The role of cGAS-STING signalling in liver diseases

Ruihan Chen,1 Jiamin Du,2 Hong Zhu,* Qi Ling1,*

Summary
The recently identified novel cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS) activates the downstream adaptor protein stimulator of interferon genes (STING) by catalysing the synthesis of cyclic GMP-AMP. This in turn initiates an innate immune response through the release of various cytokines, including type I interferon. Foreign DNA (microbial infection) or endogenous DNA (nuclear or mitochondrial leakage) can serve as cGAS ligands and lead to the activation of cGAS-STING signalling. Therefore, the cGAS-STING pathway plays essential roles in infectious diseases, sterile inflammation, tumours, and autoimmune diseases. In addition, cGAS-STING signalling affects the progression of liver inflammation through other mechanisms, such as autophagy and metabolism. In this review, we summarise recent advances in our understanding of the role of cGAS-STING signalling in the innate immune modulation of different liver diseases. Furthermore, we discuss the therapeutic potential of targeting the cGAS-STING pathway in the treatment of liver diseases.

Keywords: cGAS-STING signalling; innate immune response; viral hepatitis; nonalcoholic fatty liver disease; liver injury; hepatocellular carcinoma

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Introduction
Pattern recognition receptors (PPRs) are expressed in cell membranes, organelle membranes and even serum and are widespread in mammals.1 PPRs can detect intra- and extracellular pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) and in turn activate the immune response in the host.2 Dissociative nucleic acid molecules have been described as “danger signals” that engage in the initiation of innate immune defence.3 Hence, recognition of pathogen-derived or endogenous nucleic acids plays a crucial role in the innate immune response, which is the front-line defence against either microbial pathogens (DNA viruses, retroviruses, bacteria, parasites, etc.)3 or abnormal autologous cells (senescent cells, damaged cells, aberrant cells, etc.)3 in mammals. After release from microbial pathogens or abnormal cells, single-stranded RNAs or DNAs (ssRNAs or ssDNAs), double-stranded RNAs or DNAs (dsRNAs or dsDNAs), RNA-DNA hybrids, and cyclic dinucleotides (CDNs) can be sensed by nucleic acid sensors.5-7 Subsequently, a series of cascading reactions are triggered.

Several DNA sensors have been discovered that mediate the DNA-stimulated innate immune response, such as Toll-like receptor 9 (TLR9), absent in melanoma 2 (AIM2) and cyclic GMP-AMP (cGAMP) synthase (cGAS).5-7 The most well-known PPRs, the Toll-like receptor (TLR) family, are expressed on various innate immune cells.5,8 One of the best-studied DNA sensors, TLR9 is anchored in the endosomal membrane and has high sensitivity for the hypomethylated cytidine-phosphate guanosine region on bacterial or viral DNA,9 thus generating the corresponding innate immune response. AIM2 was identified as a cytoplasmic DNA receptor in 2009.6 After sensing dsDNA, AIM2 links to the adaptor molecule apoptosis-associated speck-like protein, which contains a caspase activation and recruitment domain within its pyrin domain.6 Then, AIM2 forms an inflammasome/pyroptosome to activate both NF-κB and caspase-1.6,10 In 2013, Chen et al.7 first discovered cGAS, which belongs to the nucleotidyltransferase superfamily. The second messenger cGAMP, whose production is catalysed by cGAS, binds to STING, thereby triggering the IFN-I pathway. The identification of cGAS solved the mystery of which molecules upstream of STING compose the STING-dependent IFN-I pathway.

Recognition of cGAS-STING signalling has grown rapidly over the last decade, with many studies demonstrating that cGAS-STING signalling is involved in various diseases including inflammation, infection, autoimmune diseases, metabolic disorders, and tumours.11-13 Recent studies in the liver have shown that cGAS-STING signalling is involved in diverse functions in viral hepatitis, non-alcoholic fatty liver disease (NAFLD), liver injury and hepatocellular carcinoma (HCC).14-16

In this review, we summarise the role of cGAS-STING signalling in multiple liver diseases, including both infectious and non-infectious diseases, and discuss the potential therapeutic implications of cGAS-STING signalling in liver diseases, especially in viral hepatitis and HCC. Furthermore, we emphasise the importance of cGAS-STING...
signalling in the innate immune response of the liver, and finally, we discuss the outlook of future research on cGAS-STING signalling and its significance for clinical translation in liver diseases.

**Molecular mechanism of cGAS-STING signalling**

The structure and signalling mechanisms of cGAS-STING have been extensively reviewed in recent years. Herein, we briefly introduce the molecular mechanism of cGAS-STING signalling in Fig. 1. cGAS structurally contains 3 dsDNA-binding sites and tends to recognise canonical B-form DNA in a completely sequence-independent manner. Upon recognition of dsDNA, cGAS molecules cross-link with each other to develop dimers or multimers, leading to the activation of cGAS. cGAS not only recognises exogenous DNA originating from pathogens but also acutely senses self-DNA from a variety of different subcellular localisations, such as cytoplasmic chromatin, micronuclei and mitochondria. A recent study found that cGAS can even bind CDNs. cGAS senses diverse DNA molecules, thereby providing a potent molecular foundation for the abundant function of cGAS in the innate immune system.

Using ATP and GTP as substrates, cGAS catalyses the formation of linear 2′-5′-linked dinucleotides and subsequent 3′-5′ phosphodiester linkage via cGAS-dependent cyclisation. Upon 2′3′ cGAMP binding, STING, an endoplasmic reticulum (ER)-residing adaptor, undergoes conformational changes and forms STING oligomers. In addition to 2′3′ cGAMP, STING recognises bacterial CDNs. However, 2′3′ cGAMP, which binds STING with its structurally unique 2′5′ linkages, has greater affinity than other stimuli comprising conventional 3′5′ linkages, such as bacterial CDNs or 3′-5′cGAMP. The STING oligomer is trafficked to the Golgi via the ER–Golgi intermediate compartment. Oligomerisation of STING upon ligand binding drives the transphosphorylation of TANK-binding kinase 1 (TBK1) in the signalling domain. pTBK1 subsequently phosphorylates STING and interferon regulatory factor 3 (IRF3, IRF3 dimers and is translocated to the nucleus where it initiates transcription of IFN-β, which phosphorylates IκB kinase (IKK), which phosphorylates IκB and induces translocation of NF-κB to the nucleus, where it transcribes a plethora of cytokines such as interferon (IFN)-β, interleukin (IL)-6 and tumour necrosis factor (TNF). The promoter region of cGAS contains 2 adjacent IFN-sensitive response elements; as a consequence, IFN-1 can enhance the expression of cGAS through positive feedback.

