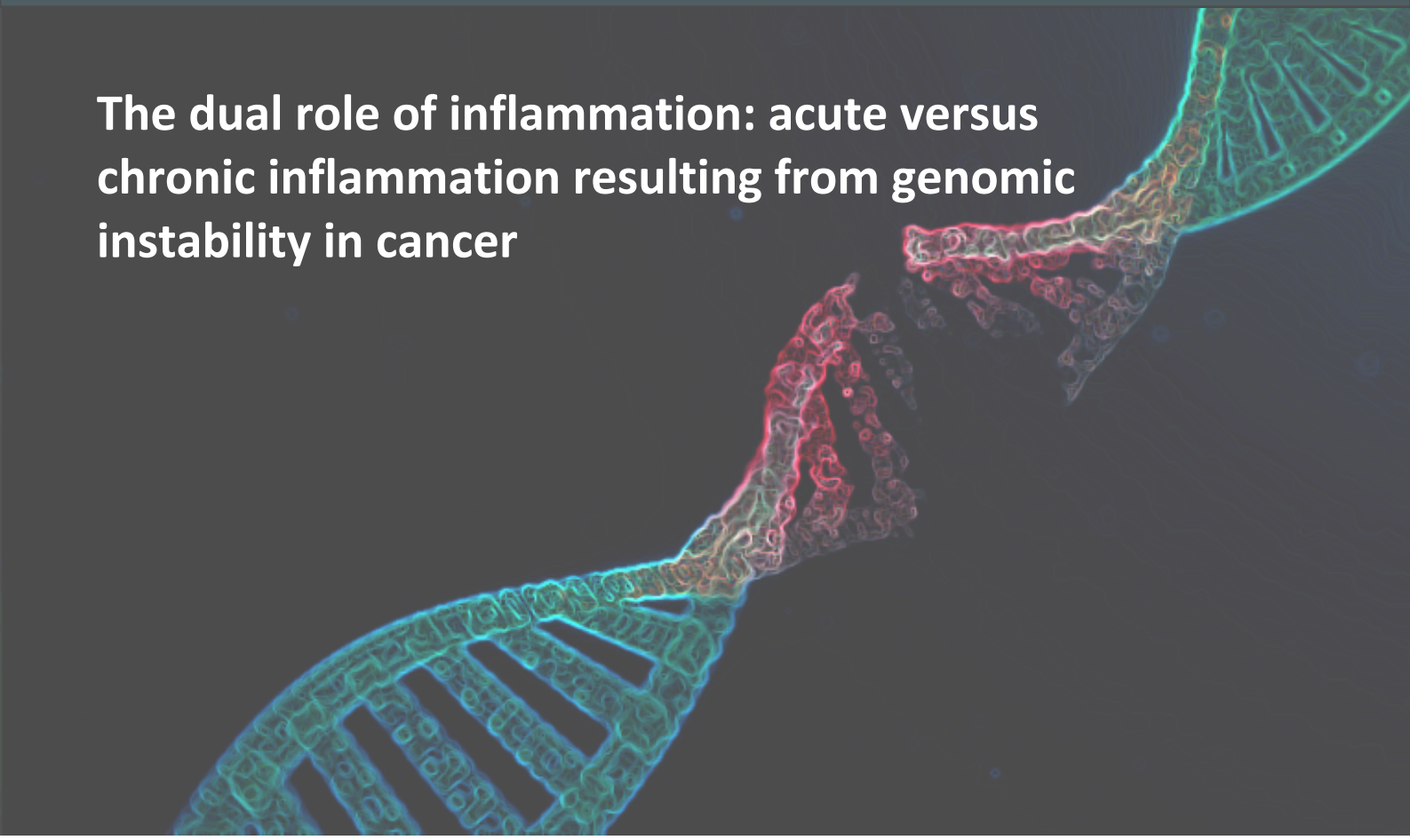
The dual role of inflammation: acute versus chronic inflammation resulting from genomic instability in cancer



Author: Lisanne Koll

S-number: S5004675

Supervisor: Prof. Dr. Marcel van Vugt

University Medical Centre Groningen (UMCG)

Department: Medical Oncology

Date of submission: 29-11-2023

Figure adapted and modified from GowingLife, Oxford, England

university of groningen / faculty of science and engineering

Abstract

Genomic instability is one of the most recently introduced hallmarks of cancer and a common feature of almost all human cancers. Genomic instability involves changes in the genome including structural and numerical alterations. For cancer cells to acquire the necessary set of mutant genes for tumorigenesis, they often boost their mutation rates. This increased mutagenicity is attained through increased responsiveness to mutagenic agents, breakdown of one or more components of the genomic maintenance machinery, or a combination of both factors. A higher mutation rate causes cells to lose their ability to maintain genome integrity, resulting in genome instability.

Increasing evidence has demonstrated the susceptibility of cancer cells to be recognized by the immune system due to defects in genome maintenance. Both genomic and chromosomal instability can lead to the release of DNA into the cytoplasm, both directly or through the formation and rupture of micronuclei. Cytoplasmic DNA is sensed by cytoplasmic sensors such as *cyclic GMP-AMP synthase* (cGAS), which results in a type 1 interferon (IFN) response through the activation of *stimulator of interferon genes* (STING). This type I interferon response induces an acute anti-tumor immune response. However, when acute inflammatory signaling becomes chronic through consistent inflammation, it leads to potential pro-tumorigenic effects, causing aggressive metastatic tumor growth.

A gap persists in our current knowledge regarding the molecular differences between acute and chronic inflammation resulting from genomic instability. This essay aims to investigate the intricate association between genomic instability and inflammation, specifically focusing on understanding the mechanisms through which acute inflammation promotes anti-tumor responses, whereas chronic inflammation promotes pro-tumorigenic immune responses. The mechanisms associated with acute and chronic inflammatory responses in cancer as a result of genomic instability, along with its downstream consequences, will be explored. Moreover, the crosstalk between inflammation and genomic instability will be discussed, focusing specifically on how chronic inflammation contributes to genomic instability. Lastly, the effects of STING agonists as an immunotherapeutic approach to elicit anti-tumor responses will be discussed. Gaining insights into the dual inflammatory effects as a result of genomic instability will allow for better understanding, improvement, and safety of future drug design for STING-mediated cancer treatments.

Nomenclature

ABZI	-	Amidobenzimidazole
AID	-	Activation-induced cytidine deaminase
BRCA1	-	*Breast cancer gene 1*
BRCA2	-	*Breast cancer gene 2*
CDN	-	Cyclic dinucleotide
$CD8^+$ T cells	-	Cytotoxic T lymphocytes
cGAMP	-	2',3'- cyclic GMP-AMP
cGAS	-	*Cyclic GMP-AMP synthase*
CIN	-	Chromosomal instability
DC	-	Dendritic cell
diABZI	-	Dimer ABZI
DSB	-	Double-strand break
dsDNA	-	Double-strand DNA
HR	-	Homologous recombination
ICI	-	Immune-checkpoint inhibitor
IFN	-	Interferon
IKK	-	IkB kinase
IL-6	-	Interleukin 6
iNOS	-	Inducible nitric oxide synthase
MDSC	-	Myeloid-derived suppressor cell
NF-kB	-	Nuclear factor (NF) - kB
NK cell	-	Natural killer cell
PARPi	-	Poly-ADP ribose polymerase inhibitor
PD-L1	-	Programmed dead-ligand 1
RIG-I	-	*Retinoic acid-inducible gene I*
SASP	-	Senescence-associated secretory phenotype
SSB	-	Single-strand break
STAT3	-	Signal transducer and activation of transcription 3
STING	-	*Stimulator of interferon genes*
TBK1	-	TANK-binding kinase-1
TME	-	Tumor microenvironment
TNF-α	-	Tumor necrosis factor alpha

Table of contents

1. Introduction

One of the most recently introduced hallmarks of cancer is genomic instability, a common feature of almost all human cancers[1,2]. Various mechanisms contribute to the occurrence of genomic instability, including impaired mitotic chromosome separation, replication stress induced by oncogenes, collision events between replication and transcription machinery, inherited or acquired defects in DNA repair, and DNA-damaging anticancer therapies[3]. Genomic instability involves changes in the genome including structural and numerical alterations. During the development of structural genomic instability, random mutations are generated that include chromosomal rearrangements[1]. However, numerical abnormalities result in the gain or loss of entire chromosomes, also termed chromosomal instability (CIN), which leads to aneuploidy[2,3]. For cancer cells to acquire the necessary set of mutant genes for tumorigenesis, they often boost their mutation rates[2,4]. This increased mutagenicity is attained through enhanced responsiveness to mutagenic agents, breakdown of one or more components of the genomic maintenance machinery, or a combination of both factors[1,5]. A higher mutation rate causes cells to lose genome integrity maintenance, resulting in genome instability. Overall, genomic instability and resulting mutability equip cancer cells with genetic alterations that drive tumor progression[1].

