The abscopal effect: a sense of DNA damage is in the air

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Tumor metastasis is a singularly important determinant of survival in most cancers. Historically, radiation therapy (RT) directed at a primary tumor mass was associated infrequently with remission of metastasis outside the field of irradiation. This away-from-target or "abscopal effect" received fringe attention because of its rarity. With the advent of immunotherapy, there are now increasing reports of abscopal effects upon RT in combination with immune checkpoint inhibition. This sparked investigation into underlying mechanisms and clinical trials aimed at enhancement of this effect. While these studies clearly attribute the abscopal effect to an antitumor immune response, the initial molecular triggers for its onset and specificity remain enigmatic. Here, we propose that DNA damage–induced inflammation coupled with neoantigen generation is essential during this intriguing phenomenon of systemic tumor regression and discuss the implications of this model for treatment aimed at triggering the abscopal effect in metastatic cancer.

History and frequency of the abscopal effect

The conception of the abscopal effect is credited to the British scientist Robin Mole, who in 1953 brought forward the notion that "irradiation of a mammal [has] an effect at a distance from the volume irradiated" (1). This terminology was later adopted by oncologists who described very rare cases of remission of metastases outside the field of irradiation from the 1970s onward (2, 3). Systematic literature searches have been carried out elsewhere and estimate that the number of reports that described abscopal effects in response to radiation therapy (RT) prior to 2014 does not exceed 47 individual cases (4, 5). This number increased markedly with the introduction of immune checkpoint inhibition (ICI) therapy in combination with RT (4, 6). Several attempts have been made to quantify the frequency of the abscopal effect in response to RT/ICI treatment. For example, analysis of ten nonrandomized studies of metastatic melanoma patients treated with RT/ICI between 2014 and 2019 revealed evidence for abscopal tumor remission in an average of 34.3% of cases (range 18%–63%; 62 total cases exhibiting an abscopal effect) (7). A similar meta-analysis of eight metastatic melanoma studies between 2009 and 2017 reported abscopal effect in mean 26.5% cases (range 10%–63%; 65 total cases exhibiting an abscopal effect) (8). Despite these encouraging observations, a randomized examination of the incidence of the abscopal effect in response to RT/ICI specifically is needed to ultimately determine the frequency of this phenomenon. In fact, one such randomized trial of RT/ICI in metastatic head and neck squamous cell carcinoma showed evidence of the abscopal effect in zero of 32 cases (9). Further insight into the actual occurrence of abscopal responses may be gained by several upcoming randomized trials of RT and ICI combination treatments, which have been designed to systematically collect data on the incidence of responses outside of the radiation field in metastatic lung cancer (10, 11).

While it is clear that there are more recorded cases of the abscopal effect with the introduction of ICI, these are likely limited to a fraction of patients, and perhaps to specific cancer types. In accordance, many recorded instances of the abscopal effect occurred in melanoma and lung cancer, which commonly exhibit high lymphocyte infiltration and mutation rates (12). These emerging data point to the use of RT/ICI therapy in particularly immunogenic cancer subtypes, as this may preferably enable abscopal effects. Since this realization, an increasing focus has been placed on the determination of clinical parameters and treatment conditions that promote systemic responses to DNA-damaging therapies. Retrospective analyses have shown that abscopal tumor regression correlates with increased $CD8^+$ cytotoxic T lymphocyte (CTL) infiltration and decreased $CD8^+FoxP3^+$ regulatory T cells (Tregs) (13–15). Incidence of the abscopal effect also correlates negatively with lymphopenia (16). However, high-dose RT is associated with the depletion of patient immune cells. In line with this, one of the observations made was that fractionated RT is associated clinically with instances of the abscopal effect (17). In addition to this, highly targeted administration of RT by procedures such as stereotactic RT may further protect patient lymphocytes and promote the abscopal effect (18, 19). Investigation of the role of RT fractionation has been carried out in preclinical mouse models, which reinforce the notion that RT spread over at least two treatment sessions favors abscopal tumor regression (20, 21) in contrast to single high-dose RT, which favors Treg development and therefore likely promotes cancer progression (22). Targeted clinical trials are needed to stratify RT regimens that favor the onset of abscopal remission for specific cancer types, as discussed in recent focused reviews on the topic (23, 24). Nevertheless, existing retrospective analyses suggest that patients exhibit the abscopal effect in situations in which RT does not deplete all lymphocytes, CTL/Treg ratios are favorable, and immune stimulation is achieved by fractionated RT.

