Review

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Cyclic GMP-AMP Synthase in Cancer Prevention

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Cyclic GMP-AMP (cGAMP), synthesized by cGAMP synthase (cGAS), serves as a secondary messenger that modulates various cellular processes, including cell proliferation, cell death, immune response, and inflammation. cGAS is activated upon detecting cytoplasmic DNA, which may originate from damaged genomic and mitochondrial DNA or from viral and bacterial infections. The presence of DNA in the cytoplasm can trigger a substantial inflammatory reaction and cytokine production via the cGAS-STING signaling pathway. Consequently, specific inhibitors targeting this pathway hold significant potential as chemopreventive agents. In this review, we explore the potential effectiveness of modulating cGAS activity. We discuss the role of cGAMP, the mechanism of action for distinguishing between self and foreign DNA, and the possible functions of cGAS within the nucleus.

Key Words Cell proliferation, Cell death, Immune response, Inflammation, Chemoprevention

INTRODUCTION

Cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) synthase (cGAS) is an enzyme found extensively in humans and other organisms [1]. It plays a pivotal role in the innate immune response by detecting foreign DNA, especially from viruses and bacteria [2-4], as well as damaged or aberrant self-DNA [5-7]. When cGAS encounters these foreign or abnormal DNA molecules within a cell, it initiates a signaling cascade that activates the immune system to counter the perceived threat [2-7]. However, the specific roles of cGAS in human cancers are not yet fully understood. Contrary to traditional views of its involvement in immune responses, recent studies suggest that cGAS predominantly localizes in the nucleus [8-12]. Nonetheless, the function of nuclear cGAS remains largely unexplored. In this review, we will provide a brief overview of the potential effects of targeting cGAS in chemoprevention.

DETECTION, ACTIVATION, AND cGAMP SYNTHESIS

cGAS is an enzyme that plays a pivotal role in the innate immune response by synthesizing cGAMP. This synthesis occurs in response to the detection of foreign or abnormal DNA molecules in the cytosol, which is a common occurrence during viral infections or when the cell's own DNA is damaged [2-7]. cGAS, predominantly located in the cytoplasm, binds to double-stranded DNA (dsDNA) in a sequence-independent manner [13]. The interaction between cGAS and dsDNA is facilitated by the attractive forces between the positively charged surface of cGAS and the negatively charged phosphate groups of the DNA backbone [14]. Binding to DNA induces a conformational change in cGAS, leading to the activation of the enzyme. This activation results in the formation of an active catalytic site within cGAS, which then catalyzes the synthesis of cGAMP through a process known as cyclic dinucleotide (CDN) synthesis. This reaction involves the formation of a 2',5'-phosphodiester linkage, joining the 2'-OH group of GTP with the 5'-phosphate of ATP. Once synthesized, cGAMP is released from cGAS into the cytosol [15-19].

DISTINGUISHING SELF OR FOREIGN DNA BY cGAS

One of the most fascinating aspects of cGAS functionality is its capacity to differentiate between self and foreign DNA in eukaryotic cells [20]. Typically, host DNA is sequestered within the nucleus or mitochondria; thus, the presence of DNA in the cytosol often signifies cellular stress, nuclear damage, infection, or cell death [21,22]. Structural distinctions between foreign DNA, originating from bacterial infections,

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and mammalian nuclear DNA include the degree of DNA packaging mediated by specific proteins such as histones and the methylation status of CpG motifs, which are generally unmethylated in bacterial DNA. DNA released during robust viral or bacterial infections undergoes processes like endocytosis-mediated endosome and lysosomal fusion, resulting in the exposure of naked DNA, which lacks protein compaction and membrane protection. In contrast, the nucleus and mitochondria compartmentalize their DNA, isolating it from the cytosol [23-26]. Another distinguishing factor between foreign and self-DNA involves specific proteins that preferentially bind to DNA in the cytosol. For instance, Toll-like receptor 9 (TLR9) has the ability to recognize unmethylated CpG motifs. Located in the endosomal compartment of immune cells such as dendritic cells and B cells, TLR9 targets DNA released from bacteria or viruses during phagocytosis or endocytosis [27,28]. However, cGAS and TLR9 function in distinct cellular locations and contexts [29]. Upon activation, cGAS synthesizes the secondary messenger cGAMP, activating stimulator of interferon genes (STING) and leading to the production of type I interferons and inflammatory cytokines (Fig. 1). Conversely, TLR9, upon recognizing CpG DNA, signals through the MyD88 adaptor protein, activating NF-κB and interferon regulatory factor 3 (IRF3), which then triggers the production of type I interferons and pro-inflammatory cytokines [30-32]. Although cGAS and TLR9 do not directly interact in terms of physical binding or direct signaling pathways, they synergistically contribute to the immune response by detecting viral infections and intracellular bacteria, thereby facilitating an early immune response.

