REVIEW

The cGAS–cGAMP–STING pathway connects DNA damage to inflammation, senescence, and cancer

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Detection of microbial DNA is an evolutionarily conserved mechanism that alerts the host immune system to mount a defense response to microbial infections. However, this detection mechanism also poses a challenge to the host as to how to distinguish foreign DNA from abundant self-DNA. Cyclic guanosine monophosphate (GMP)–adenosine monophosphate (AMP) synthase (cGAS) is a DNA sensor that triggers innate immune responses through production of the second messenger cyclic GMP-AMP (cGAMP), which binds and activates the adaptor protein STING. However, cGAS can be activated by double-stranded DNA irrespective of the sequence, including self-DNA. Although how cGAS is normally kept inactive in cells is still not well understood, recent research has provided strong evidence that genomic DNA damage leads to cGAS activation to stimulate inflammatory responses. This review summarizes recent findings on how genomic instability and DNA damage trigger cGAS activation and how cGAS serves as a link from DNA damage to inflammation, cellular senescence, and cancer.

Introduction

Genomic instability, a major driving force of cancer and age-related diseases, poses an ominous threat to human health and longevity. Nevertheless, several stringent and intricate cellular programs maintain genome integrity and prevent cells from becoming malignant. Cells may return to normal function if the genetic lesions are successfully repaired, enter a state of permanent cell-cycle arrest known as senescence if the damage is persistent but tolerable, or undergo programmed cell death to destroy an intolerably damaged genome.

Although DNA damage response (DDR) was long thought to mainly regulate genome integrity and cell fates, accumulating evidence indicates that genomic instability also triggers inflammatory response (Fig. 1). In tissue culture systems, DNA damaging agents such as topoisomerase inhibitors and ionizing irradiation induce the expression of type I IFNs and other cytokines (Fenech and Morley, 1986; Schlegel et al., 1986; Coppé et al., 2008; Rodier et al., 2009; Brzostek-Racine et al., 2011; Fenech et al., 2011; Kondo et al., 2013; Ahn et al., 2014b; Lan et al., 2014; Härtlova et al., 2015; Xia et al., 2016a; Harding et al., 2017; Luthra et al., 2017). The degree of inflammatory gene induction by genomic DNA damage is usually lower than that induced by DNA transfection or viral infection. Nonetheless, cells that sustain nuclear DNA damage also become more resistant to viral infections (Mboko et al., 2012; Härtlova et al., 2015; Luthra et al., 2017). Consistent with these in vitro findings, in vivo studies revealed that chemotherapy (Sistigu et al., 2014) and radiation treatment (Burnette et al., 2011; Lim et al., 2012; Deng et al., 2014) induce type I IFN signaling in tumors to promote antitumor immunity.

In addition to inducing cytokines, DNA damage also enhances the expression of ligands of natural killer (NK) cells such as NKG2D ligands (Gasser et al., 2005; Lam et al., 2014). These surface proteins attract NKG2D-positive NK cells and activated CD8 T lymphocytes to target damaged cells for elimination by the immune system (Bauer et al., 1999). The expression of NKG2D ligands is likely a result of type I IFN induction by DNA damage (Zhang et al., 2008; Lam et al., 2014).

Recent studies have provided mechanistic insights into how DNA damage induces type I IFNs and other immune-regulatory cytokines (Erdal et al., 2017; Glück et al., 2017; Harding et al., 2017; Mackenzie et al., 2017; Yang et al., 2017). A cytosolic DNA sensing pathway has emerged as the major link between DNA damage and innate immunity (Fig. 2). DNA normally resides in the nucleus and mitochondria; hence, its presence in the cytoplasm serves as a danger-associated molecular pattern (DAMP) to trigger immune responses. Cyclic guanosine monophosphate (GMP)–adenosine monophosphate (AMP) synthase (cGAS) is the sensor that detects DNA as a DAMP and induces type I IFNs and other cytokines (Sun et al., 2013). DNA binds to cGAS in a sequence-independent manner; this binding induces a conformational change of the catalytic center of cGAS such that this enzyme can convert guanosine triphosphate (GTP) and ATP into the second

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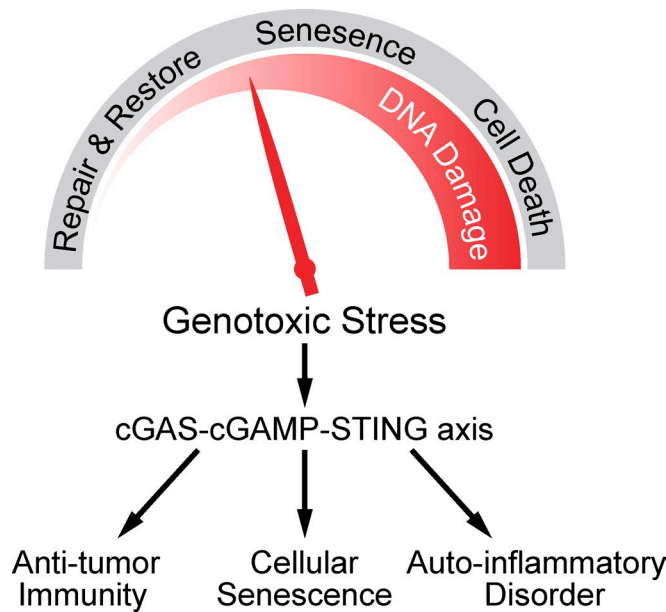
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Figure 1. Inflammatory response is another biological outcome of genomic instability. Genotoxic stress leads to DNA damage repair, cellular senescence, and cell death in a manner that depends on the severity of the DNA damage. The cGAS–cGAMP–STING pathway is activated by DNA damage to mediate antitumor immunity, senescence, and inflammatory responses.

messenger cyclic GMP-AMP (cGAMP; Wu et al., 2013). The cGAMP produced by cGAS contains two phosphodiester bonds: one between the 2'-hydroxyl group of GMP and 5'-phosphate of AMP and the other between the 3'-hydroxyl of AMP and 5'-phosphate of GMP (Ablasser et al., 2013; Diner et al., 2013; Gao et al., 2013a; Zhang et al., 2013). This cGAMP molecule, termed 2'3'-cGAMP, is an endogenous high-affinity ligand for the adaptor protein Stimulator of IFN Gene (STING, also known as MITA, MPYS, and ERIS; Ishikawa and Barber, 2008; Jin et al., 2008; Zhong et al., 2008; Sun et al., 2009).