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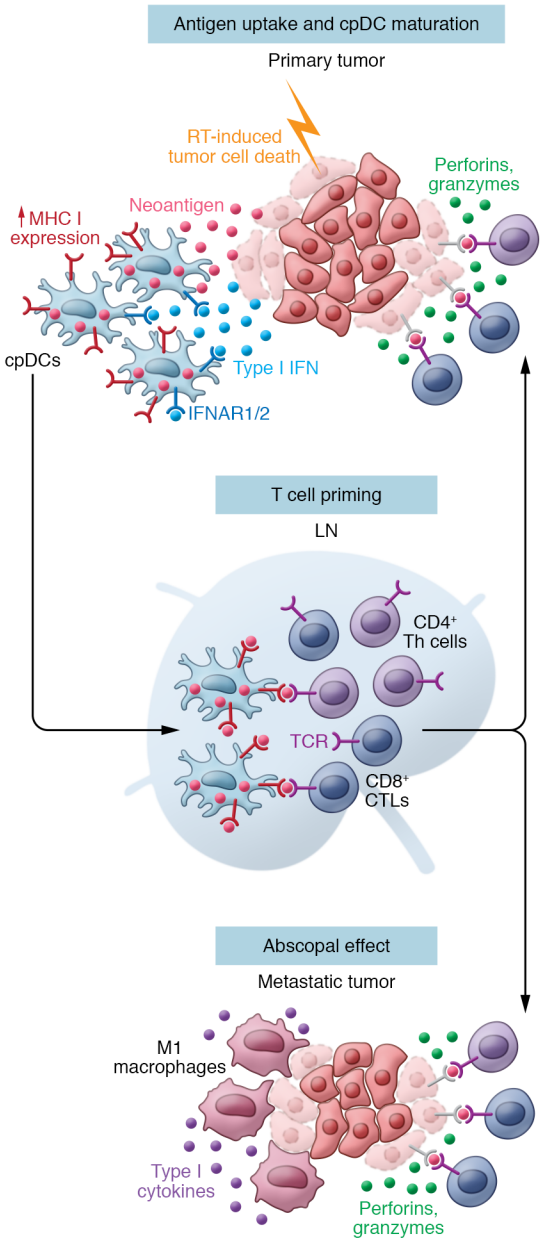

Figure 1. Overview of the antitumor immune response during the abscopal effect. Antigen generation occurs upon tumor cell death due to RT and neoantigen (pink) uptake by cross-priming dendritic cells (cpDCs). Type I inflammation is triggered with associated cytokine secretion (type I interferon, blue), which upregulates MHC class I molecules on cpDCs. These cells migrate to lymph nodes from the site of the primary tumor and present tumor-associated antigens to naive T cells. This gives rise to a population of tumor-reactive $CD4^+$ and $CD8^+$ T cells, as depicted, and these cells are released systemically. At the primary tumor mass, tumor-reactive T cells are the main effectors carrying out the abscopal effect. This includes release of cytotoxic molecules (green) by $CD8^+$ T cells upon recognition of tumor antigen (pink). This is aided by costimulatory release of cytokines such as IL-2 by $CD4^+$ T cells upon antigen recognition, which promote T cell proliferation and effector function. M1 macrophages contribute to tumor cell killing by direct phagocytosis and secretion of type I inflammatory cytokines (purple). Importantly, given that immune cells are primed in lymph nodes and released systemically, these activities also take place at distal metastases where tumors exhibit the same antigen, which gives rise to the abscopal effect.

The abscopal effect is an antitumor immune response

Murine models of the abscopal effect have been pursued, and as with the characterization of many other immunological phenomena, such studies have been vital for definition of underlying mechanisms. Early on, a landmark report demonstrated that abscopal tumor regression is not observed in immunodeficient mice (25). This established that the abscopal effect must be mediated by an antitumor immune response. Similarly, direct responses to RT in primary tumors are also mitigated in immunodeficient mice (26, 27). Generally, immune responses against tumor cells are thought to be specific to neoantigen generated by protein overexpression or sequence alterations in malignant cells (28). The main effectors of tumor cell killing are CTLs, which rely on neoantigen presentation by dendritic cells (DCs). Since tumor neoantigens are not present within DCs, such antigens are presented by specialized cross-priming DCs (cpDCs; Figure 1). In the context of the abscopal effect, this response is likely dependent on RT-induced cell death, which initiates antigen cross-presentation in DCs by activation of TLR4 (29) and type I interferon signaling (30). In addition to this, type I interferon signaling increases the number of tumor-associated DCs capable of cross-presentation (31). Neoantigens are presented via class I MHC molecules on cpDCs. Again, RT directly induces upregulation of these molecules and therefore enhances cross-presentation (32, 33). Depletion of cpDCs impairs the abscopal effect in mouse models of multiple cancer types (34), indicating that these cells are necessary for abscopal antitumor immune action. Taken together, these findings indicate that cross-presentation of neoantigen by DCs and tumor-reactive CTLs plays a major role in adaptive immune activation in the context of the abscopal effect, and RT may trigger this on multiple levels.

