Research on cGAS STING Signaling Pathway

Yutong He

University of California, Davis, Davis, CA 95616, USA

Abstract. The cGAS-STING signaling pathway serves as a pivotal component of innate immunity, enabling the detection of cytoplasmic DNA to initiate immune responses against pathogens, tumors, and cellular damage. This pathway is activated when cyclic GMP-AMP synthase (cGAS) binds to double-stranded DNA (dsDNA), catalyzing the synthesis of the second messenger 2′3′-cGAMP. cGAMP subsequently binds to the endoplasmic reticulum adaptor STING, triggering its oligomerization, translocation to the Golgi apparatus, and recruitment of TBK1 and IRF3. Phosphorylated IRF3 induces type I interferons (IFN-I), which orchestrate antiviral and antitumor responses. Recent studies highlight the dual roles of this pathway: it not only defends against viral infections (e.g., herpesvirus, poxvirus, and HIV) and suppresses tumorigenesis by recognizing genomic instability but also contributes to autoimmune disorders (e.g., systemic lupus erythematosus) due to aberrant self-DNA sensing. In cancer, cGAS-STING activation can paradoxically promote metastasis in certain contexts, underscoring its context-dependent complexity. Clinically, targeting this pathway has emerged as a therapeutic strategy, with cGAS inhibitors (e.g., aspirin-mediated acetylation) and STING agonists/antagonists under development. However, challenges persist, including systemic toxicity, drug delivery efficiency, and balancing immune activation versus suppression. This review synthesizes current advances, unresolved questions, and translational prospects of the cGAS-STING pathway in health and disease.

Keywords: cGAS-STING; Signaling Pathway; Innate Immunity; Cancer.

1. Introduction

Innate immunity is crucial in defensing against various pathogenic microorganisms. The host recognizes and interacts with pathogen-associated molecular patterns (PAMPs) of pathogens through pattern recognition receptors (PRRs), triggering a signaling cascade. This response involves the release of pro-inflammatory cytokines, chemokines, and type I interferons (IFN-I) to combat the infection caused by the pathogen [1, 2]. The detection of foreign DNA is important for protecting organisms from extracellular pathogens. Cyclic GMP-AMP synthase (cGAS) is a cytosolic DNA sensor that activates innate immune responses [3]. The binding of cGAS to double-stranded DNA (dsDNA) activates its catalytic activity and the production of 2′3′ cyclic GMP–AMP (cGAMP), which is a second messenger molecule that can induces the production of type I interferons to defend against pathogenic infections [4,5].

2. Basic Mechanism of cGAS-STING Pathway

Once the nucleotide-binding domain detects dsDNA, cGAS undergoes a conformational change, promoting the synthesis of cyclic GMP-AMP (cGAMP). cGAMP is a cyclic dinucleotide that can bind to the STING molecule on the endoplasmic reticulum membrane, causing a conformational change in STING and leading to the formation of STING tetramers and higher-order oligomers. This conformational change induces STING to translocate from the endoplasmic reticulum to the Golgi apparatus through the ER-Golgi intermediate compartment [6]. Structural studies have shown that the formation of STING tetramers acts as a signal to recruit and activate TANK-binding kinase 1 (TBK1) in the Golgi apparatus [7]. The activation of TBK1 then trans-phosphorylates the C-terminal domain of STING, recruiting interferon regulatory factor 3 (IRF3) [8]. Subsequently, IRF3 is phosphorylated and activated by TBK1, forming a dimer that translocates to the nucleus, where it functions as a transcription factor to induce the expression of IFN genes, encoding interferon-β (IFN-β) [9]. IFN-β is a crucial cytokine that activates Janus kinases and signal transducers and activators of transcription (STATs), thereby initiating a positive feedback loop to stimulate the expression of

various immune response genes, which mediate processes such as viral defense and autoimmune regulation [10].

