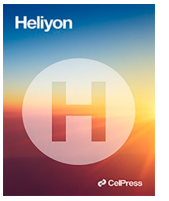
Heliyon 10 (2024) e24029

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Review article

Advances in crosstalk among innate immune pathways activated by mitochondrial DNA

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ARTICLE INFO

Keywords:
mtDNA
TLR9
NLRP3 inflammasome
cGAS-STING signaling pathway

ABSTRACT

Mitochondria are not only the power plant for intracellular oxidative phosphorylation and ATP synthesis, but also involved in cell proliferation, differentiation, signaling and apoptosis. Recent studies have shown that mitochondria play an important role in other pathophysiological functions in addition to cellular energy metabolism. Mitochondria release mitochondrial DNA (mtDNA) as a damage-associated molecular pattern (DAMP) to activate Toll-like receptor 9 (TLR9), NOD-, LRR-, and pyrin domain-containing 3 (NLRP3) inflammasome and cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) innate immune signaling pathways against foreign pathogenic microorganisms. The innate immune response not only promotes antimicrobial immune defense and regulates antiviral signaling, but their overactivation also induces the onset and progression of inflammatory diseases. In this paper, we review the role of mtDNA in the activation of innate immune signaling pathways and the crosstalk among innate immune signaling pathways activated by mtDNA, providing clues for the study of inflammatory diseases caused by mtDNA cytoplasmic translocation.

1. Introduction

After pathogenic microorganisms infect host, the organism develops innate and adaptive immune responses. The innate immune response constitutes the first defense of the organism against pathogenic attack through the release of non-specific bactericidal active substances and the activation of inflammatory signaling pathways in the early stage. The host recognizes pathogen-associated molecular patterns (PAMPs) such as nucleic acids, proteins, bacterial lipopolysaccharide (LPS) and flagella of pathogenic microorganisms through pattern-recognition receptors (PRRs) [1,2]. In addition, PRRs recognize damage-associated molecular patterns (DAMPs) such

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https://doi.org/10.1016/j.heliyon.2024.e24029
Received 20 April 2023; Received in revised form 17 December 2023; Accepted 2 January 2024
Available online 3 January 2024

as their own proteins or nucleic acids that are released when cells are damaged [2].

Mitochondria were first discovered in the 19th century and existing studies generally agree that mitochondria evolved over time from intracellular symbiotic bacteria and play an important role in the regulation of cellular homeostasis [3]. Mitochondria are the main sites of oxidative phosphorylation and ATP production in the cell, regulating a variety of biological processes within the cell [4]. When internal or external factors cause mitochondrial stress, mitochondria attempt to repair damaged mtDNA, degrade damaged proteins and lipids, and perform mitochondrial unfolded protein reactions to ensure normal mitochondrial structure and function. Once these rescue measures fail, mitochondria release mitochondrial matrix containing mtDNA [5]. mtDNA, which is located on the inner mitochondrial membrane as a genetic gene of mitochondria, is an important DAMP and contains a large amount of unmethylated CpG sequences [6]. Recent studies have shown that mtDNA is involved in a variety of innate immune signaling pathways, such as TLR-9, NLRP3 inflammasome and cGAS-STING pathways, by activating molecular defense mechanisms or inducing damage to the organism [7].The three innate immune signaling pathways activated by mtDNA are inextricably linked to each other.

In this paper, we review the close association between mtDNA and innate immunity and the crosstalk among the three widely studied innate immune signaling pathways activated by mtDNA, with a view to providing strategies for the prevention and treatment of inflammatory diseases mediated by the mtDNA-innate immune axes.

2. mtDNA

Mitochondria are involved in the regulation of cellular energy metabolism, apoptosis, calcium homeostasis, and reactive oxygen species (ROS) in cells [8]. Under normal physiological conditions, mitochondrial DNA is encapsulated in a mitochondrial bilayer structure. mtDNA is not a simple plasmid-like structure, super-resolution imaging shows that it is a dense nucleoid consisting of a number of proteins such as mitochondrial transcription factor A (TFAM), mitochondrial single-stranded DNA binding protein (mtSSB) and DNA polymerase γ [9]. Human mtDNA is approximately 16.6 kb long and consists of an outer-loop heavy (H) strand and an inner-loop light (L) strand. The H strand encodes 2 rRNAs, 14 tRNAs and 12 polypeptide genes, while the L strand encodes 8 tRNAs and 1 polypeptide gene. In addition, mtDNA has a short three-loop structure called the mtDNA control region (D-loop), which is the main control site for mtDNA expression [10]. Because of the lack of histone protection and the proximity to mitochondrial reactive oxygen species (mtROS), mtDNA is prone to oxidative damage, and an increase in the amount of damaged mtDNA to a certain level causes mitochondrial dysfunction and consequently cellular dysfunction and even tissue and organismal lesions, leading to disease [11]. In addition, mtDNA released from mitochondria into the cytoplasm and extracellular compartments activates TLR-9, NLRP3 inflammasome, and cGAS-STING innate immune signaling pathways, resulting in the production of interferons and inflammatory cytokines, and these signaling pathways interact with each other.