Upon activation, cpDCs migrate to lymph nodes and engage with naive T cells to give rise to tumor-specific CTLs (35). T cell priming occurs by neoantigen presentation on class I MHC by cpDCs, secondary activation signals, and engagement with $CD4^+$ T cells in lymph nodes (36). Upon release of tumor-reactive CTLs to the periphery, such cells are capable of infiltrating tumor tissue by extravasation. This step presents a considerable barrier to abscopal responses since tumor tissue is often inaccessible to CTLs. In fact, increased CTL accessibility to the tumor site is strongly correlated with antitumor immunity (37, 38). RT makes tumor tissue more accessible to CTLs. For example, RT triggers the production of the CTL attractant chemokine CXCR16 (39, 40). In addition to this, RT induces changes in the tissue architecture by upregulation of ICAM-1 and VCAM-1 (41), which promote vasculature permissive for CTL tumor extravasation. Therefore, while CTLs may reach irradiated primary tumor cells more readily, metastatic sites are far less accessible, thus limiting abscopal tumor remission. Effective tumor cell lysis is achieved by recognition of neoantigen by CTLs and cell killing via cytotoxic degranulation or induction of tumor cell apoptosis (42). However, evidence suggests that oftentimes, tumor cell surface expression of the inhibitory signal molecules PD-1, PD-L1, and CTLA-4 potently counteracts this process (43, 44). Prominently, monoclonal antibodies against these inhibitory molecules have been utilized extensively to stimulate CTL-mediated tumor cell killing (45). This inhibition

of immune checkpoints is thought to be one of the reasons underlying the increased incidence of abscopal responses upon RT/ICI treatment. In summary, tumor cell killing during the abscopal effect likely depends on CTL infiltration into the primary tumor mass and distal metastases, and this is augmented by ICI.

Another emerging mode of tumor cell killing during the abscopal effect is phagocytosis. Accumulating evidence suggests that this process is carried out by M1-like macrophages (M1-MΦ), a cell subset capable of inflammatory cytokine secretion and phagocytosis (46, 47). Two independent studies of mouse models showing RT/ICI-induced abscopal remission in metastatic non–small cell lung cancer and melanoma detected significant M1-MΦ populations at the site of tumor remission (48, 49). In addition to this, impairment of recruitment by genetic deletion of the main MΦ chemokine attractant CCL2 resulted in decreased RT-mediated abscopal responses in mice (50). Strikingly, there are specific settings where RT/ICI abscopal responses are entirely dependent on M1-MΦ phagocytosis and do not rely on CTL-mediated cell killing (51). These data using mouse models indicate that such cells may be important in the clearance of tumor cells during the abscopal effect. In support of this notion, one clinical trial showed that RT in combination with administration of GM-CSF, a potent activator of M1-MΦ development, increased the incidence of abscopal remission in various metastatic solid tumors (52). In addition, the alternative cell fate in MΦ development, namely M2-MΦ, is well known to inhibit antitumor immune reactivity (53). Therefore, it is likely that bias toward M1-MΦ development in the tumor counteracts the genesis of suppressive M2-MΦ cells and is beneficial for abscopal remission. This leads to a model whereby abscopal effects rely on tumor clearance mediated by both primed CTL activity and phagocytosis via tumor-associated M1-MΦ cells (Figure 1). The specificity of antitumor reactivity is conferred by T cell–mediated neoantigen recognition in both modes of tumor cell killing, since M1-MΦ cells are not capable of sensing antigen.

DNA damage can initiate the abscopal effect

Understanding the onset of the abscopal effect is imperative for the development of treatments aimed at stimulating it in a predictable manner. RT is a well-documented trigger of DNA damage, and this has taken center stage in efforts to define the underlying mechanism of the initiation of the abscopal effect. DNA damage upon RT occurs via two primary mechanisms: direct breakage of DNA by high-energy photons and the generation of free radicals (54). Perhaps the most detrimental form of DNA damage is the generation of double-strand breaks (DSBs) in genomic DNA. Under steady-state conditions, DNA strand breaks are repaired during a prolonged period of cell cycle arrest, enacted by the checkpoint signaling kinases ATM and ATR, that prevents progression of cells with DNA damage into mitosis. This delay in cell cycle advancement allows additional time for a myriad of DNA damage response (DDR) proteins to faithfully repair the genomic lesions (55). Cell cycle checkpoint disruption commonly occurs in cancer cells, or can be pharmacologically impaired by small-molecule inhibitors of ATR (56–58), thus allowing unrepaired damage to persist during mitosis and become mis-segregated into the cytoplasm. Such damaged DNA is proinflammatory when exposed to the cytoplasm, where it can be recognized by pattern recognition receptor (PRR) molecules that canonically respond to pathogenic nucleic acids following bacterial or viral infection (59). DNA damage and checkpoint responses also impact transcription of noncoding RNA elements, with loss of p53 notably resulting in a robust increase in retroelements following DSB induction (60, 61). This resulting deregulation of RNA molecules is another potential source of immune activation upon RT through RNA-sensing PRR responses (62). Therefore, DNA damage likely has a broad effect on the immunogenicity of self-DNA and -RNA throughout the cell, and it has become apparent that such molecules are triggers of innate immune responses.