STING forms a transmembrane homodimer that localizes to the ER and binds cyclic dinucleotides (CDNs), such as cGAMP and the bacterial second messengers c-di-GMP and c-di-AMP (Burdette et al., 2011; Wu et al., 2013). cGAMP binding induces a conformational change in STING (Gao et al., 2013b; Zhang et al., 2013), which subsequently translocates from the ER to the Golgi apparatus (Ishikawa and Barber, 2008; Saitoh et al., 2009). This process is thought to liberate the STING carboxyl terminus to subsequently recruit and activate TANK-binding kinase 1 (TBK1) and IFN regulatory factor 3 (IRF3) via a phosphorylation-dependent mechanism (Tanaka and Chen, 2012; Liu et al., 2015). STING also activates NF-κB, which functions together with IRF3 to turn on the transcription of type I IFNs and other cytokines (Fig. 2).

The molecular details of the cGAS–STING pathway and its pivotal roles in eliciting effective immunity against various microbial pathogens have been extensively reviewed elsewhere (Xiao and Fitzgerald, 2013; Cai et al., 2014; Wu and Chen, 2014; Chen et al., 2016b; Ma and Damania, 2016; Tao et al., 2016; Kato et al., 2017). In this review, we primarily focus on its emerging function in mediating DNA damage–induced inflammatory responses. We further discuss recent findings

on how the cGAS–STING axis contributes to the outcome of autoinflammatory diseases, senescence, and cancer.

cGAS senses cytoplasmic DNA as a consequence of nuclear DNA damage

DNA damage in the nucleus results in the accumulation of cytoplasmic DNA, notably in the form of micronuclei (Fig. 2). Micronuclei are small, DNA-containing organelles that resemble satellites to the primary nuclei (Fenech et al., 2011). They are products of chromosome damage as a result of genotoxic stress and chromosome missegregation in subsequent cell division. For instance, centromere-deficient chromosome fragments may result from the error-prone nonhomologous end-joining (NHEJ) repair and are unable to segregate normally, thereby forming micronuclei outside the newly formed nuclei. Similarly, whole chromosomes left behind by the spindle because of centromere hypomethylation or kinetochore dysfunction also end up as micronuclei after mitosis (Fenech et al., 2011). Since their early identification as "Howell-Jolly bodies" more than a century ago, micronuclei have been strongly associated with DNA damage and were adopted as a sensitive biomarker of genotoxicity (Fenech and Morley, 1985, 1986; Schlegel et al., 1986). The cytokinesis-block micronucleus (CBMN) assay, which scores the frequency of micronuclei, has been a widely used method to assess the genotoxicity of different chemical or radioactive mutagens (Fenech, 2007).

Micronuclei are originally formed with a nuclear envelope (NE), but more than half the micronuclei lose compartmentalization after mitosis as their NE ruptures (Hatch et al., 2013). NE breaks were found to negatively correlate with lamina integrity because lamin networks critically contribute to NE structural resilience (Vargas et al., 2012; Hatch et al., 2013). It was recently reported that NE of the nucleus can be rapidly repaired by the endosomal sorting complexes required for transport III (ESCRT-III) complex (Denais et al., 2016; Raab et al., 2016), and this mechanism may provide similar protection to NEs of micronuclei. These observations imply that micronuclei NEs may have compromised lamina function or membrane repair capacity so that it ruptures easily, although the detailed mechanisms underlying NE rupture await further study. A consequence of micronuclei rupture is that chromosomal DNA become accessible to cGAS (Bartsch et al., 2017; Dou et al., 2017; Glück et al., 2017; Harding et al., 2017; Mackenzie et al., 2017; Yang et al., 2017).