1.1 Genome maintenance

To maintain the integrity of the genome, cells are dependent on mechanisms that regulate DNA repair and replication[7]. Genome maintenance systems that identify and resolve DNA defects ensure that spontaneous mutation rates are typically low in each round of cell division[1]. Every stage of the cell cycle is monitored for mistakes by mechanisms such as cell cycle checkpoints, and DNA repair systems[7,8]. Mutations related to DNA repair genes have been extensively studied as a significant key mechanism underlying genomic instability[9]. A widely described example of mutations in DNA repair genes linked to predisposed cancer development is mutations in *breast cancer genes 1 (BRCA1)* and *2 (BRCA2)*[2]. BRCA1 and BRCA2 play crucial roles in DNA damage repair via homologous recombination (HR). When BRCA1 and/or BRCA2 are inactivated, homologous double-stranded break (DSB) repair is compromised, a mechanism crucial for maintaining genome integrity[10].
Increasing evidence has demonstrated the susceptibility of cancer cells to be recognized by the immune system due to defects in genome maintenance. For example, cancer cells are recognized by immune cells through the presentation of neo-antigens on the cell surface induced by genomic rearrangements or point mutations[11]. However, tumor cells evade immune clearance through evolving mechanisms that inhibit inflammatory signaling[3]. Contrastingly, multiple studies have indicated that genomically unstable cancers are associated with a poor prognosis. This is accompanied by varying degrees of immunogenicity ranging from moderate to high[12–14]. Therefore, it is important to investigate the effect of genomic instability on inflammation and its role in downstream processes.

1.2 Genomic instability as a source of cytoplasmic DNA

Interestingly, cytoplasmic DNA can also induce an immune response and trigger the activation of immune cells[15]. Upon genomic instability or CIN, DNA can be released from the nucleus into the cytoplasm through micronuclei formation, which are structures that contain extranuclear DNA[3,16]. Subsequently, these micronuclei are ruptured through collapsing of the micronucleus membrane. Disrupted micronuclei are associated with defects in replication and transcription, which result in high levels of DNA damage. Therefore, micronuclei are an important source of cytoplasmic DNA[3]. In addition, DNA can also be released directly from the nucleus. For example, through the release of single-stranded DNA fragments from stalled replication forks in cells lacking SAMHD1, which is a replication fork-protector factor[17]. Another source of cytoplasmic DNA is the direct release of

mitochondrial DNA and RNA upon mitochondrial DNA damage[18]. Cytoplasmic DNA is subsequently sensed by cytoplasmic sensors such as *cyclic GMP-AMP synthase* (cGAS) and *retinoic acid-inducible gene I* (RIG-1), which are DNA sensors associated with genomic instability[19]. Both cGAS and RIG-1 induce an inflammatory signaling response leading to the translation and release of type I Interferons (IFN) through the activation of *stimulator of interferon genes* (STING). This type I interferon response induces an acute anti-tumor immune response[3]. However, when acute inflammatory signaling becomes chronic through consistent inflammation, it leads to potential pro-tumorigenic effects, causing aggressive metastatic tumor growth[20]. Together, this suggests the dual nature of inflammation in tumor development and progression as a consequence of genomic instability.

A gap persists in our current knowledge regarding the molecular differences between acute and chronic inflammation resulting from genomic instability and their respective downstream consequences. This essay aims to investigate the intricate association between genomic instability and inflammation, specifically focusing on understanding the mechanisms through which acute inflammation promotes anti-tumor responses, whereas chronic inflammation promotes pro-tumorigenic immune responses. Together, this essay is expected to contribute novel perspectives to the complex interplay between genomic instability, acute and chronic inflammation, and their distinct roles in cancer development. Moreover, the effects of existing therapeutic approaches are discussed to mimic the anti-tumor effects of acute inflammation.

2. Anti-tumor roles of acute inflammation as a result of genomic instability in cancer

Acute inflammation is defined as an immediate response to sudden damage in the body that lasts for only a short period (hours/day). Upon injury, soluble mediators, such as chemokines, cytokines, and acute-phase proteins, are released to recruit innate immune cells, playing an active role in acute inflammation[21]. In the context of genomic instability, an acute inflammatory response is associated with acute elevated levels of DNA damage, triggered by external stimuli[3]. However, the specific nature of the acute inflammatory response that results from genomic instability remains unclear. In this chapter, the possible mechanisms associated with the induction of an acute inflammatory response in cancer as a result of genomic instability are discussed, along with its downstream consequences.

2.1 DNA sensing mechanism of the cGAS/STING pathway

Recognition of DNA as an immune-stimulating molecule represents an evolutionarily conserved mechanism that allows the initiation of a rapid innate immune response against microbial pathogens[22]. In sharp contrast to normal cells, cancer cells are often rich in cytosolic double-stranded DNA (dsDNA), which can be derived from various sources such as exogenous, mitochondrial, and genomic origins[22]. As mentioned in the introduction, genomic instability can cause the release of cytoplasmic DNA from micronuclei, stalled replication forks, and direct release of mitochondrial DNA as a result of mitochondrial damage. Cytoplasmic DNA can be sensed by DNA sensors such as cGAS or RIG-1, which are associated with genomic instability. DNA sensing by cGAS is followed by the cGAS/STING inflammatory signaling pathway[19].

Originally, the cGAS/STING pathway served as an evolutionarily preserved protective mechanism against pathogenic infections, as both homologs of cGAS and STING were found to exist in eukaryotes and prokaryotes[23]. cGAS/STING acts as a defense mechanism through the activation of immune surveillance by mounting an immune response against microbial pathogens (e.g., viruses) invading human cells[24]. Over the last few years, the expanded role of cGAS/STING signaling in other mechanisms such as cancer, autophagy, and DNA repair has been more widely described[22]. In short, cGAS is activated in the presence of cytoplasmic DNA or RNA by forming a cGAS-DNA dimer, which leads to a conformational change. In this conformation, cGAS plays a catalytic role in generating a second messenger, 2',3'-cyclic GMP-AMP (cGAMP), by catalyzing ATP and GTP[22]. Subsequently, in the endoplasmic reticulum, cGAMP activates STING, leading to oligomerization and inducing the formation of tetramers that are translocated to the Golgi compartments[25,26]. In the Golgi, STING is palmitoylated and recruits and activates TANK-binding kinase-1 (TBK1), leading to the phosphorylation of STING. In turn, phosphorylated STING recruits interferon regulatory factor 3 (IRF3) for activation[27,28]. IRF3 is translocated to the nucleus, where it performs its transcription function by facilitating the expression of IFN1 and immune-stimulated genes[22,24]. At the same time, STING activates IkB kinase (IKK) which mediates the expression of nuclear factor kB-driven (NF-kB) inflammatory genes[22]. The above-described mechanism of cGAS/STING signaling upon the generation of cytoplasmic DNA from various sources is illustrated in Figure 1.

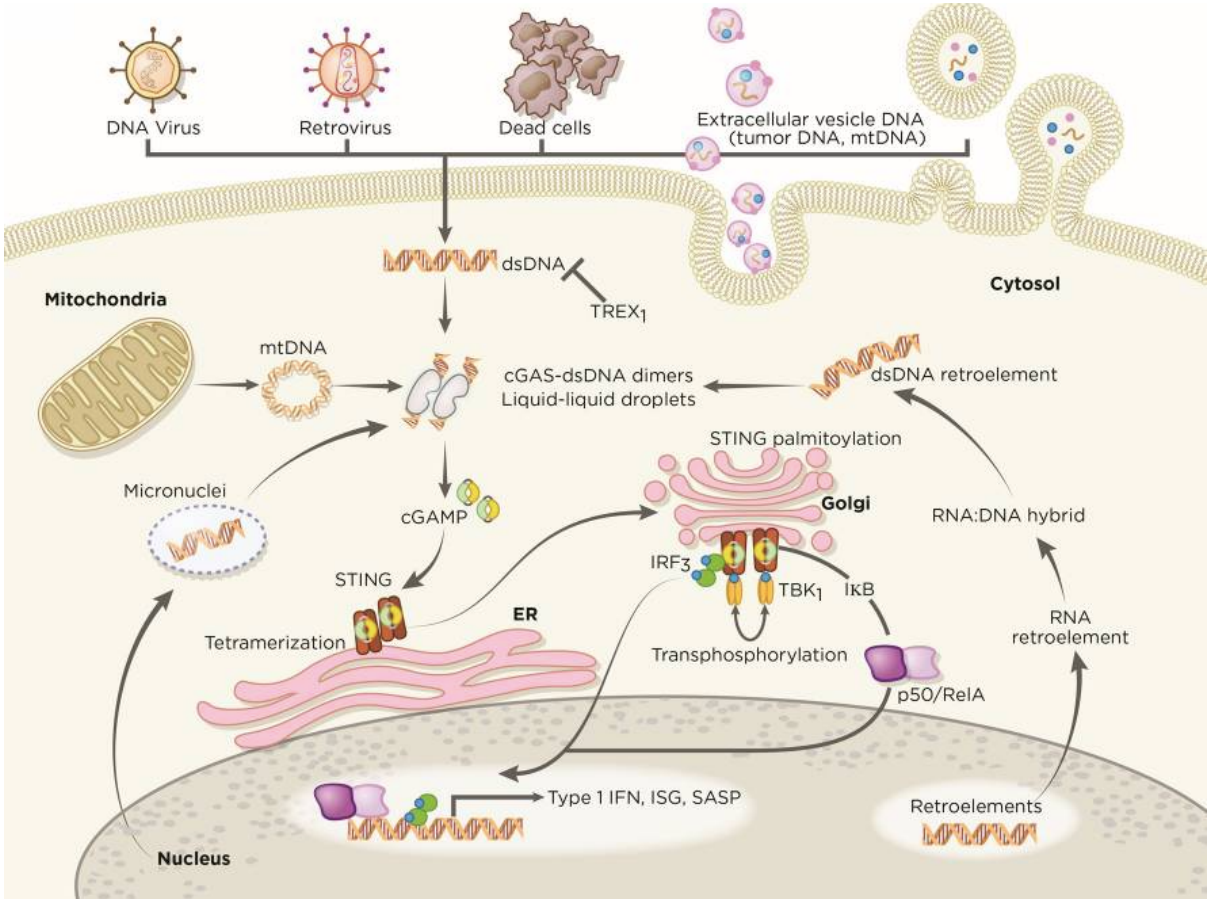


Figure 1: Various sources generate cytoplasmic DNA which activates the cGAS/STING pathway leading to the expression of type 1 interferon and immune-stimulated genes (Figure retrieved from Won & Bakhoum et al. 2020[22]).

