

Review

# The Role of cGAS-STING in Age-Related Diseases from Mechanisms to Therapies

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**ABSTRACT:** With aging, the incidence of age-related diseases increases. Hence, age-related diseases are inevitable. However, the mechanisms by which aging leads to the onset and progression of age-related diseases remain unclear. It has been reported that inflammation is closely associated with age-related diseases and that the cGAS-STING signaling pathway, which can sense the aberrant presence of cytosolic DNA during aging and induce an inflammatory response, is an important mediator of inflammation in age-related diseases. With a better understanding of the structure and molecular biology of the cGAS-STING signaling axis, numerous selective inhibitors and agonists targeting the cGAS-STING pathway in human age-related diseases have been developed to modulate inflammatory responses. Here, we provide a narrative review of the activity of the cGAS-STING pathway in age-related diseases and discuss its general mechanisms in the onset and progression of age-related diseases. In addition, we outline treatments targeting the cGAS-STING pathway, which may constitute a potential therapeutic alternative for age-related diseases.

**Key words:** cGAS-STING, age-related diseases, cardiovascular diseases, neurological disorders, neoplasms, mechanisms, therapies

Aging is an intrinsic feature of mammals associated with a gradual functional decline over time, leading to the progressive loss of physiological integrity and deterioration of multiple organ systems, a critical cause of many age-related diseases [1]. Age-related diseases are diseases that occur at quadratically increased incidence rates in the adult population with advancing age [2]. Although the biological causes of the aging process remain unknown, there are some common cellular and molecular traits known as aging hallmarks [3]. DNA integrity and stability are continuously affected by exogenous and endogenous agents throughout life, leading to the accumulation of genetic damage and genomic instability [4, 5]. Although genomic stability systems exist, the genetic damage generated during the aging process will exceed their limited repair capacity.

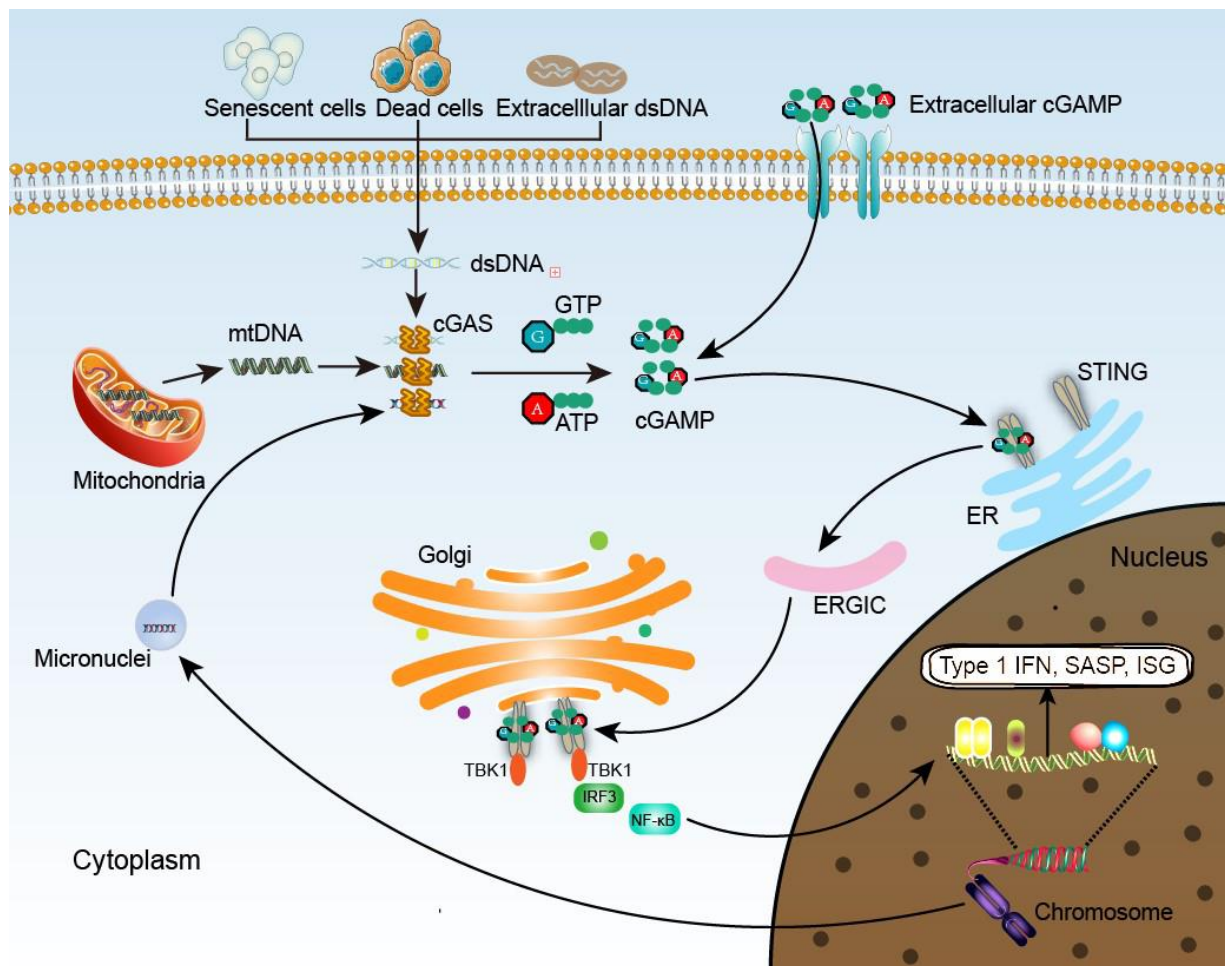
This will eventually lead to the accumulation of excess genetic damage in cells and aging. Genomic stability systems include mechanisms that repair most of the damage to nuclear DNA, ensure the integrity of mitochondrial DNA (mtDNA), and maintain the length and functionality of telomeres [6-8]. Furthermore, the efficacy of the respiratory chain degenerates as cells and organisms age, resulting in mitochondrial dysfunction. Under normal conditions, cellular autophagy or mitophagy can eliminate aberrant cytosolic DNA via the action of nucleases in the cytosol and endolysosomal compartments. However, aging cells have autophagy or mitophagy defects, and the combination of mitochondrial dysfunction and autophagy or mitophagy defects may be responsible for several age-related diseases [9].

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Genomic instability and mitochondrial dysfunction in aging are associated with the stochastic release of dsDNA from the nucleus and mitochondria into the cytoplasm [10, 11]. The aberrant presence of cytosolic dsDNA is recognized as a danger-associated molecular pattern (DAMP) by the DNA sensor cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase (cGAS) [12]. The C-terminal part of human cGAS consists of DNA-binding sites (one primary site and two additional sites) that can bind to the sugar-phosphate backbone of DNA. The catalytic pocket of the enzyme rearranges, and the conformation of cGAS changes to provide optimal interaction with the substrates when DNA binds to the primary site. DNA binding to these two additional sites is important in the formation of the minimal active enzymatic unit that acts on GTP and

ATP, inducing the synthesis of cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) [13]. cGAMP functions as an endogenous second messenger and is detected by the stimulator of interferon genes (IFN) genes (STING) located in the endoplasmic reticulum (ER) membrane [14, 15]. When GAMP binds to STING, the STING conformation changes and STING is transferred from the ER to the Golgi apparatus through the ER-Golgi intermediate compartment (ERGIC), where it is activated [16]. Activated STING polymerizes and activates TANK-binding kinase 1 (TBK1). In addition, TBK1 recruits and phosphorylates IFN regulatory factor 3 (IRF3), resulting in its dimerization, nuclear translocation, and interaction with nuclear factor  $\kappa$ B (NF- $\kappa$ B) to trigger the synthesis of IFN and inflammatory cytokines (Fig.1) [13, 17].



**Figure 1. Overview of the cGAS-STING pathway in age-related diseases.** cGAS is a cytosolic DNA sensor that detects dsDNA from senescent cells and dead cells, and some extracellular dsDNA, mtDNA and DNA in the micronuclei are also the main sources of cytosolic DNA. After combining with DNA, cGAS interacts with GTP and ATP, inducing the synthesis of cGAMP. In addition to intracellular synthesis, extracellular cGAMP can also enter the cell and acts as a second messenger, binding to STING located in the ER. Activated STING then transfers to the Golgi via ERGIC, where it can truly function, interacting with TBK1, IRF3, and NF- $\kappa$ B and mediating the production of type I IFN, SASP and ISG, promoting the inflammatory response and exerting biological effects.