Immunogenicity of RT-induced cytoplasmic DNA

Micronuclei are generated upon RT and other forms of DNA damage, as well as by agents that disrupt the mitotic spindle (63). Indeed, the correlation between micronucleus formation and irradiation is so tight that it has been used to monitor accidental exposure to radiation in atomic power plant workers (64, 65). Micronuclei arise in response to mitotic errors including improper attachment to tubulin fibers or the centriole as well as unrepaired chromosome breakage resulting in fragments without functional kinetochores (66, 67). Collectively, such events result in the aberrant occurrence of chromosomal DNA in the middle of the mitotic plane at telophase, which results in a failure to incorporate this DNA into the primary nucleus. An abnormal nuclear envelope forms, which is prone to rupture and exposure of DNA to the cytoplasm (68). This exposure is reminiscent of the presence of pathogen DNA in the cytoplasm and, as such, triggers an innate immune response (Figure 2). Indeed, if cells divide in the presence of damaged DNA, micronuclei trigger a type I interferon and NF-κB response dependent on the DNA-sensing PRR cGAS (69, 70). Nucleosomes are poor cGAS activators in cells (71–74). Therefore, immune signaling likely occurs as deregulated DNA replication, and repair in micronuclei results in damaged nucleosome-free DNA, which is then exposed to the cytoplasm in the subsequent mitosis or via micronuclear envelope rupture (75). Upon cGAS activation, paracrine cyclic dinucleotide cGAMP is synthesized and triggers type I inflammation dependent on phosphorylation of TBK1, the ER-associated transducer STING, IRF3, and NF-κB (76–79). The importance of this process for the induction of the abscopal effect was demonstrated in STING$^{-/-}$ mice, which were impaired in micronucleus-mediated immune induction and abscopal tumor regression upon treatment with RT/ICI (69, 70, 80). Moreover, progression through mitosis is critical for DNA damage–induced antitumor immune responses (75). In summary, exposure of RT-induced micronuclear DNA to the cytoplasm is a potent trigger of type I interferon signaling, and this likely contributes to the onset of the abscopal effect.

Another source of proinflammatory self-DNA is exposure of the mitochondrial genome (mtDNA) to the cytoplasm. Release of mtDNA has been described in detail for cells undergoing apoptosis, where it triggers type I inflammation (81). This is important since RT is well known to induce apoptotic cell death in the field of irradiation, and therefore, proinflammatory mtDNA release from tumor cells is likely to play an important role in the initiation of antitumor immunity during the abscopal effect. Several studies in

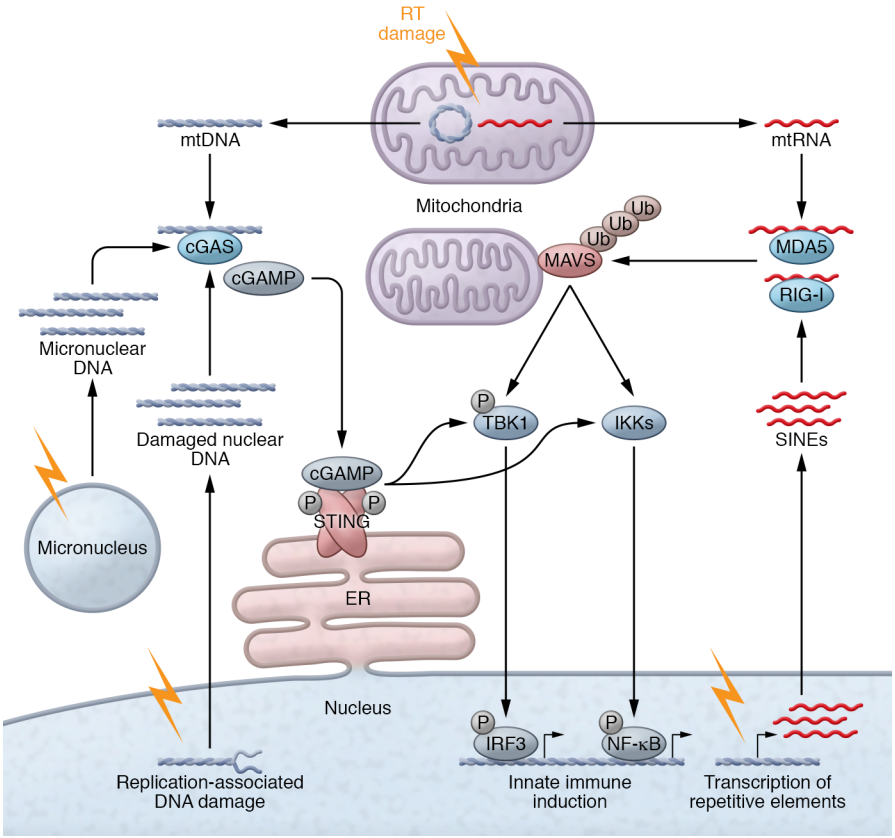

Figure 2. Sensing of self-nucleotides upon DNA damage triggers innate immunity. Cytoplasmic DNA may arise upon DNA damage in the main nucleus where replication intermediates are cleaved during repair and released into the cytoplasm. Other sources of cytoplasmic DNA include the release of mitochondrial DNA (mtDNA) and micronuclear DNA. Such molecules have been shown to be recognized by cGAS, which facilitates the generation of cGAMP and subsequent downstream signaling by activation and phosphorylation of ER-associated STING. This triggers a phosphorylation cascade involving many factors, including TBK1 and various IKK family proteins. Similarly, DNA damage results in the release of RNA into the cytoplasm as a result of deregulated transcription, especially of repetitive elements including SINEs, as well as the release of mtRNA. These molecules are sensed by RIG-I and MDA5, depending on their length, and trigger immune signaling by engagement with mitochondrial membrane–associated MAVS. Upon activation, MAVS is polyubiquitinated by factors such as TRIM25 and engages in a phosphorylation cascade similar to that of activated STING. Ultimately, both of these result in the phosphorylation and nuclear translocation of IRF3 and NF-κB, which induces transcription of innate immune effector genes.