DNA damage also induces the accumulation of cytoplasmic DNA into "speckles," which are less aggregated than micronuclei (Ahn et al., 2014b; Lan et al., 2014; Härtlova et al., 2015; Shen et al., 2015; Erdal et al., 2017). Cytoplasmic DNA speckles contain mostly single-stranded DNA (ssDNA; Härtlova et al., 2015; Shen et al., 2015; Erdal et al., 2017), but may also contain small amounts of double-stranded DNA (dsDNA; Shen et al., 2015). ssDNA in general induces very little type I IFNs compared with dsDNA (Ishii et al., 2006) because cGAS doesn't bind ssDNA. However, stem-loop–forming ssDNA sequences in HIV reverse transcripts were shown to have a high potency of cGAS activation (Herzner et al., 2015). Accumulation of ssDNA in cells deficient in the Trex1 exonuclease is also associated with chronic IFN induction and autoinflammatory phenotypes (Yang et al., 2007; Stetson et al., 2008; Gao et al., 2015; Wolf et al., 2016). Among
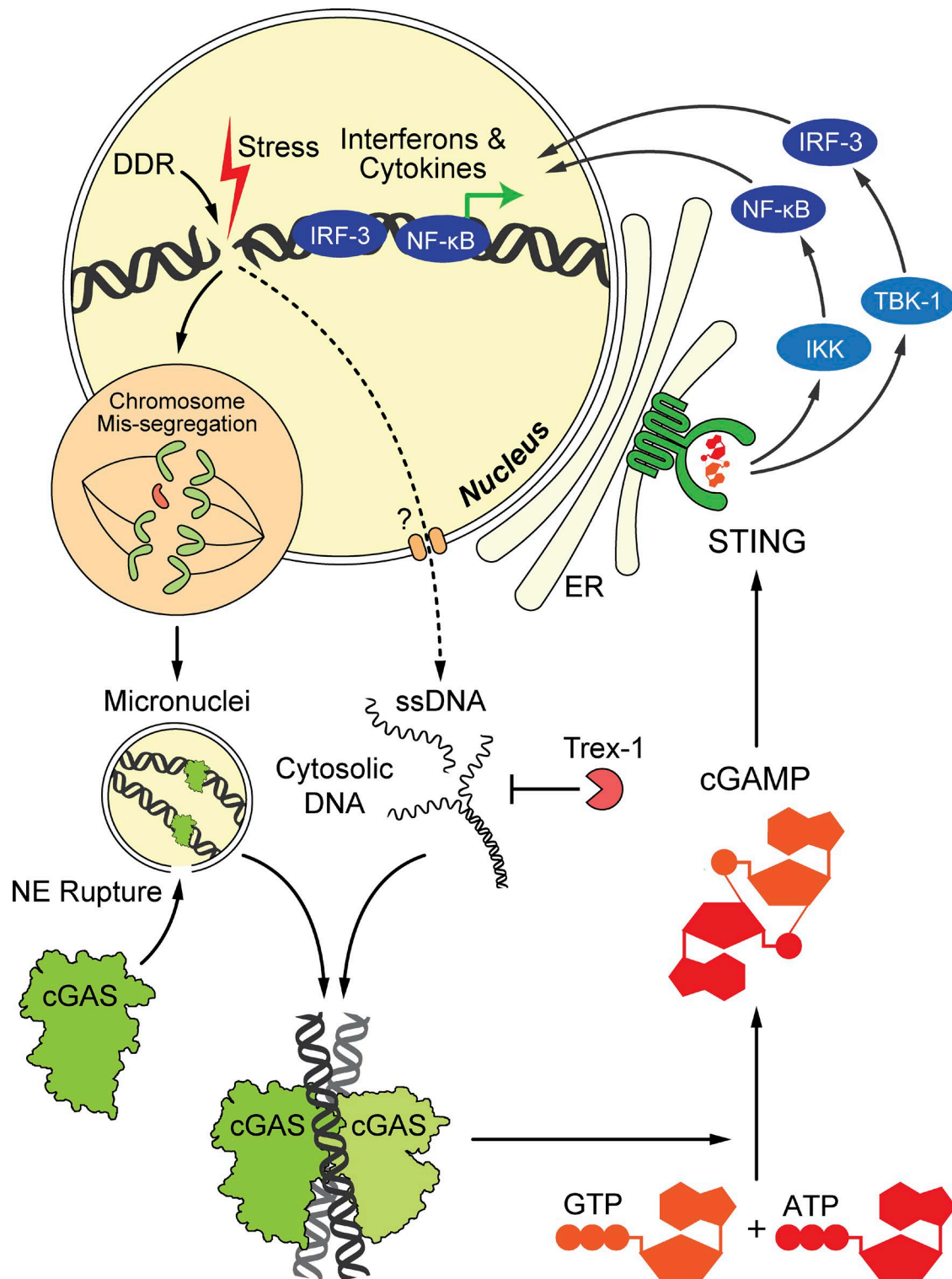
Figure 2. **The cGAS–cGAMP–STING pathway detects cytoplasmic DNA after DNA damage and activate type I IFNs and other cytokines.** Like DDR, the immune response is induced by various forms of genotoxic stress, ranging from ionizing radiation, DNA-damaging drugs, oxidative stress, oncogenic signaling, telomere shortening, and chromosome missegregation to viral infections and activation of endogenous retroelements. Nuclear DNA damage can generate cytoplasmic DNA in two possible ways. First, certain genomic damage causes chromosome to missegregate in subsequent cell division; the chromosome failing to partition into the new nuclei will form micronuclei. When the nuclear evelope (NE) of micronuclei ruptures, the DNA content is exposed to cGAS surveillance. Second, nuclear DNA damage can also generate ssDNA in the cytoplasm in a less understood process (dashed line and question mark). Such cytoplasmic DNA is degraded by Trex1, the loss of which leads to cGAS activation. Active cGAS dimerizes to synthesize cGAMP from GTP and ATP. cGAMP acts as a second messenger to activate STING on the ER surface. STING then activates transcription factors IRF3 and NF-κB via kinases TBK1 and IKK, respectively. IRF3 and NF-κB translocate into the nucleus to elicit the expression of IFNs and other cytokines. Damage to mitochondria or mitochondrial DNA can also lead to accumulation of mitochondrial DNA in the cytosol, resulting in cGAS activation and sterile inflammation (not depicted).

the ssDNA species generated after DNA damage, those that form double-stranded secondary structures may activate cGAS. Trex1 has also been shown to degrade nicked dsDNA (Chowdhury et al., 2006), and such DNA may accumulate in Trex1-deficient cells to activate cGAS.

Little is known about how DNA damage leads to DNA accumulation in the cytosol. MUS81 is an endonuclease that may play a role in this process. The MUS81-EME1 complex belongs to the family of structure-specific endonucleases that resolve interstrand DNA structures such as stalled replication forks and Holliday junctions (Dehé and Gaillard, 2017). MUS81 is required for genomic integrity and tumor suppression, as *mus81*⁻/⁻ mice display increased chromosomal aberrations in T cells and are predisposed to lymphomas and other cancers (McPherson et al., 2004). It was found that MUS81 is required for the generation of cytoplasmic DNA in prostate cancer cells (Ho et al., 2016). The amount of cytoplasmic DNA positively correlates with MUS81 protein level and the number of MUS81 nuclear foci, suggesting that MUS81 is engaged in the process that converts nuclear DNA into cytoplasmic forms. Consistently, MUS81 promotes type I IFNs and thus immune rejection of prostate tumor (Ho et al., 2016). In contrast, however, it was also reported that MUS81 is a negative regulator of spontaneous type I IFN production (Laguette et al., 2014). Thus, the exact roles of MUS81 in regulating cytoplasmic DNA sensing remain to be elucidated. A recent study reported that extracellular telomere repeat DNA (ECTR), generated by the alternative lengthening of telomeres (ALT) mechanism, accumulates in the cytoplasm of ALT cell lines and activates IFN-β via the cGAS–STING pathway (Chen et al., 2017). As MUS81 is necessary for ALT (Zeng et al., 2009), the cytoplasmic DNA derived from MUS81 activity may include ECTR DNA.

