# cGAS-STING signaling in cancer: good or bad news?

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# Abstract

Maintaining genomic stability is crucial for cells to protect them from genomic errors caused by exogenous and endogenous sources. Contrary, genomic instability is one of the hallmarks of cancer, which allows tumor cells to acquire the genetic changes that drive tumor progression. This genomic instability also leads to the release of DNA into the cytoplasm through multiple mechanisms, which triggers the activation of immune responses through the cGAS-STING pathway. cGAS-STING signaling normally acts as a defence mechanism against pathogens, such as bacteria and viruses. Particularly, cGAS-STING is part of the innate immune response and activation of this pathways results in increased expression of type I interferon (IFN) genes and pro-inflammatory cytokines.

In genomic instable cancer cells, DNA damage repair defects and the consequent cytoplasmic self-DNA have been found to induce the activation of the cGAS-STING pathway, which modulates tumor growth, tumor immune evasion and determines treatment outcome. However, the same pathway has recently also been found to play a role in tumor promoting responses and metastasis, which suggests a negative disease outcome. Clearly, these results form a paradox on the role of cGAS-STING signaling in cancer biology.

In this essay, the current literature regarding positive and negative effects of cGAS-STING signaling on tumors is reviewed, in which a distinction is made between intracellular effects of STING signaling in cancer cells versus the effect of this pathway on the tumor microenvironment. Furthermore, the potential of cGAS-STING activation in cancer therapy is discussed, based on observations from studies utilizing STING-inducing therapies (e.g. radiotherapy and chemotherapy) and STING agonists.

In conclusion, the current literature remains inconclusive if cGAS-STING signaling has a positive or negative effect on tumorigenesis. The downstream signals of this pathways, i.e. IFN signaling and canonical and non-canonical NF-κB signaling, might have a distinct effect on tumorigenesis and be the cause of this differences. More likely, the outcome of cGAS-STING signaling is context-dependent, meaning that factors including the genetical background and signaling duration determine the effect of cGAS-STING signaling on tumor behaviour.

# Abbreviations

| | |
|---|---|
| cyclic guanosine monophosphate-adenosine monophosphate | (cGAMP, cyclic GMP-AMP) |
| cGAMP synthase | (cGAS) |
| stimulator of interferon genes | (STING) |
| interferon | (IFN) |
| nuclear factor κB | (NF-κB) |
| microsatellite instability | (MSI) |
| hereditary nonpolyposis colon cancer | (HNPCC) |
| chromosomal instability | (CIN) |
| breast cancer susceptibility 1 | (BRCA1) |
| double-stranded DNA | (dsDNA) |
| adenylate triphosphate | (ATP) |
| guanosine triphosphate | (GTP) |
| interferon regulatory factor 3 | (IRF3) |
| IFNα receptor | (IFNAR) |
| Janus kinase | (JAK) |
| signal transducer and activator of transcription | (STAT) |
| interferon-stimulated genes | (ISGs) |
| tumor necrosis factor | (TNF) |
| STING-associated vasculopathy with onset in infancy | (SAVI) |
| senescence-associated secretory phenotype | (SASP) |
| cytosolic chromatin fragments | (CCF) |
| programmed death protein 1 | (PD-1) |
| cytotoxic T lymphocyte-associated protein 4 | (CTLA4) |
| PD-1 ligand | (PD-L1) |
| antigen-presenting cells | (APCs) |
| dendritic cells | (DCs) |
| natural killer cells | (NK cells) |
| reactive oxygen species | (ROS) |
| myeloid-derived suppressor cells | (MDSCs) |
| DNA damage response | (DDR) |
| poly(ADP-ribose) polymerase | (PARP) |
| checkpoint kinase 1 | (CHK1) |
| chimeric antigen receptor | (CAR) |
| cyclic di-GMP | (cdGMP) |
| Three-prime repair exonuclease 1 | (Trex1) |

# Contents

# 1. Introduction

Cancer is a worldwide burden on health. Globally, 24.5 million cases of cancer were reported in 2017 and the mortality was estimated at 9.6 million cancer-related deaths[1]. Cancer is a rather complex disease with multiple (sub)types of cancers originating from a variety of tissues and organs[1,2], which is exemplified by the divers targets for therapies[3]. To simplify the complexity of this disease and support cancer research Hanahan and Weinberg proposed the 'Hallmarks of cancer'. Their framework suggested the organization of the capabilities acquired by normal cells, to become tumorigenic and malignant cells, into defined hallmarks[2]. Initially, six hallmarks were characterized: sustaining proliferative signaling, evading growth suppressors (i.e. resisting anti-growth signals), potentiating replicative immortality, evading apoptosis/resisting cell death, inducing angiogenesis (i.e. stimulating blood vessel growth for the nutrient supply of tumor cells), and inducing tissue invasion and metastasis (Fig. 1)[2,4]. In their subsequent review in 2011, the list of Hanahan and Weinberg was extended with two emerging hallmarks: evading immune destruction and reprogramming of energy metabolism. Additionally, two enabling hallmarks were proposed as key characteristics of cancer cells that facilitate the acquisition of the other hallmarks: genomic instability and inflammation[4]. Apart from characterizing and visualizing the mechanisms playing a role in cancer pathogenesis, the hallmarks are increasingly being used as therapeutic targets[4,5].