2.2 Acute genomic stressors inducing acute inflammation

Notably, cGAS/STING signaling establishes a link between genomic instability and the recognition of self-DNA, which leads to an innate immune response triggered by cytoplasmic DNA originating from either ruptured micronuclei or DNA directly secreted from the nucleus[15]. Radiotherapy, genotoxic chemotherapy (e.g., cisplatin), and intrinsic DNA damage caused by inactivated DNA repair proteins, are all acute genomic stressors that cause DNA damage[22,29]. Importantly, both radiotherapy and genotoxic chemotherapy have been demonstrated to produce micronuclei as a result of their DNA-damaging mechanisms. Subsequently, this leads to the release of cytosolic DNA, which activates the cGAS/STING pathway, resulting in inflammatory signaling and suppression of tumor growth[30,31]. Moreover, inhibition of poly-ADP ribose polymerase (PARPi), which is normally associated with DNA repair mechanisms, leads to genomic instability as its inhibition results in unrepaired DNA breaks. DNA damage induced by PARPi also induces the formation of micronuclei and cellular DNA, which subsequently activate the cGAS/STING pathway in tumor cells and neighboring dendritic cells (DCs), leading to tumor cell death[32]. An example of an intrinsic DNA damaging process caused by inactivated DNA repair proteins is the loss of the *BCRA2* gene, which is involved in DSB repair through HR. Inactivation of BRCA2 induces the formation of cGAS-positive micronuclei, leading to the activation of the cGAS/STING pathway. As a result, a type I interferon response is induced, which is associated with limited tumor cell viability in an experimental setting[33]. Together, all the above-described acute genomic stressors promote anti-tumor effects through type 1 IFN signaling as a result of acute activation of the cGAS/STING pathway. The molecular mechanisms underlying this phenomenon are discussed in more detail in the next paragraph.

2.3 Anti-tumor effects by acute activation of cGAS/STING pathway

In preneoplastic cells, detection of cytosolic DNA by the cGAS/STING pathway plays an important role in initiating immune activation, followed by an anti-tumor response that influences both tumor cell-intrinsic mechanisms and extracellular mechanisms involving neighboring cells[22]. This anti-tumor response is mediated through IFN signaling by immune infiltrating cells, such as DCs, natural killer (NK) cells, and cytotoxic T lymphocytes ($CD8^+$ T cells)[22,34]. In addition to IFN signaling, activation of the cGAS/STING pathway induces the secretion of growth factors, proteases, pro-inflammatory cytokines, and chemokines, collectively termed senescence-associated secretory phenotype (SASP) (Figure 2). SASPs also play an important role in restricting tumorigenesis[35,36]. Inflammatory products of the cGAS/STING pathway can either inhibit tumor growth via tumor-cell intrinsic mechanisms or recruit other immune cells for antitumor killing[22,36]. Moreover, through an unclear process, tumor DNA can also be released into the cytosol of DCs[37]. Subsequently, cGAS/STING signaling is also activated in DCs leading to IFN secretion and tumor antigen presentation, resulting in the activation of NK and $CD8^+$ T cells to generate an anti-tumor killing response[37,38]. Lastly, tumor cells are also able to export extracellular cGAMP, which is imported into DCs by the folate transporter SLC19A1, leading to cGAS/STING signaling in DCs, which subsequently induces DC maturation and recruitment of effector immune cells to eliminate tumors[22,39] (Figure 2). Taken together, acute activation of the cGAS/STING pathway by acute genomic stressors leads to advantageous anti-tumor effects. However, upon chronic cytoplasmic DNA release, cancer cells have developed strategies to overcome the tumor-suppressive effects of acute activation by driving tumorigenic programs[22]. The mechanisms associated with tumorigenesis as a result of chronic inflammation are discussed in the next chapter.

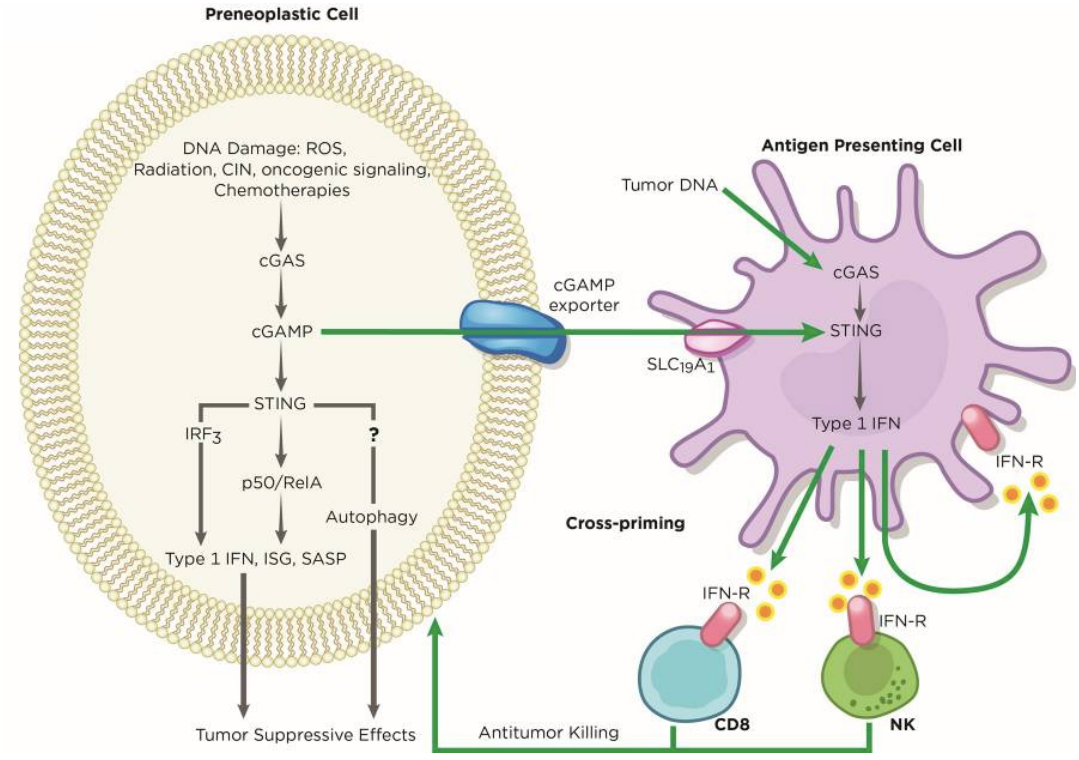


Figure 2: Autonomous and non-autonomous anti-tumor mechanisms of the cGAS/STING pathway activated by acute genomic stressors (Figure retrieved from Won & Bakhoum et al. 2020 [22]).

3. Tumor-promoting roles of chronic inflammation as a result of genomic instability in cancer

Chronic inflammation is characterized by an ongoing, active inflammatory response, resulting in tissue destruction and irreversible tissue remodeling. Diverse transcription factors, cytokines, and chemokines play roles in the pathogenesis of chronic inflammation and are involved in carcinogenesis[39]. In the previous chapter, it was mentioned that when cytoplasmic DNA is released over a long period, cancer cells develop strategies to overcome the tumor-suppressive effects of acute inflammation. Importantly, increasing evidence demonstrates that long-term signaling of the cGAS/STING pathway is associated with tumor-promoting functions and induction of an immunosuppressive tumor microenvironment (TME)[22]. However, it remains unclear how the tumor-promoting role of cGAS/STING signaling outweighs its anti-tumorigenic activity[29]. In this chapter, evidence demonstrating tumor-promoting effects as a consequence of chronic inflammation is discussed.

3.1 Chronic inflammation as a result of numerical genomic instability

Based on increasing evidence, it is considered that the cGAS/STING pathway might play a dual role in tumor development[20,40]. Typically, genomically unstable and aggressive tumors can hijack cGAS/STING signaling, promoting carcinogenesis[37,41]. Overall, it is believed that chronic STING signaling primarily contributes to malignant transformation by generating an immunosuppressive TME and supporting tumor metastasis[41]. As mentioned earlier, genomic instability involves changes in the genome including structural and numerical alterations. The effects of chronic inflammation on CIN, which involves numerical alterations, are becoming clearer. CIN has the potential to release DNA into the cytoplasm after micronuclei rupture, which initiates chronic inflammatory signaling through consistent activation of the cGAS/STING pathway. Consequently, the downstream NF-kB pathway is also consistently activated, leading to enhanced migration and cancer cell invasion[20,42]. In Chapter 2.1, it was described that in addition to the expression of IFN1 induced by STING-mediated IRF3 activation, STING also activates IkB kinase (IKK), which mediates the expression of NF-kB-driven inflammation genes[22]. The NF-kB pathway is well-known for its role in inflammation and tumor progression, which supports cell survival and produces a variety of cytokines and chemokines[43]. Moreover, it has recently been reported that inactivation of the cGAS/STING pathway specifically hinders the survival capability of triple-negative breast cancer cells exhibiting CIN. The authors demonstrated that CIN initiates interleukin 6- (IL-6) signal transducer and activation of transcription 3 (STAT3) (IL-6-STAT3)- mediated signaling, which relies on the cGAS/STING pathway and downstream non-canonical NF-kB pathway, suggesting its pro-tumorigenic traits[44]. The findings of this study revealed that cells exhibiting CIN depend on a cGAS-mediated inflammatory response needed for their survival, which might explain why cGAS and STING are hardly inactivated in primary cancers[20,45].

