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Mitochondrial DNA leakage: underlying mechanisms and therapeutic implications in neurological disorders

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Abstract

Mitochondrial dysfunction is a pivotal instigator of neuroinflammation, with mitochondrial DNA (mtDNA) leakage as a critical intermediary. This review delineates the intricate pathways leading to mtDNA release, which include membrane permeabilization, vesicular trafficking, disruption of homeostatic regulation, and abnormalities in mitochondrial dynamics. The escaped mtDNA activates cytosolic DNA sensors, especially cyclic gmp-amp synthase (cGAS) signalling and inflammasome, initiating neuroinflammatory cascades via pathways, exacerbating a spectrum of neurological pathologies. The therapeutic promise of targeting mtDNA leakage is discussed in detail, underscoