NER) and predisposes individuals to skin cancer (3). Likewise, germline mutations in the DNA repair gene ataxia telangiectasia mutated (*ATM*), can lead to increased sensitivity toward ionizing radiation, humoral and cellular immunodeficiency, and predisposition to cancer (3). Individuals with AT have approximately 60–180 times increased risk of developing cancer that primarily includes lymphomas and leukemia (3). The predisposition to cancer is most likely associated to impaired DNA damage repair given ATM's role in DNA damage repair and cell-cycle progression (3, 4). Germline mutations in *BRCA* genes, which are involved in homologous recombination (HR), predispose the individual to breast and ovarian cancer. Approximately 5%–7% of all hereditary breast cancers have *BRCA* gene mutations (5).

Complementing surgery, current treatment modalities in cancer include strategies such as ionizing radiation, alkylating agents, and platinum-based chemotherapy all of which induce excessive DNA damage (1). The ensuing DNA damage warrants effective DNA repair capacity, which may be limited in tumor cells. Compared with normal cells, cancer cells have a higher accumulation of DNA damage and build-up of replicative stress due to faulty cell-cycle checkpoint activation (1, 2). In a normal cell, such mutational burden would mean cell death. However, cancer cells utilize mutagenic repair pathways to their advantage and escape death (2). Because cancer cells already have a higher mutational load, targeting their repair capacity provides a therapeutic window where the cytotoxicity of anticancer agents can be boosted. On the basis of the nature of the DNA lesion, single- (SSB) or double-strand breaks (DSB), the cell invokes specific mechanisms to repair the damage. Summarized below are a variety of repair mechanisms that are employed by cells depending on the type of DNA lesion.

Major SSBs repair pathways

SSBs are highly common in cells and are efficiently repaired through specific SSB repair processes (6). NER removes lesions that could potentially lead to helix distortion, particularly those induced by UV. In eukaryotes, it involves the removal of a 24–32 nucleotide stretch of DNA directed by endonuclease activity and restoration by DNA polymerase activity (6). Base excision repair (BER) recognizes DNA bases damaged by oxidation, deamination, and alkylation. DNA glycosylase initiates BER by recognizing and removing damaged bases, which are processed by the APE-1 endonuclease and later restored through DNA polymerase and a ligase (7). Mismatch repair (MMR) recognizes incorrectly paired bases, and recruits repair proteins to damaged sites. Exonuclease 1 (EXO1) removes the mismatch, then polymerase D fills the gap and seals the nick through DNA ligase1 (LIG1; ref. 8). The direct repair mechanism reverses oxidative lesions created by methylating agents. This is a single-step process mediated by the enzymatic activity of methylguanine methyltransferase (MGMT) that removes the alkyl group from the oxidative lesion in

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DNA. Following repair, MGMT undergoes rapid self-degradation (6). Unrepaired SSB in proliferating cells can lead to the collapse of DNA replication forks and might even result in the formation of DSBs (1).

Cellular kinase processes in DSBs repair

Immediate response to genotoxic stresses leading to DSBs is carried out by the damage-sensing MRN (MRE11-RAD50-NBS1) complex. The MRN complex plays a key role in activating ATM, a DNA-damage signaling kinase (9). ATM responds to DNA damage throughout the cell cycle and is mainly responsible for phosphorylation of the histone H2A variant H2AX at serine 139 to generate phosphorylated H2AX (γH2AX). γH2AX formation at sites of DSBs makes it a surrogate marker for DSBs (10, 11). Other phosphatidylinositol-3-OH-kinase–like family members including DNA-PKcs (the catalytic subunit of DNA-dependent protein kinase) and ataxia telangiectasia and Rad3-related (ATR) also have redundant functions in phosphorylating H2AX. ATM also phosphorylates checkpoint kinase 2 (CHK2; ref. 12). ATM-CHK2 signaling is important to ensure activation of DSB repair through the accumulation of DNA repair proteins and chromatin-remodeling complexes (9, 11).

Major DSB DNA break repair pathways

DSBs are created by genotoxic agents commonly used in the treatment of cancer. For example, radiotherapy, radiomimetics, bifunctional alkylators, topoisomerase inhibitors, and replication inhibitors can all lead to DSBs (**Fig. 1**). DSBs caused by these agents can be resolved through the following DSB repair pathways.