Senescent cells accumulate with age. Although these cells undergo a stable cell cycle arrest and cannot duplicate, they can produce a complex secretome, including cytokines, chemokines, growth factors, and proteases. This is called the senescence-associated secretory phenotype (SASP)[18]. The functions of different SASP members differ greatly and are associated with autocrine and paracrine signaling, pro-tumorigenic and tumor-suppressive effects, and pro- and anti-inflammatory signaling [19]. The cGAS-STING pathway plays an indispensable role in cellular senescence, as cellular senescence induced by oxidative stress, DNA-damaging drugs, and oncogene activation is dependent on it [20, 21].

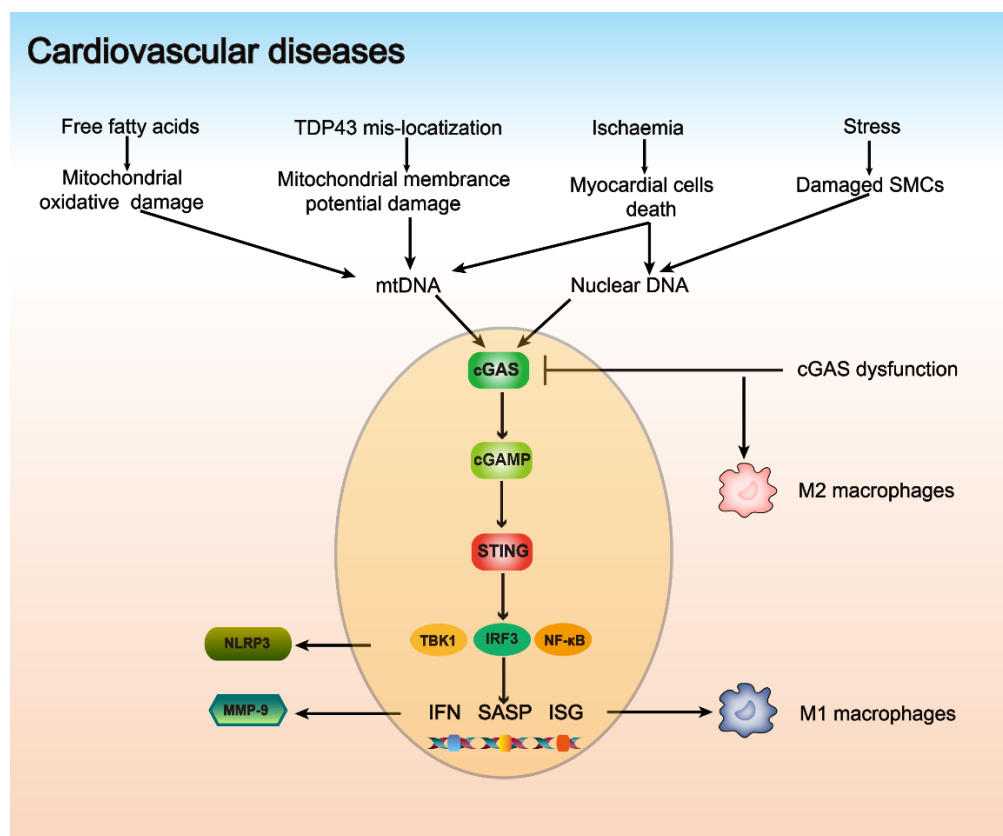
Senescent cells secrete SASP, such as IFN and interleukin (IL), in a cGAS-dependent manner, providing a critical paracrine signal to maintain cellular senescence. In addition, SASP can recruit immune cells to modulate the tissue immune microenvironment and monitor and eliminate tumor cells [22]. However, SASP is a double-

edged sword; it can also promote tumorigenesis by inducing the proliferation, transformation, invasiveness, and metastasis of epithelial cells [23, 24].

Given the important roles of the cGAS-STING pathway in cellular senescence and age-related diseases, we critically evaluated the mechanisms of cGAS-STING in age-related diseases, as well as novel therapeutic approaches targeting this pathway.

### Cardiovascular diseases (CVDs)

CVDs are the leading cause of death, accounting for almost 31% of all deaths worldwide. In 2016, it was estimated that 17.9 million people died of CVDs [25]. Although epidemiological evidence illustrates that advanced age promotes CVDs, the complete mechanisms of how aging drives CVD progression remain unclear [26]. Recent studies have indicated that the cGAS-STING pathway plays an important role in CVDs (Fig. 2) [27].



**Figure 2. The cGAS-STING pathway in cardiovascular diseases.** Free fatty acids can cause mitochondrial oxidative damage, and TDP43 mislocalization results in mitochondrial potential damage. This damage to mitochondria leads to the leakage of mtDNA into the cytoplasm. Damaged SMCs and myocardial cells release nuclear DNA; in addition, dead myocardial cells release mtDNA into the cytoplasm due to ischemia. This cytosolic dsDNA activates the cGAS-STING pathway and induces the production of NLRP3, MMP-9 and M1 macrophages (proinflammatory and antimicrobial), and inhibition of cGAS promotes the formation of M2 macrophages (healing, profibrotic, and anti-inflammatory).

### Cardiac hypertrophy

Myocyte enlargement induced by prolonged and abnormal hemodynamic stress is a characteristic of pathological cardiac hypertrophy, which usually presents with hypertension. The complex responses of fibrosis, capillary thinning, increased production of proinflammatory cytokines, cellular dysfunction, and maladaptive epigenetic changes in pathological cardiac hypertrophy are major predisposing factors for maladaptive cardiac remodeling, cardiac dysfunction, and heart failure [28, 29].

TBK1/IRF3 signaling is associated with mechanical overload-induced cardiac hypertrophy [30]. The expression of STING was found to be increased both in the pathological cardiac hypertrophy heart tissues of patients and mice and correlated with the upregulated type I IFNs. STING knockout or deletion in mice remarkably alleviates cardiac hypertrophy by reducing cardiomyocyte size, ameliorating cardiac function, and decreasing hypertrophic markers, inflammatory factors, and fibrosis. Furthermore, STING knockdown abrogated the factors secreted by cardiomyocytes *in vitro*, which are associated with collagen accumulation in cardiac fibroblasts. These findings demonstrate that the cGAS-STING pathway plays an important regulatory role in the pathogenesis of pathological cardiac hypertrophy [31].

Free fatty acids generated by injections of streptozotocin and a high-fat diet (HFD) in diabetes models can impair mitochondrial function. In addition, the leakage of mtDNA into the cytosol activates the cGAS-STING pathway in mice. Its activation subsequently promotes the formation of the nucleotide-binding oligomerization domain-like receptor pyrin domain containing 3 (NLRP3) inflammasome in the hearts of diabetic mice and the secretion of proinflammatory cytokines related to cardiac pyroptosis and chronic inflammation, ultimately promoting pathological cardiac hypertrophy [32]. HFD can induce cardiac dysfunction and promote maladaptive cardiac remodeling via chronic activation of the cGAS-STING signaling axis. Protein kinase B (Akt) and AMP-dependent protein kinase (AMPK) are closely related to cardiac energy metabolism and the maintenance of cardiac homeostasis. Double knockout of cardiac Akt isoform (Akt2) and AMPK accentuated the HFD-induced increases in heart weight, cardiac hypertrophy, myocardial apoptosis, cardiomyocyte contractile, and oxidative stress. Akt2-AMPK double ablation enhanced the HFD-induced activation of the cGAS-STING signaling pathway and promoted the production of proinflammatory cytokines. Cardiac function was improved, and myocardial hypertrophy was reduced after inhibiting cGAS or STING [33, 34]. The remarkable

mitochondrial oxidative damage to cardiomyocytes in patients with chronic kidney disease (CKD) causes mtDNA leakage, which activates the cGAS-STING-NFκB pathway and NFκB-transactivated ornithine decarboxylase (ODC1)-putrescine metabolic flux, inducing cardiac hypertrophy. Inhibition of the myocardial mitochondria-STING-NFκB-ODC1 axis using genetic or pharmacological approaches significantly alleviated CKD-associated cardiac hypertrophy [35].

### Atherosclerosis (AS)

AS is a chronic inflammatory disease that affects the large- and medium-sized arteries. Early in AS, scavenging macrophages clear atherogenic lipoproteins and lead to the accumulation of intracellular lipids and the formation of foam cells. The residual lipid from the dead foam cells is deposited in the arteries along with the proliferation of the fibrous matrix and smooth muscle cells, forming the characteristic plaques of AS. Plaque rupture is the most common cause of coronary artery thrombosis and can result in heart attack and stroke [36-38]. Inflammation has been recognized as an important mediator of the onset and progression of AS [39].

