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1 **STING signaling and sterile inflammation**

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3

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10

11 **Abstract**

12 Innate immunity is regulated by a broad set of evolutionary conserved receptors to
13 finely probe the local environment and maintain host integrity. Besides pathogen
14 recognition through conserved motifs, several of these receptors also sense aberrant
15 or misplaced self-molecules as a sign of perturbed homeostasis. Among them, self-
16 nucleic acid sensing by the cyclic GMP-AMP synthase (cGAS)/stimulator of interferon
17 genes (STING) pathway alerts on the presence of both exogenous and endogenous
18 DNA in the cytoplasm. We review recent literature demonstrating that self-nucleic acid
19 detection through the STING pathway is central to numerous processes, from cell
20 physiology to sterile injury, auto-immunity and cancer. We address the role of STING
21 in autoimmune diseases linked to dysfunctional DNase or related to mutations in DNA
22 sensing pathways. We expose the role of the cGAS/STING pathway in inflammatory
23 diseases, neurodegenerative conditions and cancer. Connections between STING in
24 various cell processes including autophagy and cell death are developed. Finally, we
25 review proposed mechanisms to explain the sources of cytoplasmic DNA.

26

27 Keywords: STING, cGAS, self-DNA, sterile inflammation, autophagy, type I IFN

28 **Introduction**

29 Supported by Charles Janeway's central model of innate immunity based on pathogen
30 recognition through conserved motifs(1), host detection of pathogen-derived nucleic
31 acids by pathogen recognition receptors (PRRs) is a common and effective strategy to

32 sense invading microorganisms and initiate innate and adaptive immune responses(2).
33 PRRs are also involved in context-dependent recognition of self-nucleic acids, either
34 nuclear DNA (nDNA) or mitochondrial DNA (mtDNA)(3-6). This is in line with Polly
35 Matzinger's model proposing that in numerous instances what ultimately matters for a
36 host is to detect danger independently of its external or internal origin to mount an
37 immune response. Cell stress or damage results in the production and/or the release
38 of host-derived danger signals(7). Apart from mitosis, cell DNA is confined within the
39 nucleus or mitochondria. DNA in the cytoplasm, from microbial origin (e.g. following
40 viral or bacterial infection) or from the host own genetic material, informs of a potentially
41 deleterious situation and the latter constitutes an endogenous danger signal.
42 Among PRRs involved in self-nucleic acid recognition, an increasing interest has
43 emerged for STimulator of Interferon Genes (STING), also known as TMEM173,
44 MPYS or MITA(6, 8-10). STING is an endoplasmic reticulum (ER)-located PRR, which
45 does not directly bind to DNA. Its activating ligands are cyclic dinucleotides (CDNs).
46 CDNs are produced as second messengers by microorganisms (11, 12) or synthesized
47 by the enzyme cyclic GMP-AMP synthase (cGAS) in response to binding either host-
48 or pathogen-derived cytosolic double-stranded (ds)DNA(6, 13-15). While displaying no
49 apparent DNA sequence specificity(16), cGAS activity is limited by spatial distribution
50 of its substrate as well as its degradation by host DNases. cGAS binds to nDNA(17) or
51 mtDNA(18) that can be continuous, fragmented or supercoiled(17). Crystal structure
52 analysis of mouse cGAS revealed that it functions as a dimer, with each protein
53 catalytic domain binding to an 18 base pairs (bp) double-stranded (ds) DNA at two
54 different sites, forming a 2:2 complex(19, 20). While initially described as a cytoplasmic
55 protein, a recent study found that cGAS binds to the inner leaflet of the plasma
56 membrane to regulate its activity and prevent overactivation from genotoxic stress(21).
57 Other studies proposed that cGAS resides predominantly in the nucleus of various
58 mouse and human cell lines, as well as human peripheral blood mononuclear cells
59 (PBMC)-derived macrophages and dendritic cells(22, 23). However, its baseline
60 activation in the nucleus is prevented by poorly accessible nucleosomal state of
61 nDNA(20, 22, 24). Thus, cGAS cellular localization appears unclear and additional
62 studies are required.
63 DNA binding to cGAS triggers ATP and GTP conversion into cyclic guanosine
64 monophosphate–adenosine monophosphate (cGAMP). cGAMP is the canonical CDN

65 that binds and activates STING (**FIGURE 1**). cGAMP binding to STING elicits a
66 conformational shift and its dimerization as well as its translocation to the ER-Golgi
67 intermediate compartment (ERGIC). STING dimers recruit TANK-binding kinase 1
68 (TBK1), which phosphorylates STING on Ser366 to serve as a docking site for
69 interferon regulatory factor 3 (IRF3) and its phosphorylation by TBK1. STING also
70 leads to nuclear factor κ B (NF- κ B) activation. IRF3 and NF- κ B transcription factors lead
71 to the production of type I interferons (IFNs) and other cytokines involved in host
72 immune responses(6, 8, 25, 26). Interestingly, recent studies showed that STING
73 stimulation can also lead to type III IFN production (also known as IL-28/IL-29 or IFN λ)
74 (27-29). Besides its roles in the regulation of gene expression, STING induces non-
75 canonical autophagy through its direct interaction with the microtubule-associated
76 protein light chain 3 (LC3), a key initiator of autophagy that cycles between the nucleus
77 and cytoplasm(25, 30-32). Importantly, STING-induced autophagy mediates the
78 clearance of cytosolic DNA(30). STING also regulates various cell death processes
79 including lysosomal cell death(33), apoptosis(34) and necroptosis(35). Of note, in
80 addition to cGAS, other cytosolic receptors (e.g. DDX41, IFI16) can sense DNA or
81 CDNs and activate STING(36-38). The cGAS/STING pathway is important to control
82 viral and bacterial pathogen infection(39, 40) as well as for immune surveillance(41,
83 42). STING is ubiquitously expressed in a variety of tissues including lungs, liver,
84 kidney, heart and spleen(8). STING is expressed in both innate and adaptive immune
85 cells (e.g. macrophages(8), dendritic cells(8), natural killer cells(43), CD4⁺ and CD8⁺ T
86 lymphocytes(44) and B lymphocytes(45)). It is also expressed by nonhematopoietic-
87 derived cells including endothelial cells(46), epithelial cells(47) and neurons(48).
88 Here, we detailed known mechanisms to explain DNA access to the cytoplasm, briefly
89 review STING allelic variants and then focus on STING biology in the context of self-
90 DNA sensing associated with autoimmunity, cancer or sterile inflammatory settings.