3. Mechanism of mtDNA release

The mtDNA is located in the mitochondrial matrix and must cross two barriers, the inner mitochondrial membrane and the outer mitochondrial membrane, to enter the cytoplasm [12]. Currently, many studies have shown a link between mitochondrial outer membrane permeabilization (MOMP) and mtDNA release. The Bcl-2 family of pro-apoptotic proteins, Bax and Bak, oligomerize at the mitochondrial outer membrane, induce MOMP, and mediate the release of cytochrome C and caspase activation, which ultimately causes apoptosis [13]. It is not clear about how the inner mitochondrial membrane is permeabilized and whether its permeability process is regulated.

The mechanism of mtDNA release in living cells is not uniform. At sublethal doses of stimulation, MOMP occurs only in a limited number of mitochondria and does not lead to cell death, a phenomenon known as minority MOMP that leads to DNA damage and genomic instability and mediates pro-inflammatory signaling to inhibit the growth of multiple pathogens in vivo [14,15]. Another possible mechanism is that oxidative stress stimulation is not sufficient to activate Bak and Bax proteins but leads to oligomerization of voltage-dependent anion channels (VDAC), which form large pores in the outer mitochondrial membrane and mediate the release of mtDNA fragments [16,17]. In addition, mtDNA interacts with positively charged residues in the N-terminal structural domain of VDAC1, promoting oligomerization of VDAC1 and forming a feed-forward loop that further increases the release of mtDNA [17].

Another potential mechanism for mtDNA release is through the mitochondrial permeability transition pore (mPTP), and cyclophilin D (CypD) is thought to be essential for the formation of mPTP [18,19].

4. mtDNA activates TLR9

Twenty-three years ago, Hemmi et al. [20] discovered that TLR9, a new member of the Tolls family, recognizes unmethylated CpG DNA and mediates its immunostimulatory activity. TLR9 is a type I transmembrane protein consisting of an N-terminal structural domain, a single transmembrane helix, and a C-terminal structural domain. MyD88 is the bridging molecule between TLR9 and other TLRs [21]. When the organism is stimulated by pathogenic microorganisms or injury, TLR-9 recognizes mtDNA released from mitochondria, migrates from the endoplasmic reticulum to the endosomal membrane, activates immune cells such as human polymorphonuclear neutrophils and macrophages, and exerts innate immunomodulatory effects by recruiting MyD88 to promote p38 MAPK phosphorylation in these immune cells, which subsequently causes the release of a series of inflammatory factors [22]. Pretreatment of neutrophils with the TLR9 antagonist oligodeoxynucleotide 2088 inhibits mtDNA-induced matrix metalloproteinase-8 (MMP-8) release and p38 MAPK activation [23,24]. Trauma or hemorrhagic shock allowed mtDNA to enter the circulatory system, recruit MyD88 via TLR9 to activate p38 MAPK, and release inflammatory factors such as TNF-α and IL-6, leading to the development of

systemic inflammatory response syndrome and organ damage [25]. Xie et al. [26] found that exogenous mtDNA reduced H9c2s cell viability through the TLR9-MyD88-MAPK pathway, and intravenous injection of mtDNA aggravated myocardial ischemia-reperfusion injury and increased infarct size in rats by activating this signaling pathway.

mtDNA also activates TLR9-MyD88-NF-κB signaling pathway to trigger inflammatory responses. It was found [27] that mtDNA induced acute lung injury by activating the TLR9-MyD88-NF-κB signaling pathway and causing the secretion of pro-inflammatory factors. Acute myocardial infarction led to increased release of circulating mtDNA, causing cardiomyocyte injury through activation of the TLR9-MyD88-NF-κB signaling pathway [28]. Data have shown that exosomes carrying plasma derived mtDNA are increased in patients with chronic heart failure. The inflammatory response is triggered through the TLR9-MyD88-NF-κB signaling pathway and the inflammatory effect is strongly correlated with the number of mtDNA copies in exosomes [29]. Increased mtDNA release in LPS-induced sepsis amplified the systemic inflammatory response syndrome by regulating the TLR9-MyD88-NF-κB pathway [30]. Dai et al. [31] found that the TLR9-MyD88-NF-κB signaling pathway is involved in finasteride treatment of collagenous arthritis by shifting the balance of Th1/Th2/Treg toward the Th2 type. Therefore, blocking the mtDNA-mediated TLR9-MyD88-MAPK/NF-κB pro-inflammatory pathway may be the key to treating inflammatory diseases.

Interestingly, foreign bodies also cause local trauma and tissue damage via mtDNA-TLR9-MyD88-NF-κB. It was found that tracheal intubation led to neutrophil migration and necrosis and that sustained release of mtDNA promoted TLR9-MyD88-NF-κB-dependent IL-8 release [32]. Another study [33] found that Foley catheter damages the bladder, leading to the release of mtDNA and the migration of neutrophils into the bladder cavity, causing TLR9 and NF-κB is activated. Therefore, inhibition of mtDNA-TLR9-MyD88-NF-κB signaling activation may help to combat medical device-induced mucosal injury, pain, and infection.