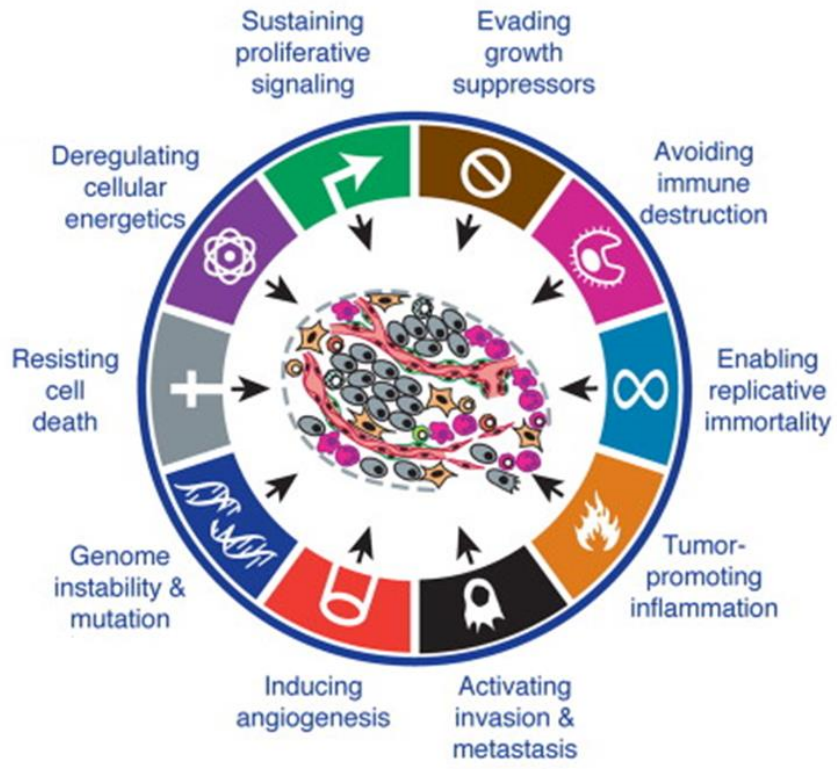


***Figure 1.*** *Hallmarks of cancer, consisting of: 1) six hallmarks originally described in 2000; sustaining proliferative signaling, evading growth suppressors, potentiating replicative immortality, evading apoptosis, promoting invasion and metastasis and inducing angiogenesis. 2) two emerging hallmarks; evading immune destruction and energy metabolism deregulation. 3) two enabling hallmarks; genomic instability and tumor-promoting inflammation. (adapted from Hanahan and Weinberg, 2011[4]).*

Cancer cells often have a (increased) genomic instability[4,6]. Accordingly, tumor cells are characterized by an accumulation of mutations and the rise of genetic alterations, which result in the associated selection process leading to more aggressive cells[6,7]. Although there are still gaps in the knowledge regarding the mechanisms behind genomic instability, especially for non-hereditary cancers, multiple forms of instability have been identified[8]. Genomic instability can present in various forms:

1. Changes at the nucleotide level, including increased base pair mutation frequencies and microsatellite instability (MSI). Microsatellites are repetitive genomic loci and the number of these repeats is altered in MSI[8,9]. In hereditary nonpolyposis colon cancer (HNPCC) mismatch repair genes have been found to regulate genomic stability, while mutations in these genes result in MSI[10]. An example of the role of increased base pair mutations in cancer is MYH-associated adenomatous polyposis. Mutations in base excision repair gene *MYH* have been found to lead to increased frequencies of somatic G:C→T:A transition mutations, resulting in a predisposition to colorectal tumors[11].

2. Changes at the chromosome level. Frequently, cancers present with genomic instability in the form of significant changes in chromosome structure and number[7,12]. These significant aberrations can be subdivided in: a) chromosomal instability (CIN), characterized by whole-chromosomal mis-segregation and, b) structural genomic instability, characterized by deletion and translocation of parts of the chromosomes[13]. For example, the presence of mutations in breast cancer susceptibility 1 (BRCA1) and BRCA2 in cells have been found to present with structural genomic instability[14-16]. BRCA1/BRCA2 deficiency was shown to be associated with an increased amount of micronuclei, which are thought to arise due to progression through mitosis with unresolved genomic instability[14,17]. These micronuclei contain (parts of) chromosomes, surrounded by its own nuclear envelope[9]. Apart from being a source of DNA damage accumulations, the micronuclei are fragile and their nuclear envelope are prone to collapse, which results in the release of DNA in the cytosol[9,18]. In addition to the rupture-prone micronuclei, a variety of other mechanisms have been found to induce the release of cytosolic DNA in cancer, including DNA repair deficiencies and replication errors[9,19]. This implies an important role of genomic instability and cytosolic DNA in cancer.

The other enabling hallmark proposed by Hanahan and Weinberg was tumor-promoting inflammation. Furthermore, advances in research have let to the recognition of tumor as organs that maintain their own tumor microenvironment[4]. This tumor microenvironment comprises multiple cell types and components, including immune cells, extracellular matrix, growth factors and cytokines[20]. These factors are known to influence the tumorigenesis, among them components of the immune system[4,21,22]. Tumors have been observed to poses infiltrations of immune cells, which in some cases were correlated with improved prognosis[21,22]. However, tumor cells commonly have been found to have employed mechanisms to circumvent the anti-tumor effects of the immune system, e.g. by suppressing immune responses[5,23]. Additionally, cancer cells have even been found to utilize components of the immune system to promote tumor growth and metastasis[24,25]. Thus, tumor cells are thought to communicate with immune cells and other components of its microenvironment to promote tumorigenesis.

One of the immune pathways that recently has been characterized to influence tumorigenesis is the cyclic guanosine monophosphate-adenosine monophosphate (cGAMP, also cyclic GMP-AMP) synthase (cGAS)-stimulator of interferon genes (STING) pathway[24,26-28]. Interestingly, cGAS is found to accumulate in cells with cytosolic DNA originating from collapsed micronuclei. This suggests a link between the genomic instability of cancers and the immune modulating effect of cancer cells[29]. The role of cGAS-STING in tumor tissues is controversial, showing both tumor promoting and anti-tumor effects in previous studies[24,26,27]. Multiple factors have been suggested to influence the end-result of cGAS-STING activation, such as the duration of the signaling[9,24]. Moreover, the cGAS-STING signaling might have a different effect in the (immune) cells of the microenvironment then on the tumor cells, causing this controversiality.

## Aim of this essay

The aim of this essay was to review and discuss the current literature on how cGAS-STING modulates disease outcome in cancers and the implications for cancer treatment. After briefly describing the cGAS-STING pathway and its role in non-tumor cells, anti-tumor and tumor promoting effects of cGAS-STING signaling will be discussed in relation to signaling duration and cellular targets. Eventually, these findings will be discussed in the context of translation to therapeutic implications.