91

92 *DNA access to cytoplasm*

93 Host DNA, which resides within the nucleus or mitochondria, may be released into the
94 cytoplasm following numerous processes, including genomic DNA instability,
95 mitochondrial stress and endosomal/lysosomal rupture (**Figure 2**). For instance,
96 nDNA-containing micronuclei rupture(17, 49) and mitochondrial stress(50-53) lead to
97 DNA release in the cytoplasm and cGAS activation. MtDNA stress elicited by

98 mitochondrial transcription factor A (TFAM) deficiency induces its release into the
99 cytosol and activates the cGAS/STING pathway leading to type I IFN-dependent ISG
100 expression and increased antiviral capacity(26). DsDNA from dying cells stimulates
101 STING pathway in surrounding cells, suggesting that extrinsic phagocytic DNA
102 improperly processed within lysosomes access the cytosol(54, 55). The mechanisms
103 by which self-DNA from neighboring cells becomes accessible for intracellular DNA
104 sensors remain uncertain. Several context-dependent pathways have been reported,
105 such as IgG- or HMGB1-bound DNA internalization following interaction with FcγRIIa
106 or receptor for advanced glycation end products (RAGE), respectively(56). The
107 antimicrobial peptide LL37 was shown to transport extracellular DNA into the
108 cytoplasm of human primary monocytes triggering STING activation(57). IL-10-family
109 member IL-26 binds to genomic, mtDNA or neutrophil extracellular traps (NETS) DNA
110 and traffic them into the cytosol of human myeloid cells activating STING(58).
111 Radiotherapy-induced cytosolic dsDNA accumulation in cancer cells activate the
112 cGAS/STING pathway to promote type I IFN production-dependent protective effect,
113 notably by recruiting BATF3-dependent DCs(59). It was shown that exosomes-
114 containing DNA from cancer cells can be transferred to the cytoplasm of DCs(59).
115 Besides chemotactic properties following their binding to chemokine receptors
116 (CXCR), several chemokines display other functions. In particular, CXCL4 and
117 CXCL10 have been shown to activate endosomal TLR9. CXCL4 binds to self and
118 foreign DNA to form liquid crystalline complexes amplifying TLR9-mediated IFN-α
119 production in systemic sclerosis(60). Neutrophil-derived CXCL10 binds to commensal
120 skin microbiota DNA and trigger TLR9/type I IFN-dependent innate repair responses
121 in injured skin(61). It remains to be determined whether CXCL4- or CXCL10-DNA
122 complexes can activate intracellular sensors such as cGAS.
123 cGAMP transfer from producing cells to neighboring ones through gap junction
124 promotes STING activation and antiviral immunity independently of type I IFN
125 signaling(62). cGAS-derived cGAMP in tumor cells diffuses to neighboring non-
126 cancerous cells through gap-junction channels to activate STING, contributing to the
127 recruitment of protective tumor-infiltrating immune cells such as NK cells(63).
128

129 ***STING allelic variants***

130 There are several STING allelic variants in the general population(64). The most
131 commons are R232H and R71H-G230A-R293Q (HAQ). Their prevalence varies
132 among ethnic populations. On average, about 15% and 20% of worldwide population
133 carries at least one copy of R232H or HAQ variants, respectively(64-66). Alterations in
134 STING biology induced by these point mutations are not fully clear and apparent
135 discrepancies have been reported. Using ectopic overexpression systems, human
136 HAQ variant exhibited a decreased response to exogenous CDNs(64) while its
137 response to 2'3'cGAMP was either normal(64) or absent(65). STING R232H showed
138 reduced responsiveness to most exogenous CDNs although its response to
139 2'3'cGAMP was relatively normal(64, 65) or reduced(67). Interestingly, it was shown
140 that R293Q STING variant displays protective effects against obesity-associated
141 cardiovascular disease(68) and tobacco-induced aging-associated diseases(69). In
142 contrast, gain-of-function point mutations in the gene encoding STING (e.g. V155M)
143 are responsible for STING-associated vasculopathy with onset in infancy (SAVI), a rare
144 inflammatory and autoimmune condition(70, 71).

145

146 ***STING and autoimmune or autoinflammatory diseases***

147 STING-associated vasculopathy with onset in infancy (SAVI)

148 In 2014, it was discovered that point gain-of-function mutations in *TMEM173* (i.e.
149 N154S, V155M, and V147L) from six unrelated children cause SAVI(70). SAVI is an
150 autoinflammatory disease with early-onset systemic inflammation, cutaneous
151 vasculopathy and interstitial lung disease(70, 72). Children PBMCs display constitutive
152 STING activation, leading to higher transcription baseline for *IFNB1*, *IL6* and *TNF* as
153 well as interferon-stimulated genes (ISGs) such as *CXCL10* as compared to PBMCs
154 from healthy controls. In contrast, baseline and cGAMP-induced transcription levels of
155 *IFNA4*, *IFNG* and *IL1B* were similar between patients and controls(70). The same year,
156 V155M point mutation in STING was also found in patients with familial lupus-like
157 phenotypes(71). STING mutant spontaneously localizes in the Golgi of patient
158 fibroblasts and is constitutively active in the absence of exogenous 2'3'-cGAMP in
159 vitro(71).

160 Two STING knock-in mouse strains corresponding to mutations found in SAVI patients
161 have been generated. Heterozygous V154M STING (equivalent to V155M in patients)

162 and heterozygous STING N153S (equivalent to N154S in patients) mouse strains both
163 display severe combined immunodeficiency disease (SCID) phenotype and
164 thymocytes impairment at early stages(73-75). Interestingly, STING N153S-
165 associated lung disease is T lymphocyte-dependent but does not require IRF3/IRF7
166 nor type I IFN signaling(76). STING-associated vasculopathy also develops
167 independently of IRF3 in mice(74).

168

169 [Bloom syndrome](#)

170 Bloom syndrome is a rare autosomal recessive genetic disorder caused by mutations
171 in the BLM gene encoding BLM RecQ-like helicase(77), which maintains DNA stability
172 during cell replication. BLM protein deficiency or lack of protein activity leads to
173 increased mutations. Bloom syndrome is characterized by short stature, cancer
174 predisposition, genomic instability and accumulation of micronuclei. A recent study
175 showed that BLM-deficient fibroblasts display constitutive ISG up-regulation in a
176 cGAS/STING/IRF3-dependent mechanism(78). Further investigation are required to
177 determine the exact contribution of the STING pathway in Bloom syndrome.

178

179 [Multiple sclerosis](#)

180 Multiple sclerosis (MS) is a demyelinating condition that can affect both the brain and
181 spinal cord. It was shown that STING activation attenuates experimental autoimmune
182 encephalitis (EAE) utilized as an MS model by attenuating effector T cell infiltration and
183 inducing a dominant T regulatory (Treg) response(79). Systemic treatments with DNA
184 nanoparticles or CDNs activate the STING/type I IFN pathway enhancing indoleamine
185 2,3 dioxygenase (IDO) enzyme activity in dendritic cells promoting Treg cells(79).
186 Another study showed that FDA-approved antiviral drug ganciclovir (GCV) induces a
187 STING-dependent type I IFN response inhibiting inflammation in cultured myeloid cells
188 and in EAE model(80). Together, STING pathway appears to be an important regulator
189 of microglial reactivity and neuroinflammation with possible beneficial therapeutic
190 effects for MS patients.