HR

HR depends on a homologous sister chromatid DNA strand for repair and hence requires cell-cycle progression into the S–G_2-phase (**Fig. 2**). An early step of DSB repair involves recruitment of the MRN nuclease. DNA end resection is coordinated by MRN proteins resulting in single strand 3′ overhangs (reviewed in ref. 13). The MRN complex aids in the recruitment of p53 binding protein 1 (53BP1) and BRCA1. BRCA1 and C-terminal binding protein-interacting protein (CtIP) promote DNA end resection leading to HR (14). During the G_1-phase of cell cycle, replication timing regulatory factor 1 (RIF1) is recruited to DSBs by 53BP1 (15, 16). Together, 53BP1 and RIF1 antagonize HR by inhibiting accumulation of BRCA1 at damage sites favoring non-homologous end joining (NHEJ). However, during the G_2-phase of cell cycle, RIF1 accumulation is antagonized by BRCA1, leading to a switch to HR (15, 16). Replication protein A (RPA) protects the single strands and facilitates the recruitment of HR proteins like BRCA1, BRCA2, partner and localizer of BRCA2 (PALB2), RAD51, X-ray repair cross-complementing protein 2 (XRCC2), and XRCC3. During the recombination process, the 3′ overhangs invade the sister chromatid to form a hetero-duplex (13). End processing is completed by excision repair cross-complementing group 1, and DNA end gaps are filled by DNA polymerase. This is a high-fidelity repair mechanism and does not generate mutations during the repair process (13, 17).

NHEJ

NHEJ is the other major pathways that repairs DSBs in mammalian cells. NHEJ involves rapid end-ligation of broken DNA ends regardless of the stage of the cell cycle (**Fig. 2**). NHEJ in mammalian cells requires the Ku70/80 heterodimer, DNA-PKcs, XRCC4 (X-ray repair cross-complementing protein 4), DNA ligase IV (LIG4), Artemis, and XRCC4-like factor (XLF; reviewed in ref. 18). The broken DNA ends are tethered by the Ku70/80 heterodimer. DNA-PKcs is recruited to

the repair site forming the DNA–PK complex. DNA-PKcs can be auto-phosphorylated or phosphorylated by ATM (19). The DNA–PK complex phosphorylates H2AX, XRCC4, LIG4, and XLF. The end ligation process through NHEJ needs DNA end-processing and activity of DNA polymerases mu and lambda, which can be error-prone (18). Ligation also needs the DNA ends to be altered, and such alteration can include removal of damaged nucleotides, and addition or removal of undamaged nucleotides. While NHEJ is a predominant DNA DSB repair pathway in mammalian cells following radiation, this repair process can lead to chromosomal rearrangements (18, 20).

Alternative NHEJ pathways

Alternative NHEJ pathways (Alt-NHEJ) were first discovered in Ku70-deficient yeast cells. Compared with classical NHEJ, alternative NHEJ is 20-fold less efficient (21, 22). The microhomology-mediated end joining (MMEJ) repair pathway exists as a backup when NHEJ repair proteins like DNA-PKcs and Ku70/80 are compromised and HR is limited. This alternative NHEJ pathway is reliant on micro-homologous regions of 5–25 base pairs. Unlike the canonical NHEJ pathway, MMEJ always results in losses of sequence (22). Therefore, this pathway leads to excessive genomic deletions and chromosomal translocations.

DSB repair proteins serve important functions in cancer cell survival. Even a single DSB has the ability to cause cell death if left unrepaired. In several tumor types like breast cancer, lung cancer, leukemia, and lymphoma, a high proportion of DSB repair genes are overexpressed when compared with other DNA repair pathways (23). As such, it is imperative to understand how DSB repair can be inhibited in tumor cells, so that current cancer therapies can be more effective. The tumor cells' response to DNA damage is governed by the integration of complex cellular and environmental cues. Therefore, in the ensuing sections of this review, we will put into context the impact of microenvironmental factors in directing DSB repair pathways.

Microenvironment and DSB DNA repair

Hypoxia and DSB repair

Hypoxia is characterized as a low oxygen condition prevalent in the tumor microenvironment. The oxygen availability in hypoxic regions is diminished due to rapid tumor proliferation and poor vasculature (24). Most advanced solid tumors, including those of the breast, head and neck, pancreatic, lung, brain, prostate, and cervix, have hypoxic regions. The presence of hypoxia clinically correlates with poor prognosis in patients (25). The HIF-1α transcription factor is upregulated in response to hypoxia; this activates expression of several target genes that collectively orchestrate increased angiogenesis, metabolic reprogramming, and survival (26). In normoxic conditions, oxygen-activated proline hydroxylase (PHD) regulates HIF-1α through posttranslational modifications. PHD hydroxylates HIF-1α at its two proline sites and marks it for degradation. Hydroxylated HIF-1α is then ubiquitinated by an E3 ligase, called von Hippel Lindau protein, leading to its degradation by the 26S proteasome (24). In contrast, lack of oxygen in hypoxic conditions inactivates hydroxylation reactions leading to rapid accumulation of HIF-1α and HIF-2α, which can heterodimerize with HIF-1β and bind to the hypoxia response element sequence in target genes (27).