# 2. cGAS-STING pathway in non-tumor cells

## cGAS-STING pathway and its downstream signaling

Cells possess multiple receptors and sensors to recognize DNA in the cytosol, including cGAS[9,30]. cGAS is a sensor for cytosolic double-stranded DNA (dsDNA), residing mainly in the cytosol[30]. The efficacy of cGAS activation has been found to depend on the length of the DNA fragment[31]. Upon its activation, cGAS catalyses the synthesis of cGAMP, utilizing adenylate triphosphate (ATP) and guanosine triphosphate (GTP) as substrates[28,30]. Subsequently, cGAMP directly binds to STING[28], which promotes innate immune signaling mechanisms (Fig. 2)[32]. Although STING is, in resting phase, predominantly located in the endoplasmic reticulum, it traffics to the Golgi apparatus upon activation[32-34]. Following its activation, STING interacts with a IκB kinase, TBK-1, resulting in the phosphorylation of STING[32,35]. This TBK-1-induced phosphorylation causes the recruitment of interferon regulatory factor 3 (IRF3), enabling the phosphorylation and activation of IRF3 by TBK-1[35]. The activation of IRF3 induces the expression of type I interferons (IFNs), IFN-α and IFN-β[32,36]. Through the binding to and activation of their receptors IFNα receptor 1 (IFNAR1) and IFNAR2, these IFNs initiate the activation of the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway and, thereby, drive the transcription of interferon-stimulated genes (ISGs)[37].

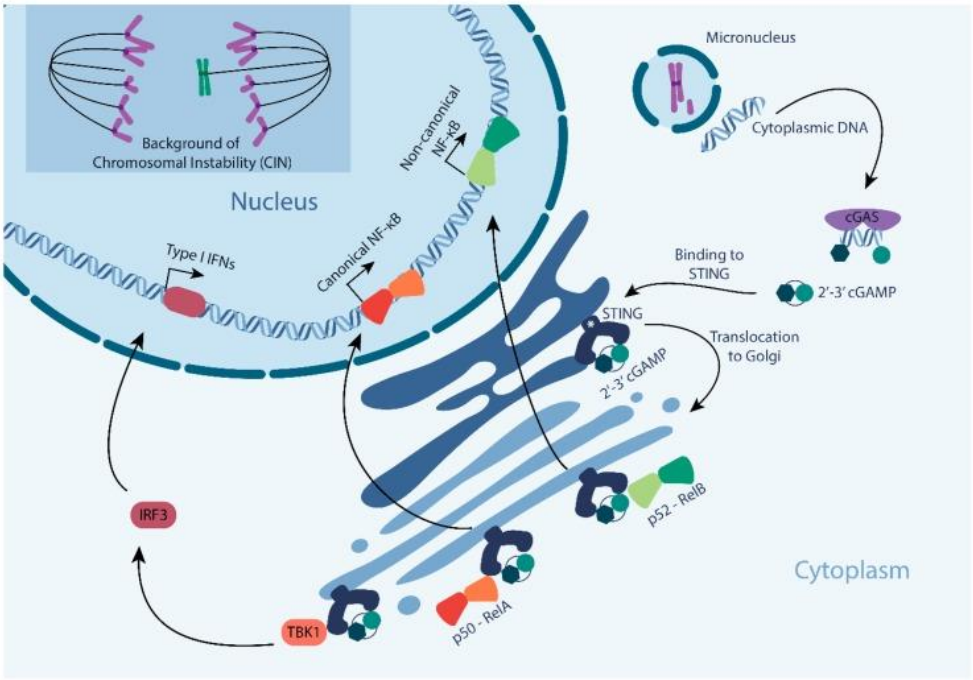


***Figure 2.*** *Components of the cGAS-STING signaling pathway. Factors like CIN and micronuclei cause the release of cytosolic DNA in cancer cells, which is recognized by cGAS. Through the synthesis of cGAMP, cGAS induces STING activation. STING signals through three pathways: type I IFN signaling, as well as canonical and non-canonical NF-κB signaling. (Retrieved from Hong, et al.*[38]*).*

Other innate immune signaling pathways activated by STING are the canonical and non-canonical nuclear factor κB (NF-κB) pathways[32,39]. The transcription factor family NF-κB consist of five proteins: RelA, RelB, c-Rel, p105/p50, and p100/p52. Among them, p105 and p100 are precursors of p50 and p52, respectively[40]. The canonical pathway is characterized by the degradation of the prototypical IκB protein IκBα and the subsequent release of NF-κB dimers, in particular the RelA-p50 heterodimer[40,41]. Pathogen-associated molecules and proinflammatory cytokines are among the inducers of this canonical NF-κB signaling[40]. Contrary, the non-canonical NF-κB pathway does not involve the degradation of the NF-κB inhibitor IκBα, but this signaling pathway is defined by the processing of p100 to p52 and the following release of p52 containing

NF-κB dimers. This pathway is induced by more defined factors such as the specific tumor necrosis factor (TNF) cytokine family members BAFF, lymphotoxin-β, or CD40 ligand[40,41].

## cGAS as dsDNA sensor in infections and autoimmune diseases

In normal physiological conditions, cGAS is a crucial sensor for DNA originating from pathogens[30,42-44]. First of all, cGAS has been found to induce IFN-β expression in infections of intracellular bacteria *Chlamydia trachomatis* and *Listeria monocytogenes*[42,43]. Secondly, the sGAS-STING pathway, and the subsequent activation of IRF3 activation and IFN responses, have been found to be induced by DNA viruses, such as herpes simplex virus 1 and Sendai virus[28,45]. Models deficient in parts of this pathway have illustrated the importance cGAS-STING in the induction of immune responses in viral infections. For instance, correlated with reduced IFN-β induction and increased susceptibility to infections of vaccinia virus, Sendai virus and vesicular stomatitis virus[30,32]. Finally, RNA viruses have also been found to influence (parts) of the cGAS-STING pathway, which is exemplified by the observation that both Dengue virus and influenza A virus target STING to promote viral deficiency of STING or cGAS have been infection[46,47].

