Drug Design, Development and Therapy

Dovepress
Taylor & Francis Group

Open Access Full Text Article

REVIEW

Regulation and Function of the cGAS-STING Pathway: Mechanisms, Post-Translational Modifications, and Therapeutic Potential in Immunotherapy

Yuhan Chen[1,*], Si Yue[1,*], Lingyan Yu[1,*], Jinghao Cao[1], Yingchao Liu[1], Aoli Deng[1], Yajuan Lu[1], Jing Yang[1], Huanjuan Li[1], Jing Du[1], Jun Xia[1], Yanchun Li[2], Yongming Xia[3]

[1]Laboratory Medicine Center, Department of Clinical Laboratory, Zhejiang Provincial People's Hospital (Affiliated People's Hospital), Hangzhou Medical College, Hangzhou, Zhejiang, People's Republic of China; [2]Department of Clinical Laboratory, Affiliated Hangzhou First People's Hospital, School of Medicine, Westlake University, Hangzhou, Zhejiang, People's Republic of China; [3]Department of Hematology, Yuyao People's Hospital, Yuyao, Zhejiang, People's Republic of China

*These authors contributed equally to this work

Correspondence: Jun Xia; Yongming Xia, Email andisky_005@126.com; xym20246@163.com

Abstract: Autoimmune diseases arise when the immune system attacks healthy tissues, losing tolerance for self-tissues. Normally, the immune system recognizes and defends against pathogens like bacteria and viruses. The cGAS-STING pathway, activated by pattern-recognition receptors (PRRs), plays a key role in autoimmune responses. The cGAS protein senses pathogenic DNA and synthesizes cGAMP, which induces conformational changes in STING, activating kinases IKK and TBK1 and leading to the expression of interferon genes or inflammatory mediators. This pathway is crucial in immunotherapy, activating innate immunity, enhancing antigen presentation, modulating the tumor microenvironment, and integrating into therapeutic strategies. Modulation strategies include small molecule inhibitors, oligonucleotide therapies, protein and antibody therapies, genetic and epigenetic regulation, cytokine and metabolite modulation, and nanoscale delivery systems. Post-translational modifications (PTMs) of the cGAS-STING pathway, such as phosphorylation, acetylation, ubiquitination, methylation, palmitoylation, and glycosylation, fine-tune immune responses by regulating protein activity, stability, localization, and interactions. These modifications are interconnected and collectively influence pathway functionality. We summarize the functions of cGAS-STING and its PTMs in immune and non-immune cells across various diseases, and explore potential clinical applications.

Keywords: cGAS, STING, immunity, immunotherapy, post-transcriptional regulation

Introduction

Autoimmune diseases arise from the human immune system's attack on self-antigens. In most countries, the number of patients diagnosed with autoimmune diseases has steadily increased from 1985 to 2015. A means increase of 19.1%, 43.1% and 12.5%, 7.9% in the worldwide incidence and prevalence of autoimmune diseases is evidenced by the annual incidence and prevalence increases, respectively.[1] In the quest to combat autoimmune diseases, medical researchers have put forth an array of therapeutic approaches. These encompass strategies aimed at inhibiting B cell functionality and autoantibody generation,[2] curbing T cell proliferation and activation,[3] depleting B cell populations,[4] modulating B cell signaling pathways,[5] and suppressing the production of inflammatory cytokines.[2] These methods continue to be considered primary interventions for autoimmune diseases. Moreover, there is a burgeoning interest in the exploration of gene therapy as a groundbreaking alternative.[6] In addition, the modulation of gut microbiota has demonstrated

Received: 22 October 2024
Accepted: 24 February 2025
Published: 11 March 2025

potential in improving the general well-being of individuals with immune-mediated disorders, offering a promising new frontier in immunotherapy.[7]

For many organisms, detecting foreign DNA is a pivotal aspect of immunity. Mammals have evolved an array of sensing strategies to identify foreign pathogens, with recent focus on DNA recognition-based strategies. In part, this is mediated by cyclic GMP-AMP synthase (cGAS) and the stimulator of interferon genes (STING).[8] The host cell's cGAS protein senses the pathogen's DNA and subsequently synthesizes the second messenger cGAMP via this pathway. The activation of cGAMP induces a series of conformational alterations and spatial rearrangements within the STING protein structure. STING engages and activates the cytoplasmic kinases IKK and TBK1, which in turn activate the transcription factors NF-κB and IRF3, respectively. These activated transcription factors, NF-κB and IRF3, subsequently migrate into the nucleus and collaborate to promote the expression of IFNs and other cytokines.[9]

The cGAS-STING pathway plays an important role in host defense against pathogens, as well as sterile inflammatory diseases, such as autoimmune disease, neurodegenerative disease, metabolic disease, and cancer.[10] The cGAS-STING signaling pathway, given its pivotal roles in both physiological and pathological processes, is subject to intricate regulatory mechanisms and fine-tuning at various levels to preserve the delicate balance necessary for maintaining cellular homeostasis.[11] With the deepening of research, an increasing number of inhibitors and activators targeting the cGAS-Sting pathway are being identified by investigators.[12,13] The ability to modulate this pathway to control the onset and progression of diseases has become feasible, providing a more substantial theoretical foundation for the development of clinical therapeutics. STING agonists can function as vaccine adjuvants in immunotherapy to enhance anti-tumor and anti-viral effects, as well as to augment the upregulation of antigen-presenting molecules, thereby bolstering immune surveillance. They can also be conjugated to specific antibodies to target antigens, serving as precision weapons for the destruction of pathogens. Research on the cGAS-STING signaling pathway and the role of post-translational modifications (PTMs) in its regulation is reviewed in this article. As part of this pathway, we also summarize the functions of two key transcription factors (nuclear factor kappa52 light-chain-enhancer of activated B cells (NF-B) and interferon regulatory factors (IRFs)).[14] We summarize the functions of cGAS-STING and describe the PTMs of cGAS-STING signaling in specific immune and non-immune cells under various disease states and discuss how our understanding of cGAS-STING signaling can be translated into clinical applications.

Pathway of the cGAS-STING

The discovery of the cGAS-STING pathway stands as a pivotal achievement in immunology. In 2008, the research team led by Glen N. Barber conclusively established that STING operates as the upstream regulator of TBK1/IRF3 within the endoplasmic reticulum. This discovery delineated STING's vital role in orchestrating the downstream NK-kB and IRF3 signaling pathways, thus playing a critical antiviral role within the immune system.[15] Firstly, various forms of DNA from viruses, dying tumor cells, or the nucleus and mitochondria are released and enter the cytosol. Subsequently, cGAS acts as a cytosolic DNA sensor, recognizing abnormal DNA and utilizing ATP and GTP to produce the second messenger cGAMP, a cyclic dinucleotide.[16,17] cGAS serves as the primary cytosolic sensor for detecting and binding to double-stranded DNA (dsDNA), single-stranded DNA, and DNA-RNA hybrid duplexes. Both dsDNA and DNA-RNA hybrids are capable of activating cGAS, triggering the synthesis of 2'3'-cGAMP. The recognition of dsDNA by cGAS is length-dependent, with a bias towards longer dsDNA molecules. To achieve a comparable immune response, short double-stranded DNA (dsDNA) fragments with less than 20 base pairs necessitate a concentration 50 to 250 times greater than that required for longer dsDNA fragments exceeding 50 base pairs.[18] Upon binding to STING on the endoplasmic reticulum, 2'3'-cGAMP induces oligomerization and activation of STING, resulting in the formation of dimers.[19] This dimerization is pivotal for the innate immune system's self-activation and serves as a key regulatory step in the subsequent signal transduction pathway.[20] The kinase TBK1 is activated once STING is activated outside the endoplasmic reticulum, where it is engaged. After TBK1 is phosphorylated, IRF3 and NF-B are translocated to the nucleus, where type I interferons and pro-inflammatory cytokines are produced[21] (Figure 1).

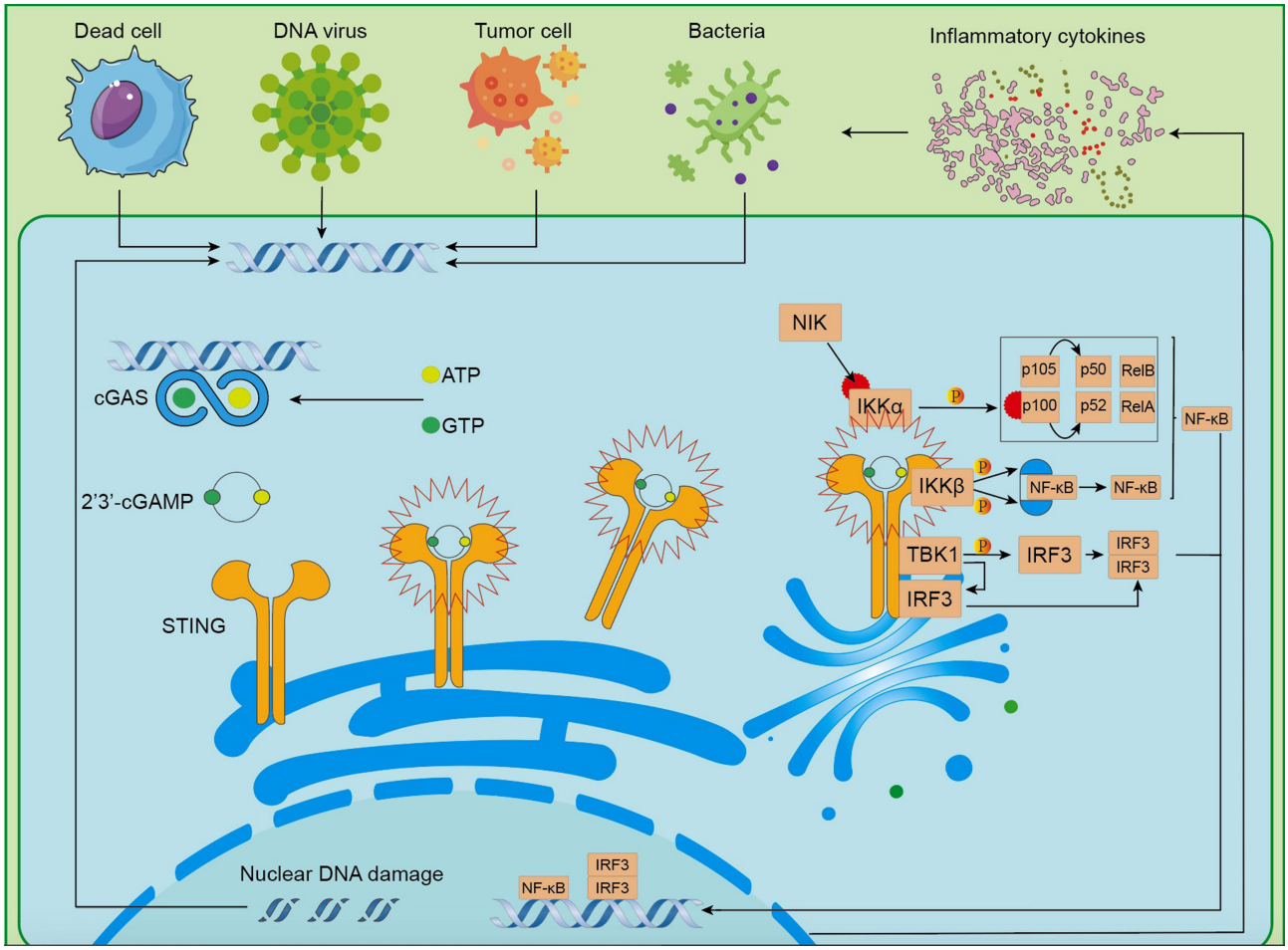

Figure 1 The cGAS-STING-TBK1 signaling pathway. DNA derived from dead cells, viruses, tumor cells, bacteria, and self-damaged DNA binds to the cytoplasmic DNA sensor cGAS. In the presence of ATP and GTP, cGAS produces the second messenger cGAMP, which then binds to STING on the endoplasmic reticulum. Subsequently, STING undergoes a conformational change and translocates to the Golgi apparatus, where it associates and activates kinases IKK and TPK1, leading to the phosphorylation of NF-κB and IRF3, respectively. Phosphorylated NF-κB and IRF3 translocate to the nucleus, where they initiate transcription and trigger the immune response.