mice demonstrated that cGAS sensing of self-DNA and immune activation is mediated by activation of cpDCs through uptake of tumor mtDNA (82–84). In addition, abscopal tumor remission was augmented with increased mtDNA release into the cytoplasm of tumor cells (85). In summary, mtDNA may present an additional source of immunogenic DNA, which is sensed in the cytoplasm during the initiation of the abscopal effect.

RT-induced DNA lesions result in replication fork stalling and fork collapse. Intact DNA repair pathways are capable of counteracting this, and impairment of such pathways may result in proinflammatory release of damaged genomic DNA into the cytoplasm. Evidence for this model is currently limited to studies of tumor cell lines. For example, excessive replication fork stalling in HEK293T cells depleted of the DNA repair protein SAMHD1 results in abundant cytosolic ssDNA, which induces type I immune signaling (86). In addition, it was proposed that defective retention of nuclear ssDNA upon knockdown of the key DNA repair factors RAD51 and RPA resulted in immunostimulatory release of these molecules into the cytoplasm, albeit the mechanisms of DNA transport from the nucleus to cytoplasm were not explained (87). Similarly, another report in HEK293T cells showed that induction of cytosolic ssDNA and dsDNA by RT was dependent on impaired replication fork progression and RAD51-dependent fork rescue (88). However, given that cGAS is not activated by ssDNA (89), the identity of the PRR involved in the sensing of cytoplasmic, damaged self-ssDNA remains to be determined. Further, the relevance of replication-stress associated DNA breakage and leakage of damaged ssDNA from collapsed forks due to impaired DNA repair remains to be investigated. Indeed, PARP inhibitor–induced STAT1 phosphorylation in BRCA1 mutant cells still required passage through mitosis (69), suggesting commonality with RT-stimulated immune responses. Nevertheless, the eventual cytoplasmic destination of such inflammatory molecules may well play a role in the onset of the abscopal effect upon RT, where replication forks stall and collapse frequently.

Finally, in light of the proinflammatory nature of cytoplasmic self-DNA, negative regulators of these molecules have been investigated as putative gatekeepers, in turn, of the abscopal effect. For example, the cytoplasmic nuclease TREX1 has been shown to degrade cytoplasmic DNA and inhibit innate immune induction upon DNA damage. Indeed, TREX1 overexpression impaired cpDC activation and antitumor CTL priming in mice, ultimately abrogating abscopal responses upon RT/ICI (90). Similarly, cytoplasmic DNA originating from mitochondria upon DNA damage insult is removed by autophagy (91). Genetic ablation of autophagy in tumor cells resulted in markedly increased abscopal responses in mice (85), suggesting that autophagy acts as another negative regulator by removing proinflammatory self-DNA. Thus, negative regulators of cytoplasmic DNA also limit the abscopal effect.

In summary, DNA damage resulting in the accumulation of cytoplasmic DNA from micronuclei, mitochondria, or genomic replication stress may be an activator of the innate immune response during abscopal tumor remission (Figure 2).

Immunogenicity of aberrant self-RNA upon DNA damage

DNA damage impacts on transcription. RNA polymerase II is degraded upon DNA damage (92), and transcription is silenced on chromatin in *cis* to DSBs (93, 94), decreasing gene expres-