The hypoxic tumor microenvironment confers tumor cells with resistance to chemo- and radiotherapy (28). In hypoxic conditions, tumor cells are less prone to ionizing radiation–induced damage than in normal conditions. Hall and colleagues compared doses needed for sensitizing mammalian cells under hypoxic versus normoxic conditions and found that it takes a dose of 1 Gy to sensitize 99% cells under
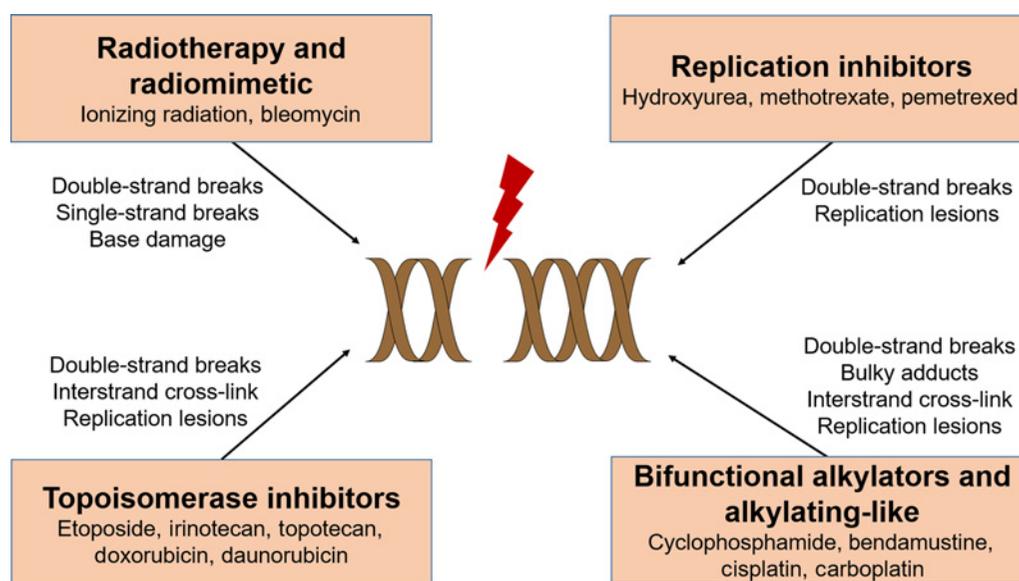
Figure 1.
DSBs-inducing agents used for the treatment of cancer.

normoxic conditions; however, only 50% of hypoxic cells are sensitized at the same dose (29). Ionizing radiation creates DNA damage through a process that involves free radical formation. These free radicals create a variety of DNA lesions. In hypoxia, low oxygen levels compromise the generation of reactive oxygen species (ROS), and tumor cells are therefore more resistant to radiotherapy (30). Similarly, chemotherapeutic drugs are less effective in hypoxic conditions due to their low efficacy in the absence of oxygen (28).

Hypoxia drives genetic instability through the impairment of DSB repair via transcriptional, translational, and epigenetic regulation of several important repair proteins like DNA-PKcs, Ku70/80, BRCA1, and RAD51 (31). Hypoxia regulates DSB repair and has distinct effects depending on the chronic (longer than 24 hours) versus acute (shorter than 24 hours) state of hypoxia. In response to acute hypoxia, ATM-CHK2 signaling is activated and leads to increased cell survival (32). In

Figure 2.
Cellular DSBs are repaired mainly through HR and NHEJ; both mechanisms involve distinct repair proteins. Tumor microenvironment factors like hypoxia, inflammation, immune cells, genotoxic stress, and cellular metabolites influence the DSB repair in the cell.