3. Role of cGAS-STING in Immune Response

3.1 The Role in Viral Infectious Diseases

The cGAS-STING pathway plays a dominant role in the early immune response following viral infection. Viral DNA, as the main pathogen-associated molecular pattern (PAMP), interacts with cGAS, activating the cGAS-STING pathway, which leads to the production of type I interferons and a subsequent series of antiviral responses. Existing studies have shown that some DNA viruses, including herpes virus, poxvirus, adenovirus, and human papillomavirus (HPV), can all be inhibited by type I interferons through this pathway [11, 12].

Except DNA viruses, RNA viruses also can activate the cGAS pathway. Human immunodeficiency virus (HIV), a typical RNA retrovirus, has its cDNA recognized by cGAS, which subsequently stimulates the STING-TBK1 signaling pathway, triggering the host's innate immune response [13].

3.2 The Role in Tumors

Cancer cells have a feature of chromosomal instability (CIN). CIN induces the formation of micronuclei, which have fragile membranes that easily rupture, causing the accumulation of DNA in the cytoplasm and subsequently activating the cGAS-STING signaling pathway [14]. Studies found that many cancer cells overexpress cGAS to recognize cytoplasmic DNA and produce cGAMP, which stimulates the secretion of type I interferons (IFN) [15]. Activation of the cGAS-STING pathway triggers the release of senescence-associated secretory phenotypes, including pro-inflammatory factors, chemokines, and proteases, thereby inhibiting tumor growth [16]. However, tumor cells can also exploit the cGAS-STING pathway to further promote tumor growth and metastasis. Research has shown that when dsDNA breaks occur in cancer cells, cGAS is translocated to the nucleus, thereby inhibiting homologous recombination repair, maintaining CIN, and promoting tumor growth [17].

Overall, the role of the cGAS-STING pathway in cancer is complex and multifaceted, influenced by factors such as tumor type, host immune status, the type of activated cells, therapeutic interventions, and the degree of cGAS-STING activation. More research is needed to discover new therapeutic strategies that maximize the anti-cancer effects of the cGAS pathway while minimizing its pro-cancer effects.

3.3 The Role in Autoimmune Diseases

However, cGAS cannot distinguish between self-DNA sequences and foreign DNA sequences. Abnormal activation of the innate immune pathway can lead to a strong IFN-I response, which in turn leads to autoimmune diseases. There are cytoplasmic exonucleases (DNases) to eliminate unnecessary DNA in the cytoplasm, maintaining the level of self-DNA below the activation threshold of cGAS, thereby preventing the occurrence of autoimmune responses. To date, four types of DNases have been identified, including DNase I, DNase IL3, DNase II, and DNase III, also known as three prime repair exonuclease 1 (TREX1). Among them, TREX1 was the first gene found to be associated with Aicardi-Goutières syndrome (AGS). AGS is a genetic systemic inflammatory disease, commonly associated with elevated levels of type I interferons in patients. Its clinical manifestations include symptoms resembling congenital viral infections, white matter lesions in the brain, microcephaly, intellectual impairment, and even death in children [18, 19]. Mutations in the human TREX1 gene lead to elevated levels of type I interferons and central nervous system inflammation. Similarly, Trex1−/− mice exhibit multi-organ inflammation that shortens lifespan and higher interferon levels. Studies have shown that knockout of the cGAS, Sting, or type I interferon receptor genes in mice rescued the inflammatory response caused by Trex1 deficiency [20]. The loss of the

TREX1 gene is also associated with systemic lupus erythematosus (SLE). Studies have found that polymorphisms in TREX1 are a marker of susceptibility to SLE. SLE is a common autoimmune disease that affects multiple organs, often characterized by elevated type I interferon (IFN) levels and defects in the clearance of apoptotic cells. The levels of dsDNA in the serum of SLE patients are significantly higher than in healthy controls. Recent research has shown that SLE patients have significantly elevated levels of cGAS, cGAMP, and immune-stimulating factors in their peripheral blood. The cGAS-STING signaling pathway, as a DNA sensor pathway, has become a key mechanism in the initiation and pathogenesis of autoimmune diseases [21,22].