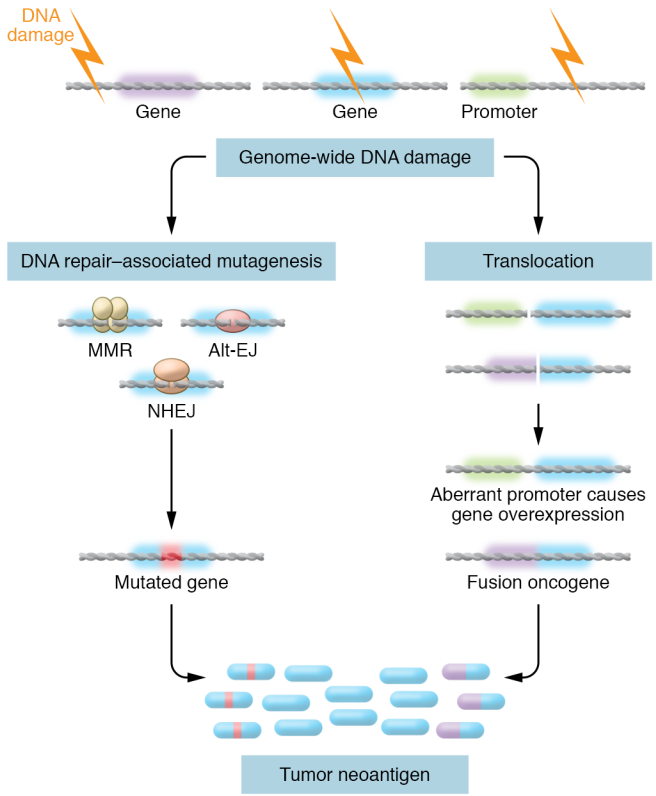

Figure 3. Mechanisms of neoantigen generation upon DNA damage. Damaged DNA may be intergenic, or between genes and promoter sequences. Damaged DNA may be repaired by end-joining pathways upon pharmacological impairment or HR deficiency (left pathway). Such repair is associated with sequence changes due to the fact that Alt-EJ and MMR result in small indels at the site of repair. In addition to this, repair by NHEJ involves end processing by factors such as Artemis, and this also results in small sequence changes at the site of ligation. Together, such repair mechanisms result in changes in nucleotide sequence at the site of repair. The resulting missense mutations are capable of producing changes in amino acid sequence that may give rise to neoantigen. In a complementary or alternative mechanism, breakage of DNA at two distinct loci may give rise to promoter translocation and aberrant overexpression of a given antigen (right pathway). These pathways are likely not exclusive events and may give rise to tumor neoantigens targeted by the immune system during the abscopal effect.

sion globally. Similarly, the genesis of rRNA by RNA polymerase I is suppressed upon DNA damage (95–98). On the other hand, transcription of repetitive sequences including telomeres and short interspersed nuclear elements (SINEs) is increased upon damage (60, 99). It has been shown that cytoplasmic self-RNA is proinflammatory in the context of antitumor immunity. dsRNA is sensed by the PRRs MDA5 and RIG-I (100), which induce type I inflammatory signaling by activation of the mitochondrial adaptor protein MAVS (101). This is followed by a signaling cascade that results in the activation of ISG expression via nuclear translocation of active IRF3/7 or NF-κB (102, 103). Direct repeat Alu elements have been shown to activate MDA5 (104–106). In addition, several endogenous 5′-triphosphorylated RNAs have been shown to activate RIG-I (107, 108). While the source of endogenous proinflammatory RNA is unclear, DNA-damaging modalities activate the RNA-sensing PRR RIG-I in a manner that also requires progression through mitosis and is substantially augmented by combined loss of the G_1/S and G_2/M checkpoints in p53 mutant cells treated with ATR inhibitors (75, 109).

Such sensing of self-RNA has been described as the trigger of antitumor immunity in multiple studies using mouse models of cancer. For example, derepression of SINEs by 5-aza-2′-deoxycitidine treatment has been shown to lead to accumulation of cytoplasmic RNA, type I inflammation, and antitumor immunity (110). In this context, ADAR1 is thought to present a negative-feedback loop, which constrains antitumor immunity. Indeed, ADAR1 knockout enhances SINE-dependent antitumor immunity in mice (111). In another study, ADAR1-knockout tumors resulted in significant enhancement of antitumor immunity with a concomitant increase in CTLs and depletion of tumor M2-MΦ populations (112). Another source of self-RNA are mitochondria, where dsRNA is abundant owing to convergent transcription (113). Upon entry into the cytoplasm, such molecules have been shown to be proinflammatory by activation of MDA5 (106) and RIG-I (114). Again, 5-aza-2′-deoxycitidine has been shown to increase cytosolic mitochondrial dsRNA, leading to antitumor immunity (115). On the basis of these reports, sensing of self-RNA in the cytoplasm as a result of deregulated transcription or mitochondrial permeabilization is an effective trigger of antitumor immunity that may contribute to the innate immune induction required for the abscopal effect (Figure 2).

In the case of innate sensing of both DNA and RNA, nuclear translocation of effector transcription factors initiates secretion of cytokines, which ready the immune system for T cell priming. In both cases, NF-κB is phosphorylated and activated to induce expression of the T cell chemoattractant CXCL10 (116) as well as many other cytokines, including the inflammasome and apoptosis activator IL-1β (117–119). Engagement with cytoplasmic self-nucleotides also results in activation of IRF3, which induces interferon-stimulated genes (ISGs) including type I interferons such as IFN-β (120, 121), potently stimulating cpDC activation (122). In addition to this, the transcription factor IRF7 is markedly upregulated and activated in a positive-feedback loop, further potentiating induction of ISGs (123, 124). Many of the targets of NF-κB, IRF3, and IRF7 overlap, and activation of these factors in tandem induces rapid and strong ISG induction. The activation of these genes, triggered by sensing of self-nucleotides upon DNA damage, creates a powerful cytokine milieu, which favors antigen cross-presentation and antitumor CTL priming.