ROLE OF cGAMP IN IMMUNE RESPONSE

Post-translational modification in cGAS activation

cGAS is predominantly localized in the cytoplasm, where it serves as a sentinel for the presence of cytosolic DNA. When cGAS binds to dsDNA, it undergoes conformational changes that reveal specific protein regions, making them more amenable to post-translational modifications, such as phosphorylation [19]. This binding to dsDNA initiates the enzymatic activation of cGAS, leading to the synthesis and accumulation of cGAMP in the cytoplasm. cGAMP then interacts with the STING protein located on the endoplasmic reticulum (ER) membrane. This interaction induces a conformational change in STING, resulting in its activation [1,9,13,15,16,19]. The activated STING recruits and activates TANK-binding kinase 1 (TBK1) [33] and IκB kinase ε (IKKε) [34]. It is important to note that the phosphorylation of cGAS, particularly at hcGAS Ser305 and mcGAS Ser291, is carried out by AKT and CDK1-cyclin B complex, which further suppresses cGAS activity and cGAMP production. This sequence of events attenuates the excessive activation of cGAS in inflammatory signaling response in the presence of cytosolic DNA exposure [35,36]. Concurrently, activated TBK1 and IKKε also phosphorylate interferon regulatory factor 3 (IRF3). The phosphorylated IRF3 is then translocated to the nucleus, where it drives the transcription of genes encoding type I interferons, such as interferon-alpha and interferon-β. Type I interferons play a crucial role in initiating antiviral immune responses and act as signaling molecules that alert neighboring cells to the presence of an infection. This cascade underscores the critical role of cGAS in detecting cytosolic DNA and initiating a coordinated immune response [33,34].

STING activation and initiation of immune responses

cGAMP acts as a secondary messenger, transmitting the signal of DNA detection from cGAS to the downstream components of the immune signaling pathway. It binds to the STING protein, which is located on the membrane of the ER. This interaction between cGAMP and STING is crucial, as it triggers a conformational change in STING, a key step in activating the STING signaling pathway [1,4-6,16]. In its resting or basal state, STING assumes a closed conformation, keeping its signaling domains inactive. This inactive conformation likely involves interactions within specific regions of STING, preventing premature activation of downstream signaling. Upon

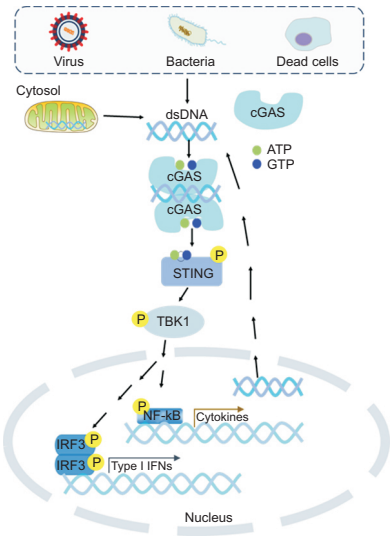

Figure 1. Canonical signaling pathways of cGAS. Cyclic GMP-AMP (cGAMP) synthase (cGAS) detects cytosolic DNA originating from external sources, such as viral and bacterial infections, as well as internal sources, including damaged DNA from the nucleus and mitochondria. When DNA is released into the cytoplasm, cGAS synthesizes cGAMP in response. cGAMP then interacts with stimulator of interferon genes (STING), leading to its dimerization and oligomerization at the endoplasmic reticulum. This activation of STING initiates two distinct signaling pathways: the proinflammatory signaling pathway via NF-κB and the immune response signaling pathway via the interferon regulatory factor 3/interferon α (IRF3/IFNα) pathway. These pathways collectively regulate a variety of cellular processes, including cell proliferation, inflammation, immune responses, and cell death.

binding with cytosolic cGAMP, the C-terminal ligand-binding domain of STING undergoes a conformational change. This alteration facilitates the dimerization or oligomerization of STING molecules, an essential process for its activation. Following dimerization, STING undergoes further modifications, including translocation from the ER to the Golgi apparatus and phosphorylation. These changes enable STING to interact with downstream signaling proteins and adaptors, such as TBK1 [37-40]. This interaction initiates a cascade of phosphorylation events, leading to the activation of transcription factors like IRF3 and NF-κB [38-40]. These transcription factors then stimulate the expression of genes responsible for producing interferons and other cytokines. Moreover, activated STING is believed to relocate from the ER membrane to a perinuclear region, enhancing its efficiency in interacting with signaling partners [41]. This activation and translocation of STING ultimately result in the production of type I interferons, such as interferon-alpha and interferon-beta, along with other proinflammatory cytokines [38-41]. These molecules are instrumental in initiating antiviral immune responses and in defending against intracellular pathogens.