Multiple studies have recently demonstrated that induction of cytokines by DNA damage is mediated through the cGAS–STING pathway (Lan et al., 2014; Härtlova et al., 2015; Bartsch et al., 2017; Erdal et al., 2017; Glück et al., 2017; Harding et al., 2017; Mackenzie et al., 2017; Yang et al., 2017). After DNA damage, cGAS is recruited to micronuclei (Glück et al., 2017; Harding et al., 2017; Mackenzie et al., 2017; Yang et al., 2017). NE rupture of micronuclei appears to precede cGAS recruitment (Harding et al., 2017; Mackenzie et al., 2017). cGAS in the micronuclei is presumably activated by the chromosomal DNA fragments to synthesize cGAMP, but direct evidence is still lacking. Interestingly, although cGAS usually localizes in the cytoplasm of nondividing cells, a majority of cGAS associates with chromatin after nuclear membrane breakdown during mitosis (Harding et al., 2017; Mackenzie et al., 2017; Yang et al., 2017). Because there is no evidence of cGAS activation during cell division, it will be interesting to determine how cGAS activity is modulated during chromatin association. Similarly, it remains unclear what form of chromatin DNA in the micronuclei may activate cGAS. Because cGAS-associated micronuclei stain positive for phosphorylated γ-H2A, a DNA damage marker (Mackenzie et al., 2017; Yang et al., 2017), DNA damage itself may contribute to cGAS sensing. In this regard, a recent proteomic study showed that cGAS is associated with a ribonuclear complex dubbed HEXIM1-DNA–protein kinase (PK)–paraspeckle components–ribonucleoprotein complex (HDP–RNP) that contains DNA-PK (Morchikh et al., 2017).

It will be interesting to determine whether this complex plays a role in the regulation of cGAS activity by DNA damage in the primary nucleus and in micronuclei.

Together, these new findings have demonstrated that the cGAS–cGAMP–STING pathway connects genotoxic stress to cytokine expression by sensing cytoplasmic DNA as a common consequence of nuclear DNA damage.

The cGAS–cGAMP–STING axis connects DNA damage to autoinflammatory diseases

Genetic deficiencies that compromise DDR functions also induce cytokines and lead to autoinflammatory diseases. An underlying mechanism is the aberrant activation of the cGAS–STING pathway. For instance, Ataxia-Telangiectasia Mutated (ATM) is a critical kinase for DDR initiation. ATM mutations result in dysfunctional V(D)J recombination and ultimately the immunodeficiency syndrome Ataxia-Telangiectasia (A-T), because V(D)J recombination is essential for antibody production and T cell development. A-T patients are also prone to autoinflammation. Elevated type I IFN signaling was associated with ATM deficiency in cultured cells (Siddoo-Atwal et al., 1996; Sugihara et al., 2011), as well as in sera of human A-T patients (Härtlova et al., 2015). The autoinflammatory phenotype of A-T is abrogated in *atm*⁻/⁻ *sting*⁻/⁻ double knockout mice and significantly reduced after cGAS knockdown, indicating that the cGAS–STING pathway plays an essential role in this disease (Härtlova et al., 2015).

Aicardi-Goutières syndrome (AGS) is an inheritable neurological disease that leads to microcephaly, intellectual retardation, and childhood death (Crow and Rehwinkel, 2009). This debilitating disease is driven by chronic IFN signaling caused by recessive mutation in one of a few genes involved in nucleic acid metabolism, such as *trex1*, *rnaseH2a*, *rnaseH2b*, and *samhd1* (Crow and Rehwinkel, 2009). For instance, the *trex1* gene encodes a 3′–5′ exonuclease that salvages DNA fragments generated from nuclear DNA damage (Yang et al., 2007; Erdal et al., 2017). Similar to human AGS patients with *trex1* deficiency, *trex1*⁻/⁻ mice also develop life-shortening inflammatory phenotypes (Crow et al., 2006a), which can be rescued by genetic ablation of either *cGas* or *sting* (Gall et al., 2012; Ahn et al., 2014a; Gao et al., 2015; Gray et al., 2015). In tissues from *trex1*⁻/⁻ mice, elevated cGAMP was detected by mass spectrometry; cGAMP detection in these mice was also dependent on wild-type cGAS, directly demonstrating that cGAS is activated in *trex1*⁻/⁻ mice (Gao et al., 2015).

RNaseH2 is a nuclear enzyme responsible for removing ribonucleotides that are misincorporated into genomic DNA (Reijns et al., 2012). Genetic defects in any of the three subunits of RNaseH2 leads to chronic DNA damage, elevated type I IFNs and perinatal lethality in mice (Hiller et al., 2012; Reijns et al., 2012; Mackenzie et al., 2016; Pokatayev et al., 2016), and the AGS phenotypes in humans (Crow et al., 2006b). In *RnaseH2b*⁻/⁻ cells, persistent genome instability promotes the formation of micronuclei, which recruit cGAS and activate type I IFN signaling (Bartsch et al., 2017; Mackenzie et al., 2017). Genetic removal of cGAS or STING can experimentally reverse the ensuing inflammation and autoimmune phenotypes in RNaseH2-deficient mice (Mackenzie et al., 2016; Pokatayev et al., 2016). AGS is also caused by mutations in human SAMHD1 (Rice et al., 2009), a

deoxyribonucleotide triphosphate triphosphatase that balances nuclear levels of deoxyribonucleotide triphosphates (Franzolin et al., 2013) and genome stability (Kretschmer et al., 2015). Although SAMHD1-deficient mice do not show any overt autoinflammatory phenotype, their myeloid cells express elevated levels of IFN-stimulated genes that are dependent on cGAS and STING (Maelfait et al., 2016). Together, these observations demonstrate the central role of the DNA-sensing pathway in mediating DNA damage–induced inflammatory diseases.

