Critical Review

The DNA damage response in immunotherapy and radiation

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Abstract

Purpose: Deficiencies in DNA damage repair (DDR) and response represent a common alteration in tumors, and exploitation of this feature using therapeutics has become more prominent.

Methods and materials: Recent work has highlighted the important interaction between DDR defects, as well as DDR targeting agents such as radiation and the immunogenicity of the tumor. This relationship emphasizes the potential for combination therapeutics with immune checkpoint inhibitors (ICI). Somatic mutations and DDR defects are some of the strongest predictors of response to ICI.

Results: This review highlights the interplay among DDR pathways, ionizing radiation, and ICI efficacy. The mechanisms of radiation immunogenicity, including the cytosolic DNA sensing cGAS/STING pathways, are also described.

Conclusions: A greater mechanistic understanding of the complex interaction between the DNA damage response and the immune system will expand the therapeutic potential of immunotherapy for patients with advanced cancer.

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Introduction

Genomic instability and mutagenesis represent one of the hallmarks of cancer, and are often critical for early tumorigenesis and subsequent tumor evolution. Sources of instability and mutagenesis include endogenous factors such as oxidative and replicative stress, loss of cell-cycle checkpoints, overexpression of damaging agents such as apolipoprotein B messenger RNA editing enzyme, catalytic (APOBEC) polypeptide—like enzymes, and exogenous factors such as cytotoxic chemotherapy agents and ionizing radiation.

Under normal conditions, a coordinated program of DNA damage sensing and appropriate repair machinery maintains genomic integrity and minimizes somatic alterations that are transferred to daughter cells during cell division. Hereditary syndromes such as Lynch syndrome (resulting from a germline defect in mismatch repair) and hereditary breast and ovarian cancer (resulting from a germline defect in homologous recombination) helped solidify the importance of DNA damage response to oncogenesis.
In the past decade, high-throughput sequencing studies have demonstrated that defects in specific DNA damage repair (DDR) pathways are increasingly prevalent and not solely limited to hereditary syndromes, further emphasizing the relevance of DDR in tumor development. Importantly, several cytotoxic chemotherapies exploit these defects to selectively target cancer cells. Depending on the nature of the repair pathway that is defective, tumors can accumulate significant somatic alterations in DNA, including point mutations, insertions and deletions, chromosomal translocations, and loss of segments or chromosomes. It is becoming increasingly clear that these somatic alterations not only facilitate oncogenesis by creating alterations in tumor-suppressor genes and onco-genes that are necessary to facilitate unregulated cellular growth, but these alterations can also alter the tumor microenvironment and inflammatory cascade.

Evasion of the immune system has also recently emerged as an important hallmark in cancer, even though therapeutics directed at activating the immune system against malignancy have failed for decades. However, the development of antibodies directed against immune checkpoint molecules, including CTLA4 and PD-1/PDL-1, have dramatically changed the treatment landscape for patients with advanced and metastatic cancer. Unfortunately, in most settings only a small minority of patients experience durable responses, emphasizing the importance of predictive biomarkers.

This review focuses on what is known about the interplay of DNA damage response and repair machinery and the immune response against the tumor. Special attention is paid to ionizing radiation and its potential to modulate DDR and the immune response in the context of immune checkpoint inhibitors (ICIs).

**Mutational landscape predicts immunotherapy response**

Somatic mutations within the tumor genome can be recognized by the immune system as foreign or non-self neoantigens, and strongly immunogenic mutations can be selected against through the process of immune-editing. The significance of neoantigen accumulation for tumor recognition by the immune system was emphasized with the rapid expansion of ICI use. In several studies the number of somatic point mutations and the resulting number of predicted neoantigens were strong predictors of response to immunotherapy and subsequent long-term survival. Biopsies from patients on treatment with anti-PD-1 therapy also demonstrated immune-editing in real time, presumably because of the pruning of antigenic subclones in the context of a hyperactive immune system.

Most studies have focused on single nucleotide variants as potential neoantigens because they are the easiest mutations to identify with current sequencing technologies and analysis pipelines, but emerging evidence suggests that other types of somatic genetic aberrations may play a similar or even greater role depending on the tumor context and histology. For example, small insertions or deletions, commonly observed with some DNA repair deficiencies, result in frameshift mutations, which are often associated with long stretches of non-self protein sequence. However, methods to reliably detect these mutations in sequencing data are still being optimized. Hence, frameshift mutations may be more antigenic than simple single nucleotide variants, although they may also be susceptible to processes such as nonsense mediated decay. Neoantigens derived from gene fusion transcripts, particularly in the context of low-mutational-burden pediatric tumors, have also been predicted, although their clinical relevance in the context of ICI remains to be seen.