addition, DSB repair proteins are involved in HIF-1α stability. Under hypoxia, activated ATM phosphorylates HIF-1α at serine 696, stabilizing HIF-1α (33). BRCA1 and DNA-PKcs both interact with HIF-1α and regulate its stability as well (34, 35). Studies in prostate cancer using NHEJ and HR reporter plasmids to assess DSB repair show that chronic hypoxia engages NHEJ and restricts HR (36). Hypoxia induces p130 dephosphorylation and nuclear localization leading to activation of HIF-independent stress signaling that facilitates formation of the repressive E2F4/p130 complex. The E2F4/p130 complex binds to the E2F site in the proximal promoter of *RAD51* and *BRCA1*, leading to decreased expression of their transcripts (37, 38). Hypoxia-mediated downregulation of Rad51 expression is also observed *in vivo* (36). There are, however, conflicting reports on the functional involvement of NHEJ in hypoxia. Meng and colleagues reported that hypoxia elicits downregulation of both, HR- and NHEJ-related RNA expression in prostate cancer (39). Another set of reports complement these observations. Tsuchimoto and colleagues report that DNA-PKcs activity and RNA expression are inhibited under hypoxia (40). Also, Lara and colleagues reported that expression of Ku70/80 is downregulated in the hypoxic regions of cervical tumors from patients (41). This is confounded by evidence from Um and colleagues that DNA-PKcs and Ku70/80 protein expression are upregulated under hypoxia and lead to increased stabilization of HIF-1α (35). Similarly, Bouquet and colleagues found that DNA-PKcs is activated under hypoxia and stabilizes HIF-1α but does not activate and recruit the XRCC4–DNA-ligase-IV complex (42). While it is likely that cellular responses of DNA repair to hypoxia are context-dependent, it is evident that hypoxia contributes significantly toward cellular choices of DSBs repair through modulating HR and NHEJ repair proteins, thereby making cells less sensitive to DNA damage.
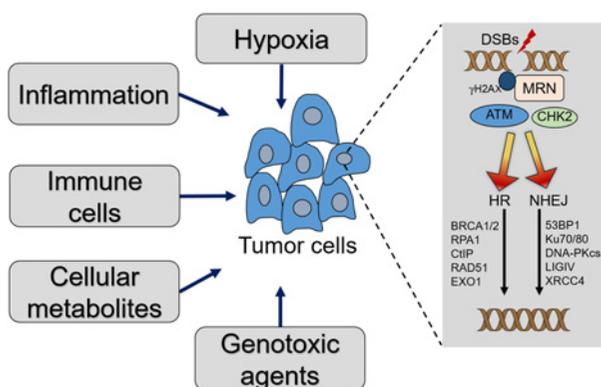
Cross-talk between DSB DNA damage response and immune cells

Studies evaluating links between DNA damage response and innate immunity discovered possible cross-talk mainly via the stimulator of interferon genes (STING) pathway. Cyclic GMP-AMP synthase

(cGAS) detects cytosolic double-stranded DNA (dsDNA) leading to the synthesis of cyclic GMP-AMP, a secondary messenger that binds to the adaptor protein STING (43). The STING protein is activated and shuttled from the endoplasmic reticulum to the perinuclear endosome where it phosphorylates TANK-binding kinase 1 (TBK1). TBK1 then activates interferon regulatory factor 3 (IRF-3) and NF-κB required for expression of type I IFN, subsequently enhancing a proinflammatory innate immune response (43, 44).

Eliciting antitumor response by innate immune cells requires cross-presentation of tumor antigens. Woo and colleagues demonstrate that the STING pathway has an important role in activating dendritic cells (DC), IFNβ expression, and priming of CD8$^+$ T cells *in vivo* (45). STING-deficient mice fail to reject immunogenic tumors compared with wild-type mice due to a defect in induction of critical factors including IL12, CXCL9, CD86, and CD40 in CD8α$^+$ DCs (45). STING activation in DCs enables antigen presentation by MHC I necessary for T-cell activation (**Fig. 3**; refs. 43, 46).

