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Non-canonical cGAS-STING Signaling: A Nexus Between Cellular Senescence and Chronic Inflammation in Aging

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Abstract. Non-canonical cGAS–STING signaling emerges from alterations in classical cytosolic DNA sensing, in which cGAS-generated 2′3′-cGAMP normally activates ER-resident STING, driving TBK1-IRF3–dependent type I/III interferon and NF-κB responses that eliminate pathogens and couple innate and adaptive immunity. In senescent and progeroid cells, de Faria and colleagues identified elevated DNA damage, cytoplasmic DNA accumulation, and SASP/ISG induction despite unchanged cGAMP levels and diminished classical STING–TBK1–IRF3 activation, with STING instead showing nuclear envelope and chromatin localization and forming alternative complexes that reprogram inflammatory and senescence-related transcription. This non-canonical mode features sustained STAT1 and NF-κB activation, robust SASP output, and organ-specific pathogenic effects on vascular smooth muscle, white adipose tissue, and connective tissues, while exerting relatively minor influence on cardiac muscle. Gulen and colleagues demonstrated that STING drives systemic low-grade inflammation in naturally aged animals, promoting inflammatory and interferon signatures in kidney, liver, and brain, microglial activation, neuronal and synaptic loss, and functional decline that can be ameliorated by pharmacological STING inhibition. Additional work links cGAS–STING activation to NLRP3-dependent IL-1β maturation in Alzheimer's disease models and to metabolic reprogramming, mitochondrial dysfunction, and deterioration of the NAD^+–Sirtuin axis. Across these contexts, pharmacological or genetic inhibition of cGAS–STING reduces SASP and ISG expression, limits inflammatory damage, and improves cellular and organismal phenotypes, highlighting non-canonical cGAS–STING signaling as a central driver of inflammation-mediated senescence and metabolic decline in aging and progeroid states.

Keywords: cGAS–STING, inflammation, aging, classical pathway, non-canonical pathway

1. Introduction

The classical cGAS-STING signaling cascade represents a cytoplasmiccytosolic DNA recognition system constructed from three principal molecular components, cGAS, 2'3'-cGAMP, and STING, each with well-defined structural and spatiotemporal properties [1-3]. cGAS is a cytosolic DNA receptor and nucleotidyltransferase that, upon binding B-form double-stranded DNA, undergoes conformational rearrangement and catalyzes synthesis of the endogenous second

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messenger 2′3′-cGAMP from ATP and GTP [2,1,3]. 2′3′-cGAMP contains one 2′-5′ and one 3′-5′ phosphodiester bond and binds STING, an ER-resident multi-pass transmembrane protein of ~379 amino acids that exists as a dimer, with high affinity [4,1,3]. Ligand engagement initiates STING translocation from the endoplasmic reticulum to the Golgiapparatus [5]. Concurrent palmitoylation occurs at Cys88/91 residues, formation of higher-order protein assemblies, and recruitment of the STING-TBK1-IRF3 molecular complex [5]. This subsequently promotes IRF3 and NF-κB phosphorylation and upregulates type I/III interferons, interferon-responsive genes, and pro-inflammatory cytokine production including TNF-α, IL-6, and CXCL10 [3,5]. The reliance upon cGAS-produced 2′3′-cGAMP, mandatory trafficking through the endoplasmic reticulum-Golgi network coupled with palmitoylation, and a dominant STING-TBK1-IRF3-interferon signaling axis collectively define the distinctive "signature" characteristic of the classical pathway [3,5].

2. Transition from classical to non-canonical cGAS-STING pathways and definition of non-canonical signaling

2.1. Discovery of non-canonical cGAS-STING activity in senescence

At the cellular level, de Faria and colleagues investigated Hutchinson-Gilford progeria syndrome fibroblasts alongside replicatively and damage-induced senescent human fibroblasts. Their analysis revealed elevated DNA damage and accumulation of cytoplasmic DNA, coupled with enhanced expression of cGAS and STING proteins, and upregulation of senescence-associated secretory phenotype (SASP) factors and interferon-related genes [6]. However, STING activation did not follow the classical pattern in these cells. cGAMP levels remained unchanged, and responsiveness to poly (dA:dT) was diminished. STING demonstrated predominant localization at the endoplasmic reticulum and the nuclear envelope and upon progerin expression, showed substantial nuclear accumulation with chromatin association, while evading translocation to perinuclear cellular compartments. Despite the provision of exogenous double-stranded DNA, ate-passage/replicatively senescent cells exhibitedreduced numbers of phospho-STING-Ser366-positive cells, diminished perinuclear STING phosphorylation, and lower phosphorylation of STING, TBK1, and IRF3 compared with young cells [6].