The Structural and Functional Exploration of cGAS in Immunology

Since its purification and identification in 2012, the exploration of the structure and function of cyclic GMP-AMP synthase (cGAS) has emerged as a significant area of interest within the realm of immunology. cGAS proteins are classified within the superfamily of nucleotidyl transferases (NTases) and are primarily responsible for recognizing exogenous or endogenously released nucleic acids, such as double-stranded DNA (dsDNA).[16] The unstructured N-terminus of cGAS is poorly conserved, and contains 130–150 amino acids, followed by a highly conserved Mab21 domain. Mab21 is part of the nucleotidyl transferase (NTase) superfamily.[22] cGAS activates upon sequence-independent interaction with double-stranded DNA (dsDNA), forming a minimal 2:2 complex with DNA ligands. By binding to ATP and GTP, cGAS is able to synthesize the cyclic dinucleotide 2′,3′-cyclic GMP-AMP (cGAMP), a cyclic dinucleotide of both 2′-5′ and 3′-5′ phosphodiester bonds.[23]

The Activation and Signaling Mechanisms of STING

STING (also known as MITA, TMEM173, MPYS and ERIS) is an endoplasmic-reticulum-anchored protein characterized by its four transmembrane segments, which are succeeded by a cytoplasmic domain responsible for ligand binding and signal transduction.[24,25] The cytoplasmic domain of STING prototypically exists as a dimer, and this dimerization is crucial for its activation. Upon binding to cGAMP, the cytoplasmic domain of STING undergoes a significant conformational shift.[26] The palmitoylation of human STING at its two cysteine residues, Cys88 and Cys91, is a critical

https://doi.org/10.2147/DDDT.S501773

modification that plays a key role in its activation and signaling efficiency.[27] Cys88 is positioned within the TM2-TM3 linker of STING, remaining internally buried, while Cys91 is surface-exposed, making it the primary site for palmitoylation. The proximity of Cys91 to TM3 implies that its palmitoylation could potentially enhance STING activation by influencing the formation and stability of the tetramer interface.[28]

NF-κB in Immune Activation and cGAS-STING Pathway

In the nuclei of activated B lymphocytes, Ranjan Sen identified a protein binding to a specific, conserved DNA sequence three decades ago.[29] As a result of this discovery, they coined the term "Nuclear Factor Binding near the light chain gene in B cells", abbreviated as NF-κB. This protein, induced during B cell maturation, plays a crucial role in both B cell activation and development. It serves as a paradigm of a rapid response factor, remaining latent within the cell until an inflammatory or other stimulus triggers a cascade of orderly responses immediately upon stimulation. Once the stimulus is resolved, the pathway returns to latency. Importantly, NF-κB activation is not limited to B cells; cells of the innate immune system are also activated through NF-κB at sites of injury or infection.[20] NF-κB induces the production of pro-inflammatory cytokines and IFN genes, exerting direct control over the former.[30] NF-κB, a transcription factor present in all cells and tissues,[31] operates within the NF-κB signaling system, consisting of two protein families: NF-κBs (activators) and IkBs (inhibitors).[17] The system consists of the NF-κB1 protein, p50, and its precursor, p105; the NF-κB2 protein, p52, and its precursor, p100; the transcription factor p65, which is encoded by the RELA gene (also known as RelA or p65); the transcription factor produced by the RELB gene (RelB); the proto-oncogene c-Rel, encoded by the REL gene (referred to as cRel or Rel); as well as the Drosophila proteins Dorsal and Dif.[32] NF-κB activation occurs in two different modes: the "canonical" and "non-canonical" pathways.[33] The primary inducers of canonical NF-κB signaling are TNF-α, interleukin-1β, lipopolysaccharide (LPS), and antigens. These molecules bind to receptors on the cell surface, initiating NF-κB signaling through the involvement of several adaptor proteins.[34] Subsequently, the released NF-κB dimers translocate from the cytoplasm to the nucleus, where they bind to the promoter regions of target genes to regulate gene expression. The activation of the non-canonical NF-κB pathway primarily relies on the disruption of the cIAP-TRAF2-TRAF3 complex, a process that is typically achieved through the degradation of TRAF3. Although certain inducers may also lead to the degradation of TRAF2 or cIAP4, the degradation of TRAF3 is the key step. The recruitment of TRAF2 and TRAF3, along with their associated cIAPs, leads to the destruction of the complex, which appears to be a common mechanism for the activation of the non-canonical NF-κB pathway by various receptors that induce this pathway.[35] Aberrant activation of the noncanonical NF-κB pathway leads to improper survival of self-reactive B cells, resulting in the production of autoantibodies linked to various inflammatory diseases. Additionally, dysregulation of noncanonical NF-κB signaling in endothelial cells can trigger abnormal chemokine secretion and the recruitment of inflammatory cells, which in turn cause excessive and chronic tissue inflammation.[36] The activation of NF-κB plays a crucial role in the cGAS-STING pathway.

The Activation of IRF3

The production of type I IFNs (TI-IFNs) stands as a crucial facet of human innate immunity against intracellular infectious agents, particularly viruses. The most abundantly expressed TI-IFNs in infected cells include interferon α (IFNα) and interferon β (IFNβ). These antiviral cytokines trigger pathways that induce the transcription of effector interferon-stimulated genes (ISGs). These translated specialized proteins significantly impede virus propagation in infected tissue or prevent it entirely. The mammalian IRF family protein, constituting nine members (IRF1-9),[37] comprises structure-related transcription factors. Type I IFNs attach to the broadly distributed IFNAR complex, a heterodimeric transmembrane receptor, which facilitates their antiviral impact. IFN-γ, unique among type II IFNs, is chiefly released by innate lymphocytes and T cells, and it interacts with the IFNGR complex to elicit a varied immune response against internal pathogens. The newly discovered type III IFNs include IFN-λ1 (IL-29), IFN-λ2 (IL-29A), IFN-λ3 (IL-28B), and IFN-λ4.[38] During a viral invasion, the transcription factor IRF3 plays a critical antiviral role. In a cell's resting state, IRF3 resides in the cytoplasm under self-inhibition. However, upon viral invasion, upstream kinases TBK1 and IKK epsilon phosphorylate multiple serine and threonine residues at the C-terminal of IRF3. This phosphorylation triggers conformational changes in IRF3. Subsequently, the C-terminal units combine to form dimers that transit from the

cytoplasm to the nucleus. There, they interact with the co-activating factor CBP (CREB Binding Protein), stimulating the expression of type I interferon.[39]

Regulation of cGAS-STING Pathway at the Post-Transcriptional Level

Protein production begins with mRNA transcription, the first step in a complex process. In order for immune effector proteins to be expressed, post-transcriptional control mechanisms are especially crucial. Post-transcriptional mechanisms that regulate immune function include the following: mRNA turnover regulation in immunity, ARE-mediated mRNA decay, non-ARE-mediated mRNA turnover, ZCCHC11-mediated stabilization of IL-6 mRNA, ZC3H12A-mediated cleavage of cytokine mRNAs, nonsense-mediated mRNA decay, cross-talk between RNA-binding proteins and microRNAs, translational control of immunity, translational silencing of ARE-containing transcripts, the IFN-γ-activated inhibitor of translation, HNRNPL-mediated translation modulation, steroid receptor co-activator 3-mediated inhibition of cytokine translation, translational control of production of lipid mediators, Indoleamine 2,3-dioxygenase/GCN2-mediated metabolic control of immune responses.[40] Following gene transcription within the nucleus, a series of essential, evolutionarily preserved modifications are necessary to produce a fully developed mRNA ready for translation in the cytoplasm. These nuclear modifications encompass the addition of a 7-methylguanosine cap at the 5' end, the removal of introns through splicing, and the truncation and addition of a poly(A) tail at the 3' end to create a mature, polyadenylated mRNA molecule.[41] Post-transcriptional regulation orchestrates various biological activities. Some studies show that the mRNA-binding proteins HuR and AUF1 respectively facilitate Nrf2-mRNA maturation, promote its nuclear export, and stabilize Nrf2-mRNA, both targeting the 3'-UTR of Nrf2-mRNA.[42] While post-translational modification has been established as a regulator of STING, the impact of post-transcriptional regulation on STING proteins remains largely unexplored. A research team uncovered the RNA-binding protein LUC7L2 as a negative regulator of DNA virus-triggered innate immune responses. LUC7L2 deficiency in mice led to resistance against lethal herpes simplex virus 1 (HSV-1) infection, reduced HSV-1 loads in the brain, and elevated STING precursor messenger RNA due to inhibited splicing and promotion of its decay, resulting in decreased STING protein levels.[43] The research discloses a negative feedback loop in the post-transcriptional regulation of STING-dependent innate immune reactions, providing an insightful model for comprehending the regulation of STING protein via post-transcriptional pathways in the context of viral and cellular DNA. It has been documented that heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNPA2/B1) recognizes viral DNA, forms homodimers, and is then demethylated at Arginine 226 by the enzyme arginine demethylase JMJD6. This sequence of events triggers the hnRNPA2/B1 translocation to the cytoplasm, where it stimulates the TBK1-IRF3 pathway, culminating in the synthesis of interferon-α/β (IFN-α/β). Simultaneously, hnRNPA2/B1 promotes the addition of N6-methyladenosine (m6A) and the nuclear-to-cytoplasmic transport of cGAS, IFI16, and STING mRNAs, which further enhances the activation of the cytoplasmic TBK1-IRF3 pathway by these molecules42.[44]

Post-Translational Modification of cGAS-STING

An increasing body of evidence highlights the pivotal role of post-translational modifications in the cGAS-STING pathway. The influence of post-transcriptional regulation on cGAS-STING remains an area of interest. The human genome is estimated to encode approximately 20,000–25,000 protein-coding genes.[45] However, the size of the human proteome surpasses this count significantly, with over 1 million proteins.[46] This disparity primarily arises from post-translational modifications (PTMs), which selectively alter proteins at the mRNA level and intricately regulate and diversify protein functions by chemically binding small molecular components to specific amino acid residues.[47] PTMs play a pivotal role in transmitting signals through phosphoric acid, acetyl, and glycosyl groups between proteins. Due to their reversible nature, these modifications serve as regulatory 'switches' in normal cellular processes that tightly control cell proliferation by governing both static and proliferative states.[48] Certain amino acid regions that regulate protein stability often become sites for post-translational modifications (PTMs). These regions, termed degrons, are modulated by PTMs acting as signals to either hasten protein degradation (PTM activates degrons) or to prevent degradation, thereby stabilizing proteins (PTM inactivates degrons). PTMs exert influence across various facets of protein function, including proteolytic stability. By adding chemical groups-such as phosphorylation, methylation, acetylation, redox-based modifications, or polypeptides-such as ubiquitination, sumoylation, or ISGylation-they significantly enrich the

proteome. As a result, PTMs are crucial in functional proteomics as they govern protein function, distribution, and interactions with both cellular and viral elements. Although many proteins are modified shortly following translation, PTMs may also take place at various stages, including after protein folding or when proteins are repositioned within the cell, thereby affecting their biological functions at these precise locations.[49] Viruses heavily rely on the host's protein synthesis machinery to facilitate viral progeny production, modulating several cellular pathways crucial for viral replication. Consequently, viruses have evolved distinct strategies to either counteract or exploit PTMs in cellular factors and many viral proteins themselves carry PTMs. Notably, PTMs are intricately involved in regulating various stages of the retrovirus viral cycle.[50] Having grasped the fundamental definition of post-translational modification, let us delve into how different PTMs can regulate proteins.