191

192 [Type I diabetes](#)

193 Type 1 diabetes (T1D), sometimes referred to as juvenile diabetes, is caused by the
194 destruction of pancreatic beta cells resulting in insufficient amount of insulin and hence

195 elevated blood sugar levels. High glucose environment increases ROS production,
196 triggering mitochondrial stress and mtDNA release in retinal cells and activating the
197 cGAS/STING pathway leading to IRF3 activation via ERK1/2-Akt-tuberin - mechanistic
198 target of rapamycin (mTOR) dependent pathways(50). Treatments with DNA
199 nanoparticles or cGAMP attenuate type I diabetes progression in non-obese diabetic
200 (NOD) female mice by inducing type I IFN-dependent IDO activity which promotes Treg
201 cell function and therefore limits autoimmunity(81). It was further shown that TBK1 in
202 monocytes from type 1 diabetes patients is important for IFN- α production in response
203 to CpG DNA stimulation(82).

204

205 Dysfunctional DNase and autoimmune diseases

206 An expected consequence of self-DNA-triggered immunity is tolerance breakdown and
207 autoimmunity(83, 84). Host cytoplasmic DNA sensing is typically prevented since it is
208 usually restricted to the nucleus and mitochondria. However, multiple situations lead
209 to the presence of misplaced DNA and autoimmunity can result from failure to properly
210 dispose it through DNase activity. Besides cell extrinsic DNase I, mammalian cells
211 express the endonuclease DNase II and the exonuclease TREX1/DNase III located
212 within lysosomes and in the cytosol in an ER tail-anchored manner, respectively. In
213 human, loss-of-function mutations in TREX1 trigger autoimmune diseases, such as
214 Aicardi-Goutières syndrome (AGS), systemic lupus erythematosus (SLE), familial
215 chilblain lupus (FCL), and retinal vasculopathy with cerebral leukodystrophy
216 (RVCL)(85, 86). Recent data suggest that STING pathway may be involved in these
217 autoimmune diseases linked to dysregulated nucleases activity(85).

218

219 *Aicardi-Goutières syndrome*

220 Aicardi-Goutières syndrome (AGS) is a neuroinflammatory autoimmune disease
221 triggered by mutations in genes encoding nucleotide-processing proteins. *Trex1*-
222 deficient mice succumb from systemic inflammation during early adulthood(87, 88)
223 whereas *DNase II*-deficient mice are embryonic lethal(89). Interestingly, lethality
224 observed in both *Trex1* or *Dnase II*-deficient mice is rescued by crossing these mice
225 to either *cGas* or *Sting*-deficient mice(55, 90). It was shown that STING-mediated type
226 I IFN production in *Trex1*-deficient mice occurs first in nonhematopoietic cells, which
227 trigger T and B lymphocytes-mediated inflammation and autoantibody production(91).

228 RNase H2 is essential to remove ribonucleotides incorporated in genomic DNA during
229 replication. Defective RNase H2 leads to AGS by promoting self-nucleic acid
230 accumulation leading to chronic type I IFN production. A knock-in mouse strain
231 containing an RNase H2 AGS mutation (G37S) shows perinatal lethality. This
232 phenotype is partially rescued when these mice are crossed with Sting-deficient
233 mice(92), confirming that STING signaling pathway is involved in AGS. Using in vitro
234 RnaseH2a G37S/G37S mouse embryonic fibroblast (MEF) cell cultures, the authors
235 confirmed that the cGAS/STING pathway triggers type I IFN and ISGs
236 upregulation(92). The exact mechanism by which defective RNase H2 leads to cGAS
237 activation remains to be determined.

238

239 *Systemic lupus erythematosus*

240 Systemic lupus erythematosus (SLE) development is classically linked to endosomal
241 toll like receptor (TLR) 7 and TLR9 nucleic acid sensors engagement and the role of
242 the cGAS/STING pathway remains elusive. PBMCs from SLE patients express high
243 levels of cGAS(93). It was recently shown that in contrast to healthy donors, most
244 monocytes from lupus patients produce IFN- α following 2'3'-cGAMP stimulation and
245 frequency of IFN- α producing monocytes positively correlates with SLE disease
246 activity(94). Interestingly, mTOR inhibition suppressed STING upregulation and IFN- α
247 production in lupus monocytes (94). In mice, *Fas*-deficient lupus-prone MRL/Mp-lpr/lpr
248 mice display systemic autoimmunity, massive lymphadenopathy, arthritis and immune
249 complex glomerulonephrosis starting at about three months of age. Crossing these
250 lupus-prone mice with *Sting*-deficient mice significantly increases autoimmunity and
251 shortens lifespans(95). The authors further show that STING-mediated protection is
252 IRF3-independent(95). Furthermore, TLR-dependent systemic inflammation following
253 2,6,10,14-tetramethylpentadecane (TMPD) stimulation is exacerbated by STING
254 deficiency which results in increased levels of pro-inflammatory cytokines and elevated
255 numbers of myeloid cells(95).

256

257 *Familial chilblain lupus*

258 Familial chilblain lupus is a rare autosomal dominant form of cutaneous lupus
259 erythematosus occurring mainly in young children, due to mutations in the *TREX1* gene
260 as well as in mutations within *SAMHD1* or *TMEM173* in rare cases(96, 97). In

261 particular, G166E heterozygous mutation in *TMEM173* was found in five patients with
262 familial chilblain lupus resulting in constitutive type I IFN production, for which the
263 Janus kinase (JAK) inhibitor tofacitinib showed promising results(96).

264

265 *cGAS/STING in inflammatory diseases*

266 Fibrosis

267 STING pathway has been linked to the regulation of fibrotic processes in various
268 tissues. Idiopathic pulmonary fibrosis (IPF) is characterized by progressive lung
269 scarring punctuated by life-threatening acute exacerbations causing shorter life
270 expectancy and a high mortality rate(98-100). It is believed that the physiopathology
271 relies on repetitive local micro-injuries leading to DNA damage, cell death and finally
272 to an aberrant repair with deposition of extracellular matrix components and
273 fibrosis(98-101). Using the classical murine model of human IPF by airway exposure
274 to bleomycin (BLM), we published recently that STING deficiency leads to increased
275 lung fibrosis in an unexpected type I IFN-independent manner indicating that STING
276 plays a protective role in limiting experimental lung fibrosis(102). In line with our
277 findings, a recent study in IPF patients showed that STING expression in PBMC
278 decreases during acute exacerbation. STING protein levels post-treatment increased
279 in patients showing clinical improvement but remained low in patients displaying
280 clinical deterioration, strongly suggesting a benefic role of STING in IPF(103). Of note,
281 elevated plasma mtDNA copy numbers in IPF patients predict death(104). A protective
282 role of STING against fibrosis has also been shown in a model of chronic
283 pancreatitis(105). Authors showed that STING deficiency promotes Th17 polarization
284 and IL-17A production therefore pancreatic inflammation and fibrosis(105).