Notwithstanding the lack of conventional classical pathway activation markers, SASP and interferon/interferon-responsive gene (ISG) transcriptional programs in senescent and progeria-derived cells exhibited dependence upon both cGAS and STING components. Genetic deletion of STING or pharmacological blockade targeting the cGAS/STING axisreducedSASP/ISG gene expression and STAT1 phosphorylation status while promoting cellular proliferative capacity [6]. In progeria mouse models, H-151 administration decelerated body weight decline, extended median lifespan, restored aortic vascular smooth muscle structural integrity and elastic fiber architecture, mitigated white adipose tissue depletion and associated metabolic dysregulation [6].

2.2. Mechanistic basis of non-canonical activation

At the mechanistic level, de Faria and colleagues integrated prior work on nuclear localization of cGAS and STING and micronuclei recognition to propose several molecular explanations for this non-canonical activity [3,6]. cGAS activity and localization are altered. cGAS is present in both cytoplasm and nucleus. Cytoplasmic cGAS responds to cytoplasmic DNA and synthesizes cGAMP, whereas nuclear cGAS is usually suppressed by the acidic regions of histones in nucleosomes. Within genotoxic stress-generated micronuclear structures, the acidic patches formed by histones

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H2A and H2B compete with double-stranded DNA for cGAS interaction, thereby resulting in, despite the presence of DNA damage and micronucleus biogenesis, only partial activation of the cGAS-STING signaling cascade [6].

STING exhibits abnormal nuclear localization and forms novel complexes with alternative interaction partners. STING localizes to the nuclear envelope and chromatin, participating in DNA damage repair, replication fork stability and transcriptional regulation, functions largely independent of cGAS or the classical TBK1-IRF3 axis. In progeria and senescent cells, enrichment of STING at the nuclear envelope and chromatin suggests that STING may assemble novel complexes with DNA damage-related or transcriptional regulatory factors. STING may reprogram inflammatory and senescence-related gene expression from within the nucleus, without relying on the classical Golgi-TBK1-IRF3 module. In addition, expression or activity of downstream components such as TBK1 or IRF3 may be impaired, may favor STING to signal preferentially through alternative pathways such as NF-κB and STAT1 [9,6].

2.3. Signal output: STAT1 bias and NF-κB cooperation

With respect to signaling characteristics, researchers have emphasized the central importance of the STAT1 axis in this non-canonical mode [6,3]. Progerin expression leads to sustained phosphorylation of STAT1 on Tyr701 and Ser727 and upregulation of STAT1, ISG15, and retinoic acid-inducible gene I proteins [6]. The STING inhibitor H-151 or the cGAS inhibitor G140 markedly reduces STAT1 activation and expression of its target genes [6]. Through the STING-STAT1 axis, the non-canonical pathway forms an amplification loop reminiscent of type II interferon signaling and is more prone to maintain chronic inflammation and senescence-associated signaling than the classical IRF3-centered type I interferon program [6,3].

NF-κB activation is tightly coordinated with STAT1-biased output and the SASP program.In replicatively senescent and ionizing radiation-induced senescent cells, phosphorylation of p65 at Ser536 coincides with robust induction of SASP genes, particularly IL-8 andIL-1β [3,6]. Loss of STING function coordinately diminishes these molecular species, indicating that non-canonical STING-mediated signaling and NF-κB pathway activation cooperatively sustain SASP transcriptional output [3,6].

2.4. Organ-specific roles in progeria models

de Faria and colleagues systematically evaluated the effects of STING inhibition across multiple organs in LmnaG609G/G609G progeria mice and delineated tissue-specific contributions of the non-canonical pathway [6]. Aortic tissue from Hutchinson-Gilford progeria syndrome mice exhibited elastic fiber fragmentation and reduction in vascular smooth muscle cell numbers. Both features were ameliorated by H-151 treatment, indicating that non-canonical STING signaling plays a primary pathogenic role in vascular smooth muscle cell degeneration and vascular aging [6].