Phosphorylation

Phosphorylation, an extensive post-translational modification, profoundly influences signal propagation.[51] The significance of this process is highlighted by the extensive genomic space allocated to kinases, with more than 500 kinase genes found in the human genome, making up approximately 1.7% of its total. The phosphosite database catalogues over 250,000 sites of phosphorylation across the proteome. Protein phosphorylation entails the attachment of a phosphate group to the hydroxyl group of serine and threonine residues, as well as, to a lesser degree, tyrosine residues.[52] Phosphorylation most commonly occurs at serine residues, with threonine and tyrosine following in prevalence, showing a frequency ratio of 11.2 to 2.5 to 1.3. Nonetheless, phosphorylation is not limited to these amino acids. Although less typical, kinases can also modify the side chains of cysteine, lysine, histidine, arginine, aspartic acid, and glutamic acid.[53] The reversible addition of a phosphate group significantly impacts the function of proteins. Orchestrated by protein kinases, this mechanism entails attaching a phosphate group (PO_4) to the polar group R of various amino acids. Consequently, this addition transforms the protein from hydrophobic to hydrophilic, facilitating conformational changes during its interactions with other molecules.[54] Specific phosphorylation sites often collaborate with ubiquitylation, and these sites display a higher level of conservation than the entire set of phosphorylation sites.[55] In the phosphorylation process, protein phosphatase plays a crucial role alongside the prominently discussed protein kinase, particularly in regulating the levels of tyrosine, serine, and threonine phosphorylation within cells. This joint regulation impacts various physiological processes, including cell growth, tissue differentiation, and intercellular communication.[56]

In innate immunity, phosphorylation plays a critical role. Previous research has revealed that STING activates IRF3 through phosphorylation by stimulating the kinase TBK1. The carboxyl terminus of STING is both essential and adequate for triggering TBK1 activation and initiating IRF3 phosphorylation. Moreover, selective disruption of the STING-IRF3 interaction abolishes IRF3 phosphorylation without impairing TBK1 activation.[57] Further research has revealed two invariant serine and threonine clusters in STING that are phosphorylated by the kinases IKK and/or TBK1 following activation. Upon phosphorylation, these proteins attach to a positively charged region of interferon regulatory factor 3 (IRF3), leading to the recruitment of IRF3 for its subsequent phosphorylation and activation by TBK1.[58] Recent structural insights have delineated the molecular basis for the association of STING with TBK1 and the subsequent phosphorylation of STING by TBK1. Mutational studies confirm the interactional architecture between TBK1 and STING, lending support to a model in which cGAMP-induced assembly of STING and TBK1 into high-order oligomers precedes TBK1-mediated phosphorylation of STING.[59] Additionally, reports indicate that the epidermal growth factor receptor (EGFR) is essential for guiding STING to endosomes, where it can phosphorylate specific tyrosine residues within STING. This localization enables its interaction with its downstream effector, IRF3.[60] Studies indicate that native STING can be phosphorylated by recombinant TBK1 at the Ser365 position of STING. This process illustrates the importance of sphingolipids and cholesterol within the Golgi membrane for STING activation. The involvement of these lipids in the innate immune response could pave the way for novel therapeutic approaches to treat inflammatory conditions induced by cytosolic DNA.[61] Recent findings show that meloxicam, a commonly prescribed nonsteroidal anti-inflammatory medication, can suppress intracellular autoimmunity and the phosphorylation of STING. This discovery not only underscores the significance of phosphorylation in the cGAS-STING pathway but also suggests that inhibitors of STING phosphorylation may serve as promising therapeutic agents for autoimmune disorders.[62]

Glycation

In protein glycosylation, sugars or glycans attach covalently to specific residues in the target protein. As one of the most prevalent and intricate post-translational modifications, protein glycosylation not only significantly expands an organism's proteome beyond its genomic encoding but also exerts profound effects on protein function, stability, subcellular localization, and other characteristics.[63] Glycation, a non-enzymatic reaction, occurs when sugars react with amino groups in proteins. Exogenous glycation forms a Schiff base via a reversible covalent bond between a reducing sugar's carbonyl group and an amino group, releasing water. Endogenous glycation, a bodily process, typically involves glucose reacting with amino acids like lysine and arginine.[64] Protein glycosylation, a sophisticated and multifaceted process, relies on approximately 200 glycosyltransferase enzymes. These enzymes govern the selection of proteins for glycosylation, the specific sites on these proteins where glycans will be added, and the particular structures of these glycans.[65] Glycosylation significantly impacts biology and disease, with IgG glycosylation's heterogeneity making it a health biomarker. Varied IgG glycosylation affects its function and disease progression, emphasizing its role in pathology.[66] Glycosylation modifications also hold significant importance in innate immunity. STING is subject to N-glycosylation by DDOST in the endoplasmic reticulum (ER) following DNA viral infection. DDOST plays a critical role in regulating the STING-dependent synthesis of antiviral type I interferons. Mutations specifically targeting DDOST-dependent N-glycosylation sites impair STING oligomerization, thereby affecting its immune responses.[67] CRISPR-Cas9 genome-wide screening revealed that sulfated glycosaminoglycan (sGAG) biosynthesis proteins play an essential role in STING activation.[68] However, comprehending the nuanced relationship between glycosylation modifications and STING proteins remains a multifaceted field that demands further in-depth investigation for a comprehensive understanding.

Ubiquitination

Post-translational modification by ubiquitination involves attaching 76 amino acid polypeptides called ubiquitins (Ub) to proteins. Initially recognized as a mechanism for targeting misfolded or short-lived proteins for proteasomal degradation, its role has expanded significantly, emerging as a pivotal regulator in protein signaling.[69] Ubiquitination is a highly accurate mechanism that marks target proteins with ubiquitin (Ub) through a cascade involving E1, E2, and E3 enzymes. This process unfolds in a three-step enzymatic sequence: initial activation of Ub by E1 in an ATP-dependent reaction, creating a thiol-ester bond between E1's active cysteine and Ub's C-terminus; subsequent transfer of activated Ub to E2's cysteinyl group, forming an E2-Ub complex; and ultimate transfer of Ub to the substrate protein by E3, either directly from E2 or following E3's prior engagement with Ub[70] (Figure 2).

The evolving understanding of ubiquitination's association with STING proteins has surfaced in recent years' research endeavors. Research investigators have utilized mass spectrometry-based approaches to demonstrate that the delivery of Tank-binding kinase 1 (TBK1) to interferon regulatory factors (IRFs), such as IRF3, following binding to cyclic dinucleotides (CDNs), is dependent on K63-linked ubiquitination at residue K224 on STING. Inhibition of K224

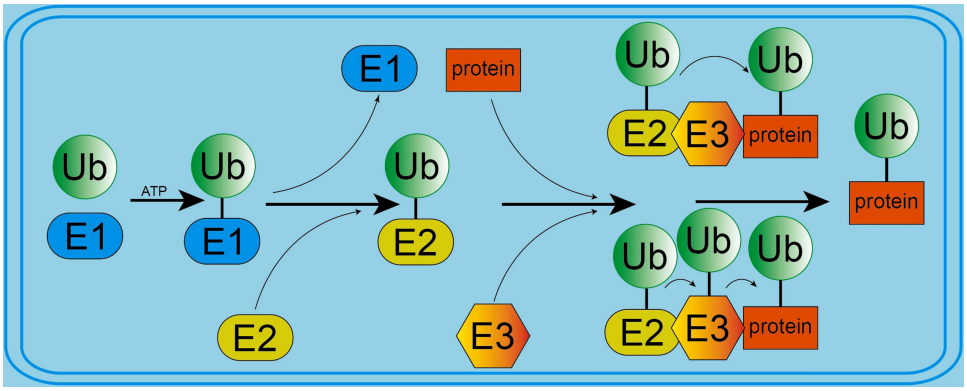

Figure 2 The conjugation of ubiquitin relies on the sequential actions of E1 activating enzymes, E2 conjugating enzymes, and E3 ligases. E1 catalyzes the ATP-dependent formation of a thioester bond between the C-terminus of ubiquitin and the active cysteine residue of E1. Subsequently, ubiquitin is transferred to the active cysteine of E2 via a thiolate intermediate, and is ultimately transferred from E2 to the target protein through the additional contribution of the E3 ligase.

https://doi.org/10.2147/DDDT.S501773

ubiquitination selectively abolishes IRF3 activation without affecting NF-κB activation.[71] In the case of viral infections, particularly PCV2, the p38-MAPK pathway is responsible for USP21 phosphorylation, thereby halting K63 ubiquitination of STING. This process inhibits the phosphorylation and nuclear translocation of IRF3, thus enhancing the organism's susceptibility to PCV2.[72] Further research has revealed that STING undergoes K63-linked ubiquitination within myeloid cells, a process that can be elicited by bacterial by-products such as c-di-GMP. Studies indicate that treating macrophages with c-di-GMP results in elevated levels of ubiquitinated STING, suggesting that c-di-GMP generated by pathogens may activate K63-linked ubiquitination, thereby maintaining STING stability in the intestinal environment.[73] Ubiquitination is a pivotal activation process in the cGAS-STING pathway of antiviral and antitumor immunity. Studies have identified Tripartite motif-containing protein 10 (TRIM10) as a key enhancer in the STING signaling cascade. TRIM10 associates with STING and facilitates K27- and K29-linked polyubiquitination at the K289 and K370 sites of STING.[74] Ubiquitination also plays a significant role in the maintenance of STING homeostasis. Investigators have found that UFL1, the sole E3 ligase for UFMylation known to date, inhibits the interaction between TRIM29 and STING, thereby reducing ubiquitination at K338/K347/K370 and subsequent proteasomal degradation. This identifies UFL1 as a key regulator in maintaining STING stability and antiviral functions.[75] Recent research has shed light on STING degradation within lysosomes, with experiments showing ubiquitinated STING on endosomal vesicles linked to the ESCRT pathway. This ubiquitination is crucial for STING degradation in macrophages. Interfering with STING's interaction with ESCRT or ubiquitin could provide a targeted strategy to boost STING's efficacy in therapy.[76] As research progresses, it is anticipated that the intricate involvement of ubiquitination modifiers in the cGAS-STING-mediated antiviral and anti-tumor immunity will be delineated with increasing clarity, shedding light on their specific roles within this vital immune pathway.

SUMOylation

SUMOylation is a dynamic and reversible post-translational modification that regulates and diversifies protein function by covalently attaching a small ubiquitin-like modifier (SUMO) to target proteins. Viruses rely on hijacking specific cellular pathways within host cells to survive and reproduce.[77] Investigators have discovered that the innate antiviral immune response is capable of being significantly suppressed, with such suppression being facilitated by the intermolecular interaction between SUMOylation in SARS2-NP and the newly identified SUMO interaction motif.[78] SUMOylation also plays a significant role in the cGAS-STING pathway. Research has shown that TRIM38, an E3 ubiquitin ligase, mediates the SUMOylation of cGAS in both uninfected cells and during the initial phase of viral infection. This SUMOylation process inhibits the polyubiquitination and subsequent proteasomal degradation of cGAS. Additionally, TRIM38 enhances the transcription of STING early in viral infection, which aids in STING activation and stabilizes the protein. However, in the later stages of infection, SENP2 de-SUMOylates cGAS and STING, leading to their degradation through the proteasomal and chaperone-mediated autophagy pathways.[79]

Acetylation

Acetylation of proteins is a primary form of post-translational modification (PTM) in eukaryotes, involving the addition of an acetyl group from acetyl coenzyme A (Ac-CoA) to specific amino acid residues within a protein chain.[80] Acetylation modifications can be categorized into two types: N-terminal acetylation and acetylation of lysine residues within proteins. N-terminal acetylation involves adding an acetyl group (-COCH3) to the Nα-group, the free amino group at the beginning of a protein chain. This is often referred to as Nα-acetylation to distinguish it from acetylation on the Nε-groups of lysine side chains. Enzymatically, N-terminal acetylation requires the action of N-terminal acetyltransferases (NATs), which interact with the acetyl donor (Ac-CoA) and the protein's N-terminus.[81] The acetylation of lysine residues within proteins, on the other hand, is generally considered to be a reversible and finely regulated process, in contrast to Nt-acetylation (Figure 3).