The mislocalization of DNA/RNA binding protein transactive response DNA-binding protein 43 kDa (TDP43) in the cytoplasm impairs the mitochondrial membrane potential, causes oxidative stress, and induces the release of mtDNA into the cytoplasm. Increased TDP43 expression in both oxidized LDL (oxLDL)-treated macrophages and peripheral blood mononuclear cells from patients with coronary artery disease (CAD) can activate inflammation via NF-κB signaling in a cGAS-dependent manner; TDP43 knockout in macrophages significantly alleviated AS progression [40]. Furthermore, it was recently demonstrated that stromal interacting molecule 1 (STIM1) regulates the cytosolic Ca<sup>2+</sup> concentration and activates the cGAS-STING pathway, promoting vascular smooth muscle cell (VSMC) proliferation and apoptosis, resulting in fragile plaque formation and AS progression [41]. Mitochondrial damage in the VSMCs of CKD patients activates the cGAS-STING pathway and promotes the expression of type I IFN, leading to loss and thinning of the fibrous cap of VSMCs. This is responsible for the vulnerability of atherosclerotic plaques, accelerated atherosclerosis development, and the high morbidity rate of CVDs in patients with CKD [42].

### Myocardial infarction (MI)

MI is characterized by myocardial cell death caused by prolonged ischemia, which is a minor event in a lifelong

chronic disease that could be the first manifestation of CAD, occurring during unstable periods of AS or recurring constantly in established MI patients. MI is a leading cause of global disability and death [43].

Massive myocardial cell death induced by ischemia during MI increases the release of nuclear DNA and mtDNA. The accumulated cytosolic DNA then stimulates the cGAS-STING pathway, inducing the production of IRF3 and its target prototypical cytokine type I IFNs (IFN $\alpha$  and IFN $\beta$ ) transcripts. The IRF3-IFN axis was activated in different populations of IFN-inducible cells in MI mice. cGAS deficiency, genetic disruption of IRF3-dependent signaling, or antibody blockade of the type I IFN receptor in mice results in the downregulation of inflammatory cytokines and chemokines, alleviated inflammatory cell infiltration, and improved cardiac function and survival after MI [44]. Furthermore, selective small-molecule STING inhibitors, C178 and H-151, could reduce pathological remodeling, improve cardiac function, and alleviate heart failure after MI by disturbing the palmitoylation of STING. Cao et al. ligated the LAD coronary artery of mice to induce MI and found that cGAS-STING consistently and significantly increased the mRNA expression of IFN-stimulated genes (ISGs), including IRF7, IFN-induced protein with tetratricopeptide repeat 1 (IFIT1), IFIT3, chemokine (C-X-C motif) ligand 10 (CXCL10), and cluster of differentiation 14 (CD14). Importantly, the expression of STING increased without influencing the expression of major proinflammatory cytokines, such as IL1 $\beta$ , IL6, IL6, and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), by the inactivation of the cGAS-STING pathway at the level of transcript abundance [45]. M1-subtype macrophages (pro-inflammatory and antimicrobial) and M2-subtype macrophages (healing, profibrotic, and anti-inflammatory) are the major cell types in early MI [46, 47]. Cytosolic DNA induced the expression of the M1 markers inducible nitric oxide synthase (iNOS) and CXCL10 via the cGAS-STING pathway. However, cGAS signaling deficiency promotes the abundance of M2 macrophages, which can promote myofibroblast angiogenesis and transformation to enhance myocardial wound repair, alleviate pathological ventricular remodeling, and improve survival. cGAS-STING pathway activation in human heart failure samples was confirmed by the dynamic changes in cGAS and CXCL10, which were consistent with those observed in mice [45].

#### ***Aortic aneurysm (AA) and dissection (AAD)***

AA is the second most common aortic disease, characterized by permanent dilation of the thoracic and abdominal aortic regions compared to normal

individuals[48]. Aortic dissection is characterized by true and false lumens of the aortic wall due to tears in the inner layer. Advanced age and vascular inflammation are two common risk factors for AA and dissection (AAD) [49]. The most important features of AAD are extracellular matrix (ECM) depletion and progressive loss of smooth muscle cells (SMCs) [50].

Significant DNA damage and leakage were observed in the aortic media and adventitia cells of patients with AAD, which resulted in the accumulation of cytosolic DNA and activation of the STING-TBK1-IRF3 pathway. This was confirmed by the upregulated expression and phosphorylation of STING, TBK1, and IRF3 in aortic tissues from patients with AAD. The STING-TBK1-IRF3 signaling pathway is critical in SMC necroptosis and apoptosis induced by ROS and cytosolic DNA. The DNA from damaged SMCs can activate the STING-TBK1-IRF3 signaling pathway in macrophages, inducing the production of matrix metalloproteinase (MMP)-9, and ultimately contributing to aortic degeneration and AAD formation. In the *Sting<sup>gt/gt</sup>* mouse model of sporadic AAD model, aortic enlargement and fiber disruption were alleviated, and the incidence of AAD was decreased in both the thoracic and abdominal aortic regions compared with the sporadic AAD wild-type mouse model. The TBK1 inhibitor amlexanox can significantly preserve the aortic structure and reduce aortic dilatation and the AAD incidence, improving survival and preventing the progression of AAD [51].

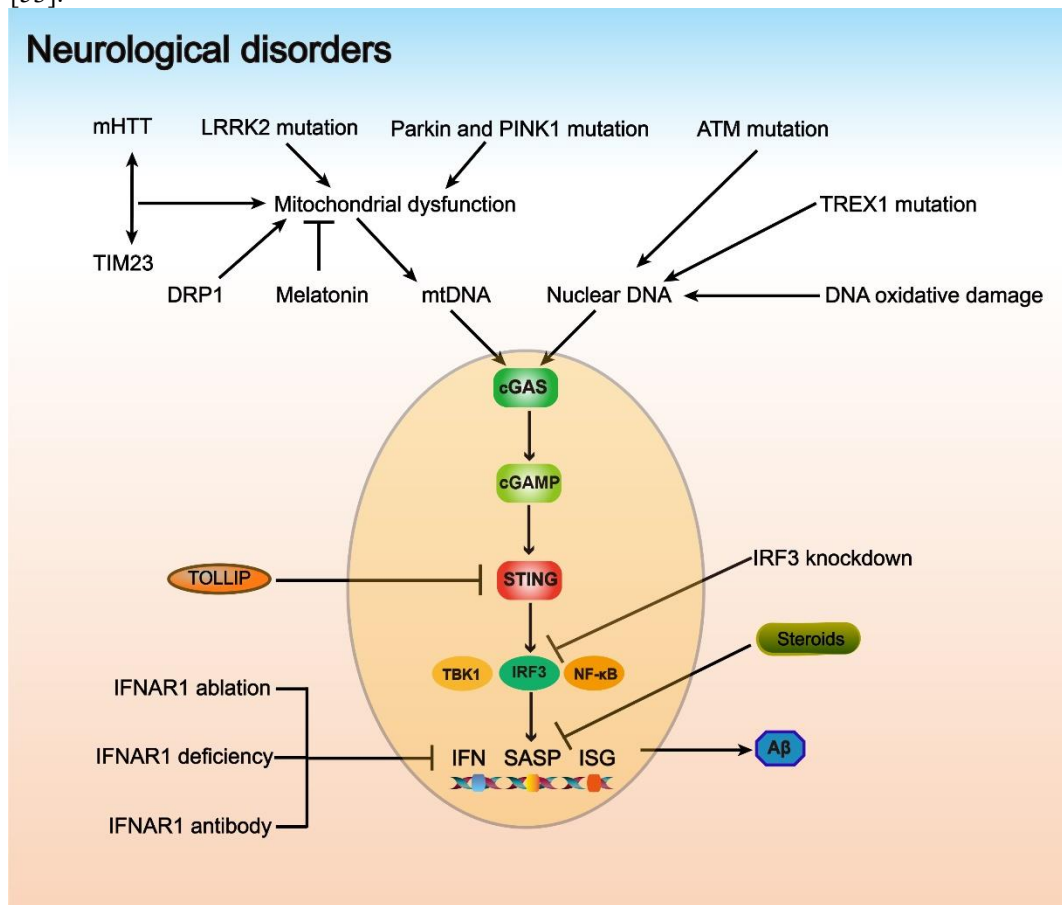
#### **Neurological disorders**

Aging is associated with several neurodegenerative diseases, and age-related brain inflammation, known as “inflammaging,” can lead to cognitive decline and deficits. The SASP phenotype induced by the cGAS-STING pathway plays an important role in neuroinflammation and age-related neurological disorders (Fig. 3) [52].

#### ***Alzheimer’s disease (AD)***

AD is the most common cause of dementia, affecting the daily activities of patients with AD. It is a major cause of dependence, disability, and mortality, and is recognized as a global public health priority. AD usually occurs in elderly individuals, resulting in insidious progressive problems in episodic memory. The representative pathological features of AD are extracellular accumulation of senile plaques containing amyloid-beta (A $\beta$ ), amyloid plaques, and neurofibrillary tangles (NFTs) containing hyperphosphorylated tau, accompanied by neuropil threads, dystrophic neurites, associated

astrogliosis, microglial activation, and cerebral amyloid angiopathy [53].