ERCC1-XPF is a structure-specific endonuclease critical for multiple genome maintenance mechanisms such as nucleotide excision repair and dsDNA break repair. Mice lacking ERCC1 display increased genome instability and chronic expression of inflammatory cytokines, leading to premature aging and early death (Melton et al., 1998; Niedernhofer et al., 2006; Karakasilioti et al., 2013). Notably, ERCC1 deficiency in humans causes the severe developmental disorder Cerebro-Oculo-Facio-Skeletal syndrome (COFS). Key features of the COFS syndrome, such as microcephaly with calcification, are similar to those of AGS and the Cockayne syndrome, the latter being caused by mutations in *ercc8* or *ercc6* (Troelstra et al., 1992; Henning et al., 1995). While the aging phenotype of ERCC1 deficiency requires NF-κB in mice (Tilstra et al., 2012), it remains to be determined whether aberrant cGAS–STING activity may also underpin these inheritable disorders.

Recent studies have shown that cGAS activation by self DNA is also linked to more common and complex diseases such as myocardial infarction (MI) and age-related macular degeneration (AMD). MI is known to drive inflammation and exacerbate lethality. The massive death of cardiomyocytes activates in heart macrophages strong type I IFN signaling that is mediated by the cGAS–STING pathway (King et al., 2017; Cao et al., 2018). Mice lacking components of the pathway, including cGAS, IRF3, and IFNAR1, had significantly improved early survival in experimental MI model, compared with wild-type animals. Similarly, cGAS also facilitates inflammation in the eyes (Kerur et al., 2017). In an Alu-RNA–induced model of macular degeneration in mice, retinal pigment epithelium (RPE) degeneration was shown to rely on caspase 4/11 and Gasdermin D of the inflammasome pathway, as well as cGAS, STING, IRF3, and IFNAR1. Although it is unclear how the cGAS–STING pathway leads to inflammasome activation in RPE cells, it has been recently reported that STING-mediated cell death can activate the NLRP3 inflammasome in some human myeloid cells (Gaidt et al., 2017).

While accumulation of cytosolic DNA through nuclear DNA damage, loss-of-function mutations of DNA degrading enzymes, or tissue damage leads to autoinflammatory diseases, gain-of-function mutations in STING are sufficient to cause an autoinflammatory disorder termed STING-associated vasculopathy with onset in infancy (SAVI; Liu et al., 2014; Melki et al., 2017). These mutations lead to constitutive STING activation and type I IFN signaling. Interestingly, the resulting vascular and pulmonary syndrome of SAVI resemble those of some DDR deficiency–associated autoinflammation, such as A-T and Artemis deficiency (Gul et al., 2017), consistent with the notion that these genetic disorders affect the same signaling pathway.

Besides damaged nuclear DNA, cGAS also responds to other self-DNA that mislocalizes to the cytoplasm and drives autoinflammation. Haplodeficiency of TFAM (transcription factor A, mitochondria), a protein that packages mitochondria DNA (mtDNA) into nucleoid, was reported to promote releases of mtDNA into the cytosol, which activate cGAS to confer IFN-mediated viral resistance (West et al., 2015). In response to apoptotic stimuli, mitochondria not only releases cytochrome *c* to activate caspases to execute apoptosis, but also mtDNA to the cytosol, which could have otherwise activated cGAS to trigger inflammation if there were no concurrent activation of caspases (Rongvaux et al., 2014; White et al., 2014). Through an unknown mechanism caspase activation in apoptotic cells inactivate the cGAS–STING pathway, thereby suppressing sterile inflammation. DNaseII is a lysosomal endonuclease that is responsible for degrading phagocytosed exogenous DNA from dead cells or expelled nuclei of developing red blood cells. Mice lacking DNaseII are embryonically lethal as a result of type I IFN–driven cell death (Kawane et al., 2003), which can be partially rescued by genetically ablating type I IFN receptor (Yoshida et al., 2005) and nearly completely rescued by deleting STING (Ahn et al., 2012) or cGAS (Gao et al., 2015). In DNaseII-deficient but not normal fetal livers, cGAMP can be detected by mass spectrometry (Gao et al., 2015). Therefore, cGAS also senses phagocytosed DNA that are inadequately digested in the lysosome. Human patients with loss of function mutations of DNaseII have recently been identified, and these patients exhibit symptoms of type I interferonopathy, including tissue inflammation and elevated anti-DNA antibodies (Rodero et al., 2017). Together, these findings strongly support the central role of the cGAS–cGAMP–STING pathway in the pathogenesis of a variety of type I IFN–mediated monogenic diseases. Therefore, components of this pathway represent promising targets for developing pharmacological intervention for AGS and other autoinflammatory disorders.

cGAS is essential for DNA damage–induced cellular senescence

Cellular senescence is a state of irreversible cell cycle arrest, induced by a variety of external or internal stress such as telomere shortening, oxidative damage, and oncogenic signaling. Although the causes of and phenotypes generated by cellular senescence are manifold, persistent DDR is thought to be the common mechanism that is critical for the establishment and maintenance of senescence phenotypes (d'Adda di Fagagna, 2008). Specifically, DDR activates the p53–p21 and p16^{INK4a}–Rb pathways to block the cell cycle and implement senescence programs.