285 On the other hand, Susztak et al. showed that patients suffering from chronic kidney
286 disease (CKD) display defective mitochondrial integrity and further linked mtDNA
287 release, STING activation and renal fibrosis(51). STING expression is upregulated in
288 human and mouse hypertrophic hearts and STING deficiency leads to decreased
289 inflammation and fibrosis in an ER stress-associated process(106). Inflammation-
290 driven liver fibrosis upon carbon tetrachloride (CCl₄) administration is a well-
291 established model of chronic liver disease. It was shown that STING/IRF3 pathway
292 mediates hepatocyte death and fibrosis independently of TLR and type I IFN
293 signaling(107). Of note, the same group showed that STING is activated by alcohol-

294 induced ER stress to trigger IRF3-dependent hepatocyte apoptosis, independently of
295 type I IFN signaling(108). Chronic silica particles inhalation triggers silicosis, a lung
296 disease with progressive interstitial fibrosis and increased risk of cancer. It was shown
297 that airway silica exposure induces both nDNA and mtDNA release and STING-
298 dependent type I IFN responses. *Sting*-deficient mice displayed significantly
299 attenuated lung inflammation(109). Thus, STING contribution to fibrosis appears highly
300 context/organ dependent and further studies are required to delineate the molecular
301 mechanisms involved.

302

303 Obesity-related diseases

304 Chronic sterile inflammation in obesity and related metabolic diseases such as type 2
305 diabetes, nonalcoholic fatty liver disease (NAFLD) and cardiovascular disease have
306 been widely demonstrated(110). A number of studies recently showed that self-DNA
307 sensing, either nuclear or mitochondrial, plays important roles in these pathologies.
308 Obesity promotes mtDNA release into the cytosol of adipocytes, which leads to cGAS-
309 STING-mediated inflammation(52). Using methionine/choline-deficient diet (MCD) or
310 high-fat diet (HFD) as murine models of non-alcoholic steatohepatitis (NASH), it was
311 shown that STING/IRF3 pathway promotes hepatocyte injury and dysfunction by
312 inducing inflammation and apoptosis as well as by disturbing glucose and lipid
313 metabolism(111). *Sting*-deficient mice display reduced HFD-induced adipose tissue
314 inflammation and insulin resistance(112). However, the cell subset(s) involved
315 remain(s) unclear. A recent study confirmed that STING deficiency attenuated
316 steatosis, fibrosis, and inflammation using both MCD and HFD murine models(53).
317 However, they pointed out that in contrast to IRF3, several studies including theirs
318 reported that STING protein is not expressed in hepatocytes of adult humans or mice.
319 They showed that hepatocytes from HFD-fed mice release mtDNA which activates
320 STING in Kupffer cells leading to TNF- α and IL-6 productions and pathology(53). The
321 role of STING in hematopoietic-derived cells in driving HFD-induced NAFLD has been
322 confirmed by bone marrow transfer experiments(113). However, STING role in non-
323 immune cells has also been confirmed. Adipose tissue chronic inflammation and
324 metabolic stress in obesity induce endothelial inflammation, which plays a key role in
325 insulin resistance. Obesity-related increase in free fatty acid induces mitochondrial
326 damage and mtDNA release, which activates the cGAS/STING pathway leading to

327 IRF3-dependent upregulation of ICAM-1 expression and endothelial
328 inflammation(112).

329

330 Myocardial infarction

331 An elegant study showed that ischemic cell death and cell debris uptake by cardiac
332 macrophages leads to cGAS/STING pathway activation and IRF3-mediated type I IFN
333 production(114). As compared to WT relatives, myocardial infarction induction in *Sting*-
334 deficient mice leads to decreased *Ifnb1* expression and a strong drop in *Cxcl10*, *Irf7*,
335 and *Iffit1* expressions(114). In contrast to about 50% mortality in WT mice, IRF3- or
336 type I IFN receptor (*Ifnar*)-deficient mice showed virtually complete protection, whereas
337 *Cgas*-deficient mice displayed partial protection and *Sting*-deficient mice did not reach
338 statistical difference(114). These data suggest that other innate immune sensors are
339 involved in IRF3 activation after myocardial infarction.

340 Upon myocardial infarction, cGAS induces iNOS and CXCL10 upregulation but not
341 pro-inflammatory mediators such as IL-1 β , IL-18, TNF- α and IL-6(115). *Cgas*-deficient
342 mice display higher regulatory capacities, including M2-like macrophages and
343 myofibroblasts in the region bordering the myocardial infarction. cGAS deficiency also
344 protects against myocardial infarction-induced adverse ventricular remodeling and
345 rupture and enhances tissue repair in the infarct region(115). Of note, it was suggested
346 that cGAS-mediated autophagy protects the liver from ischemia-reperfusion injury
347 independently of STING⁽¹¹⁶⁾.

348

349 COPD

350 Chronic obstructive pulmonary disease (COPD) is a major health issue primarily
351 caused by cigarette smoke (CS) inhalation. It is characterized by chronic bronchitis
352 and emphysema, i.e. long-term inflammation of the airways and irreversible destruction
353 of the alveolar cell wall, respectively(117). Using an acute model of CS exposure in
354 mice, we showed that CS increases self-DNA content in the alveolar space driving
355 cGAS/STING-dependent neutrophilic influx and inflammatory response(118). DNase I
356 treatment reduces CS-induced lung inflammation by limiting deleterious effects of
357 neutrophil extracellular traps (NETs) for instance in terms of protease expression⁽¹¹⁹⁾.
358 In contrast, sub-chronic CS exposure induces a reduction of STING lung expression
359 impeding subsequent response to infection(120) and COPD patients display lower

360 pulmonary IFN- β expression(121). Lower IFN levels might in part contribute to the poor
361 immune response to infection developed by COPD patients during exacerbation
362 phases.

363

364 ***cGAS/STING in neurodegenerative disorders***

365 Over the past few year, a number of important studies showed potential strong
366 involvement of the cGAS/STING pathway in neuroinflammatory processes and
367 neurodegenerative disorders. STING regulates steady-state and nerve injury-triggered
368 nociception through its signaling in sensory neurons(122). Intrathecal injection (i.e. into
369 the spinal canal) of STING agonists leads to robust antinociception in mice and non-
370 human primates (NHPs) in a type I IFN-mediated signaling on peripheral
371 nociceptors(122). *Sting* or *Ifnar*-deficient mice exhibit hypersensitivity to nociceptive
372 stimuli and increased nociceptor excitability. The exact mechanism of STING activation
373 at steady state and following nerve damage remains to be determined, potentially
374 implying cell death and self-DNA sensing.

375 Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of the central
376 nervous system (CNS) causing progressive loss of muscle control and often
377 characterized by a cytotoxic accumulation of TAR DNA-binding protein 43 (TDP-43).
378 TDP-43 accumulation in the mitochondria induces DNA leakage and cGAS/STING
379 pathway activation promoting inflammatory signaling and pathology(123). Using
380 mouse models to investigate Parkinson's disease (PD) inflammatory profile, Sliter et
381 al. showed that inflammation in *Parkin*^{-/-} and *PINK1*^{-/-} mice undergoing exhaustive
382 exercise is linked to mtDNA leakage and rescued in the absence of STING(124).
383 Serum/ glucocorticoid related kinase 1 (SGK1) is upregulated in the brains of patients
384 with various neurodegenerative disorders such as PD and pharmacological inhibition
385 of SGK1 limits NLRP3-inflammasome- and cGAS-STING-mediated inflammatory
386 pathways(125). Employing PD mouse models, it was shown that the neuroprotective
387 agent withaferin A protected against dopaminergic neuron loss in a STING-dependent
388 manner(126).