While cGAS-STING is important in the recognition of and defence against pathogens, it has also been found to play a role in multiple autoimmune diseases and other chronic inflammatory diseases. For example, a number of these diseases, such as familial chilblain lupus and STING-associated vasculopathy with onset in infancy (SAVI), are correlated with mutations in STING, resulting in increased type I IFN signaling[48]. Thus, the cGAS-STING signaling pathway is a general sensor for cytosolic DNA, playing a role in the defence against pathogens and in chronic inflammatory diseases.

# 3. cGAS-STING signaling in tumor cells

Tumors are often associated with increased levels of cytosolic DNA as a results of common cancer cell-features, such as genomic instability and micronuclei[9,29]. cGAS is also a sensor for cytosolic self-DNA within cancer cells and, accordingly, cGAS-STING signaling has been observed to take place in multiple tumors[24,29,49]. Since the cGAS-STING pathway generally induces tumor suppressive responses, it is of interest for cancer cells to modify this signaling pathway. Hence, cGAS-STING signaling has been found to be altered/defective in various cancers[50,51]. For example, in human colorectal adenocarcinoma STING signaling was found to be defective, shown by a reduced STING and cGAS expression in these tumors. Consequently, these cells react less efficient to dsDNA, illustrated by decreased production of type I IFN and pro-inflammatory cytokine IL1-β[50]. The STING signaling has been found to be suppressed in a wide variety of cancers, including lung cancers and melanomas, caused by loss-of-function mutations and epigenetic modifications of STING or cGAS promotor regions[51]. These observations that cancer cells employ features to circumvent STING/cGAS signaling towards DNA damaged cells suggest that STING is important for anti-tumor responses. Indeed, tumors have been found to modulate STING signaling to secure their survival[50,51].

## Intercellular STING signaling and cellular senescence effecting tumorigenesis

One of the intracellular mechanisms that is found to be initiated and regulated by cGAS-STING is senescence, which is accompanied by the senescence-associated secretory phenotype (SASP)[52,53]. Cellular senescence is defined as a state of permanent cell-cycle arrest, which is initiated in aging cells or in response to damage or stress. Among the triggers of this stress/damage-induced senescence are conditions that are also found in cancer cells, such as DNA damage, telomere shortening and oncogene-induced stress[52,53]. Common characteristics of senescent cells are the loss of nuclear envelop integrity and the successive occurrence of cytosolic chromatin fragments (CCF)[52,54]. These compromised nuclear envelopes were found to activate cGAS-STING signaling in senescent cells, initiated by the recognition of the CCF by cGAS[52]. Actually, cGAS was found to be fundamental for cellular senescence[53]. Additionally, the cGAS-STING pathway has been shown to have an effect on tumor development and on the secretory phenotype, SASP, of senescent cells. Utilizing STING deficient cells, it was observed that the development of hepatocellular carcinoma and induction of SASP in senescent hepatic stellate cells is dependent on STING, among others[55]. The various stimuli of cellular senescence were also found to activate cGAS-STING signaling, including irradiation, oncogene activation, CDK4 inhibition, oxidative-stress[52,53]. Since cellular senescence has been observed to arise mainly in tumors of premalignant and benign origin and not in malignant tumors, it is thought to prevent tumor progression[56-58]. This is also supported by the tumorigenesis limiting effects of senescence seen *in vitro* and *in vivo*[59,60]. Prolonged expression of the oncogene *ras* was found to induce a cell-cycle arrest identical to cellular senescence, characterized by increased levels of tumor suppressors p53 and p16. This induction of cellular senescence by oncogenic *ras* is thought to be tumor suppressive, since disruption of p53 or p16 was associated with features of neoplastic transformation, such as loss of contact inhibition and proliferation[60]. These observations suggest that cellular senescence suppresses tumorigenesis through limiting the development of cancer-like features, among others.

The cGAS-STING signaling, however, has also been observed to be involved in tumor promotive mechanisms[24]. Micronuclei are an important source of cytosolic DNA. Following the rupture of their nuclear envelope, cGAS is found to co-localise with these micronuclei[29]. One of the conditions related to the presence of a high quantity of micronuclei is CIN, where cells with high levels of CIN have been shown to possess more micronuclei compared to CIN-low cells. Consistent with the rupture-prone nature of micronuclei, these CIN-high cells were found to have increased levels of cytosolic dsDNA[24]. Interestingly, the recognition of cytosolic dsDNA within these CIN-high cells was found to activate non-canonical NF-κB signaling, accompanied by higher p52 levels, increased nuclear localization of RelB and upregulation of non-canonical NF-κB target genes. This

non-canonical NF-κB signaling, activated by CIN-induced chronic cGAS-STING signaling, was found to promote metastasis[24]. Indeed, the non-canonical subunit RelB has been found to regulate the migration and invasion of cancer cells. In *in vitro* models of prostate cancer, RelB deficient cancer cells were observed to have reduced migration and invasion abilities[61]. Thus, the increased localization of RelB in the nucleus might be one of the factors through which non-canonical NF-κB signalling promotes tumor metastasis in CIN-high cells.

Besides the observations in CIN-high cells, the cGAS-STING pathway have been implicated to promote tumorigenesis in a number of other situations. One of the main downstream signaling outputs of cGAS-STING is the upregulation of IFNs[38]. Although acute IFN signaling is thought to play a central role in anti-tumor responses, as described in the next chapter, chronic IFN signaling has been observed to contribute to the immune resistance of tumors[38,62]. Of note, because the distinction between acute and chronic is not definite in the literature, a distinction will be made here. In this essay, an *in vitro* exogenous stimulation period for more than a week or genetically modified *in vivo* models with chronic cGAS-STING signaling or predisposition for dsDNA accumulation will be categorized as chronic. In a study by Benci et al.[62] IFN signaling was found to contribute to tumor resistance towards immune checkpoint therapies targeting the receptors programmed death protein 1 (PD-1) or cytotoxic T lymphocyte-associated protein 4 (CTLA4) or the PD-1 ligand, PD-L1[62,63]. Here, sustained IFN stimulation was found to drive the resistance against checkpoint blockade plus irradiation combination therapy. This resistance was observed to be due to STAT1-related epigenetic changes, leading to increased expression of PD-L1 and T cell exhaustion (Fig. 3). Interestingly, inhibition of tumor derived IFN signaling, by JAK inhibitors or knockout of IFN receptors, reinvigorated the exhausted T cells and improved the therapeutic response of cancer cells[62].