**Key points**

- Recognition of aberrant DNA by cGAS leads to activation of cGAS-STING signalling, which triggers the innate immune response.
- cGAS–STING signalling is involved in the regulation of the innate immune responses in the liver.
- Low expression of STING in hepatocytes leads to non-clearance of HBV, and activation of cGAS–STING signalling inhibits viral infection.
- cGAS–STING signalling-induced cytokine production exacerbates the sterile inflammatory response associated with NAFLD and liver injury and suppresses the development of HCC.
- Targeting cGAS–STING signalling has potential therapeutic value for the treatment of liver diseases, especially viral hepatitis and HCC.

**Innate immunity and cGAS-STING signalling in the liver**

The liver performs functions not only in metabolism and biliary secretion but also in immune defence. The liver is supplied with blood from both the hepatic artery and portal vein, and thus it is necessary for the liver to recognise antigenic components from systemic blood circulation and the gastrointestinal tract, where pathogens and abnormal autologous cells are abundant. To facilitate the initiation of an adaptive immune response, many varieties of innate immune cells reside in the liver.

Innate immune cells in the liver can be categorised into liver-resident cells, such as Kupffer cells (KCs), liver sinusoidal endothelial cells (LSECs), and dendritic cells (DCs), and blood-borne cells, such as natural killer cells (NK cells), NKT cells, neutrophils, eosinophils and monocytes.
cells of the liver, hepatocytes constitute approximately 80% of all liver cells. In addition to their principal biochemical functions, hepatocytes are important antigen-presenting cells (APCs) in the liver. KCs are also key APCs of the hepatic immune system that express major histocompatibility complex (MHC)-I, MHC-II and costimulatory molecules simultaneously, activating T cells and initiating the adaptive immune response. The liver is the largest pool of macrophages in organisms, and approximately 80% to 90% of tissue macrophage are KCs. KCs are quiescent and located in close proximity to LSECs in blood vessels. As pioneer cells that detect diverse PAMPs and DAMPs, KCs express a broad range of immune recognition receptors. LSECs constitutively express a certain amount of programmed cell death ligand 1 (PD-L1), which is pivotal for the development of T cell tolerance. DCs predominantly migrate into the liver in an immature state via the portal vein and are located in the space of Disse. Multiple DC subsets are instrumental in maintaining the balance between tolerance and immunity in the liver. NK cells in the liver are the largest cluster of NK cells in the human body, representing 30–50% of lymphocytes in the liver. NK cells do not express specific antigen recognition receptors but respond to an array of activating receptors and inhibitory receptors to induce cytotoxicity.

Liver inflammation is triggered and sustained by the secretion of cytokines and chemokines from innate immune cells, which express diverse PRRs, including cGAS/STING. Cytokines are vital mediators of the interactions between hepatocytes and NPCs, and cytokine production is therefore essential for maintaining appropriate responses of the liver to external antigenic stimuli in a homeostatic state. Disorders in cytokine production lead to severe hepatocyte impairment, which in turn induces the development of numerous acute or chronic liver diseases. The expression of cGAS/STING in various types of liver cells is not yet clear. The level of cGAS-STING signalling varies greatly among different human immortalised hepatocytes and human hepatoma cells. NKNT-3 and Li23 cells express high levels of cGAS, while PH5CH8, HepG2 and Huh7 cells lack cGAS expression. STING expression is much higher in HepG2 cells and HepAD38 cells than in Huh7 cells. Thomsen et al. indicated that STING expression is deficient in both human and murine hepatocytes. As a consequence, hepatocytes fail to trigger the DNA-sensing pathway to initiate an efficient innate immune response, which partly explains why HBV specifically infects hepatocytes and continuously replicates in hepatocytes. Another study detected the expression of cGAS in both HBV-infected hepatoma cell lines and human primary hepatocytes. Nevertheless, based on current studies, it is relatively certain that cGAS-STING signalling in the liver occurs primarily in immune cells. Myeloid cells have higher levels of cGAS-STING signalling than human hepatocytes and are activated by HBV DNA. STING-dependent production of IFN-I was observed to be mediated by macrophages in a coculture system of macrophages and hepatocytes with stable HBV replication. In addition, the release of mitochondrial DNA (mtDNA) or dsDNA due to hepatocyte injury induces inflammation by activating the cGAS-STING pathway in NPCs, especially in liver macrophages (not only in monocyte-derived macrophages but also in KCs).

cGAS-STING signalling in liver diseases

Viral hepatitis

Since its discovery, cGAS has attracted considerable attention for its antiviral capabilities, and it is a powerful nucleic acid sensor that detects viral DNA in the cytoplasm, such as herpes simplex virus-1, human immunodeficiency virus, adenoviruses and human cytomegalovirus. cGAS additionally confers resistance to RNA viruses, especially positive ssRNA viruses. Due to the critical position of cGAS/STING in the innate immune surveillance of DNA and RNA viruses, extensive research on HBV and HCV has been carried out.

HBV is a DNA virus belonging to the Hepadnaviridae family and the predominant cause of chronic viral hepatitis. HBV is a “stealth virus” that evades recognition and offensive attack by the immune system in a wide variety of ways, leading to the chronic infection of hepatocytes; however, the mechanism of virus evasion remains unclear. As mentioned, most arguments support the idea that defective expression of STING in hepatocytes may contribute to the non-clearance of HBV by the immune system. During HBV infection, the shielding of DNA by the viral capsid, combined with the low level of cGAS/STING expression, might ultimately lead to immune escape of HBV. Nonetheless, a low level of cGAS/STING signalling is sufficient to generate a response to large amounts of naked HBV DNA. HBV also evades host immune surveillance by impairing cGAS-STING signalling.