389 The cGAS/STING pathway might also play a role in accelerated aging and
390 neurodegeneration observed in Huntington's disease (HD). Melatonin, a radical
391 scavenger expressed by neuronal mitochondria, decreases with aging and
392 neurodegeneration. Melatonin-deficient mice display increased mtDNA release and

393 activation the cGAS/STING/IRF3 pathway(127). Exogenous melatonin administration
394 in R6/2 mice as a genetic mouse model of HD alleviates cGAS/STING-mediated
395 inflammation(127). STING pathway inhibition may offer therapeutic benefits in HD by
396 limiting deleterious up-regulation of inflammatory and autophagy responses(128).
397 Finally, cGAS/STING activation delayed neurodegeneration in neonatal hypoxia-
398 ischemia in rats(129) however STING-mediated protective mechanism remains to be
399 determined.

400

401 *cGAS/STING in cancer*

402 cGAS/STING pathway in cancer settings is under deep examination in both tumor cells
403 as well as in neighboring immune and non-immune cells. Most tumors retain cGAS
404 and STING expression(130) and cancer cell cGAS recognizing cytosolic DNA
405 produces cGAMP inducing STING-dependent type-I IFN secretion(131). Acute STING
406 activation is likely to exhibit type I IFN-mediated anti-tumor effect associated with
407 cellular senescence and T lymphocyte-dependent immunity. In more advance stages,
408 chromosomally unstable tumors become tolerant to chronic cGAS-STING signaling,
409 downregulate downstream IFN signaling while maintaining alternative pathways that
410 promote tumorigenesis(130). In mice, STING deficiency facilitates development of
411 several types of tumor whereas STING stimulation favors antitumor immunity(42, 132,
412 133). Cytosolic DNA accumulation can result from the combined action of the
413 endonuclease MUS81 and PARP-dependent DNA repair as shown in prostate cancer
414 cells, leading to STING-dependent tumor rejection(134). In contrast, by generating
415 *Sting*^{S365A/S365A} mutant mouse strain that precisely ablates IFN-dependent activities,
416 Wu et al. showed that T cells in tumors undergo substantial cell death in part mediated
417 by IFN-independent STING activities promoting tumor evasion(135).

418 cGAS also elicits contrasting outcomes depending on the context. Carcinoma-derived
419 cGAMP diffuses to astrocyte through connexin 43 (Cx43) gap junctions leading to
420 STING-dependent IFN- α production acting in paracrine manner on metastatic cells to
421 support chemoresistance and tumor growth through STAT1 activation(136). DNA
422 repair following double-stranded breaks by homologous recombination prevents
423 tumorigenesis. DNA damage induces nuclear translocation of cGAS where it inhibits
424 homologous recombination and therefore promotes tumor growth(137). A recent
425 publication showed that efferocytosis blockade by inhibiting MerTK-dependent

426 apoptotic tumor cell phagocytosis promotes CD8⁺ T cell-mediated anti-tumor activity.
427 Apoptotic tumor cells clearance failure leads to secondary necrosis accompanied with
428 danger signals release. Among them, ATP and cGAMP activate P2X7 receptor and
429 STING, respectively, in neighboring CD8⁺ T cells leading to enhanced type I IFN
430 production-mediated immune activation and tumor suppression, especially at early
431 stage(138).

432 Numerous studies targeting the STING pathway have been performed to address its
433 potential use as antitumor. Intratumoral delivery of synthetic CDN derivatives induces
434 STING-dependent tumor regression as well as metastases rejection and long-lived
435 immunologic memory in a dose-dependent manner(139, 140). Intravenous CDN
436 administration also increased survival rate in mice with acute myeloid leukemia(141).
437 These results and others indicate potential strong benefit of targeting STING against
438 different types of cancer. While clinical use of immune checkpoint blockade to promote
439 anti-tumor immune responses proved to display tremendous benefit in several types
440 of cancers(142-144), most patients do not show significant improvement when these
441 checkpoint inhibitors are given as a monotherapy and thus require combined
442 chemotherapy (144). Most chemotherapeutic agents non-specifically target dividing
443 cells by blocking DNA replication leading to apoptosis(145). Besides a direct positive
444 effect by killing tumors cells, cytotoxicity and danger signals induced by
445 chemotherapeutic drugs can enhance the inflammatory environment and CD8⁺ T cell
446 activation(146). Therefore, combining immunotherapy with additional targets including
447 the cGAS/STING pathway constitutes an intense scope of investigation, recently
448 reviewed(147). The potential ability of radiotherapy to enhance immunotherapy
449 constitutes another field of investigation. While radiation doses above 12-18 Gy induce
450 DNA exonuclease TREX1 and therefore dampen cGAS activity, repeated irradiation at
451 lower doses (3 times 8 Gy) does not induce TREX1 expression and leads to increased
452 type I IFN production through the cGAS/STING pathway(148). In addition, *Trex1*
453 knockdown restores cytosolic dsDNA accumulation and ISG induction in a mouse
454 model of mammary carcinoma refractory to immune checkpoint inhibitors(148).

455 In summary, cGAS-STING-elicited outcome in cancer is largely context-dependent.
456 Transient cGAS-STING activation in innate immune cells may display anti-tumor
457 activity, whereas sustained activation might induce immune tolerance and tumor

458 growth. The use of synthetic CDNs together with anti-cancer immunotherapy may be
459 promising in response to certain types of cancers(139, 149).

460

461 **Cellular processes**

462 **STING and autophagy**

463 Autophagy is a phylogenetically conserved catabolic process induced by numerous
464 endogenous (e.g. nutrient deprivation) or exogenous (e.g. infection) cellular stress
465 conditions aiming to either promote cell survival or apoptosis of senescent cells(150).

466 Autophagy induction by STING has been first identified in the context of bacterial
467 infection. Authors showed that *M. tuberculosis* DNA recognition induces STING-
468 dependent targeting of bacteria and autophagy-mediated resistance to infection(31).

469 More recent studies suggest that STING-dependent autophagy regulation may have
470 evolved before type I IFN induction. In invertebrates such as the sea anemone

471 *Nematostella vectensis*, STING protein does not harbor the two C-terminal tail (CTT)

472 domains critical to activate IRF3 but effectively induces autophagy(30). STING triggers

473 autophagy in a TBK1- and type I IFN-independent manner(32). Upon activation,

474 STING binds to the autophagy inducing protein LC3 promoting both autophagy and
475 STING degradation(32), therefore regulating STING-mediated immune activation.