3.2 Chronic inflammation as a result of structural genomic instability

Nevertheless, it remains unclear whether the above-described mechanisms of chronic inflammatory signaling induced by CIN and its pro-tumorigenic consequences are triggered by consistent structural alterations associated with genomic instability. Evidence suggests the involvement of similar processes. Importantly, Vasiyani et al. described STING-mediated activation of NF-kB in triple-negative breast cancer cells as a consequence of doxorubicin treatment[46]. Doxorubicin is a genotoxic chemotherapeutic that intercalates into the DNA, leading to DSBs, impaired DNA replication and transcription, and cell cycle arrest[47]. The authors demonstrated that NF-kB activation resulted in IL-6 expression and activated pSTAT3, subsequently leading to enhanced cell survival and programmed dead-ligand 1 (PD-L1) expression in multiple-negative breast cancer cells. Enhanced PD-L1 expression

leads to the evasion of immune suppression. Moreover, STING expression appeared to be positively correlated with both IL-6 and PD-L1 expression in breast cancer patients. Patients with high STING expression were correlated to poor survival during chemotherapy, whereas low STING expression during chemotherapy was associated with better survival[46]. Importantly, IL6/STAT3 plays a significant role in tumor progression across various solid tumor types by inducing angiogenesis and epithelial-to-mesenchymal transition[48]. In general, higher cGAS/STING signaling was demonstrated to predict poor prognosis in patients with various cancer types in a pan-cancer study[49].

Similar to the intrinsic activation of cGAS/STING, cancer cell metastasis can also be induced in a non-autonomous manner upon chronic activation. This is particularly done by cGAMP, which has been described to transfer from the tumor cell to astrocytes through gap junctions, thereby promoting IFN and NF-kB signaling, ultimately inducing brain metastasis[50]. Additionally, upregulation of STING has been correlated with increased regulatory T cell infiltration, as well as increased immune-regulatory enzyme indoleamine 2,3-dioxygenase (IDO). IDO can exhibit tumor immune evasion and impede T cell proliferation[51,52]. Moreover, local ablative radiotherapy induced STING-mediated immunosuppressive effects through myeloid-derived suppressor cell (MDSC) recruitment via the CCR2 pathway[53]. Another example was described by Ahn et al. where they observed leakage of nuclear DNA into the cytosol of skin cancer cells following chronic administration of 7,12-dimethylbenz(a)anthracene (skin cancer carcinogen). Subsequently, this leads to STING-dependent cytokine production, skin inflammation, and eventually skin carcinogenesis in a mouse model[54]. Figure 3 presents an overview of the proposed pro-tumorigenic mechanisms induced by chronic inflammatory cGAS/STING signaling as a consequence of genomic instability.

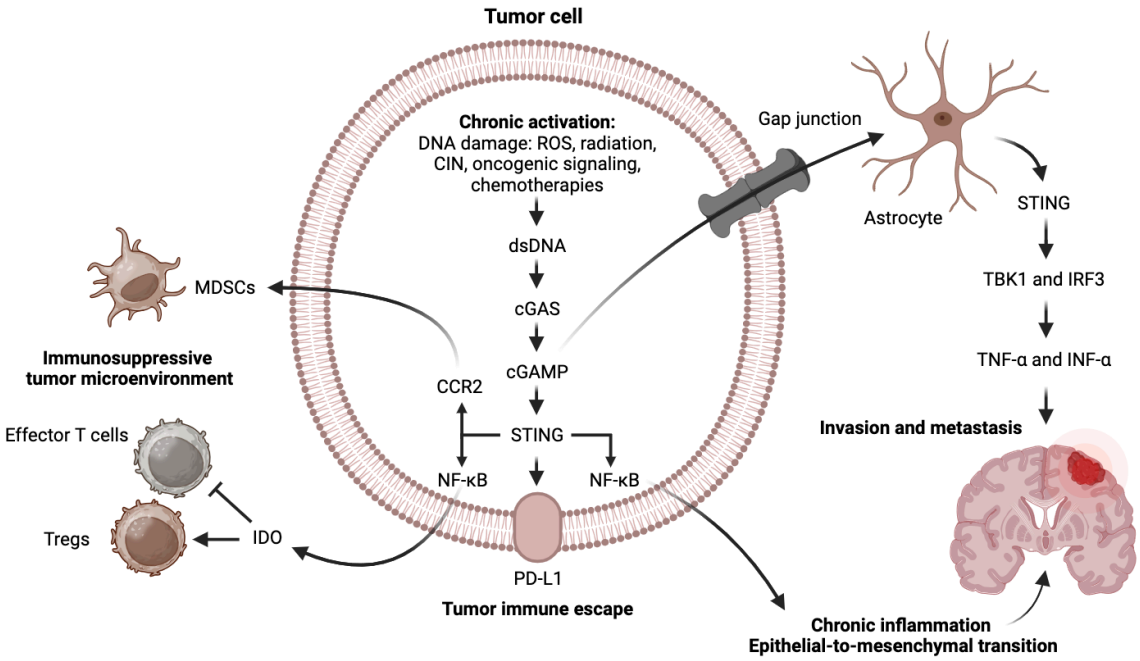


Figure 3: Proposed tumor-promoting roles by chronic activation of cGAS/STING signaling as a result of genomic instability. Retrieved and modified from Gan et al. (2022)[55] and Jiang et al. (2020)[56]. Created with Biorender.com.

Overall, the studies described above suggest that cGAS/STING signaling plays a complex role in cancer. Many more studies have provided evidence for the immunosuppressive effects associated with chronic STING signaling, however; none have provided a comprehensive depiction of the entire mechanism. Interestingly, chronic inflammation can also contribute to the induction of genomic instability, generating a positive feedback loop between inflammation and genomic instability, as discussed in the next chapter.

4. Crosstalk: How chronic inflammation results in genomic instability

In previous chapters, it was described in detail how genomic instability contributes to both acute and chronic inflammatory signaling in cancer, leading to different cellular outcomes. Importantly, both DNA damage and inflammation contribute to each other and play a collective role in cancer development. Increasing evidence has demonstrated that chronic inflammation contributes to DNA damage and genomic instability[5758]. In this chapter, the crosstalk between inflammation and genomic instability will be discussed, focusing specifically on how chronic inflammation contributes to genomic instability and subsequently results in tumor development and/or tumor progression.

Inflammation promotes mutagenesis by generating reactive oxygen and nitrogen species (RONS), potentially damaging DNA. Simultaneously, DNA damage can exacerbate inflammation, suggesting a positive feedback loop. This process is carefully regulated by various DNA repair pathways, cellular signals, and transcription factors. As a result of the complex interconnections between DNA damage, DNA repair, and inflammation, dysregulation of these mechanisms can easily lead to cancer development. Most DNA damage induced by inflammation is caused by RONS, which was originally produced by immune cells to eliminate pathogens, however; RONS can also damage adjacent human cells[57]. RONS molecules, such as nitric oxide and superoxide, are produced by innate immune cells such as neutrophils and macrophages[59,60]. Intracellular pro-inflammatory cytokines can also stimulate the production of RONS[61]. For example, tumor necrosis factor alpha (TNF-α) and IL-6, which are produced by the NF-kB pathway (described in the previous chapter to play an important role in chronic inflammation induced by genomic instability), can induce inducible nitric oxide synthase (iNOS) expression. iNOS levels are also promoted by increased hypoxia-inducible factor-1 (HIF-1) expression due to inflammation-induced hypoxia[62,63]. DNA is damaged by RONS through chemical modifications such as deamination, nucleobase oxidation, alkylation, halogenation, and breaks (DBSs and SSBs) in the strands of the phosphodiester backbone[57]. There are several DNA repair pathways that repair these types of damages to balance inflammation, DNA damage, and DNA repair. DNA is important during inflammation due to the production of large amounts of reactive chemicals and stimulation of cellular proliferation, which aims to regenerate damaged tissue[64,65]. Inflammation-induced DNA repair is mostly addressed by HR, base excision repair, and direct reversal repair pathways[57]. However, RONS can not only induce DNA damage but can also impair DNA repair activity[66]. Many studies have demonstrated disruption of DNA repair (components) as a consequence of inflammation[57,67–69]. Failure to repair DNA damage subsequently results in mutations initiating cancer development[57].