Although HBV employs several tactics to evade immune surveillance of DNA sensors, the immune responses to HBV are somewhat enhanced by the activation of cGAS- STING signalling. Both in vitro and in vivo experiments have demonstrated that activation of cGAS- STING signalling suppresses HBV replication by reducing HBV covalently closed circular DNA levels. The powerful suppression of HBV was attributed to cGAS- STING signalling-dependent release of cytokines, such as IFN-I, IFN-III and IL-6. cGAS- STING signalling activation also induces the expression of ISG56 (IFN-stimulated gene 56), which directly impairs HBV assembly and suppresses HBV RNA synthesis.

In summary, a reduced level of cGAS/STING expression in hepatocytes and the escape mechanisms of HBV that target cGAS-STING signalling could at least partially contribute to chronic HBV infection of hepatocytes. Nevertheless, activation of cGAS- STING signalling can significantly inhibit the replication of HBV. Therefore, targeting the cGAS- STING signalling pathway could be a therapeutic option for enhancing the host immune response to HBV.

HCV, a member of the Flaviviridae family, is a positive-sense ssRNA virus. Hepatitis C is less prevalent than hepatitis B, but it can similarly lead to liver fibrosis and even cirrhosis and HCC. Knocking down STING substantially reduces IFN production in HCV-transfected hepatocytes, and diminishes the inhibition of replicon replication. These observations further support the
role of STING in IFN production in HCV-transfected hepatocytes. However, the HCV NS4B protein in turn restrains IFN production by disrupting the STING-TBK1 interaction and reducing STING accumulation; hence, STING is involved in the mechanism by which HCV evades the host’s innate immune response.71-73 That said, the detailed mechanism by which cGAS-STING signalling is involved in the recognition of, and resistance to, HCV infection requires further study.

Non-alcoholic fatty liver disease
Non-alcoholic fatty liver disease (NAFLD), which can ultimately lead to cirrhosis and HCC, is now considered the most common chronic liver disease and is associated with the gradual increase in the prevalence of metabolic disorders such as diabetes and obesity.74,75 The incidence of NAFLD is now thought to be related to innate immune-mediated sterile inflammation,11 and IFNs have been shown to play an essential role in the development and progression of NAFLD.76 There is increasing evidence that cGAS-STING signalling is involved in the development of NAFLD through DNA-mediated IFN-I production.

In patients with NAFLD, hepatic STING expression has been demonstrated to be upregulated.14 The STING-IRF3 axis is involved in the activation of apoptotic pathways in NAFLD and early alcohol-related liver disease (ALD).77,78 Upregulates inflammatory pathways and induces glucose and lipid metabolism disorders.77 In line with the aforementioned studies, the accumulation of insoluble protein observed in NAFLD was found to be coupled to p62 phosphorylation, which was verified to be caused by lipotoxic activation of TBK1.79 It was demonstrated that STING activation in monocyte-derived macrophages and KCs of the liver contributes to the phosphorylation of TBK1, JNK (c-Jun-N-terminal kinase), and NF-κB.77 Liver macrophages produce TNF-α and IL-1β, which trigger inflammatory pathways in hepatocytes, as well as TGF-β1, which leads to the activation of hepatic stellate

Fig. 2. cGAS-STING signalling in liver diseases. (A) Activation of cGAS-STING signalling inhibits the production of HBV. (B) Mitochondrial DNA released from hepatocytes activates the STING-IRF3 axis, which causes fat deposition in hepatocytes. (C) Liver injury of different aetiologies activates cGAS-STING signalling, thus exacerbating liver inflammation. (D) Cytoplasmic chromatin in cancer cells triggers cGAS-STING signalling activation, leading to the induction of autophagy and the activation of apoptosis. cccDNA, covalently closed circular DNA; cGAS, cyclic GMP-AMP synthase; dsDNA, double-stranded DNA; ER, endoplasmic reticulum; HSC, hepatic stellate cell; IFN, interferon; IL-, interleukin-; IRI, ischaemia-reperfusion injury; KCs, Kupffer cells; mtDNA, mitochondrial DNA; NAFLD, non-alcoholic fatty liver disease; NPC, non-parenchymal cell; STING, stimulator of interferon genes; TBK1, TANK-binding kinase 1; TGF-β1, transforming growth factor-β1; TNF, tumour necrosis factor.
cells (HSCs). The production of cytokines results in fat deposition and fibrosis, respectively.3,4 These findings indicate a critical position of cGAS-STING signalling in NAFLD and illuminate a broader underlying connection of innate immune regulation with sterile inflammation and metabolic disorders. However, the origin of STING ligands remains unclear. Researchers have speculated that mtDNA released from hepatocytes induces the activation of the STING pathway in KCs in livers with dyslipidaemia (Fig. 2B).5,6 We hypothesise that hepatocyte damage caused by lipid oxidative stress activates cGAS-STING signalling and leads to the development of sterile inflammation, which drives the pathological process of NAFLD.

 Liver injury

Factors such as alcohol, drugs, radiation, and ischaemia/reperfusion can damage the liver. Injury-induced hepatocyte necrosis or apoptosis results in the release of nuclear DNA or mtDNA, which can behave as DAMPs to trigger the innate immune response, giving rise to sterile inflammation in the liver.51-83 As important PRRs, cGAS and STING have been found to participate in liver injury arising from various causes. Herein, we review current evidence that cGAS-STING signalling is involved in hepatic alcoholic, radiation, and ischaemia/reperfusion injury.