476 cGAMP binding induces STING translocation to the endoplasmic reticulum-Golgi
477 intermediate compartment (ERGIC) and the Golgi. STING-containing ERGIC serves

478 as a membrane source for LC3 lipidation and autophagosome formation. This form of
479 autophagy, important for the clearance of cytosolic DNA, is mediated by autophagy-

480 related gene 5 (ATG5) and Trp-Asp (W-D) repeat domain phosphoinositide-interacting

481 protein (WIPI2)(30). Furthermore, cGAS protects hepatocytes by triggering autophagy

482 independently of STING in mouse models of ischemia-reperfusion(116).

483 Cell death following replicative crisis is a senescence-independent process important

484 to prevent oncogenic transformation of pre-cancerous cells with disrupted cell cycle

485 checkpoints. It was shown that cytosolic DNA activates the cGAS/STING pathway to

486 promote macroautophagy and autophagic cell death(151). Of note, STING signaling is

487 negatively regulated by p62/SQSTM1-dependent autophagy pathway activated by

488 TBK1(152).

489 Circulating mtDNA levels and STING activation profile are increased in sepsis-induced

490 acute lung injury (ALI) patients(153). In a mouse model of sepsis, mtDNA-triggered

491 STING-mediated IFN production interferes with autophagy by preventing lysosomal
492 acidification and thus worsens pathology(153). STING-dependent autophagy
493 upregulation observed in the striatum of HD patients favors brain damage(128).
494 Radiotherapy-induced mtDNA release facilitates cGAS/STING activation and type I
495 IFN-mediated antitumor responses and autophagy induction limits this effect(154).
496 Patients with breast cancer showing increased genetic autophagy signature in the
497 tumor microenvironment display slightly decreased survival prognostic, inversely
498 correlating with mitochondrial abundance and type I IFN signaling(154).

499

500 cGAS/STING and cell death

501 There is a finely regulated and context-dependent cross-talk between several cell
502 death processes and the cGAS/STING pathway. The latter is often inhibited during
503 apoptotic processes to limit inflammation. During cell intrinsic apoptosis, mitochondrial
504 outer membrane permeabilization (MOMP) leads to mtDNA release but concomitantly
505 to caspase-9 and caspase-3 which display immunosuppressive function by repressing
506 the cGAS/STING/type I IFN pathway(155, 156). It was recently shown that caspase-9
507 and caspase-3 directly cleave cGAS and IRF3 to limit STING activation and deleterious
508 inflammation(157). In contrast, cGAS-STING pathway initiates certain form of
509 programmed cell death. Nucleosomes competitively inhibit DNA-dependent cGAS
510 activation during regular mitotic processes. However, during mitotic arrest limited
511 cGAS activation leads to mild STING-dependent IRF3 phosphorylation triggering
512 mitotic aberrations and transcription-independent induction of apoptosis (158). In
513 addition, host restriction factor SAMHD1 limits human T cell leukemia virus type 1
514 infection of monocytes via STING-mediated apoptosis and viral products interact with
515 STING to trigger an IRF3-Bax complex leading to apoptosis (159). Noteworthy, T
516 lymphocytes exhibit an intensified STING response predisposing them to apoptosis
517 (34). This effect does not appear to occur in other cell types including dendritic cells
518 and macrophages, presumably owing higher STING expression and signaling in T cells
519 as compared to other cell subsets(34).

520 Gaidt et al. showed that STING traffics through the Golgi and then through late
521 endosomes and lysosomes(33). The precise location of STING, e.g. at the outer
522 lysosomal membrane or within multivesicular bodies, remains to be determined.
523 STING in lysosomes induces lysosomal membrane destabilization leading to the

524 release of proteases such as cathepsins triggering lysosomal cell death (LCD)(33,
525 160), however the mechanism involved remains to be established. Furthermore, it
526 appears that the main function of STING targeting to the lysosome is its degradation
527 to limit its activity(161). Necroptosis is a regulated form of necrotic cell death governed
528 by RIP1/RIP3-mediated activation of MLKL. During necroptosis, the proapoptotic BH3-
529 only BCL-2 family member PUMA is transcriptionally activated in an RIP3/MLKL-
530 dependent manner. PUMA promotes the cytosolic release of mtDNA which activates
531 DAI/Zbp1 and STING leading to enhanced RIP3 and MLKL phosphorylation in a
532 positive feedback loop and thus amplifies necroptosis(35). Interestingly, basal cytosolic
533 DNA sensing by the cGAS/STING pathway is important for constitutive type I IFN
534 production and signaling, maintaining baseline ISG induction. It was shown that this
535 phenomenon is critical to reach a critical threshold of MLKL for LPS-dependent
536 necroptosis(162).

537

538 cGAS/STING and chromosomal instability/senescence

539 Chromosomal instability is a hallmark of cancer in human, associated with poor
540 prognosis, metastasis, and therapeutic failure(130). It results from errors in
541 chromosome segregation during mitosis and can cause micronuclei formation in the
542 cytoplasm. During metazoan cell division, the nuclear envelope is ruptured and if the
543 exposed chromatin is not entirely contained within the daughter cells nuclei, it can be
544 encapsulated within independent structures called micronuclei. Micronuclei rupture
545 leads to cytosolic self-DNA release and cGAS activation(17, 49). DNA damage
546 induced by monogenic autoinflammation (e.g. Rnaseh2b deficiency) or exogenous
547 DNA damage (e.g. ionizing radiation) leads to micronuclei formation which rupture
548 activates cGAS leading to ISG expression(17). This pathway is also involved in the
549 context of genotoxic cancer therapy where STING-dependent responses display
550 antitumor activities(49). A recent article showed that chromosomal instability leads to
551 increased numbers of micronuclei and cytosolic DNA, which activate the cGAS-STING
552 pathway(163). Rather than engaging type I IFN or canonical NF- κ B pathways, STING
553 activates noncanonical NF- κ B signaling linked to the upregulation of epithelial-
554 mesenchymal transition (EMT) and inflammatory genes, therefore enhancing cell
555 migratory capacity and metastasis(163).

556 cGAS has been shown to be a critical inducer of cellular senescence, a form of terminal
557 cell-cycle arrest associated with pro-inflammatory response which prevents
558 tumorigenesis and participates to the antitumor effects of radio- and chemo-
559 therapies(164). cGAS binds to cytosolic chromatin fragments in senescent cells and
560 induces STING-dependent production of senescence-associated secretory phenotype
561 (SASP) inflammatory mediators promoting paracrine senescence(165). Cytoplasmic
562 chromatin fragments are pinched off intact nuclei during senescence to activate the
563 cGAS-STING pathway(166).

564 Progeria, or Hutchinson-Gilford progeria syndrome (HGPS), is a rare autosomal
565 dominant condition beginning in childhood with striking phenotypic features of
566 premature aging. It is caused by a truncated lamin A protein (progerin) inducing nuclear
567 envelope fragility and genomic instability and fatal senescence. Progerin-mediated
568 DNA release leads to the upregulation of the cGAS/STING pathway and pathogenic
569 type IFN response(167).