**Figure 3. The cGAS-STING pathway in neurological disorders.** mHTT has a high affinity for interacting with TIM23, the interaction of which damages the function of mitochondria. Furthermore, DRP1 and LRRK2 mutations and Parkin and PINK1 mutations also cause mitochondrial dysfunction, resulting in mtDNA leakage. Melatonin stabilizes mitochondria and inhibits mtDNA release. ATM mutation, TREX1 mutation and DNA oxidative damage contribute to the increase in nuclear DNA. Activation of the cGAS-STING pathway promotes A $\beta$ -induced neurotoxicity. TOLLIP, steroids, IFNAR1 blockade (ablation, deficiency and antibody) and IRF3 knockdown inhibit the cGAS-STING pathway.

Advanced age is the greatest risk factor for AD, mainly occurring in people over 65 years of age [54]. Neuroinflammation contributes to AD pathology, as confirmed by the enhanced microglial and astroglial activation, upregulated proinflammatory cytokine levels in the brains of patients with AD, and the decreased incidence of AD in individuals treated with nonsteroidal anti-inflammatory drugs. Type I IFN aggravates neuroinflammation and promotes AD development. Inhibition of IFN signaling within the aged brain partially rescues hippocampal neurogenesis and cognitive function and re-establishes IFN1-dependent choroid plexus activity during aging [55, 56]. The choroid plexus transcriptome demonstrated an overall upregulated expression of type I IFN response genes at all ages in AD mouse models [57]. Modulation of neuroinflammatory responses by inhibiting type I IFN signaling may provide

new potential therapeutic targets in AD. Type I IFN receptor-1 (IFNAR1) is a highly specific transducer of type I IFN. Upon binding to type I IFN, IFNAR1 can trigger tyrosine phosphorylation of many proteins, including JAK, TYK2, and STAT, further regulating immune cell recruitment and inflammatory progression. Current research suggests that the removal of type I IFN signaling can reduce neuroinflammation and delay phenotypic progression. IFNAR1-deficient mice displayed a decrease in type I IFN and proinflammatory cytokines. Although astrocyte reactivity was enhanced, microglial proliferation around amyloid plaque deposition was attenuated. In addition, conditioned media from IFNAR1<sup>-/-</sup> primary glia treated with A $\beta$ 1-42 were less toxic to primary cultured neurons [58]. The expression of IFN $\alpha$  in the brains of the APP/PS1 transgenic mouse model of AD was two-fold higher than that in control

brains, and ablation of IFNAR1 and IRF3 or IRF7 knockdown protected cells against A $\beta$ -induced neurotoxicity [59, 60]. Taken together, these findings indicate that the cGAS-STING-IFN pathway plays a critical role in the onset and development of AD, and that treatments targeting this pathway can alleviate IFN-related neuroinflammation and delay the progression of AD to some extent [61].

### ***Huntington's disease (HD)***

HD is a progressive neurodegenerative disease accompanied by motor, cognitive, and psychiatric disorders. A CAG repeat expansion with a polyglutamine strand of variable length confers direct toxicity to the mutant huntingtin (mHTT) protein, the main cause of HD [62]. HD can manifest throughout life, and the typical symptoms are chorea and dystonia, incoordination, cognitive decline, and behavioral difficulties, usually occurring in middle-aged individuals [63]. CAG repeats, but not the polyglutamine strand length, determine the age at HD onset [64]. Correia et al. conducted genome-wide association studies to discover genetic modifiers of HD and demonstrated that the age of HD onset was associated with oxidative DNA damage, DNA repair, and mitochondrial function pathways [65].

mHTT has a high affinity for translocase of the inner mitochondrial membrane 23 (TIM23), and their interaction can reduce the transportation of mitochondrial matrix proteins, altering the mitochondrial proteome and leading to the profound mitochondrial dysfunction documented in HD. mHTT can enhance the activity of mitochondrial fission GTPase dynamin-related protein-1 (DRP1) by binding to DRP1, resulting in excessive fragmentation and abnormal mitochondrial distribution. Subsequently, the increase in ROS causes mitochondrial damage and massive release of mtDNA, which triggers cGAS signaling and leads to the production of proinflammatory cytokines. Exploring new drugs that block the increase in ROS and the release of mtDNA may provide novel treatment options for aging and neurodegenerative diseases such as HD. Melatonin protects neurons from mHTT-mediated neurotoxicity. However, melatonin progressively decreases with age and in HD, and is a main cause of HD onset [66, 67]. mtDNA damage of the striatum and cerebral cortex in HD mice progressively increased and was eight times more frequent than nuclear DNA damage. An age-dependent increase in mtDNA damage contributes to mitochondrial dysfunction in HD and is an early biomarker of HD-associated neurodegeneration [68]. Cytosolic mtDNA in HD mice and mHTT-expressing cells stimulates the cGAS-STING-IRF3 pathway and subsequent production of proinflammatory cytokines. Evidence of cGAS-

STING-IRF3 signaling pathway activation has also been reported in the early stage of human HD striatum, indicating that mtDNA release into the cytoplasm plays an important role in HD inflammation as an initial agent to activate the cGAS-STING-IRF3 signaling pathway [67]. Toll-interacting protein (TOLLIP) negatively regulates the Toll-like receptor (TLR) signaling pathway, which is associated with a variety of inflammatory diseases. TOLLIP is a stabilizer of STING and mediates the clearance of HD-linked polyQ protein aggregates, which can inhibit the STING-induced immune response by sequestering TOLLIP away from STING. The expression of TOLLIP decreases in the striatum of HD mice [69, 70].

### ***Parkinson's disease (PD)***

Selective neuronal loss in the substantia nigra and other brain areas is a characteristic of PD, the most common serious movement disorder and second most common neurodegenerative disease worldwide. Age is the strongest risk factor for PD. The typical syndromes of PD are rigidity, rest tremor, and bradykinesia [71, 72]. The pathological features of PD include damage to dopaminergic projections from the substantia nigra pars compacta to the caudate nucleus and putamen (striatum), and its pathological hallmarks are intraneuronal Lewy bodies and Lewy neurites [73]. Neuroinflammation is a critical cause of progressive PD. Activated microglia and upregulated proinflammatory cytokine expression were detected in both PD patients and animal models, and increased neuroinflammation damaged dopaminergic neuronal cells, resulting in PD [74, 75].

Mutations in the leucine-rich-repeat kinase 2 (LRRK2) gene in familial PD are recognized as the most common cause. Asymptomatic PD subjects with LRRK2 mutations have higher proinflammatory cytokine levels, which in turn increase LRRK2 kinase activity [76, 77]. Furthermore, LRRK2 maintains mitochondrial homeostasis, and LRRK2 deficiency in macrophages improves the basal levels of IFN. Oxidative stress mediated by purine metabolites and mitochondrial fission induced by DRP1 in LRRK2 knockout macrophages contributes to mtDNA leakage into the cytosol, which chronically activates the cGAS-STING pathway, and reduces DRP1 expression. Antioxidant treatment alleviates mitochondrial damage and decreases type I IFN expression in LRRK2 knockout macrophages [78]. Type I IFN signaling is upregulated both in the postmortem brains of PD patients and in a PD mouse model induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). IFNAR1-deficient mice displayed decreased type I IFN signaling, proinflammatory response, and dopaminergic neuron loss. Monoclonal IFNAR1 antibody

blockade treatment significantly upregulated striatal dopamine levels and improved behavior. This indicated that blocking IFNAR1 reduces MPTP-induced neuroinflammatory responses and dopaminergic cell death [79]. Parkin and PINK1 remove damaged mitochondria via mitophagy and mitigate STING-induced inflammation. Mutations in Parkin and PINK1 result in the accumulation of mtDNA mutations with age, the main cause of early onset PD. Blocking monoclonal IFNAR1 antibody treatment or STING deficiency rescued the inflammation induced by exhaustive exercise and mtDNA mutation, motor defects, and loss of dopaminergic neurons in aged Parkin-deficient mice [80].

### *Aicardi-Goutières syndrome (AGS)*

AGS is a recessive and progressive neurological disease associated with inflammation. The constitutive upregulation of type I IFN expression mediated by AGS-associated gene mutations is considered a major cause of AGS pathogenesis. Thus, ‘type I interferonopathy’ is also suggested to define the wider spectrum of diseases induced by mutations of AGS-associated genes [81, 82].