DNA damage generates neoantigens necessary for abscopal remission

Antitumor immune responses are directed against rearranged and overexpressed proteins in cancer cells, which are defined herein as tumor neoantigens (125, 126). Given that the abscopal effect constitutes such a response, neoantigen generation likely presents a crucial step in its onset. DNA damage upon RT or chemotherapy may play a key role in this process. Extensive or misrepaired DNA damage induces genomic instability, resulting in the translation of rearranged polypeptide sequences. In addition, increased intergenic instability may result in overexpression of transcripts (127), and indeed, aberrantly abundant proteins are a known source of tumor neoantigens. DNA repair

proceeds via two main pathways. During G_2/M phase of the cell cycle, the complementary sister chromatid is used to accurately copy sequence information and repair the break in error-free homologous recombination (HR) (128). On the other hand, in the absence of homologous template sequence during G_1, DNA breaks are mainly repaired via non-homologous end joining (NHEJ) (129). However, a number of other DDR pathways, such as alternative end joining (Alt-EJ), exist in cells; these are utilized when HR and NHEJ are overwhelmed or inactivated. Crucially, such alternative DDR pathways are often inherently mutagenic, and this is thought to be a source of changes in genetic sequence (130). Indeed, neoantigens frequently arise due to changes in peptide sequence (131), and inactivating mutations in canonical DDR pathways correlate with increased tumor neoantigen (132). We argue that DNA damage generates neoantigens that present the point of attack for immune cells when they become recurrent in clonally selected populations, thus allowing an abscopal effect.

Specific anticancer immune reactivity is mediated by T cell receptors on CTLs, which recognize tumor neoantigen. Detailed investigation into neoantigen identity has taken place with the aim of tumor vaccine development, and it has become clear that neoantigen may be generated via multiple pathways, all of which are dependent on DNA damage–driven changes in nucleotide sequence (Figure 3). One class of neoantigen is driven by damage-induced gross rearrangements, resulting in aberrant promoter translocation and atypically high expression of protein. This takes place by DSB and by aberrant, Polθ-mediated Alt-EJ at the site of translocation (133, 134). For example, in over 50% of prostate cancers, the transcription factor ETS is aberrantly expressed at high levels by fusion to the TMPRSS2 promoter sequence (135). Upon tumor cell lysis during early stages of prostate cancer, antitumor CTLs are primed against ETS neoantigen (136), which are later inhibited by direct action of ETS and recruitment of Tregs during the evolution of most disease instances (137). Similarly, metastatic melanoma atypically expresses high levels of the circadian clock gene BMAL1 by translocation, which again correlates with mostly exhausted CTL infiltration into the tumor (138). Similarly, intergenic DSBs and subsequent repair give rise to fusion oncogenes such as BCR-ABL or ETV6-RUNX1, which are well-documented drivers and neoantigens found in diverse cancers (139–141). Given that abundant DNA damage during RT may promote repair by Alt-EJ, we propose that chromosomal rearrangements analogous to the examples described above may generate immune target neoantigens during abscopal tumor remission.

Another frequent source of neoantigens is genetic mutation resulting in altered peptide sequence. This includes missense mutations, which lead to tumor-specific amino acid changes. Peptides corresponding to such sequence changes are recognized in cancer (142, 143), and CTL-mediated antitumor immune responses specific to such mutated protein have been recorded (144). Missense mutations have been linked to abscopal antitumor immune responses. For instance, adoptive transfer of CTLs specific to a point mutation in b-Raf resulted in abscopal regression (145). In other cases, mutated proteins are also overexpressed, potentially aiding their immunogenicity (146). Missense mutations are not the only genetic change resulting in neoantigen peptides. For example, mismatch repair (MMR) deficiency is closely associated with a robust response to ICI, and PD-1 antagonists are approved in cancers with microsatellite instability irrespective of tissue of origin (147, 148). This is the first "tumor-agnostic" approval of a cancer therapy, and in such patients, frameshift mutations are predictive of response (149), suggesting that this form of genome instability is particularly active in generating neoantigens. Interestingly, recent investigation has indicated that antitumor immunity in the context of MMR deficiency, modeled by loss of the key MMR protein MHL1, may involve excessive end resection of DSBs and aberrant DNA repair intermediates that become missegregated into micronuclei and are sensed via cGAS/STING as described above (150, 151). However, given that other MMR factors, including MHS2 and MHS6, are also predictive of antitumor immune responses upon ICI treatment in patients (152), it is more plausible that high mutation burden remains the dominant contributing factor in this phenomenon. Systematic analysis across cancer types indicates that small indels also give rise to a large proportion of neoantigen (125). Indeed, frameshift mutations in MMR-deficient cancers were implicated in the generation of neoantigens (153). Either way, during RT-induced abscopal remission, the underlying cause of these changes in sequence is DNA damage. Repair by both NHEJ and Alt-EJ frequently generates small indels at the site of repair (154, 155).