570

571 STING and inflammasome

572 Cytosolic DNA-mediated cGAS/STING activation leads to STING trafficking to the
573 lysosome, where it triggers membrane permeabilization and potassium efflux which
574 activate the NLRP3 inflammasome(33). Non-canonical functions of cGAMP in
575 regulating both priming and activation steps of the inflammasome have been reported.
576 cGAS-derived cGAMP induces type I IFN induction via STING leading to increased
577 expression of inflammasome components and cGAMP also favors inflammasome
578 activity by promoting complexes containing both NLRP3 and AIM2(168). Using protein
579 overexpression assays, it was recently shown that Herpes Simplex Virus-1 (HSV-1)
580 infection or cytosolic DNA stimulation triggers STING binding to NLRP3, promoting
581 both NLRP3 localization in the ER and attenuating NLRP3 polyubiquitination and
582 degradation(169). In addition, IL-1 β signaling on human myeloid, fibroblast, and
583 epithelial cells induce mtDNA release to activate innate immune signaling via cGAS-
584 STING(170) and mtDNA-driven cGAS activation triggers age-related macular
585 degeneration through STING-mediated non-canonical inflammasome pathway
586 involving IFN- β (171).

587

588 **Conclusion**

589 Our understanding of the mechanisms of danger signal release and sensing has
590 evolved considerably over the last years. It now becomes clear that misplaced self-
591 DNA is a potent trigger of immune activation through various DNA sensing machinery.
592 Among them, the cGAS/STING pathway has emerged as an important source of type
593 I and type III interferons as well as a critical regulator of cellular processes such as
594 autophagy and programmed cell death. Targeting the STING pathway may offer
595 tremendous therapeutic opportunities, not only in response to infection as recently
596 illustrated with SARS-CoV-2 infection(172, 173) but also in inflammatory conditions
597 and cancer settings. In addition, natural and synthetic CDNs are used as adjuvants to
598 enhance protective humoral and CD4⁺ and CD8⁺ T cells responses in a STING-
599 dependent manner(174-177).

600 **Authors' contribution**

601 Review was written by IC and NR, figures were prepared by NR.

602

603 **Conflict of interest**

604 The authors declare no competing interests

605

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612

613

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615

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1316 Figure legends

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1318 **Graphical Abstract**

1319 Illustration of the diversity of processes triggering self-DNA mediated cGAS/STING
1320 activation and subsequent pathways involved in the immune regulation of various
1321 conditions.

1322

1323 **FIGURE 1. Self-DNA-mediated activation of the cGAS/STING pathway**

1324 Numerous situations lead to the presence of nuclear DNA (nDNA) and mitochondrial
1325 DNA (mtDNA) in the cytoplasm where they bind to cGAS. DNA binding to cGAS
1326 induces the production of the cyclic dinucleotide 2',3'-cyclic GMP–AMP (2',3'-cGAMP)
1327 from ATP and GTP. cGAMP binds to STING located in the endoplasmic reticulum (ER)
1328 membrane, which undergoes a conformational shift and dimerizes. Active STING
1329 translocates from the ER to the ER-Golgi intermediate compartment (ERGIC). STING
1330 recruits TANK-binding kinase 1 (TBK1) which autophosphorylates (pTBK1). In turn,
1331 pTBK1 phosphorylates STING, which is used as a docking site for interferon regulatory
1332 factor 3 (IRF3). TBK1 phosphorylates IRF3, which functions as dimeric transcription
1333 factor to activate genes encoding for type I interferons (IFN). Active STING also
1334 induces NF-κB activation leading to the transcription of pro-inflammatory genes.
1335 Alternatively, STING-containing ERGIC serves as a membrane source for
1336 autophagosome formation in an LC3-dependent manner and fuse with lysosomes
1337 allowing STING degradation and recycling of accumulated DNA. STING protein also
1338 enters the Golgi apparatus and traffic through the cisternae and to late endosome and

1339 lysosome. Lysosomal STING is degraded and leads to membrane permeabilization
1340 and lysosomal cell death.