Thus, intracellular cGAS-STING signaling can have both tumor suppressive and tumor-promoting effects, which is suggested to be dependent on the duration of activation of the pathway.

# 4. cGAS-STING signaling within the tumor microenvironment

## Intercellular signaling of tumor-derived DNA and cGAMP

Tumor cells have also been found to induce STING signaling in surrounding cells[27,49,64-66]. In a study by Woo et al.[49] it was found that antigen-presenting cells (APCs) are also able to detect tumor-derived DNA, both *in vivo* and *in vitro*. This DNA originating from tumors was found to be transferred from cancer cells to APCs and able to induce the production of IFN-β in a cGAS-, STING-, TBK1- and IRF3-dependent manner (Fig. 3). Moreover, the activation of dendritic cells (DCs) by tumor-derived DNA induced the expression of cofactors that are crucial for T cell activation. Additionally, the cGAS-STING pathway was also observed to be required for priming a $CD8^+$ T cell response, which is demonstrated by a reduced anti-tumor T cell response in STING-deficient and IRF-3 deficient mice. This suggests a role for the cGAS-STING signaling in both innate and adaptive immune responses towards tumors[49].

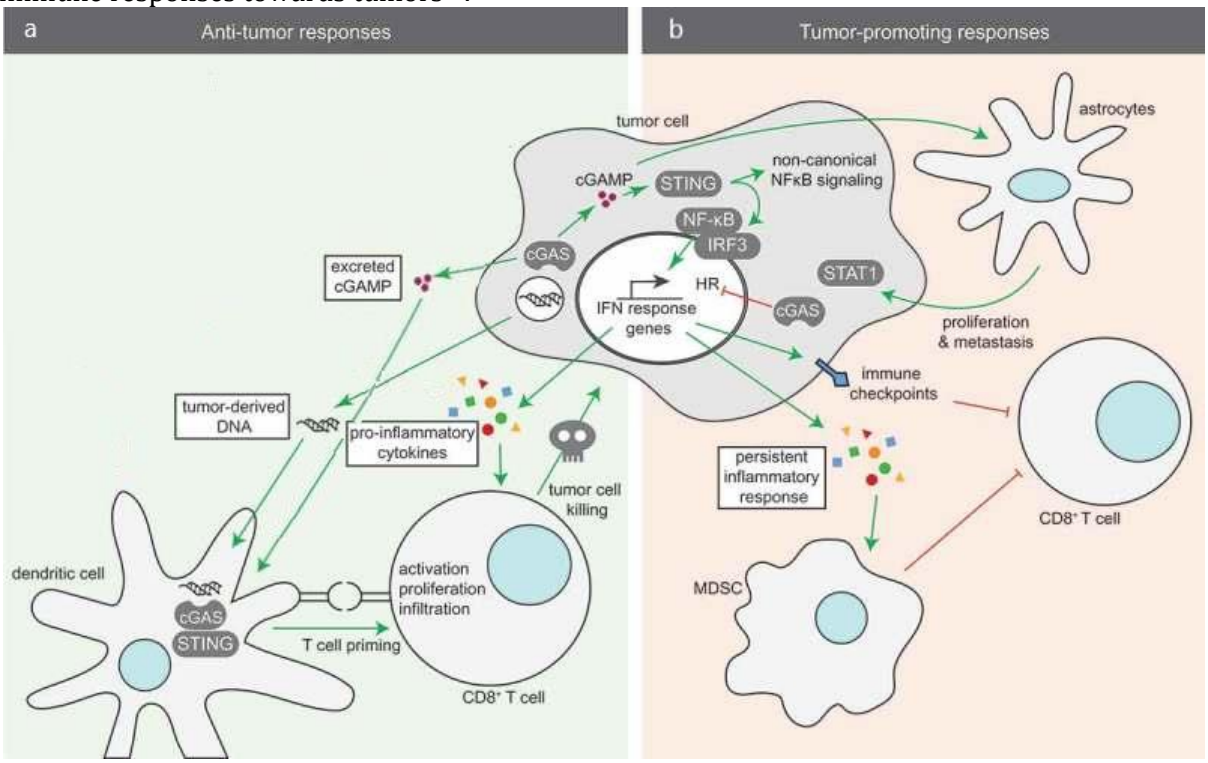


***Figure 3.*** *Some of the anti- and pro-tumor effects of cGAS-STING signaling in the tumor microenvironment. A) anti-tumor responses induced by cGAS-STING include T cell priming and activation by DCs triggered by tumor-derived DNA and cGAMP. T cells activation by DCs and STING-induced pro-inflammatory cytokines leads to tumor cell killing. B) tumor-promoting responses. (Chronic) STING signaling suppresses T cells through the upregulation of immune checkpoints and the activation of MDSC. Tumor-derived cGAMP triggers STING signaling in astrocytes, which promotes proliferation and metastasis through STAT1 signaling in tumor cells. (adapted from Talens and Van Vugt[9]).*

Like tumor-derived DNA, it is suggested that cGAMP might be transferred from tumor cells to non-tumor cells, similar to the role of cGAMP in viral infections[64]. In viral infections, cGAMP has been observed to be transferred from infected cells to uninfected cells, where this STING activator induces innate immune responses[67,68]. Accordingly, exogenous cGAMP has been found to induce STING signaling and anti-tumor responses *in vivo* and *in vitro*, such as the production of proinflammatory cytokines and activation of natural killer cells (NK cells) and DCs[27,64,65]. Among the proinflammatory cytokines induced by cGAMP are IFN-β and IFN-γ, which exert anti-tumor effects as well as anti-angiogenic effects and induce cancer cell apoptosis, respectively. Through the expression of IFN-β cGAMP is also able to activate DCs, promote the expression of co-stimulatory molecules on DCs and eventually activate $CD8^+$ T cell (Fig. 3)[27]. In the case of NK cells, their activation was found to be induced by intrinsic STING signaling as well as by downstream

STING signaling products from other cells. However, the effect of STING on tumor growth and the protection against tumors seems to be (partly) dependent on the sensitivity of tumors to NK cells[64].