In addition, oxidized mtDNA (ox-mtDNA) has been shown to induce TLR9-and IRF7-dependent interferon α expression, thereby enhancing type I interferon (IFN–I) responses in plasmacytoid dendritic cells [34].

In summary, mtDNA cytosolic release activates TLR9 receptor, which play an important role in the development of many inflammatory diseases. It is important to note that mtDNA causes sterile inflammation in a TLR9-dependent manner only in its free form and by phagocytosis into endosomes [2,35,36], so whether non-free mtDNA activates TLR9 under physiological conditions and its role in inflammation is not yet known.

5. mtDNA activates NLRP3

NLRP3 inflammasome is one of members of the NOD-like receptor (NLR) family, consisting of NLRP3, a junction protein (ASC), and caspase-1. NLRP3 inflammasome recognize a wide range of endogenous or exogenous stimuli, including bacterial, fungal, and viral DNA or RNA [37]. When mitochondrial stress occurs, mtDNA released within the cytoplasm induces the activation of NLRP3 inflammasome. Thirteen years ago, a study by Nakahira et al. [38] first linked mtDNA to NLRP3 inflammasome activation. This study found that when mouse bone marrow-derived macrophages were stimulated by LPS and ATP, excess mitochondrial reactive oxygen species (ROS) contributed to permeability changes in mitochondrial membrane potential, leading to more ROS production by mitochondrial uncoupling, promoting the release of mtDNA into the cytoplasm and inducing the activation of NLRP3 inflammasome, resulting in IL-1β and IL-18 secretion is increased.

In 2012, Shimada et al. [39] found that ox-mtDNA activated the NLRP3 inflammasome during apoptosis. In contrast, reduction of intracytoplasmic mtDNA inhibited the activation of NLRP3. TFAM gene deletion depletes more than 95 % of mtDNA in mouse macrophages, resulting in suppressed activation of NLRP3 inflammasome. However, exogenous transfection of ox-mtDNA in knockout TFAM macrophages promotes the activation of suppressed NLRP3 inflammasome [36], suggesting that ox-mtDNA effectively activated NLRP3 inflammasome.

Wu et al. [40] investigated the therapeutic effects of MitoQ (a mitochondria-targeted antioxidant) on liver injury by establishing a burn plus delayed resuscitation (B + DR) rat model and a hypoxia/reoxygenation (H/R) injury kupffer cells (KCs) model. The results revealed that B + DR caused liver injury and oxidative stress. Excessive ROS activated the mtDNA-NLRP3 pathway by disrupting mitochondrial integrity in KCs, leading to liver injury. ox-mtDNA was released into the cytoplasm during KCs apoptosis, directly activating NLRP3 inflammasome. MitoQ contributed to liver injury by scavenging ROS, maintaining mitochondrial integrity, reducing KCs apoptosis, and inhibiting mtDNA-NLRP3 pathway activation.

It has been found [41] that Atrazine, which is widely used in agricultural production and is toxic to humans, promotes ox-mtDNA formation. The release of ox-mtDNA activates NLRP3 inflammasome and induces spleen pyroptosis. Lycopene, a carotenoid and a natural bioactive component of fruits and vegetables, reduces oxidative stress and ameliorates Atrazine-induced spleen pyroptosis by depleting ox-mtDNA to prevent the activation of NLRP3 inflammasome.

New research suggests that the ROS-mtDNA-NLRP3 pathway is also involved in regulating thermogenesis in brown adipose tissue (BAT). Huang et al. [42] used BAT-specific thioredoxin-2 (TRX2) (a protein that scavenges mitochondrial ROS) deficient mice to assess the effects of the ROS-mtDNA-NLRP3 pathway in BAT on metabolism and thermogenesis. They found that TRX2 deletion improved the metabolic performance of the body by enhancing lipid uptake, thereby protecting mice from high-fat diet-induced obesity, hypertriglyceridemia, and insulin resistance. In addition, TRX2 deficiency impairs adaptive thermogenesis by inhibiting fatty acid oxidation. Mechanistically, TRX2 deletion caused an excess of mitochondrial ROS and disruption of mitochondrial integrity, which in turn led to mtDNA cytoplasmic release and activation of the NLRP3 inflammasome in BAT. In contrast, NLRP3 inhibition reversed the metabolic benefits and thermogenesis defects in BAT-specific Trx2-deficient mice fed a high-fat diet. This finding reveals a link between BAT inflammation and metabolism and broadens the functional spectrum of the ROS-mtDNA-NLRP3 pathway. Therefore, the degree of oxidative damage to mtDNA seems to be particularly important for the activation of NLRP3 inflammasome.