It is unclear how DCs uptake tumor-derived DNA, but increased genomic instability through DSB damage may increase fragmented DNA for uptake by DCs. Chromosomal instability aids in the production of cytosolic dsDNA and facilitates metastasis in a cGAS-STING–dependent manner (47). DSB-inducing agents like etoposide and DSB repair deficiency (BRCA- and ATM-deficient tumor cell lines) reportedly increase activation of type I signaling responses in a STING-dependent manner (48–50). In addition, activation of the STING pathway in DCs is essential for irradiation-induced antitumor effects. In DCs, STING is important for IFN-induction following radiation (51). Recently, DSB-induced micronuclei have also been reported to stimulate cGAS-mediated STING activation during mitotic progression (52, 53). cGAS binds to γH2AX-positive micronuclei formed by rupture of the nuclear membrane following irradiation (53).
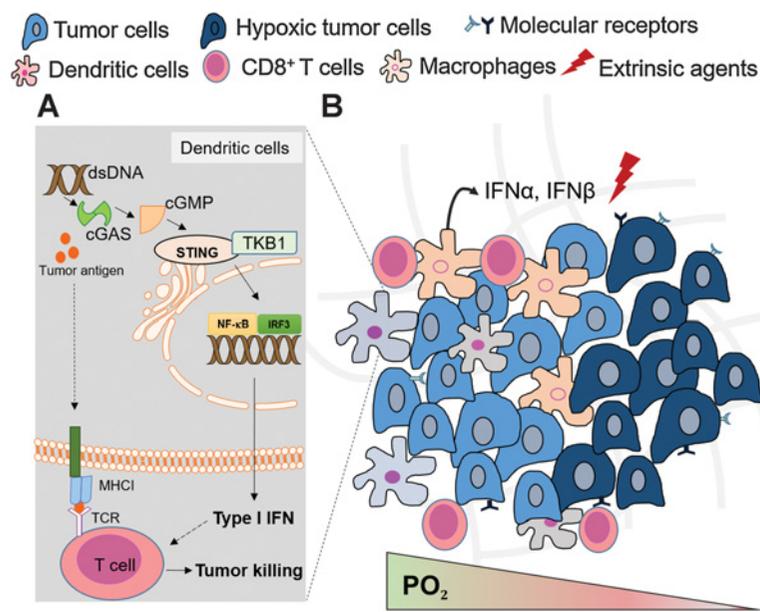
Although cytoplasmic cGAS has been well-characterized to have a role in DNA sensing in immune and tumor cells, a recent study shows that nuclear cGAS promotes tumor growth *in vivo* (54). Nuclear cGAS localizes at sites of DSBs after DNA damage, colocalizes with γH2AX, and interacts with PARP. cGAS interaction with PARP inhibits HR through downregulation of RAD51 and RPA2 (54). cGAS–STING pathway activation also has a supporting role in tumor growth and metastasis (47, 55, 56). Hence, it is important to cautiously optimize treatment conditions to target the tumor while activating the innate immune response.

Apart from cGAS, several other DSB repair proteins also are described as DNA sensors. Cytoplasmic DNA-PK and MRE11 act as sensors and activate type I IFNs in fibroblasts and in bone marrow–derived DCs, respectively (57, 58). Ku70 functions as a cytosolic DNA sensor but activates type III IFN through IRF-1 and IRF-7 (59). In DCs, RAD50 interacts with the innate immune adapter CARD9. The introduction of viral DNA or dsDNA leads to the formation of a RAD50, CARD9, and dsDNA complex that induces NF-κB signaling for IL1β production (60). These studies indicate that DNA damage repair proteins are not limited to preserving DNA integrity in the nuclear compartment, but also have important cytoplasmic functions that intersect with innate immune cell responses.

Inflammatory microenvironment and DSB repair

Inflammation plays a major role in protecting against invading pathogens and in the tissue repair process; however, prolonged inflammation can have an adverse effect if left unchecked. The persistence of chronic inflammation in the tumor microenvironment promotes tumor growth through the recruitment of macrophages and unregulated tissue repair (**Fig. 3**; ref. 61). Macrophages in the tumor microenvironment release proinflammatory cytokines and aid in genomic instability through the production of ROS and reactive nitrogen species (RNS; ref. 62). ROS generation in itself does not lead to DSBs, but ROS-induced DNA damage can create DSBs (63). ROS-induced DNA damage in transcriptionally active sites creates DNA-RNA hybrids called R-loops and needs transcription-coupled HR to resolve them (63, 64). In addition, RNS and superoxide have been found to induce HR at a chromosomally integrated direct repeat in mammalian cells (65). Interestingly, RNS-induced DSBs mediate innate immune function in macrophages (66). ROS and RNS activate ATM and DNA-PKcs by inducing DSB, which consequently promote inflammasome activation in macrophages and immune response by natural killer (NK) cells (66). Initiation of the DNA damage response in the activated macrophages requires type I IFN signaling culminating in the production of IL1β and IL18 (66). In addition to activated

Figure 3.

The solid tumor microenvironment comprising hypoxia, inflammation, and immune cells impact DSB repair pathways in cancer cells. The diagrams were drawn using motifolio drawing toolkits (www.motifolio.com). **A,** In DCs, the presence of dsDNA activates the cGAS–STING pathway and leads to activation of IRF-3 and NF-κB enhancing the proinflammatory immune response. **B,** DSB-mediated expression of cellular cytokines, chemokines, and cross-talk between stromal cells in the solid tumor microenvironment serves important function in immune cell homing and migration.

macrophages, physiologic DSBs such as VDJ recombination in developing lymphocytes serve an important function with respect to maturation, migration, and homing of immune cells (67).