In white adipose tissue, Hutchinson-Gilford progeria syndrome mice exhibitedmarked fat atrophy, smaller adipocytes, and increased mitochondrial respiration [6]. H-151 treatment preserved more visceral fat, increased adipocyte area,and restored mitochondrial respiration to approximately wild-type levels, revealing that non-canonical STING activity plays a central role in regulating cellular metabolism and survival in adipose tissue [6]. In heart, Hutchinson-Gilford progeria syndrome cardiomyocytes showed reduced mitochondrial respiration that was not significantly improved by H-151 [6]. This suggests that cardiac dysfunction may not be primarily driven by the same STING-dependent mechanism, or that distinct, tissue-specific patterns of STING–metabolism

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coupling may function differently in cardiac muscle relative to white adipose and vascular tissues [6]. Non-canonical cGAS–STING signaling coordinates inflammatory responses, cellular viability, and metabolic processes in an organ-selective manner, with significant pathogenic and pro-senescence effects observed in vascular, connective, and lipid storage tissues, while having relatively minimal effects on cardiac tissue [6].

2.5. Functional contrast with classical cGAS-STING signaling

Previous reviews have described the main functional features of the classical cGAS–STING pathway in infectious contexts. During pathogenic infection, the classical innate immune pathway rapidly identifies viral and bacterial nucleic acids, which subsequently activates the TBK1-IRF3 axis and promotes a vigorous albeit transient type I interferon production coupled with select NF-κB-dependent pro-inflammatory mediators.Its primary biological functions are to rapidly eliminate pathogens, restrict infection spread, and bridge innate immune recognition with adaptive immunity [3,5].

In contrast, the non-canonical pathway characterized by de Faria and colleagues remains continuously active under sterile, senescence-associated conditions including DNA damage, replication stress, or nuclear envelope defects [5,3,6]. This mode is characterized by low or barely detectable cGAMP production and minimal activation of the STING-TBK1-IRF3 ternary complex, but by sustained activation of STAT1 and NF-κB [5,3,6]. At cellular and organismal levels, the non-canonical pathway drives sustained SASP and ISG programs, mitochondrial and metabolic remodeling, and progressive tissue degeneration [5,3,6]. In LmnaG609G/G609G progeria mice, H-151 extended lifespan, attenuated aortic and smooth muscle degeneration and adipose tissue atrophy,demonstrating that non-canonical STING signaling promotes chronic inflammation and senescence in pathogen-free conditions, and that inhibiting it provides anti-aging benefits [5,3,6]. Functionally, the classical pathway aligns with acute, antiviral, and anti-tumor responses, whereas the non-canonical pathway is more closely associated with chronic, pro-aging, and tissue-degenerative outcomes [5,3,6].

3. Non-canonical cGAS-STING signaling and inflammation-mediated senescence response

3.1. STING as a driver of systemic inflammation in aging

Gulen and colleagues examined naturally aged mice and found that by 26 months of age, kidneys and livers exhibited pronounced inflammatory changes and type I interferon signatures [7]. Sixteen weeks of continuous H-151 administration to aged mice reduced inflammatory and ISG expression in these organs, reduced renal inflammatory cell infiltration, and decreased kidney injury markers including creatinine and urea [7]. Similarly, in Sting1-knockout aged mice, aging-associated immune markers in liver and other tissues were significantly attenuated compared with age-matched wild-type controls, indicating that STING signaling is a key driver of systemic low-grade inflammation in aged animals [7]. Functionally, H-151-treated aged mice demonstrated increased grip strength, extended treadmill endurance, improved spatial and associative memory in Morris water maze and contextual fear conditioning tests [7].

3.2. Microglial activation and brain aging

In the central nervous system, the hippocampus and cortex of aged mice exhibit clear microglial activation and astroglial responses, accompanied by reduced neuronal numbers in the CA1 region

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and decreased synaptic markers [7]. Brain tissue from aged rats contains higher levels of cGAMP than young rats, along with elevated phosphorylation of TBK1 and STING; most phosphorylated STING signal colocalizes with microglia [7]. In isolated aged microglia, transmission electron microscopy reveals disrupted mitochondrial cristae and abnormal mitochondrial morphology, accompanied byelevated cytoplasmic mitochondrial DNA (mtDNA) content [7]. Inhibition of oligomerization of voltage-dependent anion channels 1 and 3 reduces interferon-related gene expression in aged microglia, suggesting that mtDNA-driven activation of cGAS-STING is a key upstream event in the acquisition of pro-inflammatory phenotypes by aged microglia [7]. At the whole-animal level, either pharmacological inhibition or genetic deletion of STING attenuates glial responses in aged brains, restores CA1 neuronal density and synaptic protein levels, and improves cognitive performance [7].