Signaling pathways mediated by cGAS-STING also involve acetylation modification. Autoimmune diseases can be severe if the cGAS is chronically activated by self-DNA. Researchers have discovered that acetylation at the sites Lys 384, Lys 394, or Lys 414 on cGAS can inactivate cGAS. Aspirin has been found to directly enforce the acetylation of cGAS and effectively inhibit the cGAS-mediated immune response. This finding suggests that acetylation contributes to

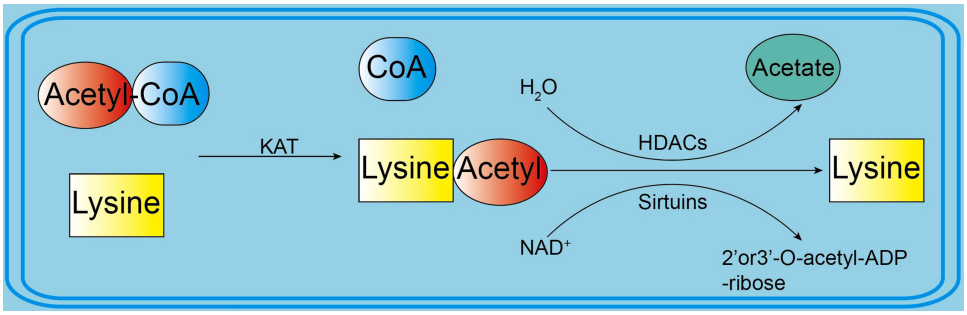

Figure 3 Regulation of reversible lysine acetylation. Lysine acetylation is mediated by lysine acetyltransferases (KATs), transferring acetyl groups from acetyl-CoA to lysine's ε-amino side chain. Non-enzymatic acetylation by acetyl-CoA also occurs. This modification is reversed by Zn^{2+}-dependent HDACs or NAD^+-dependent sirtuins. HDAC activity yields deacetylated lysine and acetate, while sirtuins produce deacetylated lysine, nicotinamide, and 2′/3′-O-acetyl-ADP-ribose.

the regulation of cGAS activity and provides a potential therapeutic approach for the treatment of DNA-mediated autoimmune diseases.[82,83] In investigating the role of STING-mediated acetylation in endometrial cancer regulation, researchers have found that HDAC3 (histone deacetylase) can bind to β-estradiol-ERα and promote the deacetylation of histone 3 lysine 4 at the STING promoter, leading to a decrease in STING expression. Blocking HDAC3 enhances STING expression, which suppresses tumor formation.[84] Divergent results were observed in a separate investigation, wherein investigators examining ischemic stroke pathology found that HDAC3 increases cGAS expression and potentiates the activation of the cGAS-STING pathway through deacetylation of p65 at lysine 122, thereby promoting its accumulation within the nucleus.[85] In the study of the mechanism by which HBV drives viral innate immune evasion, researchers discovered that HAT1 (Histone acetyltransferase 1) epigenetically modulates the acetylation of H4 K5 and H4 K12 to upregulate KPNA2 (nuclear transport protein alpha-2). This facilitates the nuclear translocation of cGAS and inhibits the cGAS-STING pathway and its downstream IFN response, leading to suppression of HBV clearance.[86] However, acetylation-related diseases extend well beyond these findings. The aspiration is for future research to offer a more comprehensive understanding of acetylation's essence, furnishing a robust theoretical foundation for treating associated diseases.

Protein Methylation

Over the past two decades, it has become firmly established that methylation constitutes a pivotal post-translational modification of proteins, with thousands of methylation sites identified across the human proteome.[87] It has been ascertained that methylation of specific proteins can lead to human diseases, such as cancer and neurological disorders, thereby signifying the biological significance and medical importance of protein methylation.[88,89] Protein arginine methyltransferases (PRMTs) and protein lysine methyltransferases (PKMTs) primarily facilitate the S-adenosylmethionine (SAM)-dependent methylation of protein substrates.[90] These catalysts mediate a nucleophilic substitution reaction, resulting in the transfer of a methyl group to the nitrogen of arginine or lysine residues, with the concomitant release of S-adenosylhomocysteine (SAH). Additionally, other protein methyltransferases that modify various peptidyl moieties including glutamate, glutamine, and histidine, as well as the N- and C-termini of proteins, utilize a similar polar mechanism.[91,92]

These methylation patterns play crucial roles in various diseases, impacting brain development, neurogenesis, synaptic plasticity, circadian rhythm, cognitive function, and stress response. Proteins associated with m6A modification significantly influence neuron-related functions, including adult neurogenesis, memory formation, cerebellar development, and axonal regeneration.[93] The importance of methylation modification in the cGAS-STING pathway has been gradually discovered by researchers in recent years. Studies have revealed that PRMT1, a type of protein arginine methyltransferase, targets cGAS at the invariant Arg133 site, leading to the inhibition of cGAS dimerization and the suppression of cGAS/STING signaling pathways within cancer cells. Critically, the suppression of PRMT1 activity through genetic or pharmacological intervention triggers cGAS/STING-mediated DNA recognition, which may upregulate the expression of type I and II interferon response genes. Therefore, the integration of PRMT1 inhibition with anti-

https://doi.org/10.2147/DDDT.S501773

PD-1 antibody treatment significantly boosts the therapeutic efficacy against tumors in vivo.[94] Certain research indicates that protein arginine methyltransferase 5 (PRMT5) serves as a direct interacting partner with cGAS, facilitating the symmetrical dimethylation of cGAS at the Arg124 position. Follow-up investigations have revealed that the PRMT5-induced methylation of cGAS mitigates the cGAS-driven antiviral immune response by reducing its ability to bind DNA. Furthermore, the oral delivery of PRMT5 inhibitors has been shown to confer significant protection to mice against Herpes Simplex Virus type 1 (HSV-1) infection and to increase the lifespan of infected animals[95] Researchers have found that methylation of IFI16 (a component of the intracellular DNA-sensing cGAS-STING complex) / IFI204 (its murine homologue) attenuates dsDNA-induced TBK1-IRF3 activation and production of interferons and chemokines. Variations in PRMT 5 expression do not alter the expression of cGAS or STING, indicating that PRMT 5 restricts activation but not the expression of components of the cGAS/STING pathway.[96] A recent study found that PRMT9 inhibition promoted DNA damage and activated cyclic GMP-AMP synthase, which is responsible for type I IFN production.[97] The methylation of the STING protein is increasingly recognized as a critical modulator of the cGAS-STING pathway.

Palmitoylation

Protein palmitoylation is a reversible lipid modification that involves the addition of palmitate, a 16-carbon fatty acid, to a cysteine residue via a thioester bond.[98] Palmitate is converted from fatty acids by fatty acid synthase (FASN). The process of palmitoylation is mediated by the zinc finger DHHC-containing protein family (encompassing ZDHHC1 to ZDHHC9 and ZDHHC11 to ZDHHC24, also referred to as DHHC1 to DHHC23), whereas the reverse reaction, depalmitoylation, is carried out by Acyl-protein thioesterases (APT1/2), palmitoyl protein thioesterases (PPT1/2), or members of the alpha/beta hydrolase domain-containing protein family, specifically ABHD17A/B/C.[99] Palmitoylation plays a significant role in various diseases. Some researchers have found that ZDHHC12-mediated palmitoylation enhances the degradation of NLRP3 via chaperone-mediated autophagy. Gain-of-function variants of NLRP3 have been identified in autoinflammatory diseases. By reducing the palmitoylation level of NLRP3 and disrupting chaperone-mediated autophagic degradation of NLRP3, the NLRP3 inflammasome is hyperactivated.[100] Evidence has shown that RAB27B, a member of the RAB family of small GTPases, interacts with ZDHHC9, a palmitoyl transferase that modifies NRAS. RAB27B regulates the c-RAF/MEK/ERK signaling pathway through the modulation of palmitoylation, thereby influencing the progression of leukemia.[101] Other researchers have found that the antimalarial drug Artemether (ART) directly targets Protein Kinase C delta (PKCδ) to inhibit its palmitoylation by blocking the binding of Zinc Finger DHHC-Type Palmitoyl transferase 5(ZDHHC5), thereby suppressing downstream neuroinflammatory signaling. This mechanism provides a promising therapeutic target for fatty liver disease.[102]

Indeed, palmitoylation also plays a significant role in the cGAS-STING pathway. Researchers have discovered that palmitoylation at the C474 residue of cGAS limits its enzymatic activity when double-stranded DNA is present. This palmitoylation is mainly catalyzed by the enzyme ZDHHC18, and the presence of double-stranded DNA enhances this modification process. Specifically, palmitoylation of cGAS diminishes its binding affinity to double-stranded DNA, which subsequently hampers the dimerization of cGAS. The negative regulation of cGAS palmitoylation by ZDHHC18 may serve as a novel regulatory mechanism for fine-tuning innate immune responses.[103] Additional studies have revealed that 4-octyl itaconate (4-OI), a derivative of itaconate, can suppress the activation of the cGAS-STING pathway. This is achieved through direct alkylation of Cys91 on STING, which in turn inhibits STING palmitoylation and oligomerization. This mechanism sheds light on how 4-OI alkylation regulates cGAS-STING function and underscores the interplay among various post-translational modifications (PTMs).[104] Research has shown that STING palmitoylation within the Golgi apparatus is essential for STING activation. Administration of the palmitoylation inhibitor 2-bromopalmitate (2-BP) effectively inhibits STING palmitoylation and consequently eliminates the type I interferon response.[105] In recent years, researchers have discovered that the interaction between STING and VDAC2 (voltage-dependent anion channel 2) occurs via the palmitoylation of STING at Cys88/Cys91. Inhibition of STING palmitoyl transferase ZDHHC using 2-bromopalmitate (2-BP) significantly impedes the growth of renal cell carcinoma (RCC) cells, both as a single agent and in combination with sorafenib. These studies have unveiled the innate immune-independent role of STING in regulating mitochondrial function and growth in RCC, providing a theoretical basis for targeting the STING/VDAC2 interaction as a therapeutic strategy for RCC.[106] An increasing body of evidence indicates that palmitoylation within the cGAS-STING

pathway is one of the key steps for pathway activation, and inhibiting this palmitoylation can ameliorate the onset and progression of diseases. This finding provides a theoretical foundation for the prevention and treatment of various diseases.

Crosstalk of Various PTM

PTM crosstalk refers to the close association and frequent mutual dependence as well as tight coordination among different PTMs.[107] Most PTM systems are fundamentally composed of two parts: the catalytic mechanisms that perform the modifications and the substrate proteins that are modified. Interactions among various PTMs may happen through one or both of these elements. The term "PTM crosstalk" in the context of catalytic mechanisms denotes the reciprocal modification of enzymes that facilitate distinct PTMs, which in turn governs their enzymatic functions.[108] An additional mode of interaction involves PTMs that converge on a common set of substrate proteins. When various PTMs act on the same substrates, they can elicit two principal effects. One is the frequent modification of identical amino acid residues on the substrate proteins by diverse PTM types at separate temporal intervals. The other situation entails the modification of different amino acid residues within the same substrate by distinct post-translational modification processes.[109]

The correlation between glycosylation and phosphorylation has garnered attention in recent research. Investigations have revealed intriguing associations that the impact of glycol-AGEs on cGAMP-induced phosphorylation of TBK1 and IRF3, pivotal proteins regulating cytokine production. Glycol-AGEs notably curbed the effectiveness of STING/TBK1/IRF3 signaling triggered by cGAMP through CD36-mediated pathways.[110] In resting cells, death-associated protein kinase 3 (DAPK3) safeguards STING from K48-chain polyubiquitination and the ensuing proteasomal degradation. Studies have indicated that following cGAMP activation, DAPK3 is crucial for triggering STING's K63-chain polyubiquitination and the assembly of the STING-TBK1 complex. Comprehensive phosphoproteomic profiling has identified a specific phosphorylation site on the E3 ligase LMO7 that is a target of DAPK3, and this site is vital for the LMO7-STING association as well as for promoting STING's K63-chain polyubiquitination.[111] Further, certain investigators have found that the asymmetric dimethylation of cGAS at residue R127, catalyzed by protein arginine methyltransferase 1 (PRMT1), enhances the association with the deubiquitinase USP7. This interaction promotes the deubiquitination and stabilization of cGAS. The stability of cGAS, mediated by PRMT1 and USP7, is pivotal in accelerating the proliferation of non-small cell lung cancer (NSCLC) cells by activating the AKT pathway. The research underscores a unique pathway regulating cGAS stability through arginine methylation, implying that the PRMT1-cGAS-USP7 axis may represent a novel therapeutic target for NSCLC.[112]

Immunotherapy Through the cGAS-STING Pathway

A growing body of research on the cGAS-STING pathway has gradually revealed that regulating this pathway can influence the occurrence and development of various diseases.