Recent research has provided strong evidence that cGAS also has an essential role in promoting cellular senescence (Dou et al., 2017; Glück et al., 2017; Yang et al., 2017). The initial clue came from spontaneous immortalization of murine embryonic fibroblasts (MEFs). When primary MEFs are serially passaged, the majority of cells gradually senesce, and only a small fraction overcome the growth crisis and become immortalized. Compared with wild-type MEFs, the *cGas*$^{-/-}$ MEFs are immortalized more rapidly, suggesting that cGAS inhibits cell proliferation (Glück et al., 2017; Yang et al., 2017). Oxidative DNA damage was previously shown to limit spontaneous immortalization (Parrinello et al., 2003). The antiproliferative effect of cGAS is ameliorated at lower oxygen levels, suggesting cGAS functions downstream of oxidative DNA damage (Glück et al., 2017). Consistent with

its role in limiting cell replication, cGAS is also required for the expression of senescence markers, such as p16[INK4a] and senescence-associated β-galactosidase (SA-β-Gal) during MEF immortalization. Therefore, cGAS plays an indispensable role in cellular senescence in response to oxidative stress. Similarly, irradiation, DNA-damaging drugs, or oncogene activation all induce cellular senescence in a cGAS-dependent manner. The activation of oncogenes, such as NRasV12, leads to DNA hyperreplication, increased replication errors, and initiation of the DNA damage response (Di Micco et al., 2006). In cells under these genotoxic stresses, cGAS is recruited to micronuclei. In oncogene-induced senescent cells, cGAMP is detectable by LC-MS (Dou et al., 2017). Therefore, cGAS senses micronuclei as the result of genomic DNA damage to promote senescent phenotypes.

How does the cGAS–STING pathway regulate cellular senescence, given that it is not an integral component of either the p53–p21 or the p16 [INK4a]–Rb pathway? A mechanism is likely through the senescence-associated secretory phenotype (SASP), which refers to the phenomenon that senescent cells produce and release a range of cytokines, chemokines, and proteases to the extracellular milieu to modulate senescent cells themselves and their microenvironment (Kuilman and Peeper, 2009). After various forms of senescing stimuli, such as oxidation, radiation, chemotherapeutic drugs, or mitogenic stress, cells produce in a cGAS-dependent manner a range of cytokines and chemokines, such as IFN-β, IL-1β, IL-6, and IL-8 (Dou et al., 2017; Glück et al., 2017; Yang et al., 2017). Some of these cytokines, namely IL-8 and IL-6, are known to feedback to the secreting cells to reinforce senescence signaling (Acosta et al., 2008; Kuilman et al., 2008). Type I IFNs are also prosenescence and antiproliferative, as they induce DNA damage and elevate the p53 level (Takaoka et al., 2003; Moiseeva et al., 2006). Therefore, the cGAS–STING pathway provides a critical paracrine signal that is necessary for sustaining cellular senescence.

Besides promoting senescence cell-autonomously, SASP can signal to the immune system and modulate the tissue microenvironment. Previously, it was demonstrated in a liver cancer model that senescent liver cells attract via SASP a variety of immune cells, specifically NK lymphocytes and neutrophils, to clear tumor cells (Xue et al., 2007). In a liver fibrosis model, SASP was shown to recruit and activate NK cells to eliminate senescent cells and resolve fibrosis (Krizhanovsky et al., 2008). The cGAS–STING pathway is also required for SASP-mediated antitumor effect. Dou et al. used an NRasV12-expressing vector to induce liver tumors and found in the STING null mice attenuated infiltration of immune cells, reduced clearance of senescent cells at the early stage, and increased tumor growth at later stages (Dou et al., 2017). Therefore, senescence can promote immune surveillance to suppress malignancy via SASP.

Although evidence cited above illustrates SASP as an antitumor component of senescence, it should be noted that SASP can also promote tumorigenesis (Coppé et al., 2010). SASP from senescent fibroblasts can strongly induce epithelium cells to proliferate, transform, and metastasize (Krtolica et al., 2001; Coppé et al., 2008). Particularly, IL-6 and IL-8 can induce invasiveness of epithelium cells (Coppé et al., 2008); matrix metalloprotease can remodel tissue microenvironment to increase the availability of growth factors (Liu and Hornsby, 2007); and vasculature endothelium growth factor (VEGF) can promote endothelium cell migration and tumor angiogenesis (Coppé et al., 2006). Therefore, SASP can mediate both positive and negative effects of senescence on cancer. Such dichotomous functions of senescence and the role of the cGAS–STING pathway in these functions should be further clarified in future studies.

Senescence also regulates stem cell functions and aging. Previously, type I IFN signaling was shown to mediate a variety of stem cell defects in telomerase-deficient mice as the result of persistent DNA damage (Yu et al., 2015). Deletion of the IFN α/β receptor 1 (IFNAR1) rescues the senescence phenotypes in Terc^{-/-} mice. As the role of IFN-β in stress-induced senescence is reminiscent of the roles of cGAS and STING (Katlinskaya et al., 2016), it would be interesting to investigate the cGAS–STING pathway in the process of stem cell senescence under stress conditions. Recent evidence shows that STING null mice display remarkably less hair graying months after irradiation (Dou et al., 2017). Radiation-induced genomic damage was previously shown to cause a loss of renewal of melanocyte stem cells (Inomata et al., 2009); therefore, STING-mediated SASP may contribute to regulating these stem cells. Remarkably, experimental elimination of p16[INK4a]-positive senescent cells in mice can attenuate aging phenotypes in organs such as the kidney and the heart, significantly prolonging mouse life span and health span (Baker et al., 2016). Given that the cGAS–STING pathway plays critical roles in cellular senescence, inhibitors of cGAS or STING may offer similar benefits to treat senescence and age-related diseases.