3.3. STING-dependent SASP across tissues and cell types

At the multi-tissue level, 26-month-old mice exhibit elevated mRNA levels of inflammatory mediators including IL-1β, IL-6, TNF-α, and C-C motif chemokine ligand 5 in kidneys and liver compared with young mice [7]. H-151-mediated STING inhibition significantly reduces these pro-inflammatory transcripts.In kidneys, IL-6, CXCL10, ISG15, and IFIT2 are suppressed, whereas IL-1β, IL-6, and TNF-αexpression is reduced in liver [7]. In the brain, aged mice exhibit substantial upregulation of β2-microglobulin, IL-1β, IL-6, TNF-α, C-C motif chemokine ligand 5, IRF7, CXCL9, CXCL10, ISG15, interferon-induced transmembrane protein 3, and additional pro-inflammatory and interferon-responsive gene transcripts, pharmacological STING blockade results in significant downregulation of these molecular markers [7].

At the cellular level, Gulen and colleagues induced senescence in WI-38 and BJ fibroblasts, BV-2 microglial cells, and adipose tissue explants from obese subjectsthrough ionizing radiation, chemotherapy, cyclin-dependent kinase 4/6 inhibitors, and replicative exhaustion [7]. Senescent cells produced large amounts of IL-6, IL-8, C-C motif chemokine ligand 2, CXCL10, and other inflammatory factors [7]. STING inhibition with H-151 or small interfering RNA significantly suppressed expression and secretion of these cytokines and ISGs at cellular and tissue levels, without affecting classical senescence markers including p16 and p21 [7].

Across diverse senescence induction paradigms, ionizing radiation exposure, bleomycin treatment, abemaciclib administration, and replicative exhaustion, senescent cellular populations maintained elevated levels of prototypical SASP mediators (IL-6, IL-8, C-C motif chemokine ligand 2, CXCL10) and interferon-stimulated gene products including IFITfamily members and ISG15 [7]. In cells already in a steady-state senescent phase, a brief course of the STING inhibitor H-151 (10 days) reduced SASP and ISG levels, but had minimal effect on senescence markers including CDKN2A, lamin B1, p21, phosphorylated retinoblastoma protein, or the proportion ofβ-galactosidase-positive cells [7]. These findings indicate that STING is required mainly for maintenance rather than initiation of senescence [7].

In human adipose tissue explants, greater omentum fat from obese patients contained abundantsenescent preadipocytes [7]. After 6 days of culture, IL-6, IL-8, and monocyte chemoattractant protein-1 levels in tissue supernatants increased markedly [7]. Continuous addition of H-151 greatly reduced secretion of these SASP factors and decreased tissue-level SASP and ISG transcription [7]. Based on these observations, Gulen and colleagues proposed a model wherein, within senescent cellular populations, the cGAS-STING axis constitutively engages IRF3 and NF-κB-interferon signaling cascades through detection of cytoplasmic nucleic acid species, encompassing DNA fragments originating from micronuclear compartments and mitochondrial

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sources, thereby sustaining SASP transcriptional expression [7]. In turn, SASP factors such as TNF-α further promote mtDNA leakage and nuclear DNA damage, enhancing cGAS activation and establishing a positive feedback loop [7].

3.4. The STING-NLRP3-IL-1β axis as a central inflammatory loop

In Alzheimer's disease models, Chung and colleagues showed that a STING-NLRP3 axis drives IL-1β maturation in aged and diseased microglia and contributes to the maintenance of neuroinflammation [8]. In primary mouse microglia, amyloid-βor cGAMP administration individually elicits partial activation of the STING-STAT1 axis, ISG15 expression, and inducible nitric oxide synthase production. Comprehensive IL-1β maturation (p17 proteolytic fragment) necessitates concurrent exposure to both amyloid-β and cGAMP [7,8]. Under these conditions, amyloid-β acts as a priming signal for the NLRP3 inflammasome and cGAMP provides the activation signal, leading to cleavage of pro-IL-1β into mature IL-1β, consistent with NLRP3 inflammasome activation [7,8]. Addition of the STING antagonist H-151 completely blocks formation of mature IL-1β, indicating that IL-1β production in this setting depends on the STING-NLRP3 pathway [7,8].