6. mtDNA activates cGAS-STING signaling pathway

In addition to interacting with TLR-9 and NLRP3 inflammasome, mtDNA activates cGAS-STING signaling pathway. The release of mtDNA into the cytoplasm during cell death or mitochondrial damage activates cGAS and catalyzes the synthesis of cGAMP from ATP and GTP [43]. cGAMP binds as a second messenger and activates STING on the endoplasmic reticulum [44], and activated STING binds to TANK binding kinase 1 (TBK1), which drives phosphorylation of interferon regulatory factor 3 (IRF3), thereby inducing IFN-I as well as interferon-stimulated genes (ISGs) expression, ultimately enhancing the innate immune response [45–47]. In addition, STING binding to TBK1 activates NF-κB, which in turn induces the expression of pro-inflammatory cytokines such as TNF and IL-1β [43]. It was found that cGAS is a major sensor of viral and bacterial DNA in the cytoplasm of infected cells [44,48].

The mtDNA-cGAS-STING pathway plays an important role in pathogenic infections. Dengue virus infection causes the release of ox-mtDNA into the cytoplasm, which activates the cGAS-STING innate immune signaling pathway [49]. However, in the long game between virus and host, dengue virus achieves immune escape by encoding proteases that target cGAS and STING for degradation, thus

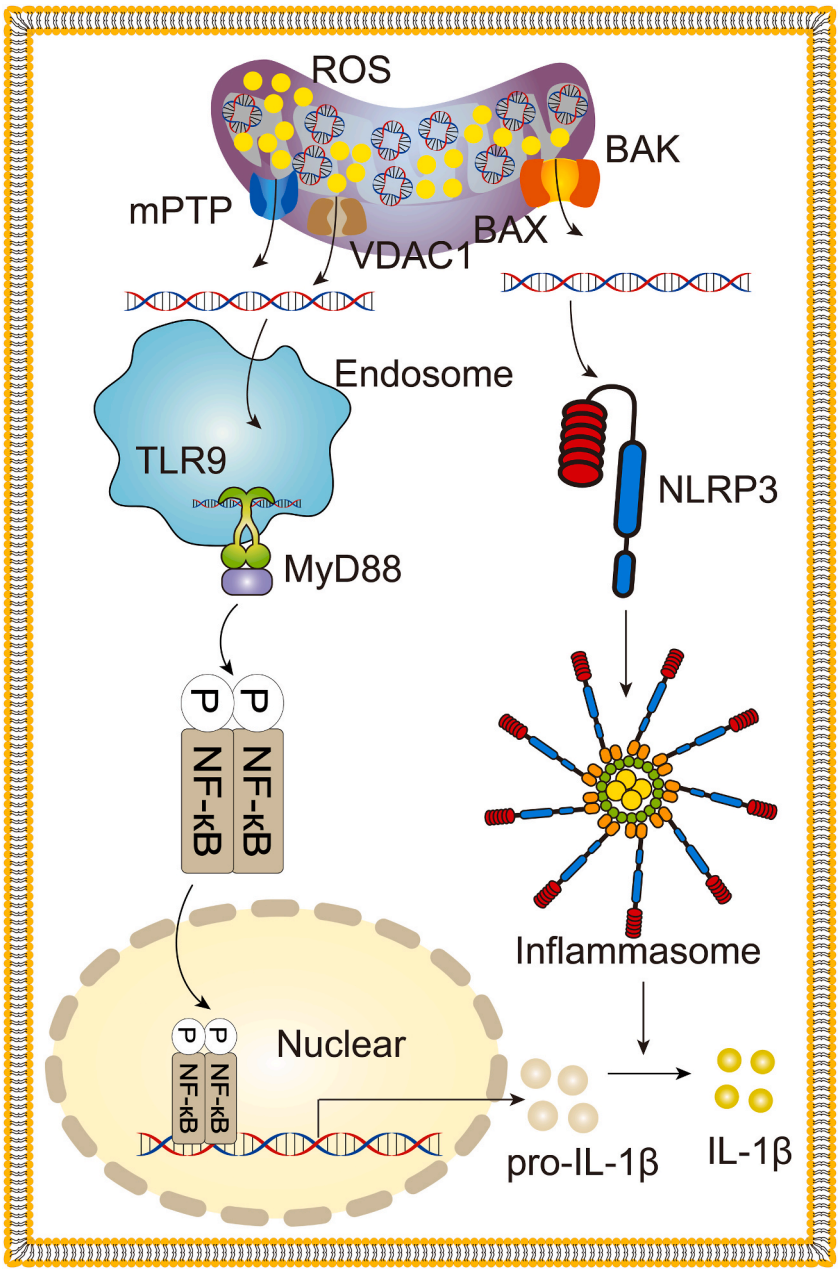

Fig. 1. Crosstalk between TLR9 and NLRP3 pathways activated by mtDNA.

ensuring the persistence of viral infection [50]. Moriyama et al. [51] found that the influenza A virus M2 protein caused the release of mtDNA into the cytoplasm in a MAVS-dependent manner and activates the cGAS-STING innate immune response to limit virus replication in the host. However, the nonstructural protein 1 (NS1) of influenza virus also binds to mtDNA to evade STING-dependent antiviral immunity.