As described in the previous chapter, chronic activation of the cGAS/STING pathway can lead to activation of downstream NF-kB. In inflammation-associated carcinogenesis, NF-kB is an important key molecule that regulates both tumor progression and tumor promotion[70]. In addition to tumorigenic effects, such as epithelial to mesenchymal transition, cell growth, proliferation, angiogenesis, invasion, and tumor cell survival, NF-kB also regulates activation-induced cytidine deaminase (AID), which is directly involved in genomic instability[70,71]. AID deaminates cytidine, which leads to mismatch or base-excision repair, subsequently resulting in mutations and eventually genomic instability[72]. Under physiological conditions, AID is only expressed in B cells and plays an important role in class-switch recombination and somatic hypermutation of immunoglobulin genes, resulting in immune diversity[70,73]. However, AID also mutates some non-Ig genes, and several studies have demonstrated that high AID expression is associated with chromosomal translocations and various oncogenic mutations in leukemia and B cell lymphoma's[74–76]. Moreover, AID expression resulting from chronic inflammation has been demonstrated to induce mutations in p53, an important tumor suppressor gene that is critical for maintaining and sensing genomic stability[77,78]. Additionally, chronic inflammation and subsequent DNA damage triggers the expression of both NF-kB and p53. NF-kB also opposes p53's function in maintaining genome stability[58,79]. Subsequently, p53's natural function to

maintain genomic instability is inhibited, leading to exacerbated effects of AID and RONS, preventing apoptosis of possible mutant tumorigenic cells[58]. Lastly, NF-kB signaling reactivates TERT expression, an enzyme involved in maintaining telomere length, preventing replicative senescence, which leads to tumorigenic proliferation and cell survival[80].

Taken together, in the course of chronic inflammation, there is a continuous release of RONS, inhibition of p53, increased AID expression, and reactivation of TERT expression. These factors, independently or in combination, promote genomic instability[81]. A schematic representation of the above-described inflammation-mediated mechanisms contributing to genomic instability is illustrated in Figure 4.

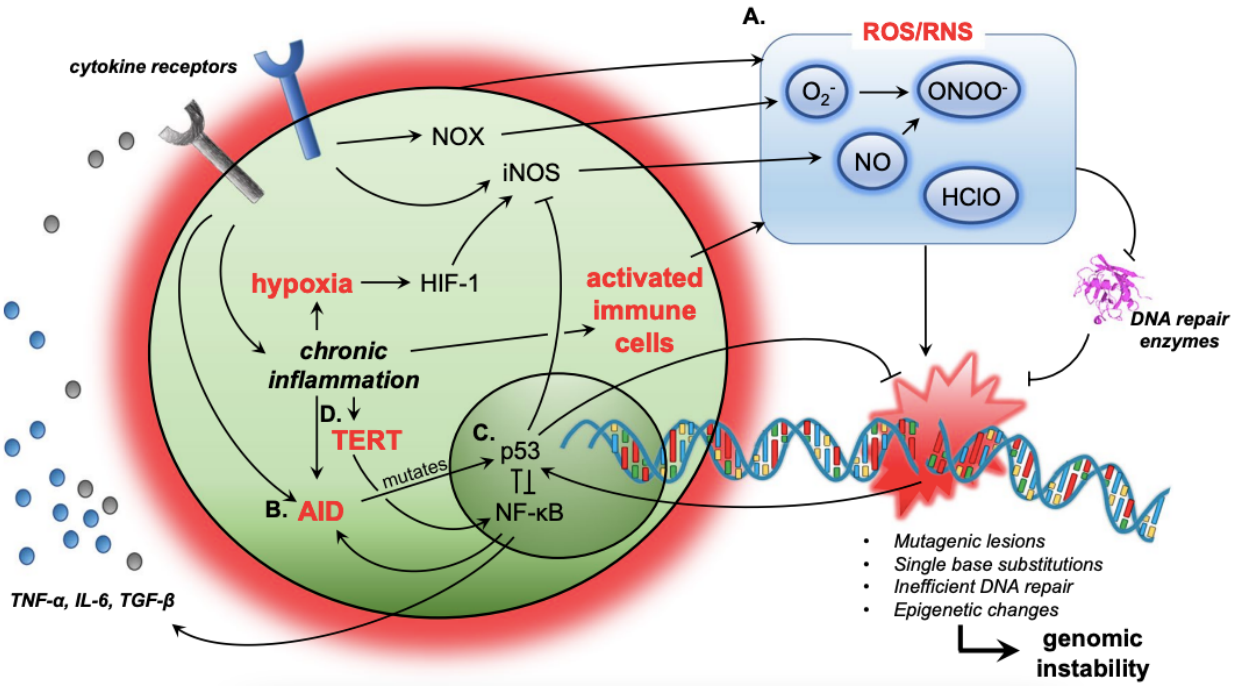


Figure 4: Inflammation-mediated mechanisms contributing to genomic instability. A) Release of RONS by immune cells. During inflammation immune cells express NOX or iNOS leading to the release of RONS. Moreover, intrinsic hypoxia in immune cells also leads to RONS release. RONS lead to DNA damage and inhibition of DNA repair enzymes, giving rise to genomic instability. B). Chronic inflammation induces high levels of AID subsequently mutating p53 inhibiting maintenance of genomic instability. C). Chronic inflammation triggers the expression of both NF-kB and p53. NF-kB in turn also opposes p53's function to maintain genome stability. D). NF-kB signaling reactivates TERT expression, preventing replicative senescence and subsequently leading to tumorigenic proliferation and cell survival. The figure is retrieved from Pua, et al (2020)[58].

5. STING agonists as an immunotherapeutic approach to elicit anti-tumor responses

All previous chapters have highlighted the possible mechanisms underlying acute and chronic inflammation induced by genomic instability, along with how chronic inflammation contributes to genomic instability. In the TME, activation of the cGAS/STING pathway can effectively initiate the cross-priming of tumor-specific antigens and enhance effector T cell infiltration[41]. In this chapter, a therapeutic approach that stimulates this activation to promote anti-tumor effects is discussed.

5.1 Overview of current STING agonists in (pre)-clinical trial

In modulating anti-tumoral responses, the anti-tumor role of cGAS/STING signaling has increased the interest of scientists in developing STING-activating agents (agonists) as a novel cancer immunotherapy[22]. Earlier, it was described that 2'3'-cGAMP is an activator of human STING, initiating a type 1 IFN signaling response. Therefore, most STING agonists are synthetic analogs of 2'3-cGAMP. These analogs include chemical modifications that enhance the anti-tumor efficacy induced by STING by generating a synthetic cGAMP that is resistant to hydrolysis[82,83]. The pharmacological activation of STING has been demonstrated to be an effective immunotherapy for cancer in diverse preclinical models[55]. Over the past years, various STING agonists have been developed. To date, multiple categories of STING stimulants have been generated, including DMXAA and its analogs, cyclic dinucleotides (CDNs) and their derivatives, and small molecule agonists[55]. Since 2019, ten STING agonists have emerged in the clinic, of which only three have released preliminary clinical results. Moreover, phase I or II clinical studies have investigated the effects of STING agonists alone or in combination with immune inhibitors such as anti-CLTA-4 or PD-1/PD-L1 inhibitors[84].

The first-generation STING agonists, MK-1454 and ADU-S100, are CDN-based compounds that are directly injected into tumors. ADU-S100, the first STING agonist in human clinical trials, has shown promising effects in melanoma, colon, and breast cancer models in a STING-dependent manner. ADU-S100 triggers $CD8^+$ T cell responses, leading to tumor regression and long-lasting anti-tumor effects[82]. Unfortunately, a phase 1 clinical trial of ADU-S100 in patients with lymphoma and advanced/metastatic solid tumors showed limited clinical activity, possibly due to its short plasma half-life of approximately 24 minutes. Of note, evidence of immune activation was observed[85]. Therefore, ADU-S100 was evaluated in combination with an anti-PD-L1 antibody. Again, this clinical trial demonstrated limited effects in triple-negative breast cancer and melanoma patients, although better results were obtained compared with monotherapy of ADU-S100[86]. Based on these limited results, enrollment in the ADU-S100 clinical trial is completely suspended[84]. The second STING agonist in clinical trial, MK-1454 showed similar effects. Both monotherapy and combination therapy with anti-PD-L1 (pembrolizumab) demonstrated limited effects in various types of cancer, of which the combined therapy demonstrated slightly better efficacy[87]. MK-1454 treatment was discontinued by 2021. The reason this first generation of STING agonists failed, is possibly due to the low penetration capability of CDN compounds. These compounds are impermeable to cell membranes, resulting in low presence in the cytosol, the location where STING is expressed[85,86,88]. Current drug development of CDN-based STING agonists focuses on designing novel molecules that overcome susceptibility to enzymatic degradation and poor penetration capabilities[84,89].

Currently, the development of amido-benzimidazole (ABZI) as a cancer treatment is a breakthrough in the field of STING agonists. Improvement of the ABZI compound resulted in the development of the ABZI dimer (diABZI), a potent non-CDN STING agonist capable of eradicating tumors in immune-competent mice following intravenous injection. Certainly, diABZI was demonstrated to be the first effective systemic STING agonist along with MSA-2, which facilitates both oral and subcutaneous

administration[90,91]. Under acidic conditions, MSA-2 generates a non-covalent pharmacologically active dimer that selectively promotes pro-inflammatory activation of the immune system in the TME. Using this strategy could prevent normal tissue toxicity due to unspecific STING activation[91]. However, the safety of diABZI and MSA-2 still needs to be considered, and clinical trials are warranted to determine their potential as immunotherapeutic in patients with cancer. Based on the current low clinical response rate, determining which patients might benefit from STING agonists is necessary[56].