cGAS ROLE IN THE NUCLEUS

Why cGAS is localized in the nucleus

Endogenous cGAS is often detected in the nucleus of cells under normal, non-infected conditions. This nuclear localization is crucial for maintaining cellular homeostasis and preventing inappropriate activation of the cGAS-STING pathway, an integral part of the innate immune response to cytosolic DNA [20]. Several hypotheses have been proposed to explain why cGAS localizes to the nucleus. Firstly, the cGAS-STING pathway is designed to detect and respond to foreign or aberrant DNA in the cytosol, typically indicative of viral or bacterial infection. In the absence of infection, nuclear localization of cGAS helps prevent unwarranted immune responses and inflammation [42]. Secondly, cGAS functions as a DNA sensor, primarily detecting cytosolic DNA, which is abnormal in healthy cells. In uninfected cells, DNA is confined to the nucleus, mitochondria, and other organelles, separated from the cytoplasm by membranes. This physical separation prevents nuclear DNA from activating cGAS. At third, sequestration of endogenous cGAS within the nucleus prevents it from inappropriately sensing self-DNA, a vital self-tolerance mechanism. This compartmentalization ensures cGAS does not encounter genomic DNA and inadvertently trigger an immune response against the host's own cells. At fourth, if cGAS were present throughout the cell, including in the cytosol, it could detect host DNA damage or mislocalized DNA fragments, potentially leading to autoimmune diseases. At fifth, during viral infection or other intracellular threats, cGAS can quickly move to the cytosol to detect and respond to foreign DNA, a key defense mechanism of the innate immune system. However, these explanations present certain complexities. Given that genomic DNA is predominantly in the nucleus, one might expect cGAS to be constantly activated there due to the dense packing of genomic DNA. Also, the significant cellular resources allocated for producing and maintaining cGAS in the nucleus seem less justified. In the nucleus, histones, which package DNA into nucleosomes, bind to cGAS, competing for binding sites and potentially inhibiting its activation. This abundance of histones might sequester cGAS, preventing its unnecessary activation [43,44]. Furthermore, the theory that cGAS primarily resides in the nucleus to avoid indiscriminate immune responses requires further examination. Cells constantly exchange materials with their environment, and DNA can enter the cytosol through mechanisms like phagocytosis [45], pinocytosis, and endocytosis [46]. The notion that nuclear-resident cGAS must relocate to the cytosol for detection poses logical challenges. It's plausible that cGAS's substantial nuclear presence serves multiple roles beyond current understanding (Fig. 2), potentially revealed through ongoing

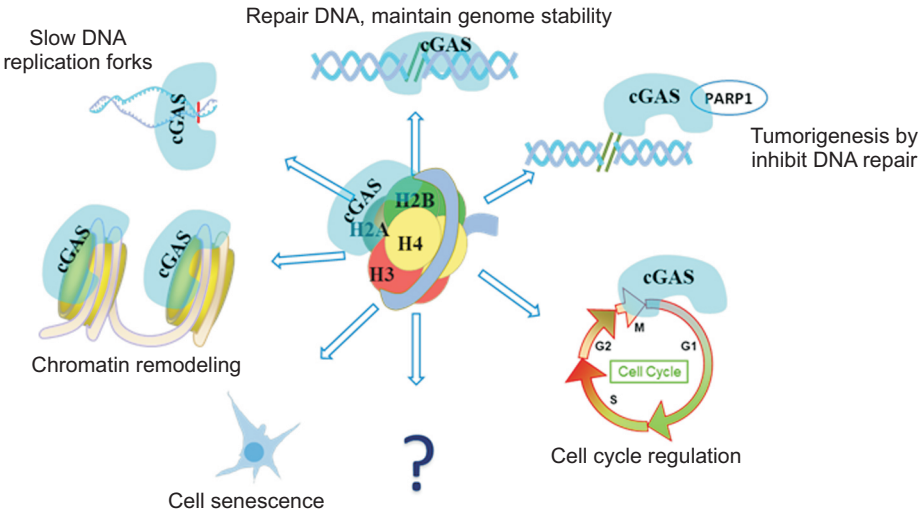

Figure 2. Roles of cyclic GMP-AMP (cGAMP) synthase (cGAS) in the nucleus. Recent studies indicate that cGAS is an abundantly found in the nucleus. Unlikely with the canonical role of cGAS at the cytoplasm in cGAMP production, cGAS also has known to involve in diverse processes occurring in the nucleus. cGAS at the nucleus involves in the formation of replication fork, DNA repair, chromatin remodeling, tumorigenesis, cell senescence, cell cycle regulation, and unrevealed other functions.

research.