It has been shown that HSV-1 and HSV-2 infection of cells causes mtDNA stress and a rapid decrease in mtDNA copy number. HSV-1 mutant strains lacking the targeted mtDNA gene are less efficient in triggering antiviral responses and expression of ISGs [52,53], which may indicate that cellular monitoring of mtDNA homeostasis is an evolutionarily beneficial mechanism that cooperates with the typical sensing of viral nucleic acids to fully participate in antiviral innate immunity. However, the host IFN-I response also enhance the pathogenesis of certain microorganisms. Unlike herpes simplex virus infection, infection of mouse macrophages by Mycobacterium bovis increases the copy number of mtDNA, leading to the release of mtDNA into the cytoplasm to induce IFN-β production, which facilitates bacterial survival. Interference with TFAM expression by siRNA inhibits the increase in mtDNA copy number and reduces

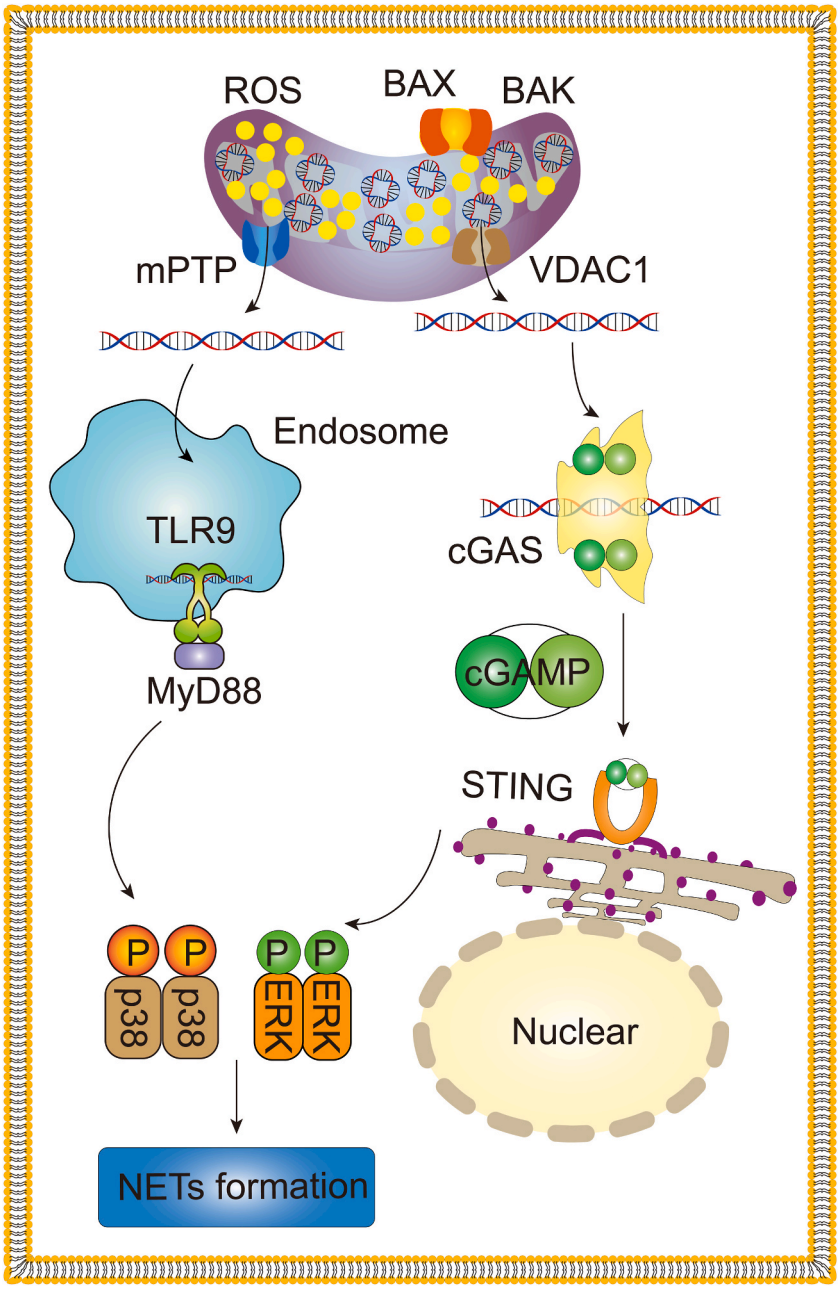

Fig. 2. Crosstalk between TLR9 and cGAS-STING pathways activated by mtDNA.

the amount of mtDNA entering the cytoplasm, thereby inhibiting bacterial survival [54]. *Mycobacterium tuberculosis* infection triggers cGAS-STING signaling and mitochondrial stress, which induces the expression of IFN-I and ISGs, thereby favoring self-survival [48,55,56]. These results suggest that mitochondrial damage and mtDNA release may be a strategy used by Mycobacterium bovis or *Mycobacterium tuberculosis*, as well as other microorganisms, to promote cGAS activation, increase IFN-I response and enhance intracellular survival.