Autoimmune Diseases

Researchers have found that rapamycin reduces lupus mononuclear cells by inhibiting mTOR STING the enhancement of expression, and suppresses the production of interferons alpha. It provides a new idea for the treatment of systemic lupus erythematosus.[113] Researchers have found that TNF-induced interferon is driven by cytosolic mitochondrial DNA (mtDNA), which depends on the cGAS-STING pathway.[114] Therefore, inhibiting the cGAS enzyme activity, which prevents the production of the second messenger molecule cGAMP, could be a novel strategy for treating inflammatory arthritis and other TNF-dependent diseases. Inhibitors of cGAMP activation of STING, including compounds such as PF-06928125, RU521, G150, and S3, may be potential therapies.[115]

Neurodegenerative Diseases

Some researchers have also found that cGAS-STING is elevated in AD mice and normalized by NR (the NAD^+ precursor nicotinamide riboside) treatment. Simultaneous cell culture experiments using microglia demonstrated that NR reduced neuroinflammation in microglia by reducing the activation of the cGAS-STING pathway.[116] Additionally, certain studies have revealed that melatonin can downregulate the cGAS/STING/IRF3 pathway in the striatum of 9-week-old R6/2 mice, a process

that involves the inhibition of caspase-1 activation and the prevention of mitochondrial DNA (mtDNA) release, consequently mitigating the symptoms of Huntington's disease in these mice.[117] Modulating these pathways may offer therapeutic options for aging and neurodegenerative diseases.

Viruses Infections

The research demonstrated that the binding of Vif (a nonstructural protein of HIV-1 that enhances viral infectivity) to SHP-1 (a cellular tyrosine phosphatase) promotes the targeting of SHP-1 to STING. This interaction results in the suppression of K63-linked ubiquitination of STING at lysine 337, which is mediated by the dephosphorylation of STING at tyrosine 162. However, the FRK inhibitor D-65495 effectively impedes the phosphorylation of Vif, thus thwarting the immune evasion tactics of HIV-1 and resistance to infection. These findings provide a molecular basis for the development of innovative therapeutic approaches to fight against HIV-1 infections.[118] The investigation uncovered that the deacetylation of β-arrestin2, a versatile signaling adaptor, at Lys171 enhances the activation of the cGAS-STING signaling pathway and promotes the secretion of IFN-β. In vitro, viral infection triggers the degradation of β-arrestin2, thereby facilitating the virus's immune evasion strategies. However, carvedilol, a β-blocker primarily prescribed for heart failure, was found to preserve β-arrestin2 expression, thereby sustaining the antiviral immune response. These findings suggest that carvedilol could be repurposed as a potential antiviral therapeutic candidate.[119]

Cancer

Research indicates that glucose serves as a cofactor for the methyltransferase NSUN2, interacting with its residues 1–28 to promote oligomerization and activation, crucial for maintaining global m5C RNA methylation. This includes the methylation of TREX2, which inhibits cytosolic dsDNA accumulation and cGAS/STING pathway activation, linked to cancer development and resistance to anti-PD-L1 therapy. The TAT-N28 peptide, by blocking glucose-NSUN2 binding, hampers NSUN2 function and TREX2 expression, inhibiting oncogenesis without disrupting normal glucose metabolism, offering a novel cancer therapy strategy.[120] Other researchers have found that both cGAS and STING in dendritic cells (DCs) also play important roles in the anti-tumor activity of live Lactobacillus rhamnosus GG (LGG) and its combination therapy.[121] Manganese (Mn) is essential for the host's defense mechanisms against cytosolic double-stranded DNA, for the innate immune system's detection of tumors, and for bolstering the adaptive immune response through the activation of the cGAS-STING pathway. Mn2+ ions facilitate the maturation of dendritic cells and macrophages, as well as the presentation of tumor-specific antigens, augment the differentiation of CD8+ T cells, stimulate natural killer (NK) cell activation, and boost the population of memory CD8+ T cells.[122] We summarize the therapeutic drugs and targets for selected diseases associated with the cGAS-STING pathway (Table 1).

In immunotherapy, the cGAS-STING pathway plays a pivotal role. Investigators developed an antibody-drug conjugate (ADC) by linking STING agonists to antibodies targeting tumor antigen EGFR. In mouse models, the STING ADC showed tolerability and strong anti-tumor effects. Combining it with anti-PD-L1 therapy further improved outcomes. The ADC selectively activates T cells, NK cells, and NKT cells in the tumor environment and promotes M2 to M1 macrophage polarization in tumors and draining lymph nodes for anti-tumor responses.[129] Accounts in scientific publications also illustrate

Table 1 Summary of Therapies for Diseases Associated With the cGAS-STING Pathway

Disease	Drug	Targeted	References
Psoriasis	C-176	STING	[123]
	Pt-CDs	STING	[124]
Ulcerative colitis	RU.521	The cyclic GMP-AMP synthase-stimulator	[125]
	Ganciclovir		[126]
Parkinson	C-176	STING	[127]
Lung cancer	Rocaglamide (RocA)	Mitochondrial DNA (mtDNA)	[128]

Notes: C-176: the STING inhibitor. Pt-CDs: Platinum-doped positively charged carbon dots. RU.521: An inhibitor of the cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway.

the synergistic approach of pairing STING agonists with anti-PD-1/PD-L1 immunotherapeutic regimens. The integration of the cGAMP/antigenic peptide-based nanosatellite vaccine SatVax with anti-PD-L1 treatment within xenograft experiments led to a surge in E7-specific CD8+ cytotoxic T cells (CTLs) and a reduction in the proportion of CD8+ Tim-3+ and CD8+ PD-1+ T cells. This effective combination therapy yielded substantial tumor suppression.[130] Additional studies indicate that imperfect homing and the transient nature of CAR (chimeric antigen receptor) T cells significantly impede their anti-tumor effects in an orthotopic model of advanced breast cancer. Conversely, the use of STING agonists like DMXAA or cGAMP markedly improves the migration and durability of Th/Tc 17 CAR T cells, thereby enhancing their therapeutic potential against solid tumors.[131] In the midst of the COVID-19 pandemic, investigators developed a pan-Sarbecovirus subgenus vaccine, which employed the RBD from the SARS-CoV-2 wild-type strain fused to human IgG Fc as the vaccine immunogen, with the STING agonist CF501 serving as the adjuvant. The CF501/RBD-Fc vaccine elicits a robust, long-lasting, and comprehensive neutralizing antibody (nAb) and T cell response, along with conferring protective immunity.[132] Concurrently, a separate group of researchers created an adjuvant featuring a stimulator of interferon genes (STING) agonist embedded within a microparticle (MP) matrix, enabling scalable production for long-lasting defense against influenza virus infections. The monomeric cGAMP Ace-DEX MP adjuvant elicited a sustained and strong immune response lasting up to a year, and in contrast to recent studies utilizing various particle formulations, it realized over a 10-fold reduction in the required cGAMP dosage.[133] Additionally, activators of the cGAS-STING pathway can also increase the expression of antigen-presenting molecules such as Tap 1, Tap 2, and MHC-I, which are upregulated by IFN, potentially enhancing tumor immune surveillance.[134]

Conclusions and Future Directions

The cGAS-STING pathway recognizes pathogen DNA and synthesizes the second messenger cGAMP, which subsequently activates downstream signaling pathways via the STING protein, thereby regulating the expression of interferons and inflammatory mediators. Post-translational modifications (PTMs) of the cGAS-STING pathway, such as phosphorylation, acetylation, ubiquitination, SUMOylation, methylation, glycosylation, and palmitoylation, finely tune the immune response by modulating protein activity, stability, localization, and interactions. Phosphorylation of serine and threonine residues on the STING protein facilitates pathway activation. STING protein on the endoplasmic reticulum undergoes N-glycosylation via DDOST, promoting its oligomerization. Moreover, the end products of glycosylation, advanced glycation end products (AGEs), can inhibit the phosphorylation of IRF3 by TBK1. Ubiquitination sites on the STING protein include K338, K347, K370, K63, K27, and K29, and ubiquitination at these sites is conducive to the activation of the cGAS-STING pathway. TRIM38 can inhibit the polyubiquitination of cGAS through SUMOylation. Acetylation sites on cGAS include Lys384, Lys394, and Lys414, and acetylation at these sites inhibits cGAS function. Methylation at the Arg133 site of cGAS inhibits its dimerization, while methylation at the Arg124 site inhibits cGAS binding to DNA. Palmitoylation of Cys88 and Cys91 on the STING protein promotes activation of the cGAS-STING pathway. In contrast, palmitoylation at the C747 site on cGAS inhibits its dimerization. 4-OI can inhibit palmitoylation of STING through alkylation (Figure 4). The cGAS-STING pathway plays a key role in the development and progression of various diseases, including autoimmune diseases, neurodegenerative diseases, viral infections, and cancer. Activation of this pathway can enhance immune responses, but over-activation may lead to chronic inflammation and autoimmune diseases. Therefore, modulating the activity of the cGAS-STING pathway is significant for the treatment of these diseases. Studies have shown that the activity of the cGAS-STING pathway can be effectively regulated by small molecule inhibitors, antibody therapies, and genetic regulation, providing new ideas for clinical treatment. By further investigating the roles of PTMs and their crosstalk within the cGAS-STING pathway, we can gain a comprehensive understanding of how these modifications regulate the activity, stability, intracellular localization, and interactions of key proteins in the pathway, thereby finely tuning the immune response. Researchers can further investigate this pathway in the following directions:

Clinical Applications of Targeted Therapies

Small Molecule Inhibitors and Activators

Develop more specific and efficient inhibitors and activators of the cGAS-STING pathway for the treatment of autoimmune diseases, viral infections, and cancer. For example, investigate how inhibiting cGAS enzyme activity can reduce inflammatory responses, or how activating STING can enhance antitumor immune responses.