Aberrant activation of the cGAS-STING signal by self-DNA can lead to an abnormal innate immune response, which is the primary pathogenic factor in AGS and causes other severe autoimmune diseases. Three-prime repair exonuclease 1 (TREX1) dysfunction has been suggested to be associated with autoimmune diseases and inflammation. Mutations in TREX1, a 3'-DNA exonuclease that degrades cytoplasmic DNA, result in DNA degradation disorders. Furthermore, cytoplasmic chromatin DNA fragments contain DNA damage, and oxidative damage to DNA can inhibit the exonuclease activities of TREX1, collectively leading to the accumulation of cytosolic DNA [83, 84]. The increased cytosolic DNA subsequently provokes the cGAS-STING pathway and induces the production of type I IFN [85]. The upregulated expression of cGAMP in the inflamed hearts of TREX1-deficient mice confirmed the activation of cGAS in vivo. The mortality and pathology of TREX1-deficient mice with IFNAR deficiency significantly improved, indicating the important role of the cGAS-STING-IFN signaling axis in TREX1 mutation. Given the close relationship between AGS and TREX1 mutations, cGAS inhibitors may be a potential therapy for patients with AGS [86-88].

### *Ataxia-telangiectasia (A-T)*

A-T is a progressive neurodegenerative disorder characterized by immune dysfunction, predisposition to cancer, cerebellar degeneration, and telangiectasia. The underlying cause of A-T is mutations in the ataxia

telangiectasia mutated (ATM) gene, which are important for dealing with DNA damage and maintaining genomic stability [89, 90]. More evidence indicates that chronic inflammation plays a critical role in the onset and development of A-T [91].

Increased numbers of micronuclei and nuclear shape abnormalities are observed in the olfactory neurosphere-derived cells (ONS) of A-T patients, and SASP genes, including IL1 $\alpha$ , IL-6, and IL-8, are upregulated. Interfering with the expression of cGAS and STING with siRNAs decreased the abundance of SASP genes. In addition, A-T brain organoid senescence is reduced, and A-T neuropathology is improved by cGAS or STING inhibition [92]. Quek et al. constructed an ATM knockout rat model with paralysis and spinal cord atrophy, which was similar to older patients and milder forms instead of cerebellar atrophy. They found that the levels of cytosolic DNA dramatically increased in the neurons and glia of ATM-deficient rats. The accumulated DNA in the cytoplasm consequently activated the cGAS-STING signaling axis, accompanied by cytokine upregulation, formation of an inflammatory microenvironment, and constitutive activation of microglia, contributing to neuronal dysfunction and death. Short-term treatment with steroids, such as betamethasone, could significantly reduce the loss of motor neurons in the spine, presumably via its anti-inflammatory function [93].

### **Neoplasms**

Inflammation induced by the cGAS-STING signaling pathway is an essential component of innate immunity that can defend against microbial infections. However, aberrant, and chronic inflammatory stimulation is responsible for some autoimmune diseases, including lethal anemia, polyarthritis, and systemic lupus erythematosus. Recent studies indicated that inflammation plays a crucial role in all phases of tumorigenesis, including onset, progression, malignant invasion, metastasis, morbidity, and mortality [94-96]. The downstream effector TBK1 of the activated cGAS-STING signaling pathway has been recognized as a critical mediator of persistent inflammatory responses that contribute to carcinogenesis (Fig. 4) [97, 98].

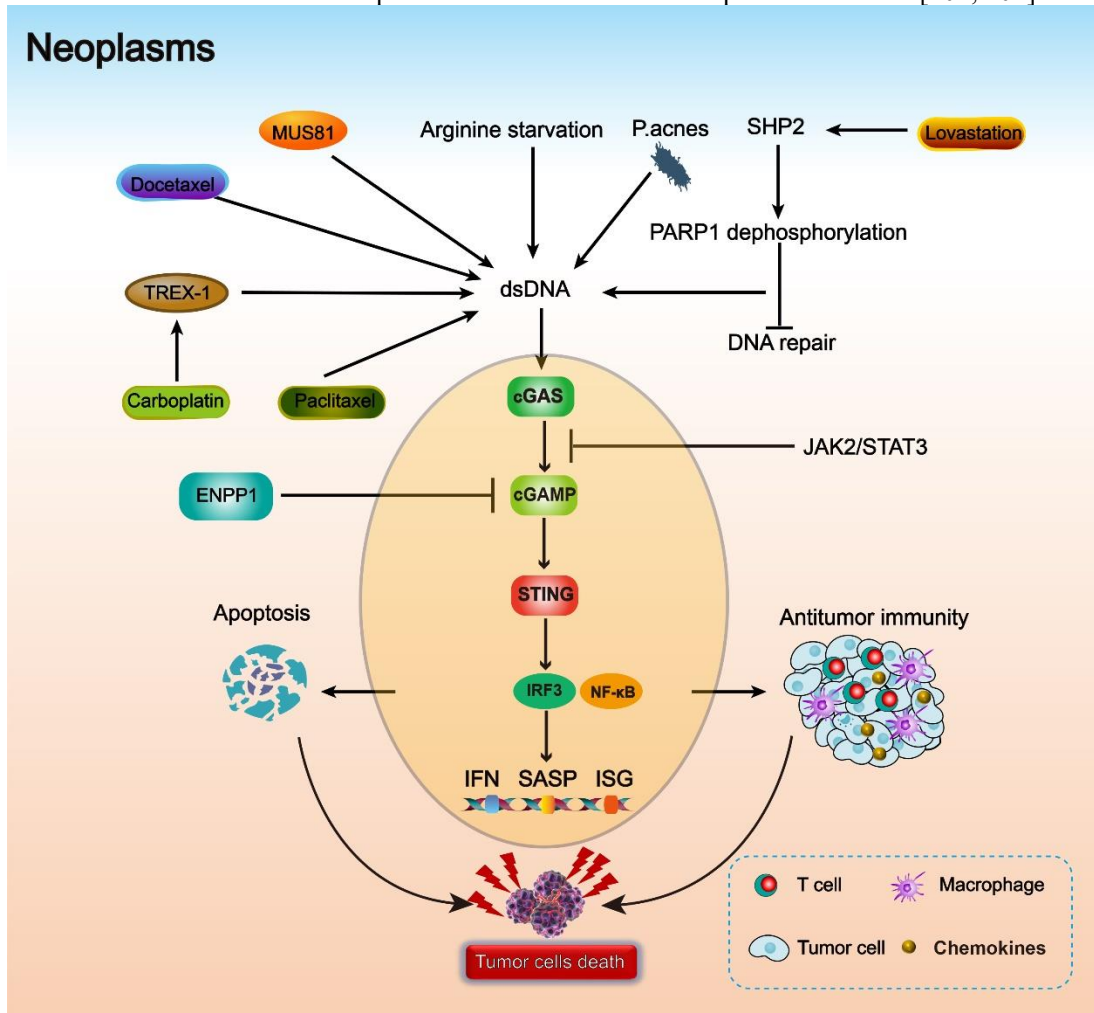
### *Colorectal cancer (CRC)*

CRC is the fourth leading cause of cancer-related deaths worldwide, accounting for approximately 10% of all annually diagnosed cancers and 9.2% of global deaths. Advanced age is a high-risk factor of CRC [99, 100]. Growing evidence suggests that chemotherapy for CRC induces multiple forms of DNA damage, of which DNA double-strand breaks (DSB) are the most severe. If left



unrepaired, DSB can lead to mutations that promote an antitumor immune response. Therefore, targeted therapy provides a new strategy for CRC to improve therapeutic efficacy and reduce the recurrence rate. Downregulation of cGAS and STING and decreased transcription of TBK1

and IFN in CRC tissues indicate that the cGAS-STING pathway is inhibited in CRC. The cGAS-STING pathway is essential for the antitumor immunity of CRC, the disruption of which may be a critical mechanism for immune escape of tumor cells [101, 102].



**Figure 4. The cGAS-STING pathway in neoplasms.** MUS81, docetaxel, paclitaxel, arginine starvation, and *P. acnes* contribute to the increase in dsDNA in the cytoplasm. Carboptatin upregulates the expression of TREX1, which increases cytosolic dsDNA. SHP2 phosphorylates PARP1 and inhibits DNA repair, resulting in the accumulation of dsDNA. Cytosolic dsDNA is recognized by cGAS, and downstream signaling pathways are activated. The activated cGAS-STING pathway induces apoptosis and antitumor immunity, and these effects together lead to tumor cell death. JAK2/STAT3 and ENPP1 can inhibit the cGAS-STING pathway.