5.2 STING agonists combined with existing cancer therapies

As described in previous chapters, cGAS/STING activation has dual inflammatory effects. Therefore, using STING agonists as a monotherapy might induce immunosuppression effects. Hence, combining STING agonists with various anti-tumor therapies might overcome these effects and promote the release and presentation of tumor antigens. Moreover, immune cold tumors lack infiltration of effector cells due to low expression of antigen-presentation markers and low mutational burden, resulting in a poor response to immune checkpoint inhibitors (ICI)[92]. Combination therapies may overcome immune deficiencies and transform immunologically cold tumors into hot tumors[93]. Chronic cGAS/STING signaling upregulates PD-L1, resulting in tumor immune escape (Figure 3). Although preclinical data demonstrated tumor regression of oral cancer when combining anti-PDL1 and STING agonists in mice[94], a combination of the STING agonist ADU-S100 and MK-1454 combined with ICI anti-PD-L1 showed limited clinical efficacy as mentioned above[86,87]. The Immunosuppressive factor IDO is also expressed as a consequence of chronic cGAS/STING activation (Figure 3), suggesting that the combination of STING agonists and IDO inhibitors may also reduce immunosuppressive effects[93]. This treatment combination is currently used in preclinical trials. A combination of diABZI STING agonist and 1-MT IDO inhibitor reduced tumor growth and inhibited MDSC infiltration, leading to effector cell recruitment[95]. Additionally, pharmacological cGAS/STING activation has improved current cancer therapies in experimental settings. cGAMP administration as a STING agonist significantly improved anti-tumor effects and resistance to radiotherapy in a colorectal cancer mouse model[38]. Moreover, brivanib, a stimulator of cGAS, improved the cGAS-mediated anti-tumor effects of genotoxic chemotherapy cisplatin, subsequently improving chemo-resistance[30].

6. Discussion

Through recognition of cytoplasmic DNA as a consequence of DNA damage arising from genomic instability, the cGAS/STING pathway orchestrates the interplay between anti- and pro-tumor effects, thereby exerting a dual impact on tumor tissue. This essay aimed to investigate the intricate association between genomic instability and inflammation, specifically focusing on understanding the mechanisms through which acute inflammation promotes anti-tumor responses, whereas chronic inflammation promotes pro-tumorigenic immune responses.
Although the specific nature of an acute inflammatory response resulting from genomic instability remains somewhat unclear, it can be appreciated that acute genomic stressors play an important role in exerting this process. Acute genomic stressors can be exogenous (e.g., radiotherapy, chemotherapy, PARPi) and endogenous (e.g., inactivated DNA repair proteins) factors, and have been demonstrated to generate micronuclei and promote anti-tumor effects by a type 1 IFN response through cGAS/STING signaling[30–33]. However, it should be noted that not all genomic stressors use the same program to activate STING signaling. For example, chemotherapeutic etoposide triggers IFN secretion via STING in a cGAS-independent manner, whereas radiotherapy and PARPi trigger IFN secretion in a cGAS-dependent manner[96]. Moreover, tumors possessing inherent DNA repair pathway defects are tolerant to exposure to cytoplasmic DNA, possibly inducing immunogenicity in a cGAS/STING/IRF3 dependent mechanism, upregulating PD-L1 expression[97]. Importantly, the basal level of cGAS/STING activation may also have an impact on its anti-tumor effects. For example, it has been demonstrated that repeated low doses of radiotherapy induce anti-tumor effects via IFN signaling through cGAS/STING. while high doses of radiotherapy activate TREX1 production, an exonuclease degrading cytoplasmic DNA. TREX1 activation subsequently inhibits anti-tumor immune effects[98].

Increasing evidence demonstrates that chronic cGAS/STING activation is associated with tumor-promoting functions and induction of an immune-suppressive TME[22]. Although the exact mechanisms and a comprehensive depiction of the entire process of chronic inflammation resulting from genomic instability remain to be determined, evidence suggests pro-tumorigenic NF-κB-mediated signaling downstream of cGAS/STING[46]. How cancer cells change the downstream circuitry of STING to mediate pro-tumorigenic effects and whether the different cellular effects vary between different cell types remain important unanswered questions. It has been hypothesized that the levels of STING expression may be involved in this transformation. Importantly, the intensity of STING signaling was shown to determine the initiation of apoptotic programs in T cells and macrophages, suggesting that adjusting STING activation may influence the selection of distinct downstream effector programs[99]. Moreover, STING activation can lead to both immune-supporting and immune-suppressive cell recruitment. However, it remains unclear which immune modulations dominate in the context of various tumor types[41]. Adding further intricacy to the narrative, chronic inflammation also contributes to genomic instability, suggesting a positive feedback loop between genomic instability and acute and chronic inflammation (Figure 5).

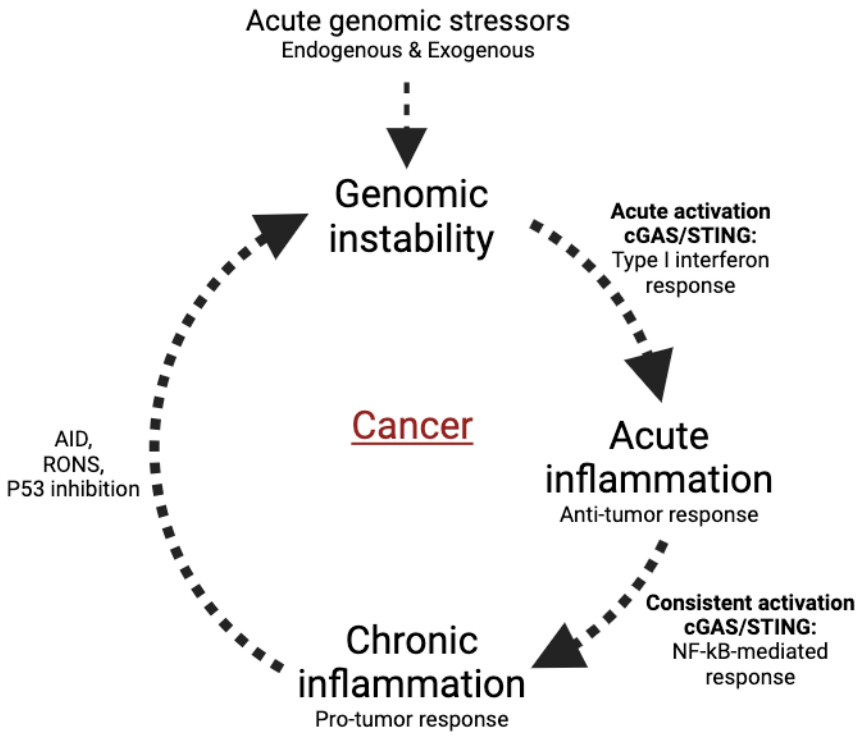


Figure 5: A proposed simplified representation of the intricate relationship between genomic instability and acute and chronic inflammation in cancer. (Figure created with Biorender.com)

The dual inflammatory effects resulting from genomic instability are of great importance for cancer therapeutics. This suggests that using STING agonists as an immunotherapeutic cancer treatment in the clinic is challenging and should be carefully considered. Currently, clinical trials utilize STING agonists to elicit anti-tumor effects[22,84]. However, consistent STING signaling by STING agonists may deteriorate clinical outcomes when tumors have already exploited STING signaling to promote malignant programs and inhibit anti-tumor effects[22]. This might also explain the pre-existing resistance and lack of clinical effects of STING agonists in late-stage tumors. In turn, STING antagonists may be a promising therapeutic approach to attenuate metastasis in these types of cancers[100]. As cGAS activates STING to drive tumorigenesis, cGAS inhibitors may also be a promising approach to repeal cGAS/STING signaling in late-stage tumors[101]. Altogether, careful patient selection is required to identify which patients benefit from STING agonists or whether they may benefit more from STING antagonists. This therapeutic response is dictated by the degree of STING activation, tumor stage, and CIN state[22]. CIN status may be an important biomarker for STING agonist administration, as high CIN levels are correlated with chronic STING activation and poor patient prognosis[20].

Overall, further exploration of the molecular mechanisms underlying cGAS/STING signaling is warranted to reveal the details of the dual role of STING-mediated inflammatory responses in various cancer types. Gaining insights into these mechanisms subsequently provides a better understanding, improvement, and safety of future drug designs for activating or inhibiting STING-mediated cancer treatment.