Gap junctions have been found to play a role in the transfer of cGAMP produced in infected cells to neighbouring cells[69]. Cells exhibiting high cGAS expression were found to be able to activate STING signaling in adjacent cGAS-low cells. Deficiency of gap junction proteins repressed this cGAMP-mediated STING activation and the subsequent phosphorylation of IRF3 and activation of IFN-β, indicating a role for cGAMP and gap junctions in activating STING signaling in adjacent cells[69]. Metastatic cancer cells have also been found to employ gap junctions for the transfer of cGAMP to other cells[66]. In the study by Chen et al.[66] brain metastases originating from brain and lung cancers were found to form gap junctions with astrocytes, the most prevalent cell in the brain. These gap junctions function as channels, enabling the transfer of cGAS-induced cGAMP from the metastatic cells to the astrocytes. Following the activation of STING, IFNα and TNFα are being produced in astrocytes. These cytokines were found to induce STAT1 and NF-κB signaling in the cancer cells, which were shown to support tumor growth and promote metastasis (Fig. 3)[66]. In line with this, migration and invasion were found to be increased in CIN-low cells upon treatment with cGAMP[24]. The above observations suggest that the transfer of tumor-derived DNA or cGAMP can have anti-tumor effects as well as tumor promoting effects.

## Paradoxical influences of SASP and IFN signaling

The induction of cellular senescence and SASP has also been found to have an effect on cells surrounding the tumor[52,70]. Senescent cells are characterized by the secretion of SASP, which includes factors like cytokines, chemokines and growth factors[52,70]. The CCF-induced activation of cGAS-STING signaling has been found to promote this SASP[52-54]. Consequently, cGAS-STING maintains paracrine senescence through the induction of ISGs and the expression of SASP factors, such as interleukin-6[52]. In line with this, sustained stimulation by the downstream cGAS-STING signaling factor IFN-β has been found to induce senescence through the induction of reactive oxygen species (ROS) production. Subsequently, this was found to induce DNA damage responses, which suggests a role for cGAS-STING in the prevention of cancer cell expansion through senescence maintenance[71]. Acute IFN signaling to surrounding cells has also been implicated to play an important role in the initiation of anti-tumor immune responses. For example, IFN is important for the activation of DCs and successive priming of tumor-specific T cells to eliminate tumors[72]. These observations show a different effect of (chronic) IFN, compared to the pro-tumor effects previously mentioned regarding immune checkpoints, which might depend on the malignancy status of the cells. The transformation of premalignant to malignant and tumor suppressive to tumor promoting effects of senescence and IFN, respectively, might be the result of the acquisition of mutation in cancers, such as mutations in tumor suppressor gene p53[60]. However, these effects could also work simultaneously, where the ratio of pro- and anti-tumor signaling determines the outcome.

## (Irradiation-induced) STING signaling between tumors and immune cells

Radiation therapy is a common treatment utilized for cancer, which is associated with the induction of anti-tumor immune responses, like type I IFN signaling. These anti-tumor responses are promoted by the rise of irradiation-induced DNA damage within the cancer cells, which evokes the activation of cGAS-STING signaling[73,74]. However, STING signaling has also been implicated to play a role in radiation therapy resistance[73]. In MC38 colon cancer cells STING signaling is activated upon irradiation, which promotes the expression of CCR2 and its ligands on myeloid-derived suppressor cells (MDSCs) and cancer cells, respectively, to mobilize the MDSCs to the tumor. Subsequently, MDSCs suppress T cell functioning and, thus, are thought to suppress anti-tumor immune responses (Fig. 3)[73]. Contrary, exogenous cGAMP activates T cells *in vivo* through the suppression of MDSCs, while inhibiting tumor growth and metastasis[75]. This suggests that cGAS-STING pathway factors can either inhibit or promote MDSCs, which might be dependent on the targeted cell type.

While DC activation induced by tumor-derived DNA and cGAMP showed to initiate tumor suppressive responses through IFN signaling in the above examples[27,49], tumor cells were also found to be able to activate non-canonical NF-κB signaling in DCs in response to irradiation[74]. Botch irradiated cancer cells and STING agonists were found to induce non-canonical NF-κB activation in a STING-TBK1-dependent manner. Irradiation induced non-canonical NF-κB was found to suppress anti-tumor responses through RelA signaling inhibition and negative regulation of IFN-β expression[74]. This implies that DCs are able to activate multiple downstream STING signals with districts effects of tumorigenesis, which might depend on the origin of the STING activating signal, in this case radiation therapy.

Taken together, cGAS-STING signaling in the tumor microenvironment can either suppress or promote tumorigenesis. The downstream effects of this pathway might depend on the target cells (DCs or MDSCs) but also the duration of the signaling has been suggested to influence its effects.

# 5. cGAS-STING pathway targeting in the cancer therapy

## STING-inducing anti-tumor therapies

Like radiation therapy, DNA-damaging therapies utilized in the fight against cancer are also associated with the activation of cGAS-STING signaling due to the rise of micronuclei and cytosolic dsDNA. For example, the chemotherapeutic agents cisplatin and etoposide trigger the production of inflammatory cytokines in a STING-dependent manner[76]. In addition, treating DNA damage response (DDR)–deficient cell lines with cisplatin promotes upregulation of PD-L1 through cGAS-STING-dependent signaling[77], suggesting that the tumor modulating effects of the cGAS-STING pathway and cisplatin might depend on the genetical background of tumors. The DDR pathway has become a target for cancer therapies in the recent years, especially in cancers containing DNA-repair deficiencies. For instance, poly(ADP-ribose) polymerase (PARP) inhibitors have been found to be effective in the therapy of breast and ovarian cancers with BRCA1 or BRCA2 defects. The combination of BRCA1/2 defects and PARP inhibitors induced chromosomal instability in these cancer cells as well as increased levels of cytosolic dsDNA and micronuclei[78,79]. Accordingly, DNA and cGAMP derived from these cancer cells were found to trigger anti-tumor immune responses through the activation of STING signaling in surrounding APC[79]. The therapeutic effect of DDR inhibitors is not limited to BRCA1/2 cancers. For example, inhibition of PARP and checkpoint kinase 1 (CHK1) was found to promote T-cell-mediated anti-tumor immune responses in small cell lung cancer models through the activation of STING signaling and the successive recruitment of T cells (Fig. 4)[80].

