The role of cGAS-STING signalling in liver diseases

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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

Received 13 March 2021; received in revised form 20 May 2021; accepted 8 June 2021; available online 24 June 2021

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.*)[4] 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.[1,4] 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.[11,12] Recent studies in the liver have shown that cGAS-STING signalling is involved in diverse functions in viral hepatitis, nonalcoholic fatty liver disease (NAFLD), liver injury and hepatocellular carcinoma (HCC).[13-16]

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

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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.[17,18] 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.[18] Upon recognition of dsDNA, cGAS molecules cross-link with each other to develop dimers or multimers, leading to the activation of cGAS.[19,20] 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.[21-24] A recent study found that cGAS can even bind CDNs.[25] 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.[26] Upon 2'3' cGAMP binding, STING, an endoplasmic reticulum (ER)-residing adaptor,[27] undergoes conformational changes and forms STING oligomers.[28-30] In addition to 2'3' cGAMP, STING recognises bacterial CDNs.[26] 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'-3'cGAMP.[26,31] The STING oligomer is trafficked to the Golgi via the ER–Golgi intermediate compartment.[27,32] Oligomerisation of STING upon ligand binding drives the transphosphorylation of TANK-binding kinase 1 (TBK1) in the signalling domain.[33] pTBK1 subsequently phosphorylates STING and interferon regulatory factor 3 (IRF3). IRF3 dimerises and is translocated to the nucleus where it initiates transcription of IFN-I.[34,35] STING also recruits IκB kinase (IKK), which phosphorylates IκBα 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).[36,37] The promoter region of cGAS contains 2 adjacent IFN-sensitive response elements; as a consequence, IFN-I can enhance the expression of cGAS through positive feedback.[38]