The NF-κB transcription factor, the master regulator of inflammation, promotes HR through interaction with CtIP–BRCA1 complexes resulting in BRCA1 stabilization (68). DSBs can lead to ATM-mediated NF-κB activation (69). ATM-mediated signaling can potentially activate NF-κB–mediated cytokine expression (IL6, IL8, etc.) and modulate functions of immune cells (70). Several cytokines like TGF-β, IL6, and TNF also activate ATM kinase (reviewed in ref. 71). In fact, TGF-β offers protective effects on cellular survival against γ-radiation–induced DSBs through eliciting NHEJ repair (72).

In addition, increased γH2AX has been observed in various stages of lung cancer development during multistep progression in an inflammation-mediated rat lung cancer model (73). Similarly, mice that were exposed to cerulein, an inducer of pancreatic inflammation, demonstrated increased cell proliferation and elevated γH2AX levels. An increase in HR DSB repair was observed in inflammation-induced DNA damage (74). These findings suggest that inflammation promotes genomic instability synergistically with replication stress–induced DNA damage.

DSB and immune checkpoint regulation

Recent studies have found that DSBs in cancer cells induce immune receptor ligand expression that can stimulate innate immune cells. Natural killer group 2D (NKG2D) receptor recognizes ligands on cancerous and infected cells (75). Expression of the NK2G2D ligand makes tumor cells susceptible to surveillance by immune cells. NKG2D ligands can activate NK cells, CD8$^+$ T cells, and γδ T cells and are rarely expressed in normal cells. NKG2D is induced when cells undergo viral infection, replicative and genotoxic stress, or malignant transformation (76). Genotoxic agents stimulate NKG2D ligand expression on mice and human tumor cell lines (77). Another activating receptor involved in NK-cell–mediated tumor cell killing is DNAX accessory molecule-1 (DNAM-1), expressed on the majority of T cells, NK cells, and macrophages following DNA damage. Both NKG2D and DNAM-1 ligands are also expressed on multiple myeloma cells in an ATM/ATR-dependent manner under conditions of genotoxic stress (77, 78). Pharmacologic inhibition of ATM, ATR, or CHK1 reduced NKG2D ligand expression on myeloma cells (78). Taken together, these evidence suggests that DSBs in cancer cells enhance expression of NKG2D ligand that serves to stimulate the innate immune response.

Furthermore, genotoxic stress like ionizing radiation increases programmed death-ligand (PD-L1) expression on tumor cells. Elevated PD-L1 expression in tumor cells corresponds with better responses to anti-PD-1 and anti-CTLA-4 therapy through immune system activation (79). This is supported by IHC staining in lung squamous cell carcinoma that shows a positive correlation between γH2AX and PD-L1 expression (80), indicating a possible link between DSBs and PD-L1 expression. Combining ionizing radiation with anti-CTLA4 therapy improved survival in patients with metastatic melanoma (79). Blockade of the PD-1/PD-L1 interaction using anti-PD-1 rescued T-cell activity and delayed tumor growth in mice (79). Sato and colleagues have recently reported a novel link between DSBs and upregulation of PD-L1 expression in cancer cells. DSBs upregulate PD-L1 expression in an ATM/ATR/CHK1-dependent manner and engage activation of STAT1/STAT3, leading to IRF1-mediated upregulation of PD-L1 (81). Similarly, *BRCA1/2*-mutated high-grade serous ovarian cancer was associated with higher association with neoantigen loads, increased CD3$^+$ and CD8$^+$ tumor-infiltrating lymphocytes, and

PD-L1 expression (82). Also, patients with metastatic melanoma harboring *BRCA2* mutations showed higher genomic mutational loads than those with wild-type *BRCA2*. Patients with loss-of-function *BRCA2* mutations showed improved survival in response to anti-PD-1 therapy (83). Overall, the use of immune checkpoint inhibitors in combination with agents that impair DSB repair may present an effective approach to treat cancers that have failed anti-PD-1 monotherapy.