Both patients with ALD and alcohol-fed mice exhibited increased levels of cGAS-STING signalling.13 ER stress following alcohol stimulation initiates STING activation and IRF3 phosphorylation, which in turn leads to hepatocyte apoptosis concurrent with early liver fibrosis.78,85 Interestingly, cGAS-driven IRF3 activation is transmitted through gap junctions between hepatocytes, thereby amplifying and extending the injurious effects of alcohol on the liver.5,84

Another study reported that cGAS−/− mice show higher levels of liver damage in response to liver ischaemia/reperfusion injury (IRI).86 while STING−/− mice6 and mice transected with STING small interfering RNA (siRNA)57 exhibited reduced liver damage. Lei et al.87 attributed the attenuation of IRI to the STING-independent induction of hepatocyte autophagy by cGAS. Liver IRI results in the release of mtDNA from hepatocytes,30,89 which may serve as ligands for STING. STING activation in aged liver macrophages generates a large array of proinflammatory cytokines by affecting the activation of NLRP3 (nucleotide-binding domain and leucine-rich repeat containing protein 3), a well-studied constituent of inflammation.56 In addition, knocking down STING in macrophages substantially mitigates the aging-related exacerbation of liver IRI.56

Similarly, radiation-induced liver injury provokes the release of large doses of dsDNA from hepatocytes. Once sensed by NPCs, dsDNA activates cGAS-STING signalling, with the subsequent production of a high level of IFN-I ultimately amplifying the hepatocyte damage generated by ionising radiation (Fig. 2C).15 There are currently no reports of a link between drug-induced liver injury and cGAS-STING signalling.

cGAS-STING signalling has also been implicated in multiple injuries and inflammatory diseases in organs other than the liver. Brain injury after ischaemic stroke is alleviated by the inhibition of cGAS activity in both cGAS-deletion models and cGAS antagonist experiments.90,91 Similarly, in ischaemic myocardial injury, the absence of cGAS leads to a better prognosis in animal models of myocardial infarction, and the deficiency of cGAS function promotes the conversion of macrophages to the reparative phenotype.92 The release of mtDNA, either as a result of acute kidney injury or chronic kidney disease, aggravates renal inflammation and accelerates the fibrotic process by activating cGAS-STING signalling.93,94 The liberation of DNA from dead acinar cells activates cGAS-STING signalling in pancreatic macrophages, thereby worsening acute pancreatitis.95

Collectively, the evidence that activation of cGAS-STING signalling exacerbates inflammation and tissue damage is robust, but the idea that cGAS activation mitigates damage by mediating the initiation of autophagy cannot be ignored. Thus, further studies are needed to explore whether cGAS aggravates tissue damage through classical cytokine production or mitigates it through autophagy and which effect predominates.

HCC

The intimate link between DNA damage and cancer is well established.60 The production of IFN-I greatly enhances the host’s capacity to resist tumour cells. The recognition of abnormal dsDNA leads to the initiation of cGAS-STING signalling and consequently to the production of IFN-I, which has antitumor effects. In addition, cGAS-STING signalling has been implicated in interactions between various immune cells in tumours, including CD8+ T cells, DCs, NK cells, and KCs,16,97-99 which are thought to be prominent in tumour immunity.100 Here, we summarise the recent progress in our understanding of the involvement of cGAS-STING signalling in HCC.

Based on an analysis of multiple databases, Qi et al.101 identified links between key genes of the cGAS-STING pathway and the HCC phenotype in human samples. For instance, X-ray repair cross complementing (XRCC)5 and XRCC6 are associated with the tumour stage, pathological grade and patient survival, and ATR and ATM are potential kinase targets in HCC. Further studies indicated the mechanism by which cGAS-STING signalling inhibits the progression of HCC. ATR inhibitors enhance the antitumor activity of radiotherapy in HCC and these effects are dependent on the activation of cGAS-STING signalling.102 Inhibition of ATM in ARID1A (AT-rich interaction domain 1 A)-deficient tumors103 causes leakage of mtDNA or replicative stress, which activates the cytoplasmic cGAS-STING pathway. Cytoplasmic chromatin-triggered cGAS-STING signalling activation has also been linked to hepatic immunosurveillance against RAS.104 Ultimately, cytokine-dependent lymphocyte infiltration inhibits tumour growth while also enhancing the efficacy of immune checkpoint blockade.105,106 STING-deficient mice with HCC exhibit larger tumours and reduced rates of autophagy and apoptosis during tumour progression – effects which are reversed by STING agonists (Fig. 2D).16 We certainly need more in-depth studies to clarify how cGAS-STING signalling functions in liver cancer, in both initiation and progression.

Other liver diseases

cGAS-STING signalling has a pathophysiological and immunological role in the development of a variety of diseases, and we hypothesise that cGAS-STING signalling may also be involved in other liver diseases, such as liver fibrosis and autoimmune liver disease. In patients with NAFLD, the expression level of STING and the stage of liver fibrosis are positively correlated.57 This correlation was attributed to the release of TGF-β1 induced by STING activation in macrophages, which stimulates HSCs, leading to fibrosis.18 However, studies of cGAS-STING signalling and liver fibrosis induced by other causes have not yet emerged. In addition, the mechanism by which cGAS-STING signalling is involved in the development and regression of liver fibrosis also warrants more detailed studies.
STING gene (TMEM173) mutations can cause an auto-inflammatory disease associated with vessels and lungs named STING-associated vasculopathy with onset in infancy (SAVI).\(^\text{106}\) Approximately 36 cases of SAVI have been reported worldwide, and only 3 patients have presented with liver disease.\(^\text{107}\) In a recent case, a 3-year-old girl with SAVI developed severe liver dysfunction after liver transplantation.\(^\text{107}\) Activation of cGAS has been linked to the development of the Aicardi-Goutières syndrome and systemic lupus erythematosus.\(^\text{108,109}\) However, there have been no reports of cGAS-STING pathway involvement in autoimmune liver disease to date. Genetic susceptibility is the primary cause of autoimmune liver disease, with dysregulation of humoral and cellular immunity resulting in pathological damage via the production of autoimmune antibodies and T-cell-mediated autoimmune responses, respectively. cGAS-STING signalling is involved at multiple points in both innate and adaptive immune regulation; hence, we believe cGAS-STING signalling is strongly implicated in autoimmune liver disease.