Thus, altered peptide sequence generated by repair of abundant RT-induced damage is another source of neoantigen during the onset of the abscopal effect (Figure 3). Such immunogenic epitopes likely complement neoantigen generated by alternative means that are largely independent of damaged DNA and its ensuing repair. This includes altered peptide sequence as a result of alternative splicing, and we direct the interested reader to a recent exhaustive review on this topic (156).

Discussion

In summary, we propose that sensing of self-nucleotides is the initial trigger of the innate immune responses underlying the abscopal effect. This hypothesis is timely considering the advent of targeted agonists of innate immune PRRs and their downstream transducers. Such drugs have shown much promise preclinically in the treatment of diverse malignancies. Multiple compounds that activate STING have been developed (157–159). Preclinical studies show that administration of PRR agonists is capable of inducing systemic, immune-mediated tumor regression (160–162). However, considerable challenges have been encountered subsequently by researchers attempting to translate these results to treatment in patients. For example, one of the first prominent candidate compounds was able to bind and activate only murine STING and not its human counterpart in clinical investigation (163, 164). Regardless, 15 more drugs are currently in phase I/II trials, mostly in combination with ICI (165), and certainly the success of such trials holds the promise of generating a treatment modality capable of inducing abscopal tumor regression. In addition, agonists of PRRs have shown promise in preclinical models. For example, TLR agonists induced CTL infiltration, M1-MΦ development, and tumor regression in several preclinical studies (29, 166, 167) but exerted protumorigenic effects in others (168, 169). Despite this context-dependent effect of TLR activation, some TLR ligands have shown promise in the clinic,

and three drugs have been licensed as monotherapy in sporadic cancers (167). Combination treatment involving such agonists with RT and/or ICI holds promise in expanding the patient population with successful induction of the abscopal effect. As suggested by others, careful analysis of the status of these molecules at baseline may be needed to determine subpopulations that benefit from PRR agonists (167), and the development of these treatments is the topic of much investigation (165, 170).

The immune induction during the abscopal effect promotes a burst of inflammatory signaling at the site of radiation, resulting in antitumor reactive T cell priming. However, persistent type I inflammatory signaling also exerts the opposite effect and has been associated with increased metastases (171). This has important implications when considering the clinical activation of abscopal tumor remission. Firstly, therapy will be dependent on triggering incremental type I inflammation in response to DNA damage by fractionated RT, allowing cpDC neoantigen uptake and CTL priming while avoiding chronic inflammation, which may be protumorigenic. This will likely require some degree of personalized RT to achieve abscopal remission in an acceptable proportion of patients, taking into account both individual susceptibility to RT-induced DNA damage and the mutational status of cytoplasmic self-nucleotide sensors in tumor cells. Successful RT in the context of the abscopal effect will have to strike the delicate balance between antitumor immune activation and protumorigenic persistent immune signaling.

If future research is successful in defining treatment conditions for activation of the abscopal effect, detailed investigation into the identity of tumor neoantigens during successful abscopal remission may take this therapy even further. Combined with the notion of tumor vaccination, one may envision abscopal cancer vaccines that further facilitate systemic remission. The recent advent of mRNA vaccines may allow rapid introduction of tumor neoepitopes predicted from tumor DNA sequencing (172). However, this task is complicated by the fact that even in situations in which cancer neoantigen arises due to DNA damage–driven sequence alterations, reactive T cells are often inhibited by antiinflammatory, tumor-specific Tregs. Indeed, such cells are found in refractory tumors. Thus, analysis that detects tumor antigen–specific T cell receptor sequence as well as T cell activation status is needed to resolve this question in the context of the abscopal effect. Another open question concerns the genesis of neoantigen during abscopal remission. Successful priming of the immune system against neoantigens resulting in abscopal remission would require that such antigen is shared between the primary irradiated tumor mass and all distal metastases. This raises the question of which antigens are essential to both primary and metastatic tumors, and when they are taken up by cpDCs. It is conceivable that neoantigens (a) arise early on during tumor evolution prior to metastasis or (b) are generated by high-probability genetic changes, which occur simultaneously in all locations. In the former case, our hypothesis that neoantigen generation is due to RT-induced DNA damage is more probable. However, the latter scenario favors the view that neoantigen generation during the abscopal effect occurs in response to endogenous damage and erroneous repair during the proliferation of malignant cells.

In summary, the type I inflammation triggered by cytoplasmic self-nucleotides alters the tumor microenvironment to allow for the presentation of neoantigens and, ultimately, the systemic immune responses that underlie the abscopal effect. Here, we hypothesize that DNA damage is critical for the abscopal effect at both the initial immune activation and priming against neoantigens. Careful dosing of direct innate immune agonists and RT is needed to establish treatment aimed at triggering the abscopal effect specifically. This also implies that retrospective analysis of the status of both DDR and innate immune signaling molecules in patients with abscopal tumor remission may enable identification of predictive markers for successful treatment. Considerable further investigation is therefore needed to effectively harness abscopal responses in a systematic manner and at the frequency needed to become standard clinical practice. Given the striking and complete remission observed in rare patients, something not possible with chemotherapy alone, it is thus imperative for fundamental research into this phenomenon to continue.

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