Literature

1. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: The next generation. *Cell* vol. 144 Preprint at https://doi.org/10.1016/j.cell.2011.02.013 (2011).
2. Negrini, S., Gorgoulis, V. G. & Halazonetis, T. D. Genomic instability an evolving hallmark of cancer. *Nature Reviews Molecular Cell Biology* vol. 11 Preprint at https://doi.org/10.1038/nrm2858 (2010).
3. Chen, M., Linstra, R. & van Vugt, M. A. T. M. Genomic instability, inflammatory signaling and response to cancer immunotherapy. *Biochimica et Biophysica Acta - Reviews on Cancer* vol. 1877 Preprint at https://doi.org/10.1016/j.bbcan.2021.188661 (2022).
4. Salk, J. J., Fox, E. J. & Loeb, L. A. Mutational Heterogeneity in Human Cancers: Origin and Consequences. *Annual Review of Pathology: Mechanisms of Disease* vol. 5 Preprint at https://doi.org/10.1146/annurev-pathol-121808-102113 (2010).
5. Jackson, S. P. & Bartek, J. The DNA-damage response in human biology and disease. *Nature* vol. 461 Preprint at https://doi.org/10.1038/nature08467 (2009).
6. Moon, J. J., Lu, A. & Moon, C. Role of genomic instability in human carcinogenesis. *Experimental Biology and Medicine* vol. 244 Preprint at https://doi.org/10.1177/1535370219826031 (2019).
7. Waters, R. Maintaining genome integrity. *EMBO Rep* **7**, (2006).
8. Barnum, K. J. & O'Connell, M. J. Cell cycle regulation by checkpoints. *Methods in Molecular Biology* vol. 1170 Preprint at https://doi.org/10.1007/978-1-4939-888-2_2 (2014).
9. Nowell, P. C. The clonal evolution of tumor cell populations. *Science (1979)* **194**, (1976).
10. Jasin, M. & Rothstein, R. Repair of strand breaks by homologous recombination. *Cold Spring Harb Perspect Biol* **5**, (2013).
11. Germano, G. *et al.* Inactivation of DNA repair triggers neoantigen generation and impairs tumour growth. *Nature* **552**, (2017).
12. Gemoll, T., Auer, G., Ried, T. & Habermann, J. K. Genetic instability and disease Prognostication. in *Recent Results in Cancer Research* vol. 200 (2015).
13. Jenzer, M. *et al.* The BRCA2 mutation status shapes the immune phenotype of prostate cancer. *Cancer Immunology, Immunotherapy* **68**, (2019).
14. Kang, K. *et al.* Genomic instability in lower-grade glioma: Prediction of prognosis based on lncRNA and immune infiltration. *Mol Ther Oncolytics* **22**, (2021).
15. MacKenzie, K. J. *et al.* CGAS surveillance of micronuclei links genome instability to innate immunity. *Nature* **548**, (2017).
16. Hatch, E. M., Fischer, A. H., Deerinck, T. J. & Hetzer, M. W. Catastrophic Nuclear Envelope Collapse in Cancer Cell Micronuclei. *Cell* **154**, (2013).
17. Coquel, F. *et al.* SAMHD1 acts at stalled replication forks to prevent interferon induction. *Nature* **557**, (2018).
18. Tigano, M., Vargas, D. C., Tremblay-Belzile, S., Fu, Y. & Sfeir, A. Nuclear sensing of breaks in mitochondrial DNA enhances immune surveillance. *Nature* **591**, (2021).
19. Chabanon, R. M. *et al.* Targeting the DNA damage response in immuno-oncology: developments and opportunities. *Nature Reviews Cancer* vol. 21 Preprint at https://doi.org/10.1038/s41568-021-00386-6 (2021).
20. Bakhoum, S. F. *et al.* Chromosomal instability drives metastasis through a cytosolic DNA response. *Nature* **553**, (2018).
21. Hannoodee, S. & Nasuruddin, D. N. *Acute Inflammatory Response*. (2023).
22. Won, J. K. & Bakhoum, S. F. The cytosolic DNA-sensing cGAS–sting pathway in cancer. *Cancer Discovery* vol. 10 Preprint at https://doi.org/10.1158/2159-8290.CD-19-0761 (2020).
23. Wu, X. *et al.* Molecular evolutionary and structural analysis of the cytosolic DNA sensor cGAS and STING. *Nucleic Acids Res* **42**, (2014).

24. Sun, L., Wu, J., Du, F., Chen, X. & Chen, Z. J. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science (1979)* **339**, (2013).
25. Zhao, B. *et al.* A conserved PLPLRT/SD motif of STING mediates the recruitment and activation of TBK1. *Nature* **569**, (2019).
26. Shang, G., Zhang, C., Chen, Z. J., Bai, X. chen & Zhang, X. Cryo-EM structures of STING reveal its mechanism of activation by cyclic GMP–AMP. *Nature* **567**, (2019).
27. Zhang, C. *et al.* Structural basis of STING binding with and phosphorylation by TBK1. *Nature* **567**, (2019).
28. Mukai, K. *et al.* Activation of STING requires palmitoylation at the Golgi. *Nat Commun* **7**, (2016).
29. Reisländer, T., Groelly, F. J. & Tarsounas, M. DNA Damage and Cancer Immunotherapy: A STING in the Tale. *Molecular Cell* vol. 80 Preprint at https://doi.org/10.1016/j.molcel.2020.07.026 (2020).
30. Liu, H. *et al.* Pharmacological boosting of cGAS activation sensitizes chemotherapy by enhancing antitumor immunity. *Cell Rep* **42**, (2023).
31. Yang, C., Liang, Y., Liu, N. & Sun, M. Role of the cGAS-STING pathway in radiotherapy for non-small cell lung cancer. *Radiation Oncology* vol. 18 Preprint at https://doi.org/10.1186/s13014-023-02335-z (2023).
32. Yi, X. F. *et al.* Dual antitumor immunomodulatory effects of PARP inhibitor on the tumor microenvironment: A counterbalance between anti-tumor and pro-tumor. *Biomedicine and Pharmacotherapy* vol. 163 Preprint at https://doi.org/10.1016/j.biopha.2023.114770 (2023).
33. Heijink, A. M. *et al.* BRCA2 deficiency instigates cGAS-mediated inflammatory signaling and confers sensitivity to tumor necrosis factor-alpha-mediated cytotoxicity. *Nat Commun* **10**, (2019).
34. Marcus, A. *et al.* Tumor-Derived cGAMP Triggers a STING-Mediated Interferon Response in Non-tumor Cells to Activate the NK Cell Response. *Immunity* **49**, (2018).
35. Yang, H., Wang, H., Ren, U., Chen, Q. & Chena, Z. J. CGAS is essential for cellular senescence. *Proc Natl Acad Sci U S A* **114**, (2017).
36. Dou, Z. *et al.* Cytoplasmic chromatin triggers inflammation in senescence and cancer. *Nature* **550**, (2017).
37. Woo, S. R. *et al.* STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. *Immunity* **41**, (2014).
38. Deng, L. *et al.* STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. *Immunity* **41**, (2014).
39. Grivennikov, S. I., Greten, F. R. & Karin, M. Immunity, Inflammation, and Cancer. *Cell* vol. 140 Preprint at https://doi.org/10.1016/j.cell.2010.01.025 (2010).
40. Liu, H. *et al.* Nuclear cGAS suppresses DNA repair and promotes tumorigenesis. *Nature* **563**, (2018).
41. Zheng, J. *et al.* Comprehensive elaboration of the cGAS-STING signaling axis in cancer development and immunotherapy. *Molecular Cancer* vol. 19 Preprint at https://doi.org/10.1186/s12943-020-01250-1 (2020).
42. Li, J. *et al.* Metastasis and immune evasion from extracellular cgamp hydrolysis. *Cancer Discov* **11**, (2021).
43. Didonato, J. A., Mercurio, F. & Karin, M. NF-κB and the link between inflammation and cancer. *Immunol Rev* **246**, (2012).
44. Hong, C. *et al.* cGAS–STING drives the IL-6-dependent survival of chromosomally instable cancers. *Nature* **607**, (2022).
45. Bakhoum, S. F. & Cantley, L. C. The Multifaceted Role of Chromosomal Instability in Cancer and Its Microenvironment. *Cell* vol. 174 Preprint at https://doi.org/10.1016/j.cell.2018.08.027 (2018).