7. TLR9 and NLRP3

Previous studies on NLRP3 have focused on cytoplasmic oxidation of mtDNA, and it has been shown that extracellular mtDNA induces the initiation and activation of NLRP3 inflammasome via TLR9-p38MAPK–NF–κB in THP-1 macrophages, which leads to lung inflammation and injury [57]. The interaction of mtDNA and TLR9, p38 MAPK phosphorylation and NF-κB activation was shown to be critical in extracellular mtDNA-induced NLRP3 inflammation initiation and activation. Although it has been previously demonstrated that TLR9 is supposed to be a key upstream molecule for the activation of NLRP3 inflammasome [58–60], there is still little evidence that can explain the mechanism between them, especially in the context of mtDNA stimulation. Therefore, this study elucidated the role of TLR9 and its downstream molecules in mtDNA-induced NLRP3 inflammation triggering and activation. Future studies could focus on validating the proportion of extracellular unoxidized mtDNA in different disease states.

Lu et al. [61] found that the mtDNA-mediated TLR9-NF-κB-NLRP3 axis is activated in oxidative stress-induced pyroptosis in human nucleus pulposus cell (NPC) in vitro. Mechanistically, Oxidative stress induces mitochondrial damage, leading to mPTP opening and excessive release of mtDNA into the cytoplasm. In addition, this study showed that TLR9 recognition and binding of mtDNA is essential for the activation of NF-κB and NLRP3 inflammasome. However, their results did not exclude a role for cGAS and AIM2 in recognizing their own DNA under oxidative stress. In short, it is unclear which factors determine the activation of DNA receptors and the signal strength or duration of these factors in activating DNA receptors.

Consistent with previous studies, it was found that the endogenous ligands of TLR9, CpG-ODN and MSU crystals, contribute to TLR9-mediated NF-κB signaling and NLRP3 inflammasome activation in mouse monocyte/macrophage cell lines. And mtDNA is responsible for TLR9 activation [62]. Thus, TLR9 is a key therapeutic target to help control uric acid-induced inflammatory diseases such as gout.

A recent study reported that ox-mtDNA is increased in myelodysplastic syndrome (MDS) plasma. Interestingly ox-mtDNA does not directly activate NLRP3 inflammasome, but indirectly activates NLRP3 through the TLR9-MyD88 pathway. this may be related to the proportion as well as spatial distribution of ox-mtDNA. In addition, extracellular ox-mtDNA also induced TLR9 redistribution to the cell surface in MDS hematopoietic stem and progenitor cells (HSPCs) [63]. This study demonstrates that TLR9 is necessary for ox-mtDNA-mediated activation of inflammasome, and therefore, blocking the ox-mtDNA/TLR9 axis is expected to be a new therapeutic tool for MDS (Fig. 1).

8. TLR9 and cGAS

Neutrophil extracellular traps (NETs) are neutrophil responses to exogenous bacteria, viruses, and other pathogens, which provide a substrate to trap and kill microorganisms [64]. mtDNA is the major structural component of NETs [65,66], which induces post-traumatic inflammation and activates neutrophils [67]. The researchers found that mtDNA and ox-mtDNA were released and induced the formation of NETs and sterile inflammation in a model of acute peripheral tissue trauma or lung injury and that Ox-mtDNA was more effective. Mechanistically, mtDNA triggered NET formation through activation of STING and TLR9 as well as ERK1/2 and p38 MAPK signaling pathways. In addition, mtDNA induces NET formation through store-operated calcium entry (SOCE) signaling [68]. Thus, STING and TLR9 may play a synergistic role in the induction of NETs by mtDNA (Fig. 2).

The relationship between TLR9 and cGAS is poorly understood and requires extensive experiments to explore.

9. NLRP3 and cGAS

Mitochondrial autophagy is a cellular adaptive response to degrade dysfunctional mitochondria through autophagosomes to maintain normal cellular function. Liu et al. [69] found that TAA- and LPS-induced acute liver injury (ALI) activated XBP1 in hepatocytes, but hepatocyte-specific XBP1 knockout mice developed more severe ALI as evidenced by increased hepatocyte pyroptosis and enhanced macrophage cGAS-STING activation. ROS and mtDNA were increased in TAA-treated hepatocytes, and mtDNA was released outside the cell. Mechanistically, hepatocyte XBP1 deficiency activates NLRP3-caspase-1-GSDMD signaling by impairing mitochondrial autophagy to increase ROS production to promote hepatocyte pyroptosis, thereby activating mtDNA-cGAS-STING signaling in macrophages. Although TAA pretreatment is dispensable in the activation of STING signaling by mtDNA, it is worthwhile to further investigate whether TAA treatment modifies mtDNA. Ox-mtDNA promotes NLRP3 inflammasome activation and inflammation, but whether ox-mtDNA activates cGAS-STING signaling still deserves further exploration. Researchers previously found that hexafluoropropylene oxide trimer acid (HFPO-TA) induced excess mtDNA release [70]. In HFPO-TA-induced hepatotoxicity, mtROS was found to be an upstream regulatory target of the cGAS-STING-NLRP3 pathway, which induces the development of hepatic fibrosis through pyroptosis in mice [71]. However, whether mtROS regulates this pathway by oxidizing mtDNA is unknown. Interestingly, in another study with opposite results, Wang et al. [72] found that XBP1 was highly expressed in macrophages of mice and patients with liver fibrosis and that XBP1 myeloid-specific deletion or pharmacological inhibition inhibited mtDNA cytoplasmic translocation and cGAS-STING-NLRP3 pathway activation by promoting BNIP3-mediated mitochondrial autophagy activation in macrophages.