Cellular metabolites and DSB repair pathway

Although not directly microenvironment derived, cellular adaptation to the microenvironment leads to the production of distinct metabolites in tumor cells. Emerging studies show that such metabolites play a critical role in DNA repair (84). Hypoxia and mutant isocitrate dehydrogenase (*IDH*) and can lead to the production of the oncometabolite 2-hydroxyglutarate (2-HG; ref. 85). Sulkowski and colleagues reported that *IDH1/2*-mutant cells are deficient in HR due to 2-HG–mediated inhibition of α-ketoglutarate–dependent dioxygenases (85). ATP-citrate lyase (ACLY), which regulates the availability of acetyl-CoA, increases nuclear acetyl-CoA localization and subsequently histone acetylation to favor HR. Histone acetylation at DSB sites promote BRCA1 recruitment and inhibits 53BP1 recruitment, thus, promoting HR-mediated DNA repair (86, 87). Interestingly, irradiation-mediated DNA damage activates DNA-PK–dependent phosphorylation of chromatin-associated fumarase, further enhancing fumarate production and DNA–PK complex at sites of DSB, resulting in increased NHEJ repair (88). Similarly following irradiation, N-acetyl-glucosamine and O-GlcNAcylation promotes histone methylation by enhancer of zeste homolog 2 (EZH2; ref. 89). This posttranslational modification by EZH2 enhances H3K27 trimethylation, an important determinant in NHEJ repair (89, 90). Pyruvate kinase M2, a master regulator of metabolic reprogramming, also promotes HR through phosphorylation of CtIP in glioblastoma (91). Irradiation induces CtIP phosphorylation and nuclear localization in an ATM-dependent manner (91). Currently, the field of cellular metabolism and how it affects cellular DNA repair is expanding. Understanding the interplay between microenvironment-induced metabolites and DNA repair defects would provide an important context for improving treatment efficacy.

Cross-talk between tumor microenvironment and SSB

Apart from the DSB repair pathway, SSB repair pathways are also impacted by the tumor microenvironment. We will briefly summarize the topic, although several reviews highlight the impact of the tumor microenvironment on SSB. Hypoxia has a long-term effect on MMR through downregulating both mRNA and protein levels of MLH1 and MSH2 (reviewed in ref. 31). NER capacity is decreased in chronic hypoxia; however, the inhibitory effect of hypoxia on NER is not conclusive (31). During inflammation, oxidative stress impairs MMR by downregulating the expression of MutS homolog 6 (92). In addition, in conditions of chronic inflammation, ROS can downregulate BER by activating the inflammasome (64). Patients with colorectal cancer with MMR deficiency show greater infiltration of T cells and higher microsatellite instability indicating a robust cross-talk between MMR and immune cells (93). In fact, tumor with high microsatellite instability is a strong indicator of responsiveness to anti-PD1/PD-L1 immunotherapy (94).

Targeting DSB repair pathways in cancer

PARP is an ADP-ribosylating enzyme that is involved in BER and alternative NHEJ (95). Inhibition of PARP increases DSBs, which are

Table 1. Different DSB repair pathway proteins and their associated inhibitors.

DSB repair proteins	DSB repair pathway	Inhibitors	References
PARP	Alt-NHEJ	Olaparib, niraparib, rucaparib, veliparib (ABT-888), talazoparib (BMN-673)	PMID: 28003236
ATM	Signaling (kinase targets: CHK2, H2AX, NF-κB, AKT, p53,IGF-1R)	KU5593, KU60019, CP466722, AZ32, AZD8055, NU7026, VE-821	PMID: 31299316, 22079189
CHK2	Effectors	Prexasertib (LY2606368), Chk2 inhibitor II (BML-277)	PMID: 27308506
MRE11	Damage sensors, resection	Mirin	PMID: 29523233
DNA-PKcs	NHEJ (kinase targets: CHK2, H2AX, p53, Artemis, Ligase IV)	NU7441 (KU-57788), NU7026, LTURM34, CC-115	PMID: 28820390
Rad51	HR	RI-1, RS-1, B02	PMID: 24856061
Ligase IV	NHEJ	SCR7	PMID: 23260137

normally repaired by HR. In *BRCA*-mutant cancers, PARP inhibitors (**Table 1**) lead to increased apoptosis due to synthetic lethality (95). Phase III clinical trials of PARP inhibitor monotherapy with olaparib or talazoparib, showed an improved progression-free survival compared with standard chemotherapy in patients with germline *BRCA*-mutated and HER2-negative advanced breast cancer (96, 97). On the basis of these results, olaparib and talazoparib are now FDA-approved for the treatment of advanced germline *BRCA*-mutated breast cancer. Similarly, olaparib, rucaparib, and niraparib are currently FDA-approved for the treatment of advanced *BRCA*-mutated and platinum-sensitive ovarian cancer (95, 98, 99). The benefit of PARP inhibitors as monotherapy in *BRCA1/2*-mutant prostate cancer is currently being investigated in clinical trials (100). As yet, there is no reliable indicator to determine the benefit of PARP inhibition beyond *BRCA* mutations, but other DNA repair defects remain an active area of investigation (100, 101). However, combination therapy is likely to yield a better response (102).