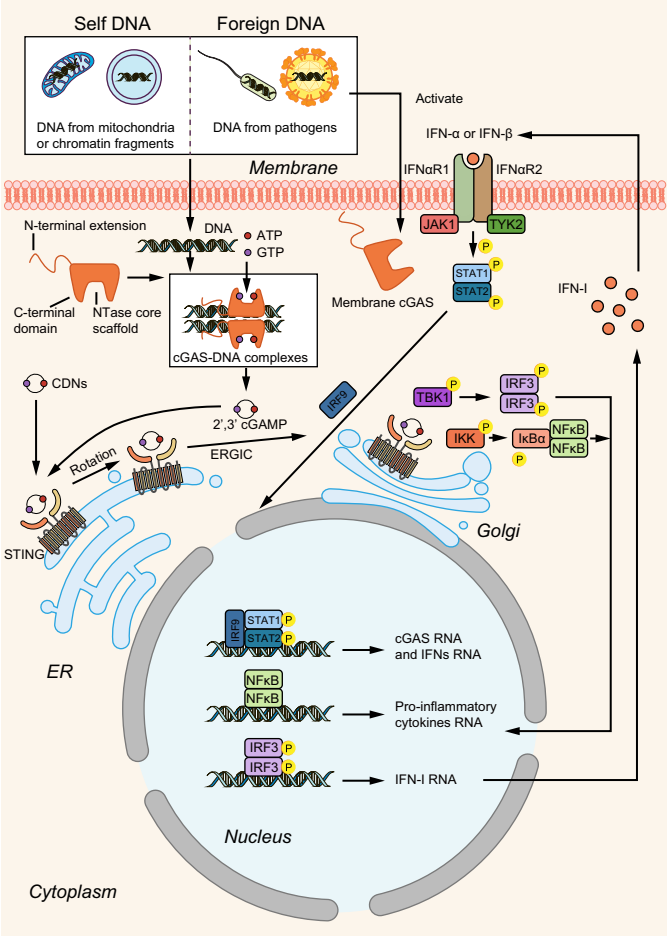

Fig. 1. Molecular mechanism of cGAS-STING signalling. The initial function of cGAS-STING signalling is to defend host cells against pathogens, and later studies have shown that this pathway is also related to inflammatory diseases and tumours. Upon recognition of DNA, cGAS-DNA complexes catalyse ATP and GTP to generate 2',3'cGAMP. STING located in the ER can be activated by 2',3'cGAMP and other CDNs. Activated STING recruits and phosphorylates TBK1 and IKK, which can phosphorylate IRF3 and IκBα. Phosphorylated IRF3 translocates to the nucleus where it triggers the transcription of the IFN-I gene. Phosphorylated IκBα recruits NF-κB and initiates the transcription of genes encoding proinflammatory cytokines. CDNs, cyclic dinucleotides; cGAMP, cyclic GMP-AMP; cGAS, cyclic GMP-AMP synthase; ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediate compartment; IFN, interferon; IKK, IkB kinase; IRF3, interferon regulatory factor 3; JAK1, Janus kinase 1; STAT1/2, signal transducer and activator of transcription 1/2; STING, stimulator of interferon genes; TBK1, TANK-binding kinase 1; TYK2, tyrosine kinase 2.

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.[39] 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.[40] 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.[39] As the parenchymal

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.[40] KCs are also key APCs of the hepatic immune system that express major histocompatibility complex (MHC)-I, MHC-II and costimulatory molecules simultaneously,[41] 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.[42] KCs are quiescent and located in close proximity to LSECs in blood vessels.[43] As pioneer cells that detect diverse PAMPs and DAMPs, KCs express a broad range of immune recognition receptors.[44] LSECs are the most populous non-parenchymal cells (NPCs) in the liver, accounting for approximately 50% of all NPCs.[45] LSECs constitutively express a certain amount of programmed cell death ligand 1 (PD-L1), which is pivotal for the development of T cell tolerance.[46] DCs predominantly migrate into the liver in an immature state via the portal vein and are located in the space of Disse.[47] Multiple DC subsets are instrumental in maintaining the balance between tolerance and immunity in the liver.[48] 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.[45,49]

Liver inflammation is triggered and sustained by the secretion of cytokines and chemokines from innate immune cells, which express diverse PRRs, including cGAS/STING.[17,45] 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.[50]

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.[51] STING expression is much higher in HepG2 cells and HepAD38 cells than in Huh7 cells.[52] Thomsen *et al.*[53] 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.[53] Another study detected the expression of cGAS in both HBV-infected hepatoma cell lines and human primary hepatocytes.[13] 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.[54] 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.[55] 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).[15,56,57]

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.[58-61] cGAS additionally confers resistance to RNA viruses, especially positive ssRNA viruses.[62] 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.[63] 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.[64] 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.[13] 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.[54] Nonetheless, a low level of cGAS-STING signalling is sufficient to generate a response to large amounts of naked HBV DNA.[54] HBV also evades host immune surveillance by impairing cGAS-STING signalling. Verrier *et al.*[13] found that the expression of cGAS and its effector genes is reduced in HBV-infected hepatocytes. HBV polymerase impairs K63 ubiquitination of STING and ultimately inhibits IFN-I production by interacting with STING, thus representing another HBV evasion mechanism (Fig. 2A).[65] STING expression in human peripheral blood monocytes is dramatically lower in patients with chronic hepatitis B than in healthy controls.[66] However, few studies have shown that HBV infection does not inhibit the expression of cGAS-STING signalling.[54,67]

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.[13,55,68] The powerful suppression of HBV was attributed to cGAS-STING signalling-dependent release of cytokines, such as IFN-I, IFN-III and IL-6.[55,69] cGAS-STING signalling activation also induces the expression of ISG56 (IFN-stimulated gene 56), which directly impairs HBV assembly and suppresses HBV RNA synthesis (Fig. 2A).[51]

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.[67]

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.[70] Knocking down STING substantially reduces IFN production in HCV-transfected hepatocytes, and diminishes the inhibition of replicon replication.[71,72] These observations further support the