However, BNIP3 knockdown reversed these effects. Moreover, macrophage XBP1-STING-NLRP3 signaling also promoted the activation of hepatic stellate cells. This suggests that mtDNA-mediated activation of cGAS-STING signaling contributes to the activation of NLRP3 inflammasome in liver fibrosis. Unique to this study is the elucidation of the activation of cGAS-STING-NLRP3 signaling in macrophages by cytoplasmic translocation of the macrophage's own mtDNA. There are two possible reasons for these different results: first, different disease models may have opposite signaling crosstalk; second, the signaling crosstalk in macrophages themselves is different from that between hepatocytes and macrophages.

In nucleus pulposus (NP) cells from patients with low back pain (LBP), oxidative stress led to the opening of mPTP and cytoplasmic overaccumulation of mtDNA induced cGAS-STING axis activation and NLRP3 inflammasome-mediated pyroptosis. mPTP inhibition effectively reduced NLRP3 inflammasome-mediated NP cell death and tissue microenvironment inflammation [73]. Thus, the cGAS-STING-NLRP3 axis provides a promising therapeutic approach for discogenic LBP. However, this study does not exclude the possibility that ox-mtDNA in NP cells directly binds to NLRP3 inflammasome, as reducing mPTP opening and cytoplasmic release of ox-mtDNA also inhibits this interaction. mtDNA also activates the AIM2 inflammasome and leads to cellular pyroptosis [74]. However, this study also does not exclude a possible role for AIM2 inflammasome in response to oxidative stress. The data suggest that the cGAS-STING axis is more sensitive to recognize cytoplasmic DNA than AIM2 [75]. Therefore, factors other than the level of

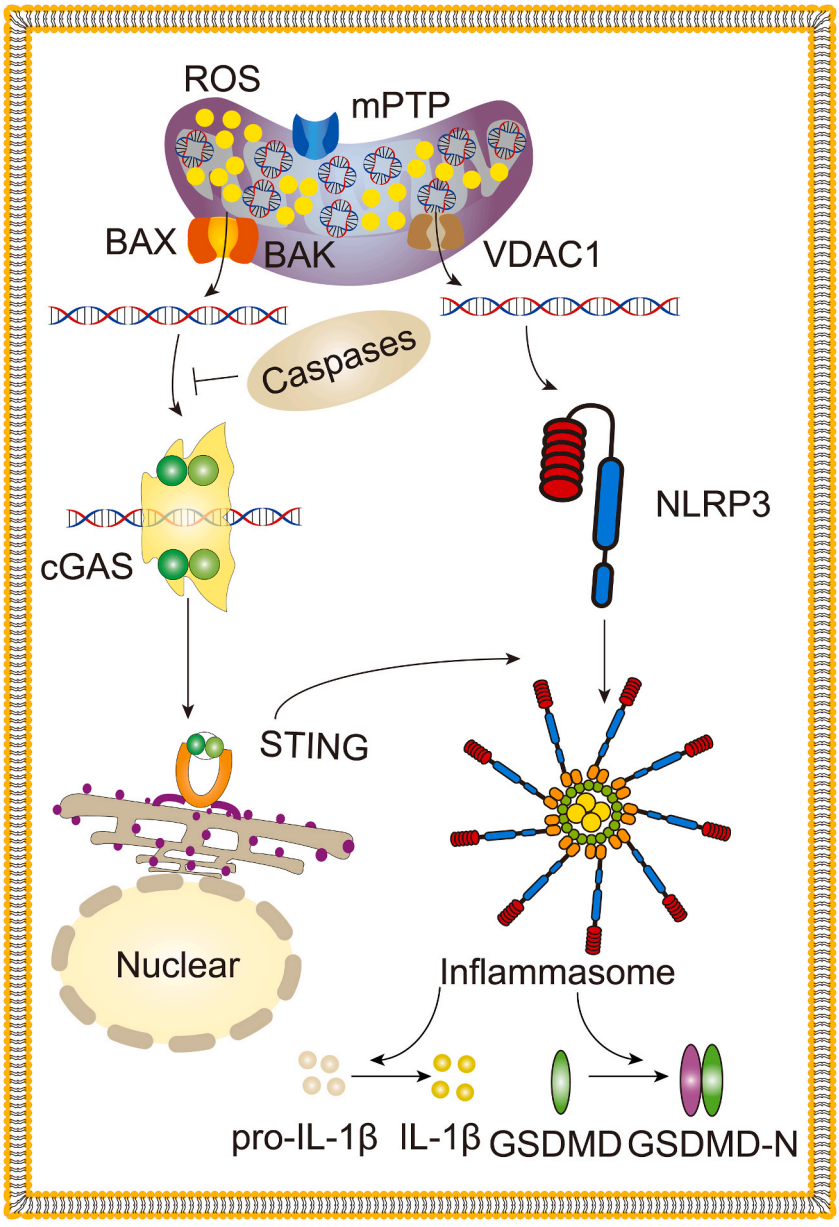

Fig. 3. Crosstalk between NLRP3 and cGAS-STING pathways activated by mtDNA.

cytoplasmic DNA affecting the signal intensity or signal duration of cytoplasmic DNA activation remain to be further investigated.