Conversely, aneuploidy and resulting copy number alterations have been associated with decreased T-cell infiltration and poorer response to ICI. However, aneuploidy is a known predictor of poor prognosis irrespective of treatment modality, and immune infiltration or tumor purity may influence the technical ability to detect aneuploidy, potentially confounding these analyses. Although the mechanism of decreased immunity and resistance to ICI resulting from aneuploidy is not well understood, possibilities include a decrease in antigen presentation, cytokine secretion, and an increased potential for immune evasion. Further work to elucidate the mechanisms of resistance associated with aneuploidy will strengthen the identified clinical association and provide important insights for targeting these tumors with immunotherapy.

Although mutation and neoantigen burden are currently being developed as potential clinical biomarkers with ongoing validation studies, microsatellite instability (MSI) has recently been granted approval by the U.S. Food and Drug Administration (FDA) as an indication for ICIs. In early mutational load studies, mutations in DNA repair genes such as *POLD1, POLE, BRCA2*, and *MSH2* were already noted to be relatively frequent and were suggested to have a potential contribution to response. MSI results from a defect in the mismatch repair pathway and can occur from either a germline mutation or a somatic alteration of the genes involved in the DNA repair pathway. The recognition that MSI tumors respond better to PD-1 blockade was first reported by Le et al in a phase 2 study of pembrolizumab in colorectal cancer, where dramatically improved response and immune-related progression-free survival were noted. This was subsequently confirmed in a larger study across multiple cancer types demonstrating that MSI, either because of an inherited germline mutation or somatic alteration, resulted in improved response to pembrolizumab.
Colorectal tumors with a mismatch-repair deficiency have long been known to be associated with rich lymphoectytic infiltrate and improved overall prognosis.\textsuperscript{34,35} MSI tumors are associated with \textgreater{}10-fold higher mutational or neoantigen load, presumably resulting in an increased ability for tumor recognition and killing by the adaptive immune system.\textsuperscript{36} MSI is particularly associated with increased insertions and deletions and resulting frameshift mutations that correlate with increasing T-cell infiltrate within the tumor, suggesting that mutations other than single nucleotide variants are likely important.\textsuperscript{30,37} The FDA approval of pembrolizumab for all MSI tumors represents the first pan-cancer FDA approval for a genomic feature and may be indicative of what future drug applications will resemble.

The potential relevance of other DNA damage response and repair defects on response to ICI is not yet fully understood, but it offers promise for improved prediction of which patients may benefit most from ICI therapy. Mutations in the proofreading domains of polymerases POLE and POLD1 are associated with profound increases in tumor mutational load, even when compared with mismatch-repair deficient tumors.\textsuperscript{4,38,39} Not surprisingly, POLE mutant endometrial cancer is associated with increased T-cell infiltration on immunohistochemistry.\textsuperscript{40} In addition, anecdotal evidence suggests that these tumors likely respond well to ICI, although a more comprehensive analysis is warranted before integration into clinical decision making.\textsuperscript{41,42}

Further evidence of the importance of DDR alteration in response to ICI was recently reported by Teo et al in a cohort of 60 patients with bladder cancer treated with PD-1/PDL-1 inhibitors on prospective trials.\textsuperscript{43} A dramatic increase in overall response rate (68\% vs 19\%) was observed in patients with deleterious mutations in a panel of DDR genes, similar to previous reports with platinum therapy in bladder cancer.\textsuperscript{44} Interestingly, the mutations found included nucleotide excision repair gene ERCC2, as had previously been reported for platinum sensitivity.\textsuperscript{45} as well as mutations in other DDR genes: ATM, POLE, and BRCA1/2.

Similarly, defects in homologous recombination, such as mutations in canonical tumor suppressor genes BRCA1 and BRCA2, have been suggested as a potential biomarker of response to ICIs.\textsuperscript{46} Germline mutations in BRCA1 or BRCA2 result in hereditary cancer predisposition, to breast and ovarian cancer in particular, and are associated with increased genomic alterations associated with a specific mutational signature.\textsuperscript{47–49} In ovarian cancer, BRCA1/2 mutations were associated with increased T-cell infiltration by genomic metrics and immunohistochemistry.\textsuperscript{50,51} BRCA1 mutant breast tumors have been associated with increased T-cell infiltration and increased staining of the PD-1 checkpoint molecule.\textsuperscript{52} However, limited response to combined immune checkpoint blockade was observed in a mouse model.

Further clinical data and analysis will likely reveal the potential complex interactions between these DDR defects and response to ICI, and cancer immunity in general. Importantly, a greater understanding of the molecular mechanisms of these interactions may offer not only biomarkers of response to existing therapeutics, it may also reveal promising targets for combination studies with ICI.