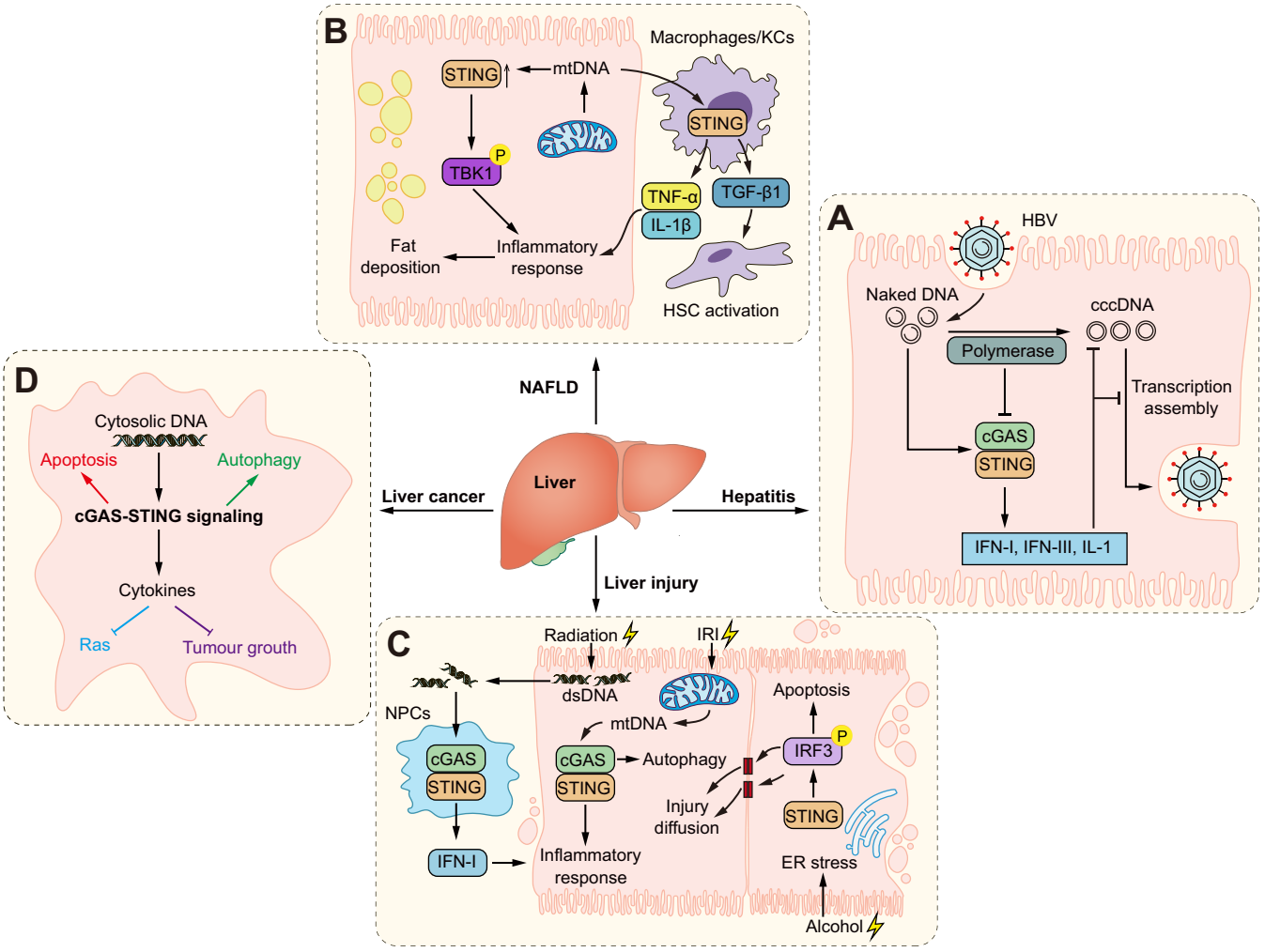

Fig. 2. cGAS-STING signalling in liver diseases. (A) Activation of cGAS-STING signalling inhibits the production of HBV. (B) Mitochondrial DNA released from hepatocytes activates the STING-IRF3 axis, which causes fat deposition in hepatocytes. (C) Liver injury of different aetiologies activates cGAS-STING 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.