When cGAS can be localized

Although cGAS is traditionally known to localize in the cytoplasm under normal physiological conditions, recent publications suggest that it is also abundantly present in the nucleus [20]. Contrary to previous understanding, cGAS can translocate from the cytoplasm to the nucleus in response to DNA fragmentation caused by DNA damage or genomic stress. This translocation triggers an immune response and the production of type I interferons, aiding in the repair of damaged DNA and the elimination of potentially harmful genetic material [47]. Additionally, often associated with aging and DNA damage from genotoxic stressors such as radiation, reactive oxygen species (ROS), and certain chemotherapeutic agents, cGAS can become activated and relocate to the nucleus [47-50]. This results in the subsequent activation of immune responses. Thus, cGAS nuclear localization can be induced under conditions of DNA damage, genomic stress, cellular senescence, or exposure to certain inflammatory signals. This movement allows cGAS to detect anomalies in nuclear DNA and initiate immune responses, contributing to the maintenance of genomic integrity and defense against intracellular threats [47,50,51]. The rationale behind the increased enzymatic activity of phosphorylated cGAS accompanying its translocation to the nucleus remains unclear. Furthermore, the exact mechanisms governing this translocation have not been fully elucidated.

Potential mechanisms how cGAS can be localized into the nucleus

Phosphorylation events significantly influence the subcellular localization of cGAS, potentially promoting its nuclear translocation and subsequent presence in the nucleus. This regulatory mechanism ensures that cGAS is appropriately activated for host defense against cytosolic DNA during interphase and remains inert to self-DNA during mitosis. Specifically, the CDK1-cyclin B complex phosphorylates human cGAS at Ser305 or mouse cGAS at Ser291, leading to the inhibition of its cGAMP synthesis ability in mitotic cells [35]. At the same time, cGAS is predominantly localized in the cytoplasm during interphase and then rapidly translocates to chromosomes upon nuclear envelope breakdown in mitosis [35]. However, specific structural rearrangements in cGAS by CDK1-mediated phosphorylation has not been fully elucidated. In inactivate state, cGAS often exists in an autoinhibited conformation, where the DNA-binding and catalytic domains are hindered or less exposed. This conformation reduces the enzyme's affinity for DNA and its activity. It is assumed that active conformation of cGAS might due to the releases the structural constraints caused by autoinhibition at the activation loop and active-site [19]. The inactivated form of cGAS also presents structural constraints that impede the optimal alignment of the active sites for catalysis, even in the presence of DNA. This phosphorylation status also affects the cGAS interaction with the protein shuttling machineries between the cytosol and nucleus. For example, cGAS phosphorylation at Tyr215 by B-lymphocyte-mediated tyrosine kinase increases the cytosolic retention, resulting in the nuclear localization suppression of cGAS [47]. DNA damage induces nuclear translocation of cGAS in a manner that is dependent on importin-α [47]. Therefore, the phosphorylation-mediated cGAS conformational changes may act as a critical factor for the interaction of nuclear transport proteins, such as importins or exportins. For nuclear import, cGAS is known to interact with importin proteins, recognizing a typical nuclear localization signal (NLS). This interaction suggests that cGAS, possessing a recognizable NLS, might engage with importin-α and subsequently importin-β for its translocation into the nucleus [47]. However, the detailed molecular mechanisms underlying the interaction between importins and cGAS have not been extensively studied. In summary, the phosphorylation of cGAS not only modulates its enzymatic activity but also appears to play a critical role in its subcellular localization, with implications for both its nuclear import and overall function in immune signaling.