**Radiation and DDR**

The interplay of ionizing radiation and local and systemic immune response against tumor has been demonstrated in several preclinical studies.\textsuperscript{53} With the rapid introduction of ICI into clinical use, exciting anecdotal evidence and, more recently, prospective studies have suggested the potential for radiation therapy to modulate responses to immunotherapy.\textsuperscript{54–57} The efficacy of radiation therapy is severely reduced in the absence of an immune response in nude mice that are deficient in B- and T-cells and is significantly dependent on local CD8 T-cell infiltration.\textsuperscript{58,59} In the clinic, patients who develop cutaneous squamous cell carcinoma in the context of immunosuppression have worse outcomes after radiation therapy than patients with an intact immune system.\textsuperscript{60}

Perhaps the strongest clinical evidence for the potential of radiation therapy combined with ICI comes from the recent publication of the PACIFIC trial.\textsuperscript{61} A large cohort of patients with locally advanced non-small cell lung cancer who received standard-of-care chemoradiation therapy were randomized to adjuvant durvalumab, a PD-L1 inhibitor, or placebo, and a dramatic increase in progression-free survival was observed in the durvalumab arm. Importantly, the benefit was exclusively observed in patients who received the ICI within 14 days of completion of radiation, emphasizing the link between DNA damage and ICI response.

The immunogenic effect of radiation is traditionally thought to be the result of increased antigen availability and presentation as a result of tumor cell death, allowing recognition by the host immune system.\textsuperscript{62} Although radiation can also be mutagenic and generate novel neoantigens, these mutations are likely to be subclonal and contribute minimally to overall tumor immunogenicity.\textsuperscript{17} Absence of the innate immune response also results in reduced efficacy of radiation therapy, suggesting the importance of activation of innate signaling pathways and alteration of the tumor microenvironment.\textsuperscript{64} Tumor irradiation results in upregulation of major histocompatibility complex class I expression\textsuperscript{65} and chemokine and cytokine secretion that promotes an inflammatory infiltrate within the tumor and draining lymph node.\textsuperscript{66,67} Type I interferon production in particular has been implicated for radiation efficacy and proinflammatory changes.\textsuperscript{68} In mouse models, systemic synergistic effects or abscopal responses
to radiation combined with ICIs have been observed, emphasizing the potential of radiation to enhance immunogenicity.69,70

The innate immune system has evolved to recognize molecular patterns associated with both extracellular and intracellular pathogens using pattern recognition receptors (PRR) within the endosome and cytosol, activating inflammatory cascades when triggered. Interestingly, recent work suggests that PRRs and their downstream signals can be directly regulated by the DNA damage response. For example, ATM (a critical kinase activated in response to DNA double-strand breaks that are the predominant result of ionizing radiation) can directly regulate PRR activation and interferon signaling.71,72 These PRRs can recognize a variety of signals that can be indicative of viral or microbial infection including DNA and RNA.

Radiation, along with some other cytotoxic drugs, has been recognized as a potent activator of immunogenic cell death (ICD). ICD is characterized by at least 3 factors, all of which have been shown to enhance DC uptake of dying tumor cells and secretion of proinflammatory cytokines such as interleukin (IL) 1β and consequently enhance DC maturation and cross-priming of CD8 T-cells.73,74

First, calreticulin translocation to the cell surface from the endoplasmic reticulum (ER) serves as an eat-me signal in response to ER stress.75 The release of HMGB1, an evolutionarily conserved nuclear protein, signals via Toll-like receptors to generate a proinflammatory expression program.76 Finally, the release of adenosine triphosphate from dying cells activates the P2XR7 receptor and NLRP3 inflammasome, resulting in IL-1β secretion and a proinflammatory cascade.77 The role of cytotoxic agents and agents targeting DDR in activating ICD and immunity is outside the focus of this review and has been reviewed elsewhere.78,79

RNA within the cytosol can be recognized by the RIG-I receptor to stimulate interferon production as a potent antiviral defense mechanism. RIG-I selectively recognizes highly structured noncoding RNA because of the lack of the 2'-omethyl cap that is present on most messenger RNA.80 Radiation has been shown to potentially activate RIG-I via accumulation of noncoding RNA in the cytoplasm, and disruption of this pathway attenuates interferon production after DNA damage.81