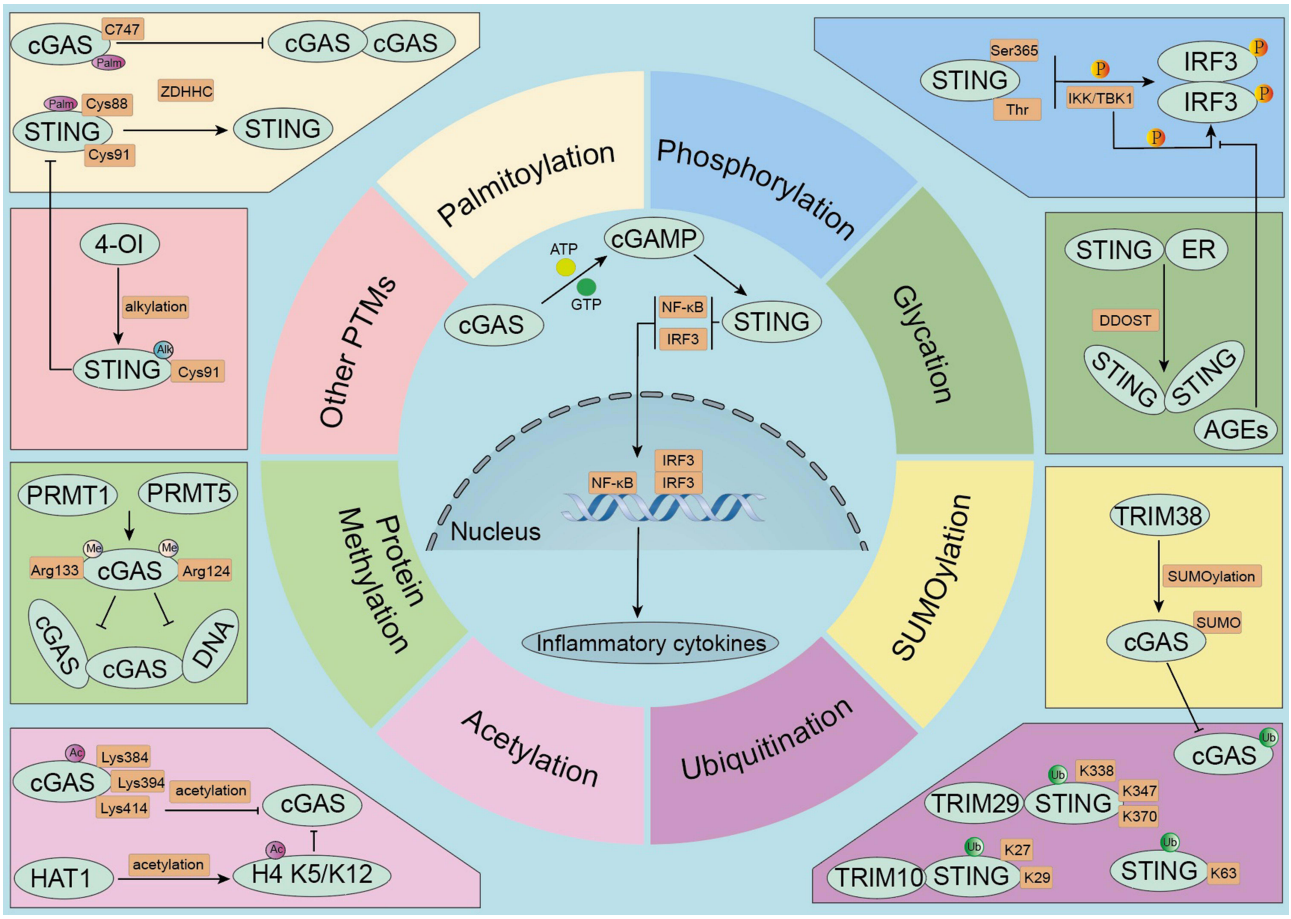

Figure 4 The roles of post-translational modifications in the cGAS-STING pathway, as well as the specific sites of these modifications.

Antibody Therapies

Develop monoclonal antibodies targeting key proteins in the cGAS-STING pathway for precision treatment. For example, develop STING agonist antibodies to enhance antitumor immune responses.

In-Depth Studies of Post-Translational Modifications

Regulatory Mechanisms of PTMs

Further investigate the specific mechanisms of PTMs in the cGAS-STING pathway, such as phosphorylation, acetylation, ubiquitination, methylation, and glycosylation, and how these modifications affect the activity of the pathway. For example, study how PRMT1-mediated methylation of cGAS affects its DNA-binding ability.

Combined Strategies for Immunotherapy

Combined Immunotherapy

Investigate the combined application of cGAS-STING pathway agonists with other immunotherapeutic strategies to improve treatment outcomes. For example, study the synergistic effects of combining STING agonists with PD-1 inhibitors in tumor treatment.

Vaccine Development

Develop vaccines based on the cGAS-STING pathway for the prevention and treatment of viral infections and cancer. For example, explore the potential of using cGAS-STING pathway agonists as vaccine adjuvants.

Cell-Specific Regulatory Mechanisms

Cell Type-Specificity

Investigate the specific regulatory mechanisms of the cGAS-STING pathway in different cell types, such as macrophages, dendritic cells, and T cells, and how these mechanisms affect immune responses. For example, study how activation of the cGAS-STING pathway in macrophages affects the secretion of inflammatory factors.

Tissue-Specificity

Investigate the specific regulatory mechanisms of the cGAS-STING pathway in different tissues, such as the liver, brain, and tumor tissues, and how these mechanisms affect disease development and progression. For example, study how activation of the cGAS-STING pathway in the tumor microenvironment affects tumor immune evasion.

Crosstalk With Other Signaling Pathways

Crosstalk With Other Pathways

Investigate the crosstalk mechanisms between the cGAS-STING pathway and other signaling pathways, such as PI3K-AKT, MAPK, and NF-κB, and how these mechanisms affect immune responses. For example, study how the crosstalk between the cGAS-STING pathway and the PI3K-AKT pathway affects cell survival and proliferation.

By delving into these research directions, it is expected that further insights into the complex regulatory mechanisms of the cGAS-STING pathway in immune responses will be gained, providing a theoretical basis for the development of new therapeutic strategies.

Funding

This research was supported by National Natural Science Foundation of China (Nos. 82202429, 82102938).

Disclosure

The authors declare that there are no conflicts of interest.

References

1. Lerner A, Jeremias P, Matthias T. The world incidence and prevalence of autoimmune diseases is increasing. *Int J Celiac Dis*. 2016;3(4):151–155. doi:10.12691/ijcd-3-4-8
2. Youinou P, Jamin C. The weight of interleukin-6 in B cell-related autoimmune disorders. *J Autoimmun*. 2009;32(3–4):206–210. doi:10.1016/j.jaut.2009.02.013
3. Ben-Ami E, Miller A, Berrih-Aknin S. T cells from autoimmune patients display reduced sensitivity to immunoregulation by mesenchymal stem cells: role of IL-2. *Autoimmun Rev*. 2014;13(2):187–196. doi:10.1016/j.autrev.2013.09.007
4. Gorman C, Leandro M, Isenberg D. B cell depletion in autoimmune disease. *Arthritis Res Ther*. 2003;5 Suppl 4(Suppl 4):S17–21. doi:10.1186/ar1007
5. Liubchenko GA, Appleberry HC, Striebich CC, et al. Rheumatoid arthritis is associated with signaling alterations in naturally occurring autoreactive B-lymphocytes. *J Autoimmun*. 2013;40:111–121. doi:10.1016/j.jaut.2012.09.001
6. Shu SA, Wang J, Tao MH, Leung PS. Gene therapy for autoimmune disease. *Clin Rev Allergy Immunol*. 2015;49(2):163–176. doi:10.1007/s12016-014-8451-x
7. Kang Y, Ren P, Kuang X, et al. Editorial: improve metabolic and autoimmune diseases by regulating gut microbiota. *Front Immunol*. 2022;13:1091805. doi:10.3389/fimmu.2022.1091805
8. Ablasser A, Chen ZJ. cGAS in action: expanding roles in immunity and inflammation. *Science*. 2019;363(6431). doi:10.1126/science.aat8657
9. Wu J, Sun L, Chen X, et al. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. *Science*. 2013;339(6121):826–830. doi:10.1126/science.1229963
10. Morehouse BR, Govande AA, Millman A, et al. STING cyclic dinucleotide sensing originated in bacteria. *Nature*. 2020;586(7829):429–433. doi:10.1038/s41586-020-2719-5
11. Decout A, Katz JD, Venkatraman S, Ablasser A. The cGAS-STING pathway as a therapeutic target in inflammatory diseases. *Nat Rev Immunol*. 2021;21(9):548–569. doi:10.1038/s41577-021-00524-z
12. Xiang W, Lv H, Xing F, et al. Inhibition of ACLY overcomes cancer immunotherapy resistance via polyunsaturated fatty acids peroxidation and cGAS-STING activation. *Sci Adv*. 2023;9(49):eadi2465. doi:10.1126/sciadv.adi2465
13. Huang A, Zhou W. Mn-based cGAS-STING activation for tumor therapy. *Chin J Cancer Res*. 2023;35(1):19–43. doi:10.21147/j.issn.1000-9604.2023.01.04
14. Gibbert K, Schlaak JF, Yang D, Dittmer U. IFN-α subtypes: distinct biological activities in anti-viral therapy. *Br J Pharmacol*. 2013;168(5):1048–1058. doi:10.1111/bph.12010
15. Ishikawa H, Barber GN. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature*. 2008;455(7213):674–678. doi:10.1038/nature07317

16. Sun L, Wu J, Du F, Chen X, Chen ZJ. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science*. 2013;339(6121):786–791. doi:10.1126/science.1232458
17. O'Dea E, Hoffmann A. The regulatory logic of the NF-kappaB signaling system. *Cold Spring Harb Perspect Biol*. 2010;2(1):a000216. doi:10.1101/cshperspect.a000216
18. Hong Z, Ma T, Liu X, Wang C. cGAS-STING pathway: post-translational modifications and functions in sterile inflammatory diseases. *FEBS J*. 2022;289(20):6187–6208. doi:10.1111/febs.16137
19. Sun W, Li Y, Chen L, et al. ERIS, an endoplasmic reticulum IFN stimulator, activates innate immune signaling through dimerization. *Proc Natl Acad Sci U S A*. 2009;106(21):8653–8658. doi:10.1073/pnas.0900850106
20. Zhang Q, Lenardo MJ, Baltimore D. 30 Years of NF-kappaB: a blossoming of relevance to human pathobiology. *Cell*. 2017;168(1–2):37–57. doi:10.1016/j.cell.2016.12.012
21. Yu Y, Liu J, Liu C, et al. Post-translational modifications of cGAS-STING: a critical switch for immune regulation. *Cells*. 2022;11. doi:10.3390/cells11193043
22. Kuchta K, Knizewski L, Wyrwicz LS, Rychlewski L, Ginalski K. Comprehensive classification of nucleotidyltransferase fold proteins: identification of novel families and their representatives in human. *Nucleic Acids Res*. 2009;37(22):7701–7714. doi:10.1093/nar/gkp854
23. Kwon J, Bakhoum SF. The cytosolic DNA-Sensing cGAS-STING pathway in cancer. *Cancer Discov*. 2020;10(1):26–39. doi:10.1158/2159-8290.CD-19-0761
24. Zhang X, Shi H, Wu J, et al. Cyclic GMP-AMP containing mixed phosphodiester linkages is an endogenous high-affinity ligand for STING. *Mol Cell*. 2013;51(2):226–235. doi:10.1016/j.molcel.2013.05.022
25. Huang YH, Liu XY, Du XX, Jiang ZF, Su XD. The structural basis for the sensing and binding of cyclic di-GMP by STING. *Nat Struct mol Biol*. 2012;19(7):728–730. doi:10.1038/nsmb.2333
26. Gao P, Ascano M, Zillinger T, et al. Structure-function analysis of STING activation by c[G(2',5')pA(3',5')p] and targeting by antiviral DMXAA. *Cell*. 2013;154(4):748–762. doi:10.1016/j.cell.2013.07.023
27. Haag SM, Gulen MF, Reymond L, et al. Targeting STING with covalent small-molecule inhibitors. *Nature*. 2018;559(7713):269–273. doi:10.1038/s41586-018-0287-8
28. Shang G, Zhang C, Chen ZJ, Bai XC, Zhang X. Cryo-EM structures of STING reveal its mechanism of activation by cyclic GMP-AMP. *Nature*. 2019;567(7748):389–393. doi:10.1038/s41586-019-0998-5
29. Sen R, Baltimore D. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell*. 1986;46(5):705–716. doi:10.1016/0092-8674(86)90346-6
30. Yu H, Lin L, Zhang Z, Zhang H, Hu H. Targeting NF-kappaB pathway for the therapy of diseases: mechanism and clinical study. *Signal Transduct Target Ther*. 2020;5(1):209. doi:10.1038/s41392-020-00312-6
31. Iwai K. Diverse ubiquitin signaling in NF-kappaB activation. *Trends Cell Biol*. 2012;22(7):355–364. doi:10.1016/j.tcb.2012.04.001
32. Bandapalli OR, Brücher BLDM, Lang F, Jamall IS. NF-κB signaling and crosstalk during carcinogenesis. *4open*. 2019;2. doi:10.1051/fopen/2019010
33. Courtois G, Fauvarque MO. The many roles of ubiquitin in NF-kappaB signaling. *Biomedicines*. 2018;6(2). doi:10.3390/biomedicines6020043
34. Guo Q, Jin Y, Chen X, et al. NF-κB in biology and targeted therapy: new insights and translational implications. *Signal Transduction and Targeted Therapy*. 2024;9. doi:10.1038/s41392-024-01757-9
35. Sun S-C. Non-canonical NF-κB signaling pathway. *Cell Res*. 2010;21(1):71–85. doi:10.1038/cr.2010.177
36. Sun S-C. The non-canonical NF-κB pathway in immunity and inflammation. *Nat Rev Immunol*. 2017;17(9):545–558. doi:10.1038/nri.2017.52
37. Al Hamrashdi M, Brady G. Regulation of IRF3 activation in human antiviral signaling pathways. *Biochem Pharmacol*. 2022;200:115026. doi:10.1016/j.bcp.2022.115026
38. Chiang HS, Liu HM. The molecular basis of viral inhibition of IRF- and STAT-Dependent immune responses. *Front Immunol*. 2018;9:3086. doi:10.3389/fimmu.2018.03086
39. Lin R, Heylbroeck C, Pitha PM, Hiscott J. Virus-dependent phosphorylation of the IRF-3 transcription factor regulates nuclear translocation, transactivation potential, and proteasome-mediated degradation. *mol Cell Biol*. 1998;18(5):2986–2996. doi:10.1128/MCB.18.5.2986
40. Ivanov P, Anderson P. Post-transcriptional regulatory networks in immunity. *Immunol Rev*. 2013;253(1):253–272. doi:10.1111/imr.12051
41. Corbett AH. Post-transcriptional regulation of gene expression and human disease. *Curr Opin Cell Biol*. 2018;52:96–104. doi:10.1016/j.ceb.2018.02.011
42. Poganik JR, Long MJC, Disare MT, et al. Post-transcriptional regulation of Nrf2-mRNA by the mRNA-binding proteins HuR and AUF1. *FASEB J*. 2019;33(12):14636–14652. doi:10.1096/fj.201901930R
43. Li C, Feng L, Luo -W-W, et al. The RNA-binding protein LUC7L2 mediates MITA/STING intron retention to negatively regulate innate antiviral response. *Cell Discov*. 2021;7(1):46. doi:10.1038/s41421-021-00277-y
44. Wang L, Wen M & Cao X. Nuclear hnRNPA2B1 initiates and amplifies the innate immune response to DNA viruses. *Science*. 2019;365(6454). doi:10.1126/science.aav0758.
45. International Human Genome Sequencing, C. Finishing the euchromatic sequence of the human genome. *Nature*. 2004;431(7011):931–945. doi:10.1038/nature03001
46. Jensen ON. Modification-specific proteomics: characterization of post-translational modifications by mass spectrometry. *Curr Opin Chem Biol*. 2004;8(1):33–41. doi:10.1016/j.cbpa.2003.12.009
47. Duan G, Walther D, Radivojac P. The roles of post-translational modifications in the context of protein interaction networks. *PLoS Comput Biol*. 2015;11(2):e1004049. doi:10.1371/journal.pcbi.1004049
48. Chen L, Liu S, Tao Y. Regulating tumor suppressor genes: post-translational modifications. *Signal Transduct Target Ther*. 2020;5(1):90. doi:10.1038/s41392-020-0196-9
49. Audagnotto M, Dal Peraro M. Protein post-translational modifications: in silico prediction tools and molecular modeling. *Comput Struct Biotechnol J*. 2017;15:307–319. doi:10.1016/j.csbj.2017.03.004
50. Biard-Piechaczyk M, Borel S, Espert L, de Bettignies G, Coux O. HIV-1, ubiquitin and ubiquitin-like proteins: the dialectic interactions of a virus with a sophisticated network of post-translational modifications. *Biol Cell*. 2012;104(3):165–187. doi:10.1111/boc.201100112