Several non-synonymous variants of STING have been reported in human populations and it has been shown that STING variants can influence CDN recognition inducing different effects.\(^\text{26,110}\) A recent study reported that genetic variants in STING are associated with graft-versus-host disease (GVHD) after allogeneic haematopoietic stem cell transplantation (aHSCT).\(^\text{111}\) Deficiency of STING in recipients attenuates CD8\(^+\) T cell-induced GVHD after aHSCT regardless of MHC matching.\(^\text{111}\) For the same reason, genetic variants in STING from either donor grafts or recipients may also be instrumental in determining immune remodelling (e.g., rejection, tolerance, GVHD) after liver transplantation.

cGAS-STING signalling: A potential therapeutic target

Accumulating evidence suggests that cGAS–STING signalling is a prospective drug target that could overcome the shortcomings of current therapeutic regimens, especially in viral hepatitis and HCC. Targeting cGAS-STING signalling may also have beneficial effects for the treatment of NAFLD and liver injury.

cGAMP, as an effective ligand for STING, greatly enhances the immune response to HBV vaccines when applied as a vaccine adjuvant (Fig. 3B).\(^\text{112}\) Compared with TLR agonists, STING agonists not only induce a more potent antiviral response but also lead to less severe inflammation and tissue damage by reducing proinflammatory cytokine responses.\(^\text{55}\) To overcome the problem of low hepatocyte STING expression and HBV DNA cloaking, Gv1001 and daunorubicin can be used to inhibit the replication of HBV by eliciting mitochondrial stress and hepatocyte DNA damage, respectively.\(^\text{113,114}\) IFN-1 generated by Gv1001 via cGAS-STING signalling can prevent HBV escape of the IFN-1-induced cell response (Fig. 3A).\(^\text{112}\) Thus, drugs and vaccines targeting cGAS-STING signalling have great potential in overcoming chronic HBV infection. Moreover, targeting cGAS-STING signalling may have extensive research prospects for attenuating HBV resistance to antiviral drugs. A recent study reported that the STING agonist cyclic di-AMP can serve as a potential adjuvant for the HCV E1E2 vaccine. Cyclic di-AMP displays a favourable dual humoral and cellular immune response, substantially improving the immunogenicity of the E1E2 vaccine (Fig. 3B).\(^\text{115}\) Additionally, a novel broad-spectrum antiviral drug, remdesivir, surprisingly reduces inflammation and lipid dysfunction in NAFLD by inhibiting STING signalling and could be a therapeutic candidate (Fig. 3C).\(^\text{116}\)

Moreover, STING agonists elicit robust antitumour effects, especially in combination with immune checkpoint therapy (PD-1/PD-L1 antibodies).\(^\text{116,117}\) Huang et al.\(^\text{118}\) engineered novel HCC-specific nanoparticles with STING-activating dendrimers, PD-L1 siRNA and IL-2 plasmid DNA and observed stronger immune cell infiltration, possibly via upregulation of IFN-1 and IL-2 and downregulation of PD-L1 (Fig. 3D).\(^\text{118}\) Additionally, deficiency of STING could potentially enhance the efficacy of DNA-virus-mediated oncolytic therapy.\(^\text{119}\) More studies on the link between cGAS-STING and tumour immunity in the liver should be pursued to maximise the potential of inducing cGAS-STING signalling with cancer-fighting drugs and to overcome the problem of drug resistance in HCC treatment.

Recent studies have indicated interactions between the cGAS-STING signalling and mammalian target of rapamycin (mTOR) signalling pathways.\(^\text{110,112-114}\) mTOR is an integral molecule downstream of the PI3K/Akt pathway and is involved in metabolism, cell proliferation, apoptosis, and autophagy. The mTOR inhibitor rapamycin has dual anti-immune rejection and tumour suppression effects and is widely used in the treatment of HCC and for liver transplantation.\(^\text{123,124}\) The interaction of mTOR and cGAS-STING signalling might also guide the individualised administration of rapamycin.

Conclusions and perspectives

In this review, we have summarised recent progress in our understanding of the role of cGAS-STING signalling in multiple liver diseases and discussed this pathway as a potential therapeutic target. Despite a large body of relevant work, many problems warranting prompt resolution remain. cGAS/STING expression in the liver is controversial, with some studies suggesting that STING can be expressed and activated in hepatocytes,\(^\text{78,85}\) while others argue that STING expression and activation occur only in hepatic immune cells.\(^\text{110,114}\) We assume that cGAS/STING is
expressed in hepatocytes at low levels and that its activation occurs under DNA stimulation. By contrast, the expression of STING in hepatic immune cells is well defined. cGAS-STING signalling likely affects the development and progression of liver diseases primarily through immune cells. Further studies at the single-cell level are necessary to elucidate whether liver-resident immune cells or blood-derived immune cells mediate the hepatic innate immune response through cGAS-STING signalling. It is also worth investigating the origin of DNA ligands in different pathological contexts to determine whether different types of DNA ligands that bind to cGAS will elicit different effects.

For diseases such as viral hepatitis and HCC, activation of cGAS-STING signalling strengthens the immune surveillance capacity of the liver. In the case of liver injury and NAFLD, the inflammatory response caused by activation of cGAS-STING signalling results in greater liver impairment, more severe liver inflammation and even fibrosis. Therefore, activation or inhibition of cGAS-STING signalling may have significant applications in the treatment of different liver diseases. In addition, novel drug delivery systems are needed to overcome the susceptibility of STING agonists to degradation and to improve delivery of these drugs to their target cells.

**Abbreviations**

aHSCT, allogeneic haematopoietic stem cell transplantation; AIM2, absent in melanoma 2; ALD, alcohol-related liver disease; APCs, antigen-presenting cells; CDNs, cyclic dinucleotides; cGAMP, cyclic guanosine monophosphate–adenosine monophosphate; cGAS, cyclic guanosine monophosphate–adenosine monophosphate synthase; DAMPs, damage-associated molecular patterns; DCs, dendritic cells; dsDNA, double-strand DNA; ER, endoplasmic reticulum; GVHD, graft-versus-host disease; HCC, hepatocellular carcinoma; HSCs, hepatic stellate cells; IFN-γ, type 1 interferon; IL, interleukin; IRF3, interferon regulatory factor 3; IRI, ischaemia refusion injury; KCs, Kupffer cells; LSECs, liver sinusoidal endothelial cells; MHC, major histocompatibility complex; mtDNA, mitochondrial DNA; mTOR, mammalian target of rapamycin; NAFLD, non-alcoholic fatty liver disease; NK cells, natural killer cells; NPCs, non-parenchymal cells; PAMPs, pathogen-associated molecular patterns; PD–1, programmed cell death protein-1; PD–L1, programmed cell death protein ligand-1; PPRs, pattern recognition receptors; SAVI, STING-asso- ciated vasculopathy with onset in infancy; siRNA, small interfering RNA; TANK-binding kinase 1; TGF-β, transforming growth factor-β; TLR, Toll-like receptor; TNF, tumour necrosis factor; XRCC, X-ray repair complementing.