These findings, combined with Gulen and colleagues' senescence studies, support IL-1β as a core SASP component [7,8]. IL-1β released via NLRP3-caspase-1 cleavage further induces expression of IL-6, TNF-α, CXCL10, and other mediators, thereby contributing to the maintenance of local and systemic chronic inflammation [7,8]. The STING-NLRP3-IL-1β signaling chain thus exemplifies how IL-1β can act as a central hub sustaining SASP-like inflammatory circuits, particularly in Alzheimer's disease models [7,8].

3.5. Self-reinforcing non-canonical STING signaling and metabolic decline

cGAS–STING activation triggers SASP in aging tissues, and SASP cytokines including IFN-β, IL-6, and TNF-α, canvia autocrine and paracrine effects, further increase DNA damage, p53 pathway activity, and cGAS–STING signaling, creating positive feedback loops that stabilize senescence over the long term [3,6]. de Faria and colleagues observed similar self-sustaining properties in several primary human fibroblast senescence models [3,6]. In replicative and ionizing radiation-induced senescent cells, even in the absence of exogenous DNA stimulation, SASP and ISG expression, as well as NF-κB and STAT1 activation, remained persistently high [3,6]. Concurrently, these cells showed ablunted response to classical poly deoxyadenine–deoxythymidine stimulation, with reduced cGAMP production, diminished STING phosphorylation, and limited perinuclear translocation [3,6]. These observations suggest that once cells enter a non-classical, STING-dependent steady state, they exhibit reduced responsiveness to classical antiviral stimuli, primarily rely on endogenous positive feedback to maintain inflammation and senescence [3,6]. Pharmacological inhibition of STING or cGAS not only reduces SASP and interferon expression but also improves the proliferative capacity of these cells [3,6].

Zhang and colleagues pointed out that STING activation is closely linked to cellular metabolism and mitochondrial function [5]. Via TBK1, STING promotes phosphorylation of phosphoribosyl pyrophosphate synthetase, increasing phosphoribosyl pyrophosphate production and thereby affecting nucleotide metabolism [9,5]. In parallel, STING associates with sterol regulatory element-binding protein and its cleavage-activating protein, and with fatty acid desaturase 2 and polyunsaturated fatty acid metabolism, indirectly altering redox balance and energy metabolism [9,5]. These changes are closely associated with altered cellular redox state and impaired

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mitochondrial function [9,5]. As the Sirtuin family of deacetylases depends on nicotinamide adenine dinucleotide (NAD^+), age-related declines in NAD^+ availability can reduce Sirtuin activity [9,5]. Li and colleagues therefore proposed that chronic cGAS–STING activation, by driving persistent inflammation, mitochondrial injury and reactive oxygen species production, may further deplete NAD^+ pools, suppress Sirtuin activity and aggravate senescence phenotypes [9]. In this framework, cGAS–STING–driven chronic inflammation and mitochondrial dysfunction are viewed as key upstream events that contribute to deterioration of the NAD^+–Sirtuin axis [9].

4. Conclusion

Non-canonical cGAS–STING signaling critically contributes to chronic low-grade inflammation, the maintenance of cellular senescence, and metabolic dysfunction associated with aging and progeroid conditions. The classical pathway, triggered by infection, strongly activates the TBK1-IRF3 axis and produces a brief type I interferon response. In senescent and progeroid cells, cGAS–STING activation often occurs with little or no detectable increase in cGAMP and a blunted STING–TBK1–IRF3 response. At the same time, STAT1 and NF-κB remain chronically active and sustain SASP and ISG expression. This shift in signaling maintains high levels of SASP and ISG expression over time and has beenobserved in Hutchinson–Gilford progeria fibroblasts, LmnaG609G/G609G mice, and naturally aging animals.In progeroid LmnaG609G/G609G mice, sustained STING activation promotes vascular smooth muscle cell loss and elastic fiber fragmentation within the aorta, alongside white adipose tissue atrophy accompanied by increased mitochondrial respiration. In naturally aged mice, STING signaling also promotes low-grade inflammation inthekidneys, liver, and brain, characterized by microglial activation, neuronal and synaptic loss, and impaired memory. Mechanistically, the constant sensing of DNA fragments from micronuclei and mitochondria in the cell's cytoplasm, paired with abnormal buildup of STING in the cell nucleus and its impact on mitochondrial function alongside declining activity in the NAD+–Sirtuin pathway, establishes a vicious cycle of ongoing inflammation and metabolic problems. Blocking STING with H-151 reduces SASP and ISG activity, lessens inflammatory damage throughout the body, improves physical strength and cognitive function, suggesting that inhibiting non-canonical cGAS–STING signaling might help treat age-related and metabolic conditions.

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