4. Clinical Applications of cGAS-STING Pathway

4.1 cGAS Inhibitors

Up to now, the reported cGAS inhibitors are mainly divided into the following two categories: one is inhibitors that competitively bind to the active site of cGAS enzyme with ATP or GTP; the other is inhibitors that inhibit the binding of dsDNA to cGAS [23]. Jiang et al. reported that acetylation modification is a key molecular event that controls cGAS activity, and found that acetylation at K384, K394 and K414 sites can inhibit cGAS activation. Firstly, the function of the simulated acetylated cGAS mutant was evaluated in cells, and the inhibitory effect of acetylation modification on cGAS was preliminarily found. Subsequently, the inhibitory effect of acetylation modification on cGAS was verified by CRISPR Cas9 gene editing technology, in vitro fixed-point acetylated protein purification system and cGAMP quantitative analysis technology. Through the analysis of cGAS acetylation modification sites, it was finally proved that aspirin can directly acetylate cGAS, and the effect of aspirin was verified at the cellular and animal levels, which can effectively inhibit the autoimmunity induced by self-DNA in AGS patient cells and mouse models [24].

4.2 STING Agonists

Studies have shown that targeting the regulation of the cGAS-STING pathway becomes an important approach in immunotherapy. Currently, several cGAS-STING pathway modulators have entered clinical research stages, such as the STING agonist IMSA101 and the cGAS inhibitor VENT-03 [25]. STING agonists can be primarily divided into cyclic dinucleotide (CDNs) agonists and non-CDNs agonists. However, CDNs are rapidly cleared in tissues, have poor delivery efficiency to the cytoplasm, and exhibit low response rates in clinical trials, which hinders their further development. Therefore, modifications to the phosphodiester bond, sugar, and base components of endogenous STING agonists to enhance their stability and therapeutic efficacy hold significant clinical application potential.

4.3 STING Inhibitors

There are two main types of STING inhibitors reported: one is covalent inhibitors that bind to Cys88 or Cys91 residues in the transmembrane region of STING protein, which can block the palmitoylation of STING and inhibit the activation of STING; the other is inhibitors that target the C-terminal ligand binding region of STING, which can competitively bind to the endogenous ligand of STING. In addition, promoting STING protein degradation can also be used to negatively regulate STING signaling pathway [26].

5. Discussion and Conclusion

In summary, the cGAS-STING pathway plays a crucial role in the occurrence and development of diseases. The discovery of the cGAS pathway has linked many major diseases to innate immunity, opening a new avenue for studying the mechanisms of human diseases and attracting widespread attention from the international academic community. Further research on the mechanism of virus evasion from cGAS-STING pathway surveillance will be of great significance for designing novel

antiviral drugs. Future research needs to further elucidate the mechanisms of the cGAS-STING pathway in various tumors and different tumor stages, and design targeted therapeutic drugs or early diagnostic biomarkers for cGAS to fully develop application prospects. Therefore, it is extremely important to clarify the different roles played by the cGAS-STING pathway in different diseases, which help understand the mechanisms of disease occurrence and provide more potential methods for disease prevention and treatment.

Although considerable progress has been made in the study of cGAS-STING signaling pathway and related modulators, there are still some problems to be solved, mainly because of the potential adverse reactions. cGAS and STING proteins are widely expressed in various tissues, which may lead to obvious adverse reactions when cGAS-STING signaling pathway modulators are used. In particular, the application of STING agonists may lead to excessive activation of the body's immune system and induce cytokine storms. Therefore, the selective delivery of cGAS-STING signaling pathway modulators to target tissues to avoid adverse reactions caused by acting on the systemic immune system as much as possible is the focus of future research. Moreover, from the perspective of structure and function, in the upstream, cGAS serves as a DNA sensor and initiates a subsequent pathway by synthesizing cGAMP; in the downstream, the recruitment and phosphorylation of TBK1 and IRF3 are now also established as one of the key mechanisms of immune activation. As a core regulatory protein, STING receives signals from host DNA, but also responds to gene mutations and endoplasmic reticulum stress. STING is involved in the regulation of various autoimmune and inflammatory diseases. Based on this, targeting STING directly may be more desirable in drug discovery than targeting the upstream cGAS or the downstream TBK1.

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