Emerging role for the cGAS/STING pathway

DNA within normal cells is typically excluded from the cytosol and contained within the nucleus and mitochondria. In the setting of microbial/viral infection and potentially in tumor cells, cytoplasmic double-strand DNA is recognized by cytoplasmic cGAS, inducing a conformational change and catalyzing the synthesis of cGAMP from adenosine triphosphate and GTP.82 Subsequently, cGAMP can bind the ER membrane adaptor STING, resulting in activation of TBK1 and downstream transcription factors IRF3 and NF-κB, which in turn results in cytokine production including tumor necrosis factor, IL-1β, IL-6, and type I interferons.83–85

Within the tumor microenvironment, STING activity and interferon production have been shown to play an important role in tumor immunosurveillance and CD8 T-cell activation. In addition to tumor-cell intrinsic cGAS-STING activity, the transfer of tumor DNA and activation of dendritic cells in the tumor and draining lymph node result in effective antigen presentation.86,87 Deficiency of cytoplasmic DNase Trex1 and DNase II in mouse models has been associated with the development of autoimmunity because of recognition of self-DNA.88,89 STING activity has been shown to be upregulated in the setting of DNA repair deficiencies including both BRCA1/2 and ATM mutant tumor-cell lines.71,90

More recently, cGAS/STING signaling has been shown to be important for response to both radiation and immune checkpoint blockade. Interferon production after radiation and the resulting antitumor-immunity was dependent on STING.91 cGAS-deficient mice are relatively refractory to PD-L1 blockade in a mouse melanoma model, and administration of STING agonists enhanced response.92 The abscopal response observed in mouse bilateral flank models with radiation to a single flank along with immune checkpoint inhibitors was also shown to be dependent on cGAS/STING signaling.93

Tumor cell intrinsic cGAS/STING activation and the resulting recruitment and activation of dendritic cells was necessary for cross-priming of CD8 T-cells against the tumor. Interestingly, the dose and fractionation schedule of radiation influenced the degree of interferon production and the resulting response, which was shown to be dependent on upregulation of the exonuclease TREX1 and subsequent depletion of cytoplasmic DNA at higher doses. The ideal dose and fractionation appear to be partially dependent on the intrinsic sensitivity of the cell line used, suggesting variability between tumors that may need to be empirically determined. This work may have important implications for optimization of radiation therapy to activate innate signaling and promote an antitumor immune response.

Recent work has also implicated the activity of the cGAS/STING pathway in response to micronuclei and chromosomal instability commonly observed in tumors.94,95 In the setting of chromosomal instability, chronic activation of the cGAS/STING pathway and perhaps an adapted response (without interferon production) can facilitate tumor invasion and metastasis and may be an important prognostic factor across cancer types.96,97 Thus activation of these pathways in some tumors may not result in a characteristic proinflammatory interferon-driven response and should be tested judiciously.
Finally, chronic interferon signaling can also ultimately lead to activation of PD-L1—dependent and PD-L1—indépendent resistance to immune checkpoint blockades.\(^9\),\(^8\) Thus, ionizing radiation—mediated activation of cytosolic DNA signaling may offer important insights in the proinflammatory potential of radiation, but the interaction is likely complex and will require further study for integration into clinical practice.

**Conclusions and future directions**

Although checkpoint blockade therapy has revolutionized the treatment of cancer, only a subset of patients may benefit from ICI at this time. Defects in DNA repair pathways and the resulting mutational load represent some of the strongest predictors of response to ICI. Unfortunately, combination immunotherapeutic strategies to date have not resulted in significant gains over single-agent strategies. In fact, the most promising combination therapy with anti—PD-1 treatment in most cancers has been in combination with cytotoxic chemotherapies, many of which are known to be dependent on the DNA damage response for efficacy. Preclinical and clinical data demonstrate that immune response plays an integral role in tumor control after radiation therapy. Interestingly, a significant part of this response is likely the result of DNA damage induced by radiation therapy, facilitating both an adaptive and innate immune response to therapy.

A better understanding of how DNA damage response interacts with tumor immunity will significantly improve our understanding of the optimal method to combine radiation therapy with current immunotherapeutic approaches. A greater mechanistic understanding of this interaction will likely offer important insights for open questions in the radiation immunology field, including the timing of radiation and immunotherapy (reviewed separately in this issue [Gundersen]) and optimal dose and fractionation (Ko), and optimal tissue targets of radiation therapy (Ellsworth).

This will also facilitate rational strategies for combining other novel therapies, including those targeting DDR pathways directly, such as PARP inhibitors. This will likely require not only preclinical investigations, but also a more careful molecular analysis of clinical samples, including a more careful assessment of DDR deficiencies in individual tumors. The important insights gained from these studies will open the door to a more personalized immunotherapy treatment approach that targets the DDR pathways with drugs and radiation in precise coordination with ICIs to maximize host immunity against the tumor.

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