Exogenous cGAMP injection into tumors can provoke the cGAS-STING pathway and promote the expression of IFN, initiating antitumor immunity [103]. Higher STING expression in patients with CRC is associated with longer overall and recurrence-free survival [104]. Zhu et al. generated a colitis-associated CRC mouse model and found that STING-deficient mice exhibited increased colonic inflammation, hyperplasia, tumor burden, and severe intestinal pathology, suggesting that STING plays an essential role in suppressing colorectal tumorigenesis [105]. Poly ADP-ribose polymerase 1

(PARP1) is dephosphorylated by src homology-2 domain-containing protein tyrosine phosphatase-2 (SHP2) when DNA is damaged. This compromises DNA repair and contributes to the accumulation of cytosolic dsDNA, resulting in activation of the cGAS-STING signaling pathway. The agonist lovastatin can enhance the activity of SHP2 and promote the chemotherapeutic effect in colon cancer by activating the cGAS-STING signaling pathway, which could be a better alternative for colon cancer treatment [106]. The cGAS-STING signaling axis may be a potential therapeutic target for CRC [107].

## Melanoma

Melanoma is a potentially fatal malignancy with an increasing incidence and mortality; it usually occurs in young and middle-aged people, and the incidence increases linearly from the age of 25 to 50 years [108-110].

Takashima et al. established a melanoma mouse model by injecting B16D8 melanoma cells into mice, which were sensitive to the cytotoxicity of natural killer (NK) cells. STING-knockout mice and STING-disrupted B16D8 cells were used to probe the critical role of STING in NK cell sensitivity in a melanoma mouse model. The proliferation and survival of STING-disrupted B16D8 cells were unaffected, and the expression of IFN $\beta$  mRNA did not increase when stimulated with cGAMP. The growth of STING-disrupted B16D8 cells was much faster than that of normal B16D8 cells in STING knockout mice, indicating that STING is involved in natural regulation and inhibits tumor proliferation [111].

As a primary epigenetic regulator, protein arginine methyltransferase 5 (PRMT5) is involved in various cell growth processes and has been reported to be associated with the development and progression of melanoma. PRMT5 methylation of IFN gamma inducible protein 16 (IFI16) and its mouse homolog IFI204, which plays a vital role in the cGAS-STING pathway, attenuates the production of IFN and chemokines induced by solute DNA, further inhibiting the activity of the cGAS-STING pathway. NLR family CARD domain containing 5 (NLRC5) is a member of a class of genes that regulate MHC I antigen presentation. The elevated NLRC5 expression enhanced MHC I cell surface expression and inhibited melanoma growth more effectively. Furthermore, upregulation of IFI16, IFI204, and NLRC5 could limit melanoma growth in mice and improve the survival of patients with melanoma. PRMT5 function is negatively associated with antitumor immunity. PRMT5 methylates IFI16/IFI204 and inhibits NLRC5 transcription, suppresses inflammation and antigen presentation, and promotes melanoma growth. PRMT5 inhibition antagonizes melanoma growth in immunocompetent mice [112]. TREX1 is essential for DNA replication and repair, but its expression is downregulated in human melanoma cell lines. Carboplatin activates the cGAS-STING pathway by upregulating TREX1 expression to inhibit proliferation and induce apoptosis in melanoma cells. Targeting the TREX1/cGAS-STING signaling axis could be a potential therapy for human melanoma [113].

## Breast tumors

Breast tumors are the most common female malignancy worldwide, accounting for 30% of all female cancers in 2021 [114]. Parkes et al. reported a molecular subtype of breast cancer with a defective DNA damage response (DDR), which demonstrated increased DNA in the cytoplasm and constitutive activation of the cGAS-STING-TBK1-IRF3 pathway [115]. The activated cGAS-STING axis promoted the proliferation of triple-negative breast cancer (TNBC) cells [116].

Chromosomal instability (CIN), a tumor hallmark, is caused by persistent errors in chromosomal segregation during mitosis. It is considered a major driver of metastasis in tumors, such as breast tumors. CIN induced by continuous errors in chromosome segregation contributes to the generation of micronuclei, which are one of the main sources of cytosolic DNA after rupture. Chronic stimulation of cGAS-STING and its downstream effector NF- $\kappa$ B due to CIN is responsible for breast tumor metastasis [117]. Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), which selectively degrades extracellular cGAMP and interferes with immune cell infiltration, is upregulated in TNBC cells with CIN, contributing to resistance to immunotherapy and tumor metastasis [118]. ENPP1 inhibitors may restore innate immune signaling and potentiate antitumor immune responses [119]. Metastatic breast tumor cells in the brain can transfer the second messenger cGAMP to adjacent astrocytes as paracrine signals, which activate STING and mediate the generation of inflammatory cytokines to promote metastatic tumor growth and chemoresistance [120]. Hong et al. found that drug-induced CIN TNBC cells could trigger IL-6 and signal transducer and activator of transcription 3 (STAT3)-mediated signaling to prevent tumor cell death, depending on the activation of the cGAS-STING pathway. Tocilizumab targets the IL-6 receptor and disturbs IL-6-STAT3 signaling, inhibiting the proliferation of TNBC cells with CIN. It has been reported that the application of immune checkpoint inhibitors, such as paclitaxel, in patients with TNBC could induce CIN and the production of micronuclei, which consequently provoked the cGAS-STING pathway. The combined use of tocilizumab with drugs mediating CIN could be a promising therapeutic target for activating IL-6 signaling in cancers, such as TNBC [121, 122].

## Prostate cancer (PCa)

PCa is the second most frequently diagnosed malignancy and the fifth leading cause of cancer-related deaths in men [123]. PCa is recognized as a heterogeneous age-related disorder; the risk of PCa increases significantly with age, and over 85% of newly diagnosed cases are

older than 60 years [2, 124]. Recent bioinformatics research has also demonstrated that senescence-related gene prognostic indices can predict PCa prognosis [125].

Cytosolic dsDNA has been detected in various human PCa cells, and dsDNA continually increases after bromodeoxyuridine labeling, demonstrating that genomic DNA is the source of cytosolic DNA in PCa cells [126]. However, STING signaling is defective in human DU145 PCa cells due to the Janus kinase 2 (JAK2)/STAT3 pathway, and STING agonists fail to provoke the cGAS-STING pathway and promote the production of type I IFN. Blocking the IL-6 or JAK2/STAT3 pathway can restore the responsiveness of DU145 cells to STING agonists [127]. DNA structure-specific endonuclease ultraviolet-sensitive 81 (MUS81) is associated with the amount of DNA in the cytoplasm of both human and mouse PCa cells. MUS81 induces the expression of type I IFNs and chemokines, promotes the immune responses of T cells, and enhances phagocytosis of PCa cells in a STING-dependent manner [126]. *Propionibacterium acnes* (*P. acnes*) is the most common microorganism in normal prostate tissues and is abundant in PCa tissues. It can activate the cGAS-STING pathway and induce the expression of IFN, which subsequently promotes PCa growth [128, 129].

Arginine synthesis deficiency, a common characteristic of cancers, causes depletion of  $\alpha$ -ketoglutarate and epigenetic silencing of metabolic genes related to oxidative phosphorylation and DNA repair via inactivation of histone demethylases. Cancer cells with arginine deficiency exhibit mitochondrial dysfunction, transcriptional reprogramming, and eventual cell death, resulting in chromatin leakage and DNA damage. This leads to cytosolic DNA accumulation and activation of the cGAS-STING pathway. Moreover, the resulting cGAS-STING activation may further enhance its killing effects. Consequently, PCa growth was inhibited in response to the upregulation of type I IFN and recruitment of immune cells. Arginine starvation can be an effective therapy for PCa by activating the cGAS-STING pathway [130]. Ma et al. combined chemotherapy with checkpoint blockade immunotherapy in PCa to investigate the therapeutic effect and found that docetaxel therapy promoted the expression of IFN, and T cell infiltration mediated by the cGAS-STING pathway in PCa patient samples. Correspondingly, docetaxel therapy enhanced intratumor-infiltrating T cells in a PCa xenograft mouse model and promoted the expression of programmed cell death-1 (PD1) and programmed cell death-ligand 1 (PD-L1), potentiating the anti-PD1 blockade therapeutic effect in PCa xenograft mice. Furthermore, prostate-specific antigen progression-free survival of the combination of docetaxel and anti-PD1 blockade was much better than

that of anti-PD1 blockade alone in a metastatic castration-resistant prostate cancer (CRPC) cohort [131].

## Conclusions and perspectives

During human life, genomic stability is challenged by various endogenous and environmental factors. Many mechanisms, collectively named DDR, maintain genomic stability, by recognizing and repairing DNA damage. However, genomic damage still accumulates when genomic damage is excessive and DDR is deficient or disrupted, leading to genomic instability [132]. Genomic instability is a hallmark of aging and a leading cause of age-related diseases, which are closely related to the aberrant presence of cytosolic DNA and activation of the cGAS-STING pathway [3, 133].