51. Sacco F, Perfetto L, Castagnoli L, Cesareni G. The human phosphatase interactome: an intricate family portrait. *FEBS Lett*. 2012;586 (17):2732–2739. doi:10.1016/j.febslet.2012.05.008
52. Czuba LC, Hillgren KM, Swaan PW. Post-translational modifications of transporters. *Pharmacol Ther*. 2018;192:88–99. doi:10.1016/j.pharmthera.2018.06.013
53. Bilbrough T, Piemontese E, Seitz O. Dissecting the role of protein phosphorylation: a chemical biology toolbox. *Chem Soc Rev*. 2022;51 (13):5691–5730. doi:10.1039/d1cs00991e
54. Ardito F, Giuliani M, Perrone D, Troiano G, Lo Muzio L. The crucial role of protein phosphorylation in cell signaling and its use as targeted therapy (Review). *Int J Mol Med*. 2017;40(2):271–280. doi:10.3892/ijmm.2017.3036
55. Swaney DL, Beltrao P, Starita L, et al. Global analysis of phosphorylation and ubiquitylation cross-talk in protein degradation. *Nat Methods*. 2013;10(7):676–682. doi:10.1038/nmeth.2519
56. Tonks NK. Protein tyrosine phosphatases: from genes, to function, to disease. *Nat Rev mol Cell Biol*. 2006;7(11):833–846. doi:10.1038/nrm2039
57. Tanaka Y, Chen ZJ. STING specifies IRF3 phosphorylation by TBK1 in the cytosolic DNA signaling pathway. *Sci Signal*. 2012;5(214):ra20. doi:10.1126/scisignal.2002521
58. Christian F, Smith EL, Carmody RJ. The regulation of NF-kappaB subunits by phosphorylation. *Cells*. 2016;5(1):12. doi:10.3390/cells5010012
59. Zhang C, Shang G, Gui X, et al. Structural basis of STING binding with and phosphorylation by TBK1. *Nature*. 2019;567(7748):394–398. doi:10.1038/s41586-019-1000-2
60. Wang C, Wang X, Veleeparambil M, et al. EGFR -mediated tyrosine phosphorylation of STING determines its trafficking route and cellular innate immunity functions. *EMBO J*. 2020;39(22):e104106. doi:10.15252/embj.2019104106
61. Takahashi K, Niki T, Ogawa E, et al. A cell-free assay implicates a role of sphingomyelin and cholesterol in STING phosphorylation. *Sci Rep*. 2021;11(1):11996. doi:10.1038/s41598-021-91562-z
62. Yu Y, Wang M, Li X-W, et al. Meloxicam inhibits STING phosphorylation and alleviates intracellular DNA-mediated autoimmune responses. *Cell Biosci*. 2023;13(1):76. doi:10.1186/s13578-023-01025-3
63. Eichler J. Protein glycosylation. *Curr Biol*. 2019;29(7):R229–R231. doi:10.1016/j.cub.2019.01.003
64. Fournet M, Bonte F, Desmouliere A. Glycation damage: a possible hub for major pathophysiological disorders and aging. *Aging Dis*. 2018;9 (5):880–900. doi:10.14336/AD.2017.1121
65. Schjoldager KT, Narimatsu Y, Joshi HJ, Clausen H. Global view of human protein glycosylation pathways and functions. *Nat Rev mol Cell Biol*. 2020;21(12):729–749. doi:10.1038/s41580-020-00294-x
66. Gudelj I, Lauc G, Pezer M. Immunoglobulin G glycosylation in aging and diseases. *Cell Immunol*. 2018;333:65–79. doi:10.1016/j.cellimm.2018.07.009
67. Tu Y, Yin X-J, Liu Q, et al. MITA oligomerization upon viral infection is dependent on its N-glycosylation mediated by DDOST. *PLoS Pathog*. 2022;18(11):e1010989. doi:10.1371/journal.ppat.1010989
68. Fang R, Jiang Q, Guan Y, et al. Golgi apparatus-synthesized sulfated glycosaminoglycans mediate polymerization and activation of the cGAMP sensor STING. *Immunity*. 2021;54(5):962–975e968. doi:10.1016/j.immuni.2021.03.011
69. Sanchez-Sanchez J, Arevalo JC. A review on ubiquitination of neurotrophin receptors: facts and perspectives. *Int J mol Sci*. 2017;18(3):630. doi:10.3390/ijms18030630
70. Ortiz de Solorzano C, García RODRIGUEZ E, JONES A, et al. Segmentation of confocal microscope images of cell nuclei in thick tissue sections. *J Microsc*. 1999;193(3):212–226. doi:10.1046/j.1365-2818.1999.00463.x
71. Ni G, Konno H, Barber GN. Ubiquitination of STING at lysine 224 controls IRF3 activation. *Sci Immunol*. 2017;2(11). doi:10.1126/sciimmunol.aah7119
72. Wu X, Wang Z, Qiao D, et al. Porcine circovirus type 2 infection attenuates the K63-linked ubiquitination of STING to inhibit IFN-beta induction via p38-MAPK pathway. *Vet Microbiol*. 2021;258:109098. doi:10.1016/j.vetmic.2021.109098
73. Shmuel-Galia L, Humphries F, Lei X, et al. Dysbiosis exacerbates colitis by promoting ubiquitination and accumulation of the innate immune adaptor STING in myeloid cells. *Immunity*. 2021;54(6):1137–1153e1138. doi:10.1016/j.immuni.2021.05.008
74. Kong L, Sui C, Chen T, et al. The ubiquitin E3 ligase TRIM10 promotes STING aggregation and activation in the Golgi apparatus. *Cell Rep*. 2023;42(4):112306. doi:10.1016/j.celrep.2023.112306
75. Tao Y, Yin S, Liu Y, et al. UFL1 promotes antiviral immune response by maintaining STING stability independent of UFMylation. *Cell Death Differ*. 2023;30(1):16–26. doi:10.1038/s41418-022-01041-9
76. Balka KR, Venkatraman R, Saunders TL, et al. Termination of STING responses is mediated via ESCRT -dependent degradation. *EMBO J*. 2023;42(12):e112712. doi:10.15252/embj.2022112712
77. Fan Y, Li X, Zhang L, et al. SUMOylation in viral replication and antiviral defense. *Adv Sci*. 2022;9(7). doi:10.1002/advs.202104126
78. Ren J, Wang S, Zong Z, et al. TRIM28-mediated nucleocapsid protein SUMOylation enhances SARS-CoV-2 virulence. *Nat Commun*. 2024;15. doi:10.1038/s41467-023-44502-6
79. Hu -M-M, Yang Q, Xie X-Q, et al. Sumoylation promotes the stability of the DNA Sensor cGAS and the Adaptor STING to regulate the kinetics of response to DNA Virus. *Immunity*. 2016;45(3):555–569. doi:10.1016/j.immuni.2016.08.014
80. Drazic A, Myklebust LM, Ree R, Arnesen T. The world of protein acetylation. *Biochim Biophys Acta*. 2016;1864(10):1372–1401. doi:10.1016/j.bbapap.2016.06.007
81. Aksnes H, Hole K, Arnesen T. Molecular, cellular, and physiological significance of N-terminal acetylation. *Int Rev Cell mol Biol*. 2015;316:267–305. doi:10.1016/bs.ircmb.2015.01.001
82. Dai J, Huang Y-J, He X, et al. Acetylation blocks cGAS activity and inhibits Self-DNA-induced autoimmunity. *Cell*. 2019;176(6):1447–1460e1414. doi:10.1016/j.cell.2019.01.016
83. Zhu T, Chen X, Qiu H, et al. Aspirin alleviates particulate matter induced asymptomatic orchitis of mice via suppression of cGAS-STING signaling. *Front Immunol*. 2021;12:734546. doi:10.3389/fimmu.2021.734546
84. Chen G, Yan Q, Liu L, Wen X, Zeng H, Yin S. Histone deacetylase 3 governs beta-Estradiol-ERalpha-Involved endometrial tumorigenesis via inhibition of STING transcription. *Cancers*. 2022;14. doi:10.3390/cancers14194718