46. Vasiyani, H. *et al.* DNA damage induces STING mediated IL-6-STAT3 survival pathway in triple-negative breast cancer cells and decreased survival of breast cancer patients. *Apoptosis* **27**, (2022).
47. Patel, A. G. & Kaufmann, S. H. How does doxorubicin work? *Elife* **2012**, (2012).
48. Johnson, D. E., O'Keefe, R. A. & Grandis, J. R. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. *Nature Reviews Clinical Oncology* vol. 15 Preprint at https://doi.org/10.1038/nrclinonc.2018.8 (2018).
49. An, X. *et al.* An Analysis of the Expression and Association with Immune Cell Infiltration of the cGAS/STING Pathway in Pan-Cancer. *Mol Ther Nucleic Acids* **14**, (2019).
50. Chen, Q. *et al.* Carcinoma-astrocyte gap junctions promote brain metastasis by cGAMP transfer. *Nature* **533**, (2016).
51. Munn, D. H. & Mellor, A. L. IDO in the Tumor Microenvironment: Inflammation, Counter-Regulation, and Tolerance. *Trends in Immunology* vol. 37 Preprint at https://doi.org/10.1016/j.it.2016.01.002 (2016).
52. Liang, D. *et al.* Activated STING enhances Tregs infiltration in the HPV-related carcinogenesis of tongue squamous cells via the c-jun/CCL22 signal. *Biochim Biophys Acta Mol Basis Dis* **1852**, (2015).
53. Liang, H. *et al.* Host STING-dependent MDSC mobilization drives extrinsic radiation resistance. *Nat Commun* **8**, (2017).
54. Ahn, J. *et al.* Inflammation-driven carcinogenesis is mediated through STING. *Nat Commun* **5**, (2014).
55. Gan, Y. *et al.* The cGAS/STING Pathway: A Novel Target for Cancer Therapy. *Frontiers in Immunology* vol. 12 Preprint at https://doi.org/10.3389/fimmu.2021.795401 (2022).
56. Jiang, M. *et al.* CGAS-STING, an important pathway in cancer immunotherapy. *Journal of Hematology and Oncology* vol. 13 Preprint at https://doi.org/10.1186/s13045-020-00916-z (2020).
57. Kay, J., Thadhani, E., Samson, L. & Engelward, B. Inflammation-induced DNA damage, mutations and cancer. *DNA Repair* vol. 83 Preprint at https://doi.org/10.1016/j.dnarep.2019.102673 (2019).
58. Pua, K. H., Chew, C. L., Lane, D. P. & Tergaonkar, V. Inflammation-associated genomic instability in cancer. *Genome Instab Dis* **1**, (2020).
59. Badwey, J. A. & Karnovsky, M. L. Active oxygen species and the functions of phagocytic leukocytes. *Annual review of biochemistry* vol. 49 Preprint at https://doi.org/10.1146/annurev.bi.49.070180.003403 (1980).
60. Lewis, R. S., Tamir, S., Tannenbaum, S. R. & Deen, W. M. Kinetic analysis of the fate of nitric oxide synthesized by macrophages in vitro. *Journal of Biological Chemistry* **270**, (1995).
61. Yang, D. *et al.* Pro-inflammatory cytokines increase reactive oxygen species through mitochondria and NADPH oxidase in cultured RPE cells. *Exp Eye Res* **85**, (2007).
62. Hoki, Y. *et al.* 8-Nitroguanine as a potential biomarker for progression of malignant fibrous histiocytoma, a model of inflammation-related cancer. *Oncol Rep* **18**, (2007).
63. Kawanishi, S., Ohnishi, S., Ma, N., Hiraku, Y. & Murata, M. Crosstalk between DNA damage and inflammation in the multiple steps of carcinogenesis. *International Journal of Molecular Sciences* vol. 18 Preprint at https://doi.org/10.3390/ijms18081808 (2017).
64. Kiraly, O., Gong, G., Olipitz, W., Muthupalani, S. & Engelward, B. P. Inflammation-Induced Cell Proliferation Potentiates DNA Damage-Induced Mutations In Vivo. *PLoS Genet* **11**, (2015).
65. Calvo, J. A. *et al.* DNA repair is indispensable for survival after acute inflammation. *Journal of Clinical Investigation* **122**, (2012).
66. Jaiswal, M., LaRusso, N. F., Burgart, L. J. & Gores, G. J. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Res* **60**, (2000).
67. Jones Larry E., J. E. *et al.* Differential effects of reactive nitrogen species on DNA base excision repair initiated by the alkyladenine DNA glycosylase. *Carcinogenesis* **30**, (2009).

68. Wei, W. *et al.* S-nitrosylation from GSNOR deficiency impairs DNA repair and promotes hepatocarcinogenesis. *Sci Transl Med* **2**, (2010).
69. Parrish, M. C. *et al.* Nitric oxide induced S-nitrosation causes base excision repair imbalance. *DNA Repair (Amst)* **68**, (2018).
70. Shimizu, T., Marusawa, H., Endo, Y. & Chiba, T. Inflammation-mediated genomic instability: Roles of activation-induced cytidine deaminase in carcinogenesis. *Cancer Science* vol. 103 Preprint at https://doi.org/10.1111/j.1349-7006.2012.02293.x (2012).
71. Marusawa, H., Takai, A. & Chiba, T. Role of Activation-Induced Cytidine Deaminase in Inflammation-Associated Cancer Development. in *Advances in Immunology* vol. 111 (2011).
72. Kumar, R., DiMenna, L. J., Chaudhuri, J. & Evans, T. Biological function of activation-induced cytidine deaminase (AID). *Biomedical Journal* vol. 37 Preprint at https://doi.org/10.4103/2319-4170.128734 (2014).
73. Muramatsu, M. *et al.* Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* **102**, (2000).
74. Pasqualucci, L. *et al.* Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. *Nature* **412**, (2001).
75. Greeve, J. *et al.* Expression of activation-induced cytidine deaminase in human B-cell non-hodgkin lymphomas. *Blood* **101**, (2003).
76. Klein, I. A. *et al.* Translocation-capture sequencing reveals the extent and nature of chromosomal rearrangements in B lymphocytes. *Cell* **147**, (2011).
77. Endo, Y. *et al.* Activation-Induced Cytidine Deaminase Links Between Inflammation and the Development of Colitis-Associated Colorectal Cancers. *Gastroenterology* **135**, (2008).
78. Lane, D. & Levine, A. p53 Research: the past thirty years and the next thirty years. *Cold Spring Harbor perspectives in biology* vol. 2 Preprint at https://doi.org/10.1101/cshperspect.a000893 (2010).
79. Tergaonkar, V., Pando, M., Vafa, O., Wahl, G. & Verma, I. p53 stabilization is decreased upon NFκB activation: A role for NFκB in acquisition of resistance to chemotherapy. *Cancer Cell* **1**, (2002).
80. Ghosh, A. *et al.* Telomerase directly regulates NF-B-dependent transcription. *Nat Cell Biol* **14**, (2012).
81. Okazaki, I. M. *et al.* Constitutive expression of AID leads to tumorigenesis. *Journal of Experimental Medicine* **197**, (2003).
82. Corrales, L. *et al.* Direct Activation of STING in the Tumor Microenvironment Leads to Potent and Systemic Tumor Regression and Immunity. *Cell Rep* **11**, (2015).
83. Li, X. *et al.* Cyclic GMP-AMP Synthase Is Activated by Double-Stranded DNA-Induced Oligomerization. *Immunity* **39**, (2013).
84. Kong, X. *et al.* STING as an emerging therapeutic target for drug discovery: Perspectives from the global patent landscape. *Journal of Advanced Research* vol. 44 Preprint at https://doi.org/10.1016/j.jare.2022.05.006 (2023).
85. Meric-Bernstam, F. *et al.* Phase I Dose-Escalation Trial of MIW815 (ADU-S100), an Intratumoral STING Agonist, in Patients with Advanced/Metastatic Solid Tumors or Lymphomas. *Clinical Cancer Research* **28**, (2022).
86. Meric-Bernstam, F. *et al.* Combination of the STING Agonist MIW815 (ADU-S100) and PD-1 Inhibitor Spartalizumab in Advanced/Metastatic Solid Tumors or Lymphomas: An Open-Label, Multicenter, Phase Ib Study. *Clin Cancer Res* **29**, (2023).
87. Harrington, K. J. *et al.* Preliminary results of the first-in-human (FIH) study of MK-1454, an agonist of stimulator of interferon genes (STING), as monotherapy or in combination with pembrolizumab (pembro) in patients with advanced solid tumors or lymphomas. *Annals of Oncology* **29**, (2018).
88. Sun, X., Zhou, X., Lei, Y. L. & Moon, J. J. Unlocking the promise of systemic STING agonist for cancer immunotherapy. *Journal of Controlled Release* **357**, (2023).

89. Amouzegar, A., Chelvanambi, M., Filderman, J. N., Storkus, W. J. & Luke, J. J. Sting agonists as cancer therapeutics. *Cancers* vol. 13 Preprint at https://doi.org/10.3390/cancers13112695 (2021).
90. Ramanjulu, J. M. *et al.* Design of amidobenzimidazole STING receptor agonists with systemic activity. *Nature* **564**, (2018).
91. Pan, B. S. *et al.* An orally available non-nucleotide STING agonist with antitumor activity. *Science (1979)* **369**, (2020).
92. Gianni, T. *et al.* Genotype of immunologically hot or cold tumors determines the antitumor immune response and efficacy by fully virulent retargeted ohsv. *Viruses* **13**, (2021).
93. Tian, Z., Zeng, Y., Peng, Y., Liu, J. & Wu, F. Cancer immunotherapy strategies that target the cGAS-STING pathway. *Frontiers in Immunology* vol. 13 Preprint at https://doi.org/10.3389/fimmu.2022.996663 (2022).
94. Dorta-Estremera, S. *et al.* Targeting interferon signaling and CTLA-4 enhance the therapeutic efficacy of anti-PD-1 immunotherapy in preclinical model of HPV+ oral cancer. *J Immunother Cancer* **7**, (2019).
95. Shi, J. *et al.* STING agonist and IDO inhibitor combination therapy inhibits tumor progression in murine models of colorectal cancer. *Cell Immunol* **366**, (2021).
96. Dunphy, G. *et al.* Non-canonical Activation of the DNA Sensing Adaptor STING by ATM and IFI16 Mediates NF-κB Signaling after Nuclear DNA Damage. *Mol Cell* **71**, (2018).
97. Parkes, E. E. *et al.* Activation of STING-dependent innate immune signaling by s-phase-specific DNA damage in breast cancer. *J Natl Cancer Inst* **109**, (2017).
98. Vanpouille-Box, C. *et al.* DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. *Nat Commun* **8**, (2017).
99. Gulen, M. F. *et al.* Signalling strength determines proapoptotic functions of STING. *Nat Commun* **8**, (2017).
100. Haag, S. M. *et al.* Targeting STING with covalent small-molecule inhibitors. *Nature* **559**, (2018).
101. Lama, L. *et al.* Development of human cGAS-specific small-molecule inhibitors for repression of dsDNA-triggered interferon expression. *Nat Commun* **10**, (2019).