Interestingly, in Bak/Bax-mediated apoptosis, activated caspases inhibit the mtDNA release-activated cGAS-STING signaling pathway, thereby inhibiting IFN-I production. Possible mechanisms include caspases cleaving and inactivating one or more components of the IFN-I production pathway, such as IRF3 [76], and, in addition, cystatinases mediating the degradation of mtDNA, thereby preventing its interaction with cGAS [77,78]. Although the exact mechanism that inhibits IFN-I production in apoptotic cells remains to be determined, it should be a dense network of multiple mechanisms. What is certain is that caspases-mediated acceleration of cell death and inhibition of IFN-I signaling are inextricably linked.

Although the crosstalk between NLRP3 inflammasome and the cGAS-STING pathway has been extensively studied, many controversial and pressing questions remain (Fig. 3).

10. Other agents

Not only mtDNA, but other agents activate innate immune signaling pathways. For example, Mn^{2+} released from mitochondria and Golgi binds directly to cGAS, which enhances cGAS enzyme activity as well as sensitivity to dsDNA, and in addition, Mn^{2+} enhances cGAMP-STING binding affinity and thus STING activity, which in turn aids in host defense against DNA viral attack [79]. Another study showed that Mn^{2+} enhances the sensitivity of cGAS to the large amount of dsDNA released by β-lapachone-induced apoptosis of tumor cells, which in turn enhances STING signaling and triggers downstream immunostimulatory signals against poorly immunogenic solid tumors [80]. In addition, Mn^{2+} induces NLRP3 inflammasome activation via the cGAS-STING-NLRP3 axis, which promotes IL-1β maturation and exacerbates neuroinflammation [81]. In addition to Mn^{2+}, Huang et al. [82] found that internalized bacterial lipopolysaccharide (LPS) activated the pore-forming protein Gasdermin D, which led to the formation of mitochondrial pores and the release of mtDNA into the cytoplasm of the endothelial cells. cGAS recognizes the mtDNA and produces cGAMP, which inhibits the proliferation of endothelial cells through the downregulation of the YAP1 signaling pathway. Another study found that LPS induced mitochondrial dysfunction in endometrial mesenchymal cells through activation of the cGAS-STING pathway, induced mtDNA leakage, and promoted endometritis [83]. In addition, Interleukin-1β [84], palmitic acid (PA)-induced Lipotoxicity [85], TNF [86], and Electronic Cigarettes [87] are all involved in a variety of physiological and pathological processes through the activation of innate immune signaling pathways by mtDNA. Therefore, we suggest that mtDNA may act as a hub for other agents to activate innate immune signaling pathways.

11. Concluding remarks

Mitochondria are multifunctional organelles that are key hubs of cellular metabolism and signaling. There is growing evidence that mitochondria are involved in the innate immune response to pathogen infection and cell injury as an important source of endogenous DAMPs. mtDNA properties such as relative hypomethylation, unique structural features and high sensitivity to oxidative damage make it a potent mitochondria DAMP that activates TLR9, NLRP3 inflammasome, cGAS and other innate immune sensors, thereby triggering pro-inflammatory processes and IFN-I responses, and playing an important role in the development of pathogenic infections and inflammatory diseases. However, a number of questions remain to be further investigated. Firstly, why are mitochondria alone among the numerous organelles in the cell so closely related to innate immunity. In addition, although there have been some studies on how mtDNA is released into the cytoplasm or outside the cell, it is still only the tip of the iceberg and controversial. Moreover, the mechanism of how mtDNA specifically activates different innate immune sensors is still unclear. Fourth, whether and how different mitochondrial stressors activate different mtDNA recognition pathways in balance. Last but not lest, the potential mechanisms of crosstalk among different mtDNA recognition pathways are poorly understood. The revelation of these questions can help us to deeply explore the role played by the mtDNA pathway in the host innate immune system, and provide new targets and ideas for the treatment of diseases caused by mtDNA molecular signaling.

Funding

This research was funded by Hunan Provincial Natural Science Fund (No.2021JJ40495, 2022JJ30526, 2022JJ30530), Clinical Medical Technology Innovation Guidance Project from Department of Science and Technology of Hunan Province (No.2020SK5181, 2021SK51812) and Scientific Research Fund Project of Hunan Provincial Health Commission (No.202105010225, 20201950), Science and Technology Research Project (Basic Applied Research Program) from Department of Science and Technology of Hengyang City (No.202150063929).

Data availability statement

No data was used for the research described in the article.

CRediT authorship contribution statement

Guangwei Tao: Writing - review & editing, Writing - original draft. **Wenyan Liao:** Writing - review & editing. **Jiafeng Hou:** Software. **Xinmiao Jiang:** Writing - review & editing. **Xin Deng:** Software. **Guodong Chen:** Writing - review & editing, Supervision. **Chengming Ding:** Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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