DNA damage activates the cGAS–cGAMP–STING pathway in cancer

Crucial links between DNA damage and cancer have long been recognized. On one hand, DNA damage can promote antitumor immunity in both natural rejection and therapies. On the other hand, genome instability is a hallmark as well as an important driving force of cancer. As the cGAS–cGAMP–STING pathway critically determines the immunological outcomes of DNA damage, it also plays prominent roles in both aspects of cancer.

Effective antitumor immunity relies on cross-presentation of tumor antigens by APCs to CD8 T lymphocytes. The activation of APCs requires type I IFN signaling initiated by innate immune sensors (Diamond et al., 2011; Fuertes et al., 2011). Recent evidence has shown that the cGAS–STING pathway, activated in APCs by cytosolic DNA, provides a critical source of such a priming signal. It was shown that the STING-deficient mice fail to reject the growth of inoculated tumor cells spontaneously (Woo et al., 2014) after local radiation therapy (Deng et al., 2014) or after immune checkpoint blockades using antibodies against PD-L1 (Wang et al., 2017) or CD47 (Xu et al., 2017). Similarly, cGas^{-/-} mice also show defective tumor rejection (Wang et al., 2017; Xu et al., 2017). In line with these findings, the cGAS–STING pathway in dendritic cells (DCs) is required to prime DCs in coculture with tumor cells for cross-presentation to activate CD8 T cells (Deng et al., 2014; Wang et al., 2017; Xu et al., 2017). Consistent with tumor suppression by the cGAS–STING pathway, treatment with exogenous cGAMP or other STING agonists can also enhance immunity and promote tumor regression (Li et al., 2013;

Chandra et al., 2014; Corrales et al., 2015; Demaria et al., 2015). Importantly, cGAMP therapy can further enhance the antitumor effect of ionizing irradiation (Deng et al., 2014) or chemotherapy (Li et al., 2016) and synergize with immune checkpoint blockade (Demaria et al., 2015; Wang et al., 2017).

How is cGAS activated in APCs? Cell death is known to occur commonly in tumors as a consequence of genomic instability, hypoxia, or other tumor-associated stresses (Lowe and Lin, 2000), providing ample DNA that may activate cGAS in APCs (Xu et al., 2017). As infiltrating APCs sample tumor tissue by phagocytosis, they also pick up tumor DNA. Through an undefined mechanism, tumor DNA may escape phagosomes and enter the cytoplasm to activate cGAS and STING (Woo et al., 2014). Together, the data suggest that cytoplasmic DNA sensing is an early and critical step in APC activation that determines the outcome of antitumor immunity.

Besides APCs, the cGAS pathway is also active in some tumor cells to promote antitumor immunity. The activity is likely triggered by increased genome instability in tumor cells. For instance, genome-derived DNA was shown to accumulate in the cytoplasm of prostate cancer cells as the result of nuclear MUS81 activity, inducing STING-dependent type I IFNs and tumor rejection (Ho et al., 2016). Radiation further promotes DNA damage and DNA sensing in tumor cells. It was shown that irradiated B16 cells can be used as a cancer vaccine to promote systematic tumor rejection in combination with immune checkpoint blockade. However, STING deficiency in the irradiated tumor cells was found to abolish the efficacy of this combination therapy (Harding et al., 2017). Therefore, DNA damage intrinsically engages the cGAS–STING pathway in tumor cells to prevent malignancy. In addition to elevating antitumor immunity, this pathway is also implicated in shaping the tumor microenvironment. *Sting*^{−/−} mice are more susceptible to colitis-associated cancer induced by chronic DNA damage and inflammation (Zhu et al., 2014; Ahn et al., 2015). Persistent activation of the cGAS–STING pathway also lead to cell death, adding another layer of resistance to tumorigenesis (Li et al., 2016; Tang et al., 2016; Gaidt et al., 2017; Gulen et al., 2017; Larkin et al., 2017). For example, 5,6-dimethylxanthenon-4-acetic acid (DMXAA), a potent agonist of mouse STING (Conlon et al., 2013), can effectively induce tumor hemorrhagic necrosis and promote tumor regression in mice (Zwi et al., 1994). Moreover, cGAMP treatment can also trigger the activation of NLRP3 inflammasome (Gaidt et al., 2017; Swanson et al., 2017). In summary, the cGAS–STING pathway acts as an intrinsic barrier to tumorigenesis by linking DNA damage to several antitumor mechanisms: immune surveillance, cellular senescence, and cell death. Therefore, this pathway represents an attractive pharmaceutical target to harness these intrinsic antitumor mechanisms for cancer therapy.

In response to the tumor suppressive functions of cGAS and STING, cancer cells frequently adapt to down-regulate these proteins to promote malignancy (Xia et al., 2016a,b). Likewise, oncogenic DNA viruses, such as human papilloma virus and adenovirus, evolved specific viral proteins to directly antagonize STING (Lau et al., 2015). As a counter measure to these immune evasive mechanisms, engineered oncolytic viruses, which preferentially replicate in tumor cells defective in type I IFN signaling, can be exploited to treat late-stage malignant cancer (Xia et al., 2016b; de Queiroz et al., 2017).

On the other side of the dichotomous relationship between DNA damage and cancer are protumor functions of the cGAS–STING pathway induced by genotoxic stress. 7,12-dimethylbenz(a)anthracene (DMBA) is a strong DNA-damaging agent that induces inflammation-driven skin carcinogenesis. As DMBA activates the cGAS–STING pathway, DMBA-treated *sting*^{−/−} mice, unlike other cancer models, are actually more resistant to the growth of DMBA-induced skin cancer (Ahn et al., 2014b). Adoptive transfer assays implicated STING in both hematopoietic and nonhematopoietic cells for tumorigenesis in the DMBA model. Another study shows that cancer cells metastasizing to the brain can also use the cGAS–STING pathway to promote tumor progression (Chen et al., 2016a). These cancer cells transfer cGAMP via gap junctions into astrocytes; subsequent paracrine signaling from astrocytes feedback to cancer cells to promote malignancy (Chen et al., 2016a). It is possible that the protumor effect of the cGAS–STING pathway may be linked in part to inflammatory cytokines, such as TNF-α. Although TNF-α can induce potent necrosis in tumors, it is also an important driver of inflammatory carcinogenesis (Balkwill, 2009). TNF-α can promote epithelium cells to survive under stress, induce blood vessel growth (Leibovich et al., 1987), and enhance metastasis (Malik et al., 1990; Orosz et al., 1993). *tnf-α*^{−/−} mice were shown to be resistant to carcinogen-induced skin cancer (Moore et al., 1999). Therefore, an active cGAS–STING axis can exacerbate certain cancers. Such findings add a note of caution to the development of anticancer strategies that aim to activate the cGAS–STING pathway.