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**Conflict of interests**

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

**Authors’ contributions**

Q.L. and H.Z. conceived of the paper; R.H.C. wrote the original draft; R.H.C. and J.M.D. generated the figures; Q.L. and H.Z. reviewed and edited the paper. All the authors agreed to the published version of the manuscript.

**Supplementary data**

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**References**

**Author names in bold designate co-first authorship**

[1] Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell 2010;140:805–820.
[2] Riera Romo M, Perez-Martinez D, Castillo Ferrer C. Innate immunity in vertebrates: an overview. Immunology 2016;148:125–139.
[3] Cao X. Self-regulation and cross-regulation of pattern-recognition receptor signalling in health and disease. Nat Rev Immunol 2016;16:35–50.
[4] Roers A, Hiller B, Hornung V. Recognition of endogenous nucleic acids by the innate immune system. Immunity 2016;44:739–754.
[5] Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immuono 2010;11:373–384.
[6] Hornung V, Abbassar A, Charrel-Dennis M, Bauermeulen F, Horvath G, Caffrey DR, et al. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature 2009;458:514–518.
[7] Sun LJ, Wu JX, Du FH, Chen X, Chen ZJ. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science 2013;339:796–799.
[8] Vollmer J. TLR2 in health and disease. Int Rev Immunol 2006;25:155–181.
[9] Farrokh S, Abbasirad N, Movahed A, Khazaee HA, Pishjoo M, Rezaei N. TLR9-based immunotherapy for the treatment of allergic diseases. Immunotherapy 2017;9:329–346.
[10] Fernandes-Alnemri T, Yu JW, Datta P, Wu J, Alnemri ES. AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. Nature 2009;458:509–513.
[11] Bai J, Liu F. The cGAS-cGAMP-STING pathway: a molecular link between immunity and metabolism. Diabetes 2019;68:1099–1108.
[12] Motwani M, Persisidis S, Fitzgerald KA. DNA sensing by the cGAS-STING pathway in health and disease. Nat Rev Genet 2019;20:657–674.
[13] Verrier ER, Yim SA, Heydmann L, El Saghir H, Bach C, Turon-Lagot V, et al. Hepatitis B virus evasion from cyclic guanosine monophosphate–adenosine monophosphate synthase sensing in human hepatocytes. Hepatology 2018;68:1695–1709.
[14] Luo X, Li H, Ma L, Zhou J, Guo X, Wuo SL, et al. Expression of STING is increased in liver tissues from patients with NAFLD and promotes macrophage-mediated hepatic inflammation and fibrosis. Gastroenterology 2018;155.1971-1984 e1974.
[15] Du S, Chen G, Yuan B, Hu Y, Yang P, Chen Y, et al. DNA sensing and associated type 1 interferon signaling contributes to progression of radiation-induced liver injury. Cell Mol Immunol 2020.
[16] Thomsen MK, Skouboe MK, Boulanar C, Vernejoul F, Lioux T, Leknes SL, et al. The cGAS-STING pathway is a therapeutic target in a preclinical model of hepatocellular carcinoma. Oncogene 2020;39:1652–1664.
[17] Hopfner KP, Hornung V. Molecular mechanisms and cellular functions of cGAS-STING signalling. Nat Rev Mol Cell Biol 2020;21:501–521.
[18] Zhang X, Bai XC, Chen ZJ. Structures and mechanisms in the cGAS-STING innate immunity pathway. Immunity 2020;53:43–53.
[19] Li X, Shu C, Yi G, Chaton CT, Shelton CL, Dao J, et al. Cyclic GMP-AMP synthase is activated by double-stranded DNA-induced oligomerization. Immunity 2013;39:1019–1031.
[20] Diner EJ, Burdette DL, Wilson SC, Monroe KM, Kellenberger CA, Hyodo M, et al. The innate immune DNA sensor cGAS produces a non-canonical cyclic dinucleotide that activates human STING. Cell Rep 2013;3:1355–1361.
[21] Heijink AM, Talens F, Jae LT, van Gijn SE, Fehrmann RSN, Brummelkamp TR, et al. BRCAl2 deficiency instigates cGAS-mediated inflammatory signaling and confers sensitivity to tumor necrosis factor-alpha-mediated cytotoxicity. Nat Commun 2019;10:100.
[22] Gluck S, Guey B, Gulen MF, Wolter K, Kang TW, Schmack NA, et al. Innate immune sensing of cytosolic chromatin fragments through cGAS promotes senescence. Nat Cell Biol 2017;19:1061–1070.
[23] White MJ, McCarthy M, McTavish D, Lane RM, Cambier JC, Herold MJ, et al. Apoptotic caspases suppress mtDNA-induced STING-mediated type I IFN production. Cell 2014;159:1549–1562.
[24] West AP, Khoury-Hanold W, Staron M, Tal MC, Pineda CM, Lang SM, et al. Mitochondrial DNA stress primes the antiviral innate immune response. Nature 2015;520:553–557.
Dansako H, Ueda Y, Okumura N, Satoh S, Sugiyama M, Mizokami M, et al. The cyclic GMP-AMP synthetase-STING signaling pathway is required for both the innate immune response against HBV and the suppression of HBV assembly. FEBS J 2016;283:144–156.

Guo F, Tang L, Shu S, Sehgal M, Sheraz M, Liu B, et al. Antimicrob Agents Chemother 2017;61.

Kizaki K, Tanaka H, Sugiyama H, Ota T, Yamada H, et al. STING expression in human hepatocytes facilitates development of HBV infection. Hepatology 2016;64:746–759.

Lauterbach-Rivière L, Bergez M, Mönch S, Qi B, Riess M, Vondran FWR, et al. Hepatitis B virus DNA is a substrate for the cGAS/STING pathway but is not sensed in infected hepatocytes. Viruses 2020;12.

Guo F, Han Y, Zhao X, Wang J, Liu F, Xu C, et al. STING agonists induce an innate antiviral immune response against hepatitis B virus. Antimicrob Agents Chemother 2015;59:1273–1281.