85. Liao Y, Cheng J, Kong X, et al. HDAC3 inhibition ameliorates ischemia/reperfusion-induced brain injury by regulating the microglial cGAS-STING pathway. *Theranostics*. 2020;10(21):9644–9662. doi:10.7150/thno.47651
86. Zhao L, Yuan H, Wang Y, et al. HBV confers innate immune evasion through triggering HAT1/acetylation of H4K5/H4K12/miR-181a-5p or KPNA2/cGAS-STING/IFN-I signaling. *J Med Virol*. 2023;95(7):e28966. doi:10.1002/jmv.28966
87. Malecki JM, Davydova E, Falnes PØ. Protein methylation in mitochondria. *J Biol Chem*. 2022;298(4):101791. doi:10.1016/j.jbc.2022.101791
88. Dai X, Ren T, Zhang Y, Nan N. Methylation multiplicity and its clinical values in cancer. *Expert Rev Mol Med*. 2021;23:e2. doi:10.1017/erm.2021.4
89. Huseby CJ, Hoffman CN, Cooper GL, et al. Quantification of tau protein lysine methylation in aging and alzheimer's disease. *J Alzheimers Dis*. 2019;71(3):979–991. doi:10.3233/JAD-190604
90. Le DD, Fujimori DG. Protein and nucleic acid methylating enzymes: mechanisms and regulation. *Curr Opin Chem Biol*. 2012;16(5–6):507–-515. doi:10.1016/j.cbpa.2012.09.014
91. Oleksiuk O, Jakovljevic V, Vladimirov N, et al. Thermal robustness of signaling in bacterial chemotaxis. *Cell*. 2011;145(2):312–321. doi:10.1016/j.cell.2011.03.013
92. Tooley CE, Petkowski JJ, Muratore-Schroeder TL, et al. NRMT is an alpha-N-methyltransferase that methylates RCC1 and retinoblastoma protein. *Nature*. 2010;466(7310):1125–1128. doi:10.1038/nature09343
93. Zhang N, Ding C, Zuo Y, Peng Y, Zuo L. N6-methyladenosine and Neurological Diseases. *mol Neurobiol*. 2022;59(3):1925–1937. doi:10.1007/s12035-022-02739-0
94. Liu J, Bu X, Chu C, et al. PRMT1 mediated methylation of cGAS suppresses anti-tumor immunity. *Nat Commun*. 2023;14(1):2806. doi:10.1038/s41467-023-38443-3
95. Ma D, Yang M, Wang Q, et al. Arginine methyltransferase PRMT5 negatively regulates cGAS-mediated antiviral immune response. *Sci Adv*. 2021;7(13). doi:10.1126/sciadv.abc1834
96. Kim H, Kim H, Feng Y, et al. PRMT5 control of cGAS/STING and NLRC5 pathways defines melanoma response to antitumor immunity. *Sci Transl Med*. 2020;12(551). doi:10.1126/scitranslmed.aaz5683
97. Dong H, He X, Zhang L, et al. Targeting PRMT9-mediated arginine methylation suppresses cancer stem cell maintenance and elicits cGAS-mediated anticancer immunity. *Nat Cancer*. 2024;5(4):601–624. doi:10.1038/s43018-024-00736-x
98. Linder ME, Deschenes RJ. Palmitoylation: policing protein stability and traffic. *Nat Rev mol Cell Biol*. 2007;8(1):74–84. doi:10.1038/nrm2084
99. Zhou B, Hao Q, Liang Y, Kong E. Protein palmitoylation in cancer: molecular functions and therapeutic potential. *Mol oncol*. 2022;17(1):3–26. doi:10.1002/1878-0261.13308
100. Wang L, Cui J. Palmitoylation promotes chaperone-mediated autophagic degradation of NLRP3 to modulate inflammation. *Autophagy*. 2023;19(10):2821–2823. doi:10.1080/15548627.2023.2187957
101. Ren J-G, Xing B, Lv K, et al. RAB27B controls palmitoylation-dependent NRAS trafficking and signaling in myeloid leukemia. *J Clin Investig*. 2023;133(12). doi:10.1172/jci165510
102. Wang Y-H, Chen X, Bai Y-Z, et al. Palmitoylation of PKCδ by ZDHHC5 in hypothalamic microglia presents as a therapeutic target for fatty liver disease. *Theranostics*. 2024;14(3):988–1009. doi:10.7150/thno.89602
103. Shi C, Yang X, Liu Y, et al. ZDHHC18 negatively regulates cGAS-mediated innate immunity through palmitoylation. *EMBO J*. 2022;41(11). doi:10.15252/embj.2021109272
104. Su C, Cheng T, Huang J, Zhang T, Yin H. 4-Octyl itaconate restricts STING activation by blocking its palmitoylation. *Cell Rep*. 2023;42(9):113040. doi:10.1016/j.celrep.2023.113040
105. Mukai K, Konno H, Akiba T, et al. Activation of STING requires palmitoylation at the Golgi. *Nat Commun*. 2016;7(1). doi:10.1038/ncomms11932
106. Zhu Z, Zhou X, Du H, et al. STING suppresses mitochondrial VDAC2 to Govern RCC growth independent of innate immunity. *Adv Sci*. 2022;10. doi:10.1002/advs.202203718
107. Venne AS, Kollipara L, Zahedi RP. The next level of complexity: crosstalk of posttranslational modifications. *Proteomics*. 2014;14(4–5):513–-524. doi:10.1002/pmic.201300344
108. Zhang Y, Zeng L. Crosstalk between ubiquitination and other post-translational protein modifications in plant immunity. *Plant Commun*. 2020;1(4):100041. doi:10.1016/j.xplc.2020.100041
109. Csizmok V, Forman-Kay JD. Complex regulatory mechanisms mediated by the interplay of multiple post-translational modifications. *Curr Opin Struct Biol*. 2018;48:58–67. doi:10.1016/j.sbi.2017.10.013
110. Nishinaka T, Hatipoglu OF, Wake H, et al. Glycolaldehyde-derived advanced glycation end products suppress STING/TBK1/IRF3 signaling via CD36. *Life Sci*. 2022;310:121116. doi:10.1016/j.lfs.2022.121116
111. Takahashi M, Lio C-WJ, Campeau A, et al. The tumor suppressor kinase DAPK3 drives tumor-intrinsic immunity through the STING-IFN-beta pathway. *Nat Immunol*. 2021;22(4):485–496. doi:10.1038/s41590-021-00896-3
112. Liu X, Zheng W, Zhang L, et al. Arginine methylation-dependent cGAS stability promotes non-small cell lung cancer cell proliferation. *Cancer Lett*. 2024;586:216707. doi:10.1016/j.canlet.2024.216707
113. Murayama G, Chiba A, Kuga T, et al. Inhibition of mTOR suppresses IFNalpha production and the STING pathway in monocytes from systemic lupus erythematosus patients. *Rheumatology*. 2020;59(10):2992–3002. doi:10.1093/rheumatology/keaa060
114. Willemsen J, Neuhoff M-T, Hoyler T, et al. TNF leads to mtDNA release and cGAS/STING-dependent interferon responses that support inflammatory arthritis. *Cell Rep*. 2021;37(6):109977. doi:10.1016/j.celrep.2021.109977
115. Yang X, Zhao L, Pang Y. cGAS-STING pathway in pathogenesis and treatment of osteoarthritis and rheumatoid arthritis. *Front Immunol*. 2024;15:1384372. doi:10.3389/fimmu.2024.1384372
116. Hou Y, Wei Y, Lautrup S, et al. NAD + supplementation reduces neuroinflammation and cell senescence in a transgenic mouse model of Alzheimer's disease via cGAS–STING. *Proc Natl Acad Sci U S A*. 2021;118(37). doi:10.1073/pnas.2011226118
117. Jauhari A, Baranov SV, Suofu Y, et al. Melatonin inhibits cytosolic mitochondrial DNA-induced neuroinflammatory signaling in accelerated aging and neurodegeneration. *J Clin Invest*. 2020;130(6):3124–3136. doi:10.1172/JCI135026
118. Wang Y, Qian G, Zhu L, et al. HIV-1 Vif suppresses antiviral immunity by targeting STING. *Cell mol Immunol*. 2022;19(1):108–121. doi:10.1038/s41423-021-00802-9

119. Zhang Y, Li M, Li L, et al. beta-arrestin 2 as an activator of cGAS-STING signaling and target of viral immune evasion. *Nat Commun*. 2020;11(1):6000. doi:10.1038/s41467-020-19849-9
120. Chen T, Xu Z-G, Luo J, et al. NSUN2 is a glucose sensor suppressing cGAS/STING to maintain tumorigenesis and immunotherapy resistance. *Cell Metab*. 2023;35(10):1782–1798e1788. doi:10.1016/j.cmet.2023.07.009
121. Si W, Liang H, Bugno J, et al. Lactobacillus rhamnosus GG induces cGAS/STING- dependent type I interferon and improves response to immune checkpoint blockade. *Gut*. 2022;71(3):521–533. doi:10.1136/gutjnl-2020-323426
122. Lv M, Chen M, Zhang R, et al. Manganese is critical for antitumor immune responses via cGAS-STING and improves the efficacy of clinical immunotherapy. *Cell Res*. 2020;30(11):966–979. doi:10.1038/s41422-020-00395-4
123. Sun X, Liu L, Wang J, et al. Targeting STING in dendritic cells alleviates psoriatic inflammation by suppressing IL-17A production. *Cell mol Immunol*. 2024;21(7):738–751. doi:10.1038/s41423-024-01160-y
124. Zhang Z, Zhou D, Li Z, et al. A nanoinhibitor targeting cGAS-STING pathway to reverse the homeostatic imbalance of inflammation in psoriasis. *Angew Chem Int Ed Engl*. 2024;63(2):e202316007. doi:10.1002/anie.202316007
125. Uthaman S, Parvinroo S, Mathew AP, et al. Inhibiting the cGAS-STING pathway in ulcerative colitis with programmable micelles. *ACS Nano*. 2024;18(19):12117–12133. doi:10.1021/acsnano.3c11257
126. Gong L-K, Yang X, Yang J, et al. Low-dose ganciclovir ameliorates dextran sulfate sodium-induced ulcerative colitis through inhibiting macrophage STING activation in mice. *Front Pharmacol*. 2022;13:1020670. doi:10.3389/fphar.2022.1020670
127. Wang B, Wang Y, Qiu J, et al. The STING inhibitor C-176 attenuates MPTP-induced neuroinflammation and neurodegeneration in mouse parkinsonian models. *Int Immunopharmacol*. 2023;124:110827. doi:10.1016/j.intimp.2023.110827
128. Yan X, Yao C, Fang C, et al. Rocaglamide promotes the infiltration and antitumor immunity of NK cells by activating cGAS-STING signaling in non-small cell lung cancer. *Int J Biol Sci*. 2022;18(2):585–598. doi:10.7150/ijbs.65019
129. Wu YT, Fang Y, Wei Q, et al. Tumor-targeted delivery of a STING agonist improves cancer immunotherapy. *Proc Natl Acad Sci U S A*. 2022;119(49):e2214278119. doi:10.1073/pnas.2214278119
130. Jiang M, Chen P, Wang L, et al. cGAS-STING, an important pathway in cancer immunotherapy. *J Hematol Oncol*. 2020;13(1):81. doi:10.1186/s13045-020-00916-z
131. Xu N, Palmer DC, Robeson AC, et al. STING agonist promotes CAR T cell trafficking and persistence in breast cancer. *J Exp Med*. 2021;218(2). doi:10.1084/jem.20200844
132. Liu Z, Zhou J, Xu W, et al. A novel STING agonist-adjuvanted pan-sarbecovirus vaccine elicits potent and durable neutralizing antibody and T cell responses in mice, rabbits and NHPs. *Cell Res*. 2022;32(3):269–287. doi:10.1038/s41422-022-00612-2
133. Gallovic MD, Junkins RD, Sandor AM, et al. STING agonist-containing microparticles improve seasonal influenza vaccine efficacy and durability in ferrets over standard adjuvant. *J Control Release*. 2022;347:356–368. doi:10.1016/j.jconrel.2022.05.017
134. Grabosch S, Bulatovic M, Zeng F, et al. Cisplatin-induced immune modulation in ovarian cancer mouse models with distinct inflammation profiles. *Oncogene*. 2019;38(13):2380–2393. doi:10.1038/s41388-018-0581-9