Inflammation induced by the cGAS-STING signaling pathway is important for the onset of many age-related diseases. However, the cGAS-STING pathway plays different roles in different age-related diseases (Table 1). Its aberrant activation can lead to pathological cell proliferation, cellular dysfunction, and death, ultimately resulting in a detrimental disease state. Activated cGAS-STING pathway-mediated inflammation increases immune cell infiltration, promotes antitumor immunity, and reduces the risk of tumor metastasis in some cancers. Downregulation or deletion of the cGAS-STING pathway may be an important cause of tumorigenesis and progression. In contrast, the cGAS-STING pathway can also promote tumor proliferation and metastasis [117, 134].

In addition to malignant age-related diseases, inflammation also plays an important role in the development and progression of benign age-related diseases such as benign prostatic hyperplasia (BPH), which mainly develops in the transition zone and enlarges with age. BPH is a hyperplastic process with an increased number of epithelial and stromal cells, but the stromal-to-epithelial ratios in BPH samples are significantly heterogeneous [135]. SASP plays a crucial role in tumorigenesis and age-related diseases. Recent studies have suggested SASP can be triggered by cGAS-STING signaling pathway via accumulation of cytoplasmic DNA in senescent cells. Given the critical role of the cGAS-STING pathway in inflammation and aging, it may also be involved in the development and progression of BPH. Choi et al. used senescent biomarker senescent-associated beta-galactosidase to detect BPH specimens and demonstrated that senescence was only presented in epithelial cells, but not in stromal cells [136]. Although senescent epithelial cells cannot proliferate anymore in BPH, we propose that senescent epithelial cells may secrete SASP via the cGAS-STING pathway to promote the proliferation of stromal cells, which could be a

potential mechanism by which aging results in BPH [137, 138]. However, given that there are no studies have been reported on the cGAS-STING pathway and BPH, further studies are needed to confirm the hypothesis.

**Table 1.** List of age-related diseases and supporting evidence associated with the cGAS-STING pathway.

Age-related disease	cGAS-STING activity	Pathogenesis	Human sample	Animal model	Therapy	Therapeutic effect	Ref	
Cardiovascular diseases	Cardiac hypertrophy	-	DCM and HCM patients	Cardiac hypertrophy mice induced by AB and Ang II	STING knockout	Alleviating inflammation, fibrosis, and attenuating cardiac hypertrophy	[31]	
		Free fatty acids	-	DCM mice induced by streptozotocin and HFD	cGAS and STING knockdown and inhibitor (Ru.521 and C176-1)	Alleviating myocardial pyroptosis, inflammation, and attenuating cardiac hypertrophy	[32]	
		Akt2-AMPK $\alpha$ 2 double ablation	-	Akt2 $^{-/-}$ and AMPK $\alpha$ 2 $^{-/-}$ mice; HFD-induced cardiac hypertrophy mice	cGAS and STING inhibitors (PF-06928215 and Astin C)	Ameliorating HFD-induced contractile dysfunction	[33]	
	Atherosclerosis	In human: upregulated of NF- $\kappa$ B, IL-6 and TNF- $\alpha$ In animal: downregulated IL-6 and TNF- $\alpha$ after TDP43 knockout	TDP43 mis-localization	CAD patients peripheral blood sample	TDP43 $^{-/-}$ mice	NF- $\kappa$ B inhibitors (PDTC and SC75741)	Alleviating AS in TDP43 $^{-/-}$ mice	[40]
	Myocardial infarction	In animal: downregulated type I IFN in IRF3 knockout mice, upregulated ISGs in WT mice	Dead myocardial cells	-	cGAS and STING deficient mice	Genetic disruptor or antibody blockade of IFNAR1	Improving heart function and survival	[44]
		In human: upregulated cGAS and CXCL10 In animal: upregulated STING, CXCL10, IFIT1, IFIT3, IRF7 and CD14	Dead myocardial cells	Failing and nonfailing hearts tissues	LAD coronary artery ligation-induced MI mice; cGAS-null mice	cGAS knockout	Improving myocardial repair after infarction	[45]
Aortic aneurysm and dissection	In human: upregulated STING, TBK1 and IRF3 In animal: upregulated STING	DNA from damaged SMCs	AAD patient aortic tissues	HFD and Ang II-induced AAD model; Sting deficient mice	TBK1 inhibitor (amlexanox)	Partially preventing AAD development	[51]	
Neurological disorders	Alzheimer's disease	-	AD patient prefrontal cortex	AD mouse model; IFNAR1 $^{-/-}$ mice	IFNAR1 ablation	Protection against A $\beta$ -induced toxicity	[57] [59]	
	Huntington's disease	Melatonin decrease	HD patient striatum samples	HD mice model	cGAS inhibitor (Ru.521); melatonin	Inhibition of cGAS-STING pathway	[67]	
		-	-	TOLLIP $^{-/-}$ mice	TOLLIP knockout	Impairing STING signaling and ameliorating HD	[70]	
	Parkinson's disease	In LRRK2 KO macrophages: cGAS, STING, IRF3, IFN and ISG	LRRK2 mutation	-	LRRK2 knockout mice	reducing DRP1 and antioxidants	Alleviating mitochondrial damage and decreasing type I IFN	[78]

	In human: upregulated IL-6, IL-1 $\beta$ , CCL2, and CCL4 In animal: upregulated cGAMP, IL-6, IFN $\beta$ 1, IL-12, IL-13, CXCL1, CCL2, and CCL4	Parkin and PINK1 mutations	-	Parkin $^{-/-}$ , PINK1 $^{-/-}$ and STING $^{-/-}$ mice	Blocking IFNAR1 or STING knockout	Alleviating inflammation and PD	[80]
Aicardi-Goutières syndrome	In animal: upregulated cGAMP and IFN	TREX1 mutation	-	TREX1 deficient mice	IFNAR1 knockout	The mortality and pathology significantly improved	[86-87]
Ataxia-telangiectasia	In brain organoid: downregulated cGAS, STING and SASP (IL-6, IL-8, IL1 $\alpha$ and CCL20) via RNA interference	ATM mutation	A-T patient nasal biopsies	-	cGAS and STING inhibitors (aspirin and H-151)	Reduction of senescence phenotypes and inflammation	[92]
	In animal: upregulated STING, TBK1, NF- $\kappa$ B, IFN $\beta$ , IL-1 $\beta$	ATM mutation	-	ATM knockout mice	Steroids (betamethasone)	Reducing the loss of motor neurons	[93]
Colorectal cancer	In cells: downregulated cGAS, STING, IRF3 and IFN	-	-	STING knockout mice	Viral oncolytic therapy	Tumor cells growth reduction	[101]
	In cells: upregulated STING and IFN after cGAMP stimulation	-	-	shSHP2 mice	SHP2 agonist (lovastatin)	Delayed DNA damage repair and enhanced antitumor immunity	[106]
Melanoma	In cell: upregulated cGAS, STING, TBK1, IRF3 and TNF after TREX1 overexpression	TREX1 decrease	Human sinonasal mucosal melanoma	-	Carboplatin	Inducing apoptosis and inhibiting proliferation	[113]
Breast tumors	In human: upregulated cGAS, STING, TBK1, IRF3	CIN	Human breast cancer samples	-	Tocilizumab	Inducing tumor cells death	[121]
Prostate cancer	In cell: upregulated IRF3 and type I IFN	Nuclear MUS81 decrease	Human PCa samples	-	-	-	[126]
	In cells: upregulated cGAS, STING, TBK1 and type I IFN	Arginine	-	Arginine-restriction xenograft mouse model	Arginine starvation	Increased immune cells infiltration and PCa growth inhibition	[130]
	In human: upregulated cGAS, STING and IFN	-	PCa patient tumor samples	CRPC mice	Docetaxel	Immune microenvironment remodeling and enhancing immunotherapy	[131]

For each age-related disease described in the text, the evidence for causality or correlation with the cGAS-STING pathway, human sample(s), animal model(s), therapy targeting this pathway and its effect are listed. cGAS, cyclic guanosine monophosphate (GMP)- adenosine monophosphate (AMP) synthase; cGAMP, cyclic guanosine monophosphate-adenosine monophosphate; STING, stimulator of interferon genes; IFN, interferon; TBK1, TANK-binding kinase 1; IRF3, interferon regulatory factor 3; IL, interleukin; TNF, tumor necrosis factor; DCM, diabetic cardiomyopathy; HCM, hypertrophic cardiomyopathy; AB, aortic banding; Ang II, angiotensin II; NLRP3, nucleotide-binding oligomerization domain-like receptor pyrin domain containing 3; Akt2, protein kinase B 2; AMPK, AMP-dependent protein kinase; TDP43, Transactive response DNA-binding protein~43 kDa; CAD, coronary artery disease; ISG, interferon stimulated gene; CXCL10, chemokine (C-X-C motif) Ligand 10; IFIT, interferon-induced protein with tetratricopeptide repeat; CD14, cluster of differentiation 14; AAD, aortic aneurysm and dissection; HFD, high-fat diet; TOLLIP, toll-interacting protein; MI, myocardial infarction; SMCs, smooth muscle cells; MMP-9, matrix metalloproteinase (MMP)-9; AD, Alzheimer's disease; IFNAR1, type I interferon- $\alpha$  receptor 1; HD, Huntington's disease; LRRK2, leucine-rich repeat kinase 2; DRP1, dynamin-related protein 1; SHP2, Src homology-2 domain-containing protein tyrosine phosphatase-2; TREX1, three-prime repair exonuclease 1; CIN, chromosomal instability; MUS81, DNA structure-specific endonuclease ultraviolet-sensitive 81; CRPC, castration-resistant prostate cancer.