Zhong W, Rao Z, Rao J, Han G, Wang P, Jiang T, et al. Aging aggravated liver ischemia and reperfusion injury by promoting STING-mediated NLRP3 activation in macrophages. Aging Cell 2020;19:e13186.

Wang X, Rao H, Zhao J, Wei A, Li X, Fei K, et al. STING expression in monocyte-derived macrophages is associated with the progression of liver inflammation and fibrosis in patients with nonalcoholic fatty liver disease. Lab Invest 2020;100:542–552.

Li XD, Wu JX, Gao DX, Wang H, Sun LJ, Chen ZJ. Pivotal roles of cGAS-cGAMP signaling in antiviral defense and immune adjuvant effects. Science 2013;341:1390–1394.

Gao DX, Wu JX, Wu YT, Du FH, Aroh C, Yan N, et al. Cyclic GMP-AMP synthase is an innate immune sensor of HBV and other retroviruses. Science 2013;341:903–906.

Anghelina D, Lam E, Falck-Pedersen E. Diminished innate antiviral response to adenovirus vectors in cGAS/STING-deficient mice minimally impacts adaptive immunity. J Virol 2016;90:5915–5927.

Mocarski E, Pajouhesh M, Spanjer J, Grabski E, Noorazanam M, et al. cGAS senses human cytomegalovirus and induces type I interferon responses in human monocyte-derived cells. PloS Pathog 2016;12.

Schoggins JW, MacDuff DA, Imanaka N, Gainey MD, Shrestha B, Etlin J, et al. Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. Nature 2013;505:691–695.

Revill PA, Tu T, Nettter HJ, Yuen LW, Locarnini SA, Littlejohn M. The evolution and clinical impact of hepatitis B virus genome diversity. Nat Rev Gastroenterol Hepatol 2020;17:618–634.

Kuipery A, Gehring AJ, Isogawa M. Mechanisms of HBV immune evasion. Trends Immunol 2013;34:446–454.

Byrne CD, Targher G. NAFLD: a multisystem disease. J Hepatol 2016;65:390–400.

Revill PA, Tu T, Nettter HJ, Yuen LW, Locarnini SA, Littlejohn M. The changing epidemiology of liver diseases in the Asia–Pacific region. Nat Rev Gastroenterol Hepatol 2018;15:765–773.

Steg P, Meerschaut T, Prinzen S, Seynaeve C, Decaux G, Ectors N. The role of IFN in the development of NAFLD and therapeutic strategies. Nat Med 2018;24:908–922.

Mohenberg M, Terczynska-Dyla E, Thomsen KL, George J, Eslam M, Gronbaek H, et al. The role of IFN in the development of NAFLD and NASH. Cytokine 2019;124:154519.
Li T, Chen ZJ. The cGAS-cGAMP-STING pathway connects DNA damage to nonalcoholic fatty liver disease. Meta- bolism 2018;81:13–24.

Petrasek J, Iracheta-Velvle A, Csak T, Satchidanand A, Kody K, Kurt-Jones EA, et al. STING-cGAMP pathway links endoplasmic reticulum stress with hepatitis apotosis in early alcoholic liver disease. Proc Natl Acad Sci U S A 2013;110:16544–16549.

Xu MM, Pu Y, Han D, Shi Y, Cao X, Liang H, et al. Dendritic cells but not macrophages sense tumor mitochondrial DNA for cross-priming in mice. Sci Immunol 2020;5.

Yi G, Brendel VP, Shu C, Li P, Panalathan S, Cheng Kao C. Single nucleotide polymorphisms of human STING can affect innate immune response to cyclic dinucleotides. PLoS One 2013;8:e77846.

Shen A, Zheng D, Luo Y, Mou T, Chen Q, Huang Z, et al. MicroRNA-24-3p alleviates hepatic ischemia and reperfusion injury in mice through the repression of STING signaling. Biochem Biophys Res Commun 2020;522:47–52.

Zhai Y, Petrowsky H, Hong JC, Busuttill RW, Kupiec-Weglinski JW. Ischaemia-reperfusion injury in liver transplantation—from bench to bedside. Nat Rev Gastroenterol Hepatol 2013;10:79–89.

Zhang X, Wu X, Hu Q, Wu J, Wang G, Hong Z, et al. Mitochondrial DNA in liver inflammation and oxidative stress. Life Sci 2019;236:116446.

Li Q, Cao Y, Dang C, Han B, Han R, Ma H, et al. Inhibition of double-strand DNA-sensing cGAS ameliorates brain injury after ischemic stroke. EMBO Mol Med 2020;12:e10102.

Liao Y, Cheng J, Kong X, Li S, Li X, Zhang M, et al. HDAC3 inhibition ameliorates ischemia-reperfusion-induced brain injury by regulating the microglial cGAS-STING pathway. Theranostics 2020;10:9644–9662.

Cao DJ, Schiattarella GG, Villalobos E, Jiang N, May HI, Li T, et al. Cytosolic DNA sensing promotes macrophage transformation and governs myocardial ischemia. Circulation 2018;137:2613–2634.

Maekawa H, Inoue T, Ouchi H, Jao TM, Inoue R, Nishi H, et al. Mitochondrial damage causes inflammation via cGAS-STING signaling in acute kidney injury. Cell Rep 2019;29:1261–1273 e1266.

Chung KW, Dillon P, Huang S, Sheng X, Shrestha R, Qiu C, et al. Mitochondrial damage and activation of the STING pathway lead to renal inflammation and fibrosis. Cell Metab 2019;30:784–789 e785.

Zhao Q, Wei Y, Pandol SJ, Li L, Hatsebton A. STING signaling promotes inflammation in experimental acute pancreatitis. Gastroenterology 2018;154:1822-1835 e1822.

Li H, Zhang H, Wu X, Ma D, Wu J, Wang L, et al. Nuclear cGAS suppresses DNA repair and promotes tumorigenesis. Nature 2018;563:131–136.

Li W, Lu L, Lu J, Wang X, Yang J, Jin J, et al. cGAS-STING-mediated DNA sensing maintains CD8+ T cell stemness and promotes antitumor T cell therapy. Sci Transl Med 2020;12.