In summary, genomic instability engages the cGAS–cGAMP–STING pathway on both sides of cancer. On the bright side, this pathway is instrumental for antitumor immunity and is an intrinsic barrier in tumor cells that blocks malignancy. On the dark side, this pathway can promote inflammation-driven carcinogenesis and metastasis. As mounting evidence suggests that the cGAS–cGAMP–STING pathway makes fundamental contributions to at least three major cancer therapies, radiation therapy, chemotherapy, and immunotherapy, it represents a promising pharmaceutical target to enhance our current treatment regimens. A major task in cancer therapies is to develop new approaches to maximize the bright side while minimizing the dark side of the contributions of the cGAS pathway in cancer.

Closing remarks and future directions

It is now well established that cGAS is a major sensor of microbial pathogens that contain DNA or generate DNA in their life cycles. New studies have illuminated an equally critical role of cGAS in the surveillance of self-DNA that mislocalizes to the cytoplasm under pathological conditions. Although decades of research have led to a good understanding of how genotoxic stress and genomic instability regulate DNA damage repair, cell cycle checkpoints, and programmed cell death, the mechanism by which DNA damage leads to inflammation and autoimmunity was not well understood until recently. In retrospect, it is logical that cGAS is well positioned to connect nuclear DNA damage to immune responses because cGAS can be activated by any dsDNA that enters the cytoplasm. It is interesting to note that in addition

to chemical and radiation damage to DNA, many other biological processes such as oncogene activation, telomere shortening, and cell division errors all impinge on DNA. Although NF-κB is well known to play an important role in mediating inflammatory responses to DNA damage (Janssens and Tschopp, 2006), the link between DNA and NF-κB was not clear. Now cGAS has emerged as an important link between DNA damage and activation of NF-κB, IRF3, and likely other transcription factors.

Many important questions remain. For example, it is not clear how cytoplasmic DNA is generated from damaged nuclear genome. Although MUS81 has been shown to regulate generation of cytoplasmic DNA from genomic DNA (Ho et al., 2016), additional factors may be required for this process. The exact cellular mechanism responsible for transporting genomic DNA fragments from the nucleus to the cytoplasm remains to be elucidated. Also unclear is how GAS is activated in micronuclei. Although cGAS was found to associate with the chromatin during mitosis (Yang et al., 2017), there is no evidence that cGAS is activated during cell cycle transition. Thus, it is important to determine how cGAS is kept inactive when it is associated with the chromosome during mitosis and how cGAS is activated by chromatin fragments within the micronuclei. How cGAS is activated in APCs by tumor DNA also requires further investigation. After APCs phagocytose tumor cells, tumor antigens are delivered from the phagosome to the cytosol for proteasomal processing and cross-presentation of antigenic peptides. It is possible that tumor cell DNA enters the cytosol along with tumor protein antigens, but details regarding the cytosolic delivery of tumor proteins and DNA remain to be delineated. As tumor DNA is also chromatinized, it is unknown whether it requires any further processing to become an active cGAS ligand.

The knowledge gained from understanding the cGAS–STING pathway in DDRs may be translated into innovative therapies for a variety of human diseases. Antagonists of cGAS or STING are potentially useful in blocking uncontrolled cytokine expression that leads to inflammation and autoimmune diseases. Because these antagonists may inhibit cellular senescence, they may also be used for treating senescence-associated diseases. Several attempts have been reported to find compounds that inhibit cGAS. So far the reported molecules are active against cGAS in vitro, but haven't demonstrated desirable potency in vivo (An et al., 2015; Hall et al., 2017; Vincent et al., 2017). A concern about using antagonists of cGAS or STING in treatments of inflammation or senescence-associated diseases is a potential increase of susceptibility to infectious diseases as well as cancer. Hopefully, redundancy in the human immune system and selection of appropriate doses of drugs that partially inhibit the cGAS–STING pathway would allow these drugs to provide therapeutic benefits to the patients without compromising their immunity to infections and tumorigenesis.

Agonists of the cGAS–STING pathway such as cGAMP and its analogues, on the other hand, can be applied to enhance antitumor immunity in conjunction with other cancer therapies. These small molecules can be further improved by preventing hydrolysis of the phosphodiester bonds (Li et al., 2014). Several STING agonists have already entered clinical trials in human, and more potent and selective agonists of STING are expected in the next few years. Although these STING agonists will strongly enhance antitumor responses, including in those "cold" tumors that are so far refractory to immune checkpoint blockade, a major concern of using these compounds systemically is the cytokine "storm," the acute production of multiple inflammatory cytokines that could cause severe toxicity and even death. Thus, it remains to be seen whether there is a sufficient therapeutic window that allows STING agonists to be used at certain doses to provide efficacious antitumor activity while minimizing immunotoxicity to patients. Innovative approaches, such as nanoparticles (Hanson et al., 2015; Luo et al., 2017; Wilson et al., 2018), viral particles (Bridgeman et al., 2015; Gentili et al., 2015), or targeted deliveries, could allow precise delivery of STING agonists to tumors to generate localized and specific antitumor immune responses and significantly improve the therapeutic window of using these compounds for cancer immunotherapies.

Acknowledgments

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