Binding partners in the nucleus

Although the binding partners of cGAS in the nucleus are not as well-characterized as its interactions in the cytoplasm, where it primarily acts as a DNA sensor, it is known that cGAS can translocate to the nucleus under specific conditions, such as DNA damage or genomic stress, and may interact with nuclear proteins [47,48]. In the nucleus, cGAS engages with histones and chromatin-associated proteins, enhancing its ability to sense and respond to chromatin structure changes or DNA damage [43]. A study utilizing cryo-EM structural analysis revealed complex interactions between two mcGAS arginines (R222 and R241) and four acidic-patch residues (E61, E64, D90, and E92) in histone H2A [43,52]. In these interactions, the cGAS residues R222 and R241, located in spatially adjacent loops, have conformations stabilized by an inter-loop hydrogen bonding network and direct side-chain interactions with the histones. R222 forms three intramolecular hydrogen bonds with H2A E61 and E64, as well as two intramolecular hydrogen bonds with the backbone atoms of cGAS K241 and R241 in the adjacent loop. Concurrently, cGAS K240 forms a hydrogen bond with E224. R241, besides interacting with R222, extends its side chain into a cavity surrounded by H2A E61, D90, and E92, forming four intermolecular hydrogen bonds and becoming fully encapsulated. Additionally, the backbone of cGAS K315 is hydrogen-bonded with the side chain of H2A R71. Together, these interactions establish two pivotal points, providing a structural basis for the observed hinge motions of cGAS relative to the nucleosome surface. This intricate molecular arrangement underscores the complexity of cGAS's interactions in the nucleus and its role in responding to nuclear DNA anomalies [43,52].

cGAS INHIBITORS POSSESSING CHEMOPREVENTIVE ACTIVITY

cGAS activation is involved in both tumor-suppressing and tumor-promoting process depend on the cancer types. cGAS detects cytosolic DNA derived from tumor cells as a result of genomic instability, which is a common characteristic in many cancers [53,54]. The cGAMP produced by cGAS activates the STING pathway, resulting in the production of type I interferons and other inflammatory cytokines. These cytokines enhance the immune system's ability to detect and eradicate cancer cells [55,56]. Additionally, the cGAS-STING pathway plays a vital role in recruiting and activating T cells, essential for the adaptive immune response against tumors [56-58]. Consequently, targeting the cGAS-STING pathway is being investigated to augment the efficacy of cancer immunotherapies, such as checkpoint inhibitors. Conversely, chronic activation of the cGAS-STING pathway can create a persistently inflammatory environment, potentially facilitating tumor growth and metastasis [58,59]. Elevated cGAS levels have been observed in various cancers, including breast, colorectal, and lung cancer. This elevation often correlates with the presence of cytosolic DNA, genomic instability, and an activated immune response within the tumor microenvironment [60]. In some studies, increased cGAS expression has been associated with advanced cancer stages and poorer prognosis, indicating a possible role in tumor progression under certain conditions [61]. For therapeutic purposes, particularly in conditions with dysregulated cGAS-STING pathways, several specific cGAS inhibitors have been identified. These inhibitors are designed to inhibit cGAS's enzymatic activity, preventing cGAMP synthesis in response to DNA and thereby modulating the immune response. RU.521, one of the first small molecule inhibitors identified for cGAS, binds directly to its active site, inhibiting cGAMP production with an IC_{50} value around 700 nM [62]. PF-06928215, similar to RU.521, binds to the catalytic domain of cGAS, inhibiting its activity [63]. A-151 is another potent and selective small molecule inhibitor of cGAS, targeting cGAS without significantly affecting other nucleotidyl transferases, with an IC_{50} value around 4.9 μM. This specificity makes A-151 a useful tool for studying cGAS's roles in cellular processes [64]. Suramin, a more general inhibitor, affects various enzymes, including cGAS. Although it inhibits cGAS activity, its broad spectrum means it influences multiple pathways. The CDN analog (G[2',5']pA[3',5']p), a non-hydrolyzable cGAMP analog, competitively inhibits cGAS by mimicking cGAMP, binding to cGAS and preventing actual cGAMP synthesis [65].

CONCLUSION

The use of cGAS inhibitors for chemoprevention is a barely studied filed focusing on how modulation of chronic inflammation. Since chronic inflammation is a major risk factor for several types of cancer and the cGAS-STING pathway is activated by in inflammatory response, cGAS inhibitor may be a promising to reduce chronic inflammation and lower the risk of cancer development. Moreover, basis on the rationale that cGAS is activated in response to the presence of cytosolic DNA which is a sign of genomic instability, the cGAS inhibitor possibly reduces the inflammatory response to genomic instability, potentially slowing down the progression of pre-cancerous lesions. Another emerging research field of cGAS-STING signaling pathway is a targeting tumor environment. Evidence that the tumor microenvironment influenced by the cGAS-STING pathway strongly suggest that inhibiting cGAS could alter the tumor microenvironment in a way that makes it less conducive to cancer growth and metastasis. Therefore, in the context of cGAS inhibitors, the idea is to modulate the immune response in a way that could prevent oncogenic processes.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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