Treatment with inhibitors or agonists targeting the cGAS-STING pathway in age-related diseases should be prudent depending on the function of this pathway in these diseases. Some drugs targeting the cGAS-STING pathway used for the treatment of diseases are listed in Table 2 [87, 103, 139-159]. However, as most of these drugs are still in the experimental/clinical trial stage, they

cannot be used in humans to address aging and age-related diseases [160]. Further studies are needed to identify ideal inhibitors or agonists targeting the cGAS-STING pathway that can be used in humans. These agents may provide new insights into and therapeutic alternatives for age-related diseases.

**Table 2.** The inhibitors and agonists of the cGAS-STING pathway.

Target	Agents	Function or property	Species specificity	Advantage	Disadvantage	Application model	Treatment effect	Ref
<b>cGAS inhibitors</b>	AMDs	Blocking dsDNA/cGAS binding	Human and mouse cGAS	Widely used with a strong safety profile	-	Trex1 <sup>-/-</sup> mouse model	Effective for the treatment of AGS mice	[87]
	EGCG	Disrupting cGAS activation	Human and mouse cGAS	Specifically inhibit inflammation caused by cGAS activation	Effect of EGCG on cGAS activity is clearly dependent on G3BP1	AGS patient cells and AGS mouse model	Effective in treating cGAS-mediated autoinflammation	[139]
	Aspirin	Inhibiting cGAS by acetylation	Human and mouse cGAS	Widely used with a strong safety profile	-	AGS patient cells and AGS mouse model	Effective in treating AGS and potentially other DNA-mediated autoimmune diseases	[140]
	RU.521	Occupying the catalytic site of cGAS	Mouse cGAS	Selective inhibition of cGAS dsDNA-dependent enzymatic activity in vitro and in cells	Poor inhibitor of recombinant human cGAS	AGS mouse model	Suppressing type I IFN expression	[141]
	G140, G150	Inhibiting cGAS activity	Human cGAS	Specific and potent small-molecule inhibitors for human cGAS	The utility for therapeutic treatment of cGAS-related human diseases is uncertain	Human monocytic THP1 cells and mouse macrophage RAW 264.7 cells	Inhibiting dsDNA-induced cGAS activity	[142]
	PAH	Inhibiting cGAS activity	Human and mouse cGAS	No side effects and toxicity with biological safety in vivo	Inhibitory mechanisms are uncertain	AGS mouse model	Ameliorating self-DNA-induced autoinflammatory responses	[143]
	Compound 25	Inhibiting cGAS activity	Human and mouse cGAS	Superior in vivo anti-inflammatory effects	Structural optimization is needed to achieve higher potency	Lipopolysaccharide-induced mouse model	Inhibiting the dsDNA-induced phosphorylation of STING/TBK1/IRF3 signaling and the mRNA expression of ISGs	[144]
	A151	Competing with DNA	Mouse cGAS	Into the damaged brain parenchyma from the blood	-	Ischemic mouse brains model	Protecting against brain damage and improving neurodeficits	[145]
	Compound 18	Competing with cGAMP	Human STING	Good oral exposure and slow binding kinetics	-	Human monocytic THP1 cells	Inhibiting cGAMP-induced IFN $\beta$ production	[146]
	Astin C	Blocking IRF3 recruitment onto the STING	Human and mouse STING	Well-tolerated compound with minimal cytotoxic side effects	The in vivo effectiveness is uncertain	Trex1 <sup>-/-</sup> BMDMs cells and Trex1 <sup>-/-</sup> mouse model	Inhibiting the expression of type I IFN and pro-inflammatory cytokines and alleviating the autoinflammatory responses	[147]
<b>STING inhibitors</b>	NO2-FA	Inhibiting STING palmitoylation	Human and mouse STING	Natural antiinflammatory mediators	Not yet used in clinical practice	Fibroblasts from SAVI patients	Inhibiting production of type I IFN	[148]
	C-178, C-176	Inhibiting STING palmitoylation	Mouse STING	In vivo inhibitory capacity is not limited by the short serum half-life	Low affinity to human STING	Trex1 <sup>-/-</sup> mouse model	Amelioration of various signs of systemic inflammation	[149]
	C-170, C-171, H-151	Blocking palmitoylation-induced	Human and mouse STING	Highly potent and selective	-	Trex1 <sup>-/-</sup> mouse model	Reducing systemic cytokine responses	[149]

	clustering of STING		small-molecule inhibitor					
	SN-011	Blocking CDN binding and STING activation	Human and mouse STING	Potent and selective inhibitor with high affinity and specificity	-	Trex1 <sup>-/-</sup> mouse model	Ameliorating autoimmune pathology and preventing death	[150]
<b>STING agonists</b>	DMXAA	Non-CDNs	Mouse STING	Potent and specific therapeutic effect on mice	Lacking the ability to activate human STING	Mouse xenotransplantation models	Induction of cytokines and disrupting tumor vascularization	[151]
	CMA	Non-CDNs	Mouse STING	Potent and specific therapeutic effect on mice	Lacking the ability to activate human STING	Mouse macrophages	Inducing IRF3 phosphorylation and Ifnb mRNA translation	[152]
	c(di-GMP)	CDNs	Mouse STING	Mobilizing abscopal immunity when combined with checkpoint modulation	Low affinity to human STING	Bilaterally-implanted TRAMP-C2 tumors mouse model	Mediating regression of injected tumors	[153]
	3'3'-cGAMP	CDNs	Mouse STING	More efficient than DMXAA in activating STING	-	Chronic lymphocytic leukemia and multiple myeloma mouse model	Inducing apoptosis and tumor regression	[154]
	ML RR-S2 CDA	CDNs	Human and mouse STING	Activating all human and mouse STING alleles	Intratumor injection is necessary to achieve maximal therapeutic effect	4T-1 colon or CT26 mammary carcinomas mouse model	Inducing tumor regression and resistant to the same tumor cell line	[103]
	ABZI	Non-CDNs	Human and mouse STING	Intravenous administration can induce adaptive CD8 T cell response in vivo	-	Syngeneic colon tumours mouse model	Strong anti-tumour activity with complete and lasting regression of tumours	[155]
	STING-LNP	Non-CDNs	Mouse STING	Efficiently delivered the STING agonist to the cytoplasm	-	B16-F10-luc2 lung metastatic mouse model	Increasing the expression of CD3, CD4, PD-1 and IFN in lung metastases	[156]
	MSA-2	Non-CDNs	Human and mouse STING	Favorable activity and tolerability profiles	-	MC38 syngeneic tumors mouse model	Inducing tumor regressions	[157]
	PC7A	Non-CDNs	Human and mouse STING	Polyvalent STING agonist with prolonged cytokine expression	-	MC38 and TC-1 tumour models	Notably extended the survival	[158]
	SR-717	Non-CDNs	Human and mouse STING	Substantial efficacy without considerable toxicity	-	B16.F10 melanomas mouse model	Reduction in tumor growth and the increased survival	[159]

cGAS, cyclic guanosine monophosphate (GMP)- adenosine monophosphate (AMP) synthase; cGAMP, cyclic guanosine monophosphate-adenosine monophosphate; STING, stimulator of interferon genes; IFN, interferon; TBK1, TANK-binding kinase 1; IRF3, interferon regulatory factor 3; ISG, interferon stimulated gene; AMDs: antimalarial drugs; EGCG: epigallocatechin gallate; PAH: perillaldehyde; BMDMs: bone marrow-derived macrophages; SAVI: STING-associated vasculopathy with onset in infancy; CDN: cyclic dinucleotide; ABZI: amidobenzimidazole.

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## Conflict of interest

All authors declare that no competing interest associated with the manuscript needs to be disclosed.

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