Several clinical trials are currently assessing the use of DSB repair protein inhibitors in combination with immunotherapy, DNA damaging agents, topoisomerase inhibitors, and chemotherapy (**Table 2**). Clinical strategies to target ATM, ATR, and CHK in combination with PARP inhibitors are in clinical trials (NCT02588105, NCT03022409,

and NCT03057145). Also, the efficacy of DNA-PKcs inhibitors in combination with radiotherapy is being tested in the clinic (NCT02516813). PARP inhibitors in combination with PD-1 inhibitors (NCT03522246), and topoisomerase inhibitors (NCT00588991) are also in trials. ATR inhibitors are also being tested in combination with PD-1 inhibitors and chemotherapy (NCT02264678).

Dinaciclib targets cyclin-dependent kinases upstream of HR and blocks phosphorylation of BRCA1 and EXO1. This activity of dinaciclib is the likely reason that it sensitizes tumor cells to the PARP inhibitor, veliparib, in a preclinical model of multiple myeloma (103). The efficacy of using dinaciclib with veliparib is currently being tested in the clinic in advanced cancers (NCT01434316). Similarly, preclinical studies showed that hypoxia compromises HR by downregulating *BRCA1* and *RAD51* (36, 37). A phase I/II trial of cediranib, a pan-VEGF 1–3 inhibitor regulated by HIF-1α, in combination with olaparib in patients with platinum-sensitive ovarian cancer, has already shown improved progression-free survival compared with olaparib alone (99, 104). Cediranib is also currently in a clinical study in combination with olaparib in prostate cancer (NCT02893917). In addition to the FDA-approved drugs, small-molecule inhibitors of CHK1/2, WEE1, ATM, ATR, and DNA-PKcs have shown promising results in preclinical studies (reviewed in ref. 105). Overall, preclin-

Table 2. Current clinical trials targeting DNA DSB repair pathway proteins as mono- and combination therapy agents.

NCT number	Conditions	Interventions	Target	Phases	Enrollment
NCT03522246	Epithelial ovarian cancer	Rucaparib, nivolumab	PARP, anti-PD-1	III	1012
NCT03602859	Ovarian cancer	Niraparib, TSR-042	PARP, anti-PD-1	III	912
NCT03330847	Metastatic triple-negative breast cancer	Olaparib, AZD6738, AZD1775	PARP, ATR, WEE1	II	450
NCT02975934	Metastatic castration-resistant prostate cancer	Rucaparib vs. abiraterone acetate or enzalutamide or docetaxel	PARP	III	400
NCT02952534	Metastatic castration-resistant prostate cancer	Rucaparib	PARP	II	360
NCT02854436	Prostatic neoplasms	Niraparib	PARP	II	301
NCT03414047	Ovarian cancer	Prexasertib	CHK1, CHK2	II	173
NCT03307785	Advanced non–small cell lung cancer	Niraparib, TSR-042, TSR-022, bevacizumab	PARP, anti-PD-1, anti-TIM-3, VEGF	I	168
NCT02124148	Colorectal and breast cancer	Prexasertib and combination	CHK1, CHK2	I	167
NCT02203513	Ovarian, breast, and prostate cancer	LY2606368	CHK1, CHK2	II	153
NCT03637491	Pancreatic and non–small cell lung cancer	Avelumab, binimetinib and/or talazoparib	anti-PD-L1, MEK, PARP	II	127

Note: Currently active or recruiting 11 clinical trials are arranged according to the number of enrollment of participants ($n > 100$) in the United States, clinicaltrials.gov last accessed on Sep 26, 2019.

evidence suggests that immune checkpoint inhibition synergizes with PARP inhibitor treatment in HR-deficient tumors; clinical trials investigating this hypothesis are ongoing.

Conclusions

Genome integrity and maintenance through repair pathways are paramount for normal cellular function. Cancer cells with mutations in one or more of these DNA repair pathways are dependent on the remainder of the functional repair pathways. Current therapies seek to target these functional DNA repair pathways in cancer for synthetic lethality. *BRCA* mutations are abundant in different cancers; hence, DSB repair pathways may present as viable therapeutic targets. In the clinics, the success of PARP inhibitors for *BRCA1/2*-mutated ovarian cancer has opened doors for exploring different strategies that combine PARP inhibitors with conventional therapies for durable responses. The role of the tumor microenvironment is an important consideration in developing therapeutics that target DNA repair–deficient cancer cells. Chronic hypoxia, inflammation, and checkpoint activation all play critical roles in facilitating these repair choices.

Another developing area of research is exploring the effects of mechanical forces, stroma, and stromal cells in the tumor microenvironment. Tumor cells experience physical stress and are in a constant cross-talk with the stromal cells and stroma; thus, it is essential to identify the nature of DNA damage and repair evoked by these factors. As such, new insights into the relationship between tumor microenvironment and DNA damage repair present a critical area of investigation to treat cancer effectively.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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