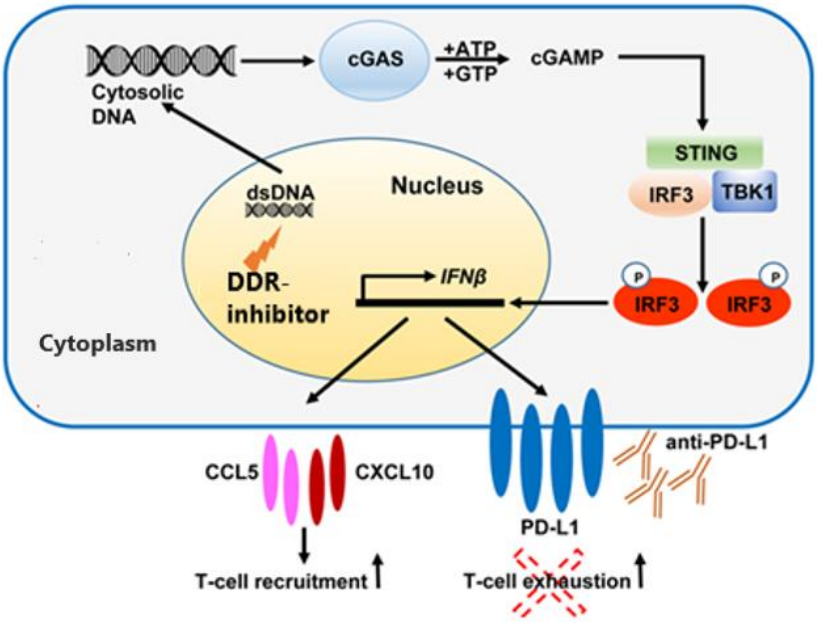


***Figure 4.*** *STING-dependent anti-tumor effects of DDR-inhibitors in tumors with DNA repair deficiencies. DDR-inhibitor treatment (e.g. PARP or CHK1 inhibitors) promotes cytosolic dsDNA, which activates cGAS-STING signaling. Subsequently, T cell are recruited and PD-L1 is being expressed on cancer cells, making the tumor more sensitive to anti-immune checkpoint therapies. (adapted from Sen, et al[80]).*

Currently, immunotherapies are increasingly being applied to cancer therapy, such as anti-immune checkpoint therapies targeting PD-L1 or PD-1[81]. The expression of PD-L1 on tumor cells is thought to suppress T cells through the PD-L1/PD interaction, which explains the interest of targeting this immune checkpoint[5]. Despite the promising results of immune checkpoint blockade, some cancer cells have been observed to be resistant to the therapy. One of the proposed causes of this resistance is reduced expression of PD-L1 by these tumor cells[80]. DDR inhibitors have been found to promote the expression of PD-L1 in small cell lung cancer and breast cancer in a STING-dependent manner (Fig. 4). Therefore, these inhibitors are thought to increase the

potency of immune checkpoint-targeted therapies[80,81]. Likewise, STING agonists themselves have been showed to contribute to the responsiveness to immune checkpoint therapies targeting CTLA4 or PD-1[63]. In a study by Fu et al.[82] synthesized cGAMP was used as a vaccine adjuvant in immunogenic tumors that were unresponsive to anti-PD-1 therapy alone. The combination of PD-1 blockade with this vaccine increased PD-L1 expression, resulting in tumor regression in previously anti-PD-1 resistant tumors[82]. The above observations suggests that activation of cGAS-STING signaling could increase therapeutic effects of other anticancer agents, such as PD-1/PD-L1 antibodies.

## Stimulating cGAS-STING signaling in anti-tumor therapy

Since cGAS-STING is an important modulator of immune responses against tumors and influences therapeutic responses, STING agonist have been applied to multiple cancer therapies[27,63]. 5-FU is a clinical chemotherapy used as anti-tumor drug for colon cancer. The combination of 5-FU with exogenous cGAMP treatment increased the efficacy of 5-FU anti-tumor treatment while reducing its side-effects[27]. The proposed underlying mechanism is cGAMP-induced activation of DCs, which results in the observed increase in $CD8^+$ T-cell levels and its related cytokines[27,65]. Indeed, cGAMP has been found to directly activate DCs *in vitro* and to induce antigen-specific cellular and humoral immune responses *in vivo*[65], indicating the relevance to use cGAMP as adjuvant or combination therapy to promote (long-term) anti-tumor immune responses. In line with this, STING agonists have been found to increase the efficacy of immunotherapies, beyond the previous mentioned immune checkpoint-targeted therapies[82,83]. An emerging therapeutic approach is chimeric antigen receptor (CAR) T cell immunotherapy, These CAR-T cells are genetically engineered to specifically target tumor antigens. Combining the treatment of CAR-T cells with the STING agonist cyclic di-GMP (cdGMP) was found to trigger APC activation and result in increased lymphocyte responses, compared to either therapies alone. Moreover, tumors were eradicated by this combination therapy and the treatment with CAR-T cells and cdGMP was suggested to promote systemic anti-tumor immunity to distant metastases[83]. Thus, STING agonists could be used to increase the efficacy of multiple immunotherapies by increasing anti-tumor immune responses.

As previously mentioned, some tumors appear to have defective STING signaling due to epigenetic modifications[51]. In colorectal adenocarcinoma cGAS expression was found to be suppressed by epigenetic modifications, such as cGAS promotor hypermethylation and histone modifications. Treating these cancer cells with demethylating agents was found to rescue them from the suppression of cGAS, suggesting that employing epigenetic modifying therapies might be beneficial in cGAS-STING defective cancers[50].