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.[57] 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

cells (HSCs). The production of cytokines results in fat deposition and fibrosis, respectively.[14] 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).[80] We hypothesise that hepatocyte DNA 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.[81-83] 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.[84] 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.[84]

Another study reported that $cGAS^{-/-}$ mice show higher levels of liver damage in response to liver ischaemia/reperfusion injury (IRI),[86] while $STING^{gt/gt}$ mice[86] and mice transfected with STING small interfering RNA (siRNA)[87] exhibited reduced liver damage. Lei *et al.*[86] 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,[88,89] 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 inflammasomes.[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.[96] 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 tumors[103] 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.[103,105] 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.[14] 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 autoinflammatory disease associated with vessels and lungs named STING-associated vasculopathy with onset in infancy (SAVI).[106] Approximately 36 cases of SAVI have been reported worldwide, and only 3 patients have presented with liver disease.[107] In a recent case, a 3-year-old girl with SAVI developed severe liver dysfunction after liver transplantation.[107] Activation of cGAS has been linked to the development of the Aicardi-Goutières syndrome and systemic lupus erythematosus.[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.[20,110] A recent study reported that genetic variants in STING are associated with graft-versus-host disease (GVHD) after allogeneic haematopoietic stem cell transplantation (aHSCT).[111] Deficiency of STING in recipients attenuates $CD8^+$ T cell-induced GVHD after aHSCT regardless of MHC matching.[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).[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.[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.[113,114] IFN-I generated by GV1001 via cGAS-STING signalling can prevent HBV escape of the IFN-I-induced cell response (Fig. 3A).[113] 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).[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).[116]

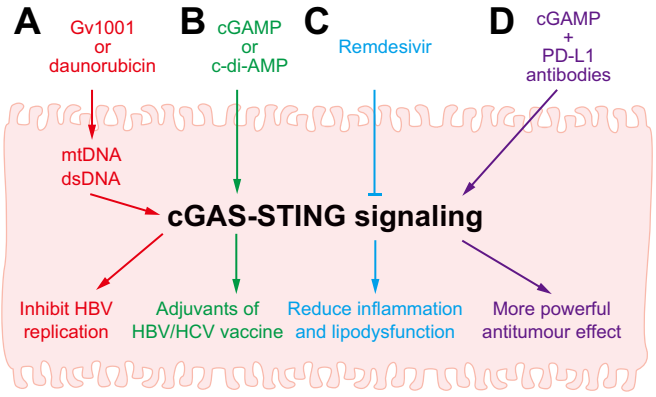

Fig. 3. Potential therapeutic agents for cGAS-STING signalling in liver diseases. (A) Gv1001 and daunorubicin inhibit the replication of HBV by eliciting mitochondrial stress (mtDNA) and hepatocyte DNA damage (dsDNA), which result in cGAS-STING signalling activation. (B) The STING agonists cGAMP and c-di-AMP serve as HBV/HCV vaccine adjuvants. (C) Remdesivir reduces inflammation and lipid dysfunction in NAFLD by inhibiting cGAS-STING signalling. (D) Coadministration of cGAMP and PD-L1 antibodies leads to a powerful antitumor effect in HCC. C-di-AMP, cyclic di-AMP; cGAMP, cyclic GMP-AMP; cGAS, cyclic GMP-AMP synthase; dsDNA, double-stranded DNA; mtDNA, mitochondrial DNA; NAFLD, non-alcoholic fatty liver disease; PD-L1, programmed cell death ligand 1; STING, stimulator of interferon genes.

Moreover, STING agonists elicit robust antitumour effects, especially in combination with immune checkpoint therapy (PD-1/PD-L1 antibodies).[16,117] Huang *et al.*[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-I and IL-2 and downregulation of PD-L1 (Fig. 3D).[118] Additionally, deficiency of STING could potentially enhance the efficacy of DNA-virus-mediated oncoviral therapy.[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.[11,120-122] 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.[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,[78,85] while others argue that STING expression and activation occur only in hepatic immune cells.[14,53] 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 regression 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-I, type I 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-associated vasculopathy with onset in infancy; siRNA, small interfering RNA; ssRNA, single-stranded RNA; STING, stimulator of interferon genes; TBK1, TANK-binding kinase 1; TGF-β1, transforming growth factor-β1; TLR, Toll-like receptor; TNF, tumour necrosis factor; XRCC, X-ray repair cross complementing.

Financial support

This work was supported by the National Natural Science Foundation of China (81771713 and 82011530442); the Zhejiang Provincial Natural Science Foundation of China (LR18H030001).

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2021.100324

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