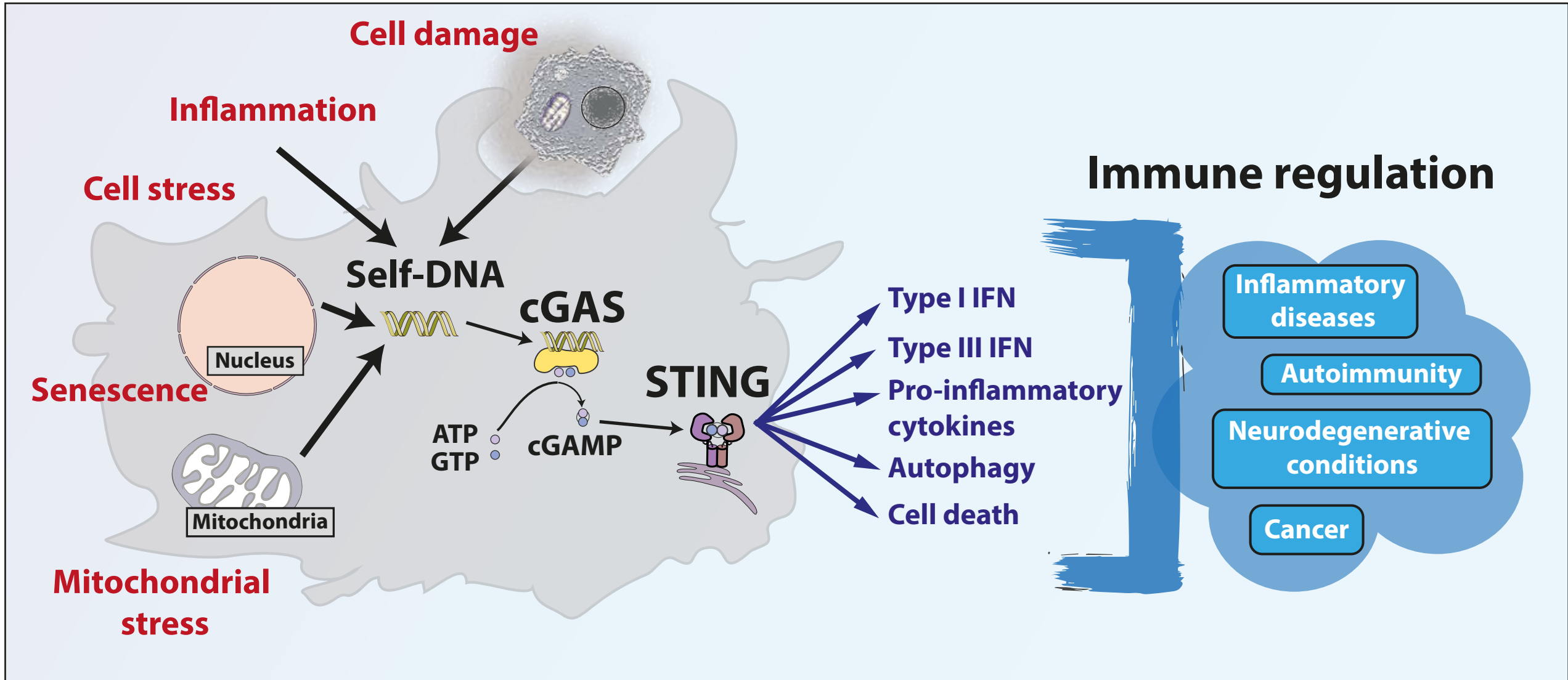
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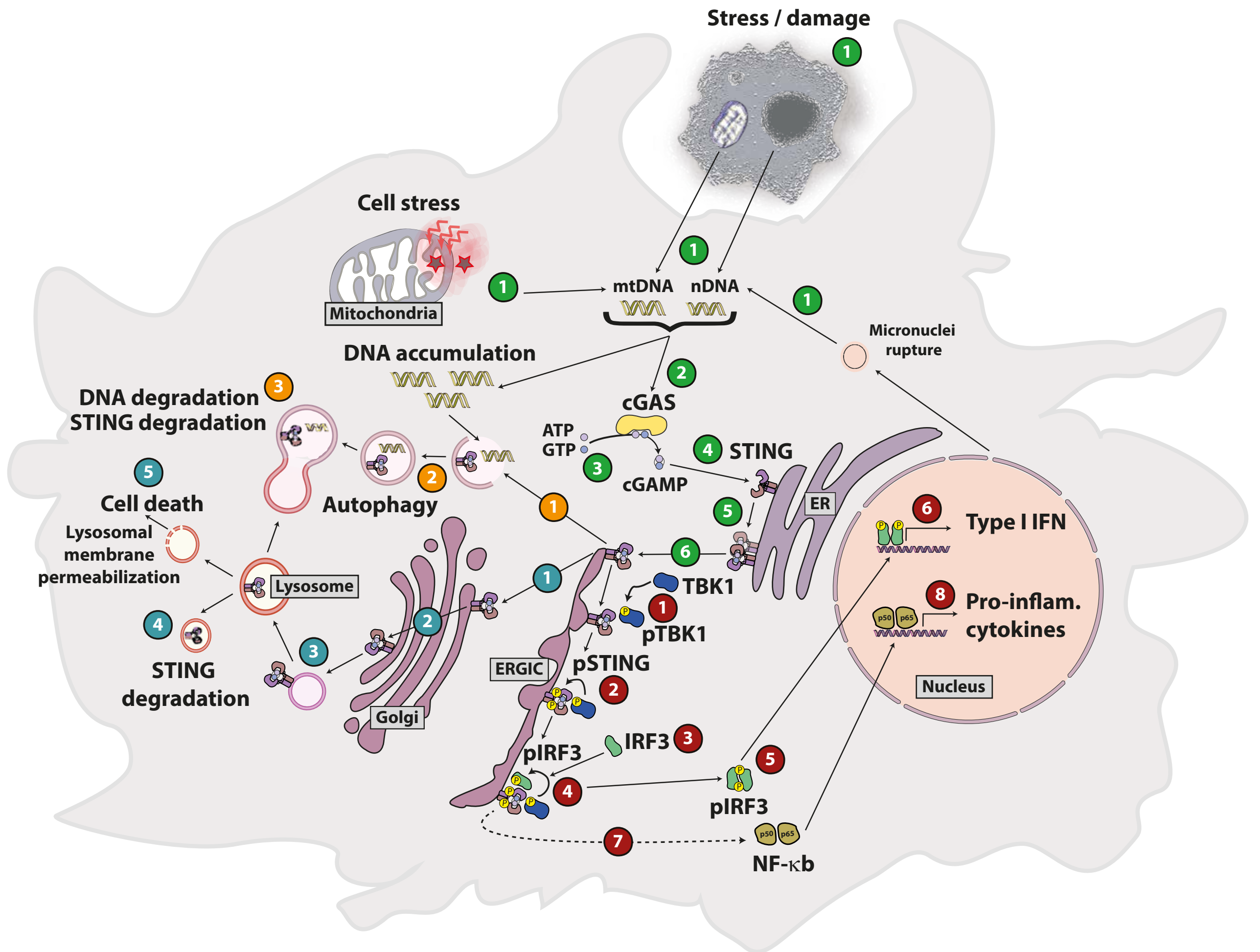
1342 **FIGURE 2. Proposed mechanism for self-DNA access to the cytoplasm**

1343 Mitochondrial stress-induced membrane permeability with mitochondrial DNA
1344 (mtDNA) leakage and chromosomal instability/senescence lead to micronucleus
1345 formation from incomplete segregation of chromatin and micronucleus rupture induces
1346 cytosolic nuclear DNA (nDNA). Multiples pathways lead to self-DNA internalization
1347 within endosomal structures and we believe that endosomal pathway perturbation
1348 leads to cytosolic delivery of DNA and cGAS/STING pathway activation. Apoptotic
1349 bodies engulfment may enable self-DNA delivery in the cytoplasm. The antimicrobial
1350 peptide LL37 and IL-26 bind to genomic DNA and induce its translocation within
1351 endosomes and/or in the cytoplasm. CXCL4- and CXCL10-DNA complexes as well as
1352 cell surface receptors FcγRIIa or receptor for advanced glycation end products (RAGE)
1353 bind to IgG- or HMGB1-bound DNA, respectively, and may lead to cGAS activation
1354 upon cytosolic DNA release. DNA-containing exosomes constitute another source of
1355 cGAS activation. Finally, cGAMP enters the cell from neighboring ones through gap
1356 junctions to directly activate STING.

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Numerous situations lead to the **1** presence of nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) in the cytoplasm where **2** they bind to cGAS. DNA binding to cGAS induces **3** the production of the cyclic dinucleotide 2',3'-cyclic GMP-AMP (2',3'-cGAMP) from ATP and GTP. **4** cGAMP binds to STING located in the endoplasmic reticulum (ER) membrane, which undergoes **5** a conformational shift and dimerizes. Active STING **6** translocates from the ER to the ER-Golgi intermediate compartment (ERGIC). STING **1** recruits TANK-binding kinase 1 (TBK1) which autophosphorylates (pTBK1). In turn, pTBK1 **2** phosphorylates STING, which is used as a **3** docking site for interferon regulatory factor 3 (IRF3). TBK1 **4** phosphorylates IRF3, which **5** functions as dimeric transcription factor to **6** activate genes encoding for type I interferons (IFN). Active STING also induces **7** NF- κ B activation leading to the **8** transcription of pro-inflammatory genes. Alternatively, STING-containing ERGIC serves **1** as a membrane source for **2** autophagosome formation in an LC3-dependent manner and **3** fuses with lysosomes allowing STING degradation and recycling of accumulated DNA. STING protein also **1** enters the Golgi apparatus and **2** traffic through the cisternae and to **3** late endosome and lysosome. Lysosomal STING **4** is degraded and **5** leads to membrane permeabilization and lysosomal cell death.