In line with the previous mentioned differences in chronic and acute STING/IFN signaling the efficacy of STING to promote T cell responses in immune checkpoint therapies has been found to be dependent on the duration and degree of STING activation. Sivick et al.[63] reported that a lower dosage and shorter treatment with a STING agonist was more favourable than high-dose administrations. While low doses resolved the tumor with the help of $CD8^+$ T cells, high dose treatment facilitated systemic STING agonist distribution and did not induce the long-term anti-tumor immunity seen in low-dose treatments[63]. Nevertheless, the effect of different levels of STING activation seems to be controversial and might depend on which factor of the pathway is used or targeted. For instance, Li et al.[27] suggested that low levels of cGAMP and fewer administration times might not be enough to induce the anti-tumor responses[27]. Meanwhile, Sun et al.[30] found that the transfection of DNA enhanced the induction of IFN-β when combined with low levels of cGAS plasmids, while this IFN-β induction was not seen at high levels of cGAS plasmids[30]. Possibly the DNA of the plasmids activated cGAS themselves and the cGAS signaling got saturated at too high dosage levels.

Overall, STING agonists have shown promising results in multiple cancers and the above observations suggest that activation of STING signaling can contribute to the efficacy of other anti-tumor therapies.

# 6. Discussion

Initially discovered for its role in the recognition of pathogens, the cGAS-STING pathway increasingly has gained interest for its role in tumorigenesis in the last years. In general, cytosolic dsDNA is thought to be the main inducer of this pathway. In this essay multiple cGAS-STING-inducing factors have been described in the tumor-context, including DNA repair deficiencies, CIN, micronuclei and DNA damaging therapies. Activation of this pathway showed to effect tumorigenesis both through intracellular signaling in cancer cells as well as through signaling in the tumor microenvironment. The effect of cGAS-STING activation has been found to vary in tumors between being tumor suppressive or promoting tumorigenesis, for which some causes were suggested, such as signal duration and defects in tumor suppressors. Nevertheless, therapeutical activation of STING signaling showed promising effects both as monotherapy and in combination with other cancer therapies, where it promoted anti-tumor responses and increased therapeutic efficacy.

A common thought in the field is that short-term signaling of the cGAS-STING pathway results in anti-tumor responses, while sustained signaling is correlated with tumor promoting effects[38,70]. Possibly, these differences are the result of the activation of other downstream signaling pathways in chronic STING signaling than in acute signaling. The anti-tumor effects of acute STING signaling have mainly been correlated with factors of the IFN signaling pathway[49,64]. Although chronic STING signaling has been found to induce IFN signaling too, sustained stimulation of STING signaling has also been found to promote tumorigenesis through mechanisms distinct from IFN signaling[24,74]. For example, in CIN-high cells the induction of metastasis has been found to be mediated by non-canonical NF-κB, while type I IFNs and canonical NF-κB were associated with improved prognosis[24]. Additionally, activation of the non-canonical NF-κB in DCs has been shown reduce anti-tumor effects of irradiation[74]. Furthermore, chronic STING signaling in Three-prime repair exonuclease 1 knockout (Trex1$^{-/-}$) mice has been shown to inhibit mTORC1 and promote tumor growth and metastasis through chronic TBK-1 activation. In these mice, TKB-1 was activated in a IRF3-independent manner, suggesting the pro-tumor effects were mediated through NF-κB or other downstream STING signaling pathways[84]. Considering the above observations, a proposed mechanism for tumor promotive effects of chronic STING signaling is the induction of non-canonical NF-κB instead of IFN and canonical NF-κB. In light of this it would be interesting to look into the development of cancer in chronic inflammatory autoimmune diseases in relation to cGAS-STING signaling.

Cancer cells are often associated with high mutation rates to support the process of tumorigenesis. Although there are thought to be general tumor-supportive genetic alterations among tumors, different tumor types have been found to have distinctive DNA mutation patterns[4]. Likewise, tumors have been observed to have various defects in the STING signaling pathway, where some tumors showed to have defects upstream of IRF3/NF-kB signaling and others were defective in NF-kB, specifically[50]. Possibly, the district mutation patterns among tumors are contributing to the different effects seen in cancer cells in response to cGAS-STING signaling. In line with this, having defective STING signaling made tumors more susceptible to oncolytic viral infections, suggesting that the occurrence of mutations in cGAS-STING genes might influence the efficacy of cancer therapies[50]. While some tumors actively suppress STING signaling, others have been found to enhance STING signaling to induce metastasis through the recruitment of (mitochondrial) DNA from surrounding cells[70]. Taken together, these findings suggest that the outcome of cGAS-STING signaling in tumors might depend on which pathways are up- and downregulated in cancer cells as well as the genetic background of these cells.

Immune cells in the tumor microenvironment can both anti-tumor effects and promote tumorigenesis[4]. These differential effects were also seen in response to the activation of cGAS-STING signaling in distinct cell types. While cGAMP induced anti-tumor responses in natural killer NK cells and DCs, the transfer of cGAMP to astrocytes through gap junctions was found induce tumor supportive responses[27,64-66]. Although the way cGAMP was presented in these settings (exogenous or through gap junctions) might have influenced the outcome, it could also be that

distinct cells vary in their response towards cGAS-STING signaling. If this is the case, therapeutic responses might be increased through pre-therapeutical characterization of the tumor microenvironment and modulation of this environment towards tumor suppressive cGAS-STING signaling.

The observations and findings depicted in this essay are just a glimpse of the whole story and more research should be done to elaborate on the effect of cGAS-STING in defined situations. Taken together, the above observations suggest that activation of cGAS-STING signaling can have beneficial effects on tumor growth, but also may contribute to increased efficacy of cancer therapies, depending on the context. Probably, cGAS-STING signaling doesn't have a general effect in all cancers, but its effects should be examined in relation to specific cancer types or on an individual level, suggesting a personalized treatment regime.

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