Xu MM, Pu Y, Han D, Shi Y, Cao X, Liang H, et al. Dendritic cells but not macrophages sense tumor mitochondrial DNA for cross-priming through signal regulatory protein alpha signaling, immunity 2017;47:395–400.

Nicolai CJ, Wolf N, Chang IC, Kirk G, Marcus A, Ndbukabo CO, et al. NK cells mediate clearance of CD8+ T cell-resistant tumors in response to STING agonists. Sci Immunol 2020;5.

Li T, Chen ZJ. The cGAS-cGAMP-STING pathway connects DNA damage to inflammation, senescence, and cancer. J Exp Med 2018;215:1287–1299.

Qi Z, Yan F, Chen D, Xing W, Li Q, Zeng W, et al. Identification of prognostic biomarkers and correlations with immune infiltrates among cGAS-STING in hepatitis B virus infection. J Hepatol 2019;70:2024–2030.

Sheng H, Huang Y, Xiao Y, Zhu Z, Shen M, Zhou P, et al. ATR inhibitor AZD6738 enhances the antitumor activity of radiotherapy and immune checkpoint inhibitors by potentiating the tumor immune microenvironment in hepatocellular carcinoma. J Immunother Cancer 2020;8.

Wang L, Wang L, Wang C, Zhao W, Ju Z, Zhang W, et al. Inhibition of the ATM/Chk2 axis potentiates cGAS/STING signaling in ARID1A-deficient tumors. J Clin Invest 2020;130:5951–5966.

Dai Z, Ghosh K, Vizioli MG, Zhu J, Sen P, Wangsteenst KJ, et al. Cyto- plasmic chromatin triggers inflammation in senescence and cancer. Nature 2017;550:402–406.

Hu M, Zhou M, Bao X, Pan D, Jiao M, Liu X, et al. ATM inhibition enhances cancer immunotherapy by promoting mtDNA leakage/cGAS- STING activation. J Clin Invest 2020.

Liu Y, Jesus AA, Mabarro B, Yang D, Ramsey SE, Sanchez CAM, et al. Activated STING in a vascular and pulmonary syndrome. N Engl J Med 2014;371:507–518.

Ishikawa T, Tamura E, Kasahara M, Uchida H, Higuchi M, Kobayashi H, et al. Severe liver disorder following liver transplantation in STING-associated vasculopathy with onset in infancy. J Clin Immunol 2021.

Gray EE, Treuting PM, Woodward JJ, Stetson DB. Cutting edge: cGAS is required for lethal autoimmune disease in the trex1-deficient mouse model of aicardi-goutieres syndrome. J Immunol 2015;195:1939–1943.

Gao D, Li T, Li XD, Chen X, Li QZ, Wight-Carter M, et al. Activation of cyclic GMP-AMP synthase by self-DNA causes autoimmune diseases. Proc Natl Acad Sci U S A 2020;117:12699–12704.

Yi G, Brengel VP, Shu C, Li P, Panalathan S, Cheng Kao C. Single nucleotide polymorphisms of human STING can affect innate immune response to cyclic dinucleotides. PLoS One 2013;8:e77846.

Bader CS, Barreras H, Lightbourn C, Copsel SN, Wolf D, Meng J, et al. STING differentially regulates experimental GVHD mediated by CD8 versus CD4 T cell subsets. Sci Transl Med 2020;12.

Ito H, Kanbe A, Hara A, Ishikawa T. Induction of humoral and cellular immune response to HBV vaccine can be up-regulated by STING ligand. Virology 2010;531:233–239.

Choi Y-M, Kim H, Lee S-A, Lee S-Y, Kim B-J. A telomerase-derived peptide exerts an anti-hepatitis B virus effect via mitochondrial DNA stress-dependent type I interferon production. Front Immunol 2020;11.

Imai H, Dansako H, Ueda Y, Sato S, Kato N. Daunorubicin, a topoisomerase II poison, suppresses viral production of hepatitis B virus by inducing cGAS-dependent innate immune response. Biochem Biophys Res Commun 2018;504:672–678.

Landi A, Law J, Hochman D, Logan M, Crawford K, Chen C, et al. Superior immunogenicity of immunogenic peptides from telomerase when adjuvanted with cyclic-di-AMP, a STING activator or arachosamtes. Vaccine 2017;35:6949–6956.

Li YN, Su Y. Remdesivir attenuates high fat diet (HFD)-induced NAFLD by regulating hepatocyte dyslipidemia and inflammation via the suppression of STING. Biochem Biophys Res Commun 2020;526:381–388.

Wang H, Hu S, Chen X, Shi H, Chen C, Sun L, et al. cGAS is essential for the antitumor effect of immune checkpoint blockade. Proc Natl Acad Sci U S A 2017;114:1637–1642.

Huang KW, Hsu FF, Qiu JT, Chen CJ, Lee YA, Chang CC, et al. Highly efficient and tumor-selective nanoparticles for dual-targeted immuno- gene therapy against cancer. Sci Adv 2020;6:eaaq5032.

Xia T, Konno H, Anh J, Barber GN. Deregression of STING signaling in colorectal carcinoma constrains DNA damage responses and correlates with tumorigenesis. Cell Rep 2016;14:282–297.

Bodur C, Kazyken D, Huang K, Ekim Ustunel B, Sirokya KO, Tooley AS, et al. The IKK-related kinase TBK1 activates mTORC1 directly in response to growth factors and innate immune agonists. EMBO J 2018;37:19–38.

Huang M, Gonuguntla VK, Dobbs N, Ali A, Palchik C, Calvvarso MA, et al. Chronic innate immune activation of TBK1 suppresses mTORC1 activity and dysregulates cellular metabolism. Proc Natl Acad Sci U S A 2017;114:746–751.

Meade N, Furey C, Li H, Verma R, Chai Q, Rollins MG, et al. Poxviruses evade cytokolic sensing through disruption of an mTORC1-mTORC2 cytokolic pathway. Cell 2019;177:1155–1166.

Zhang T, Guo J, Lu J, Chen K, Li H, Wang J. Protective role of mTOR in liver ischemia/reperfusion injury: involvement of inflammation and auto- phagy. Oxid Med Cell Longev 2019;2019:7861290.

Han J, Wang Y. mTORC1 signaling in hepatic lipid metabolism. Protein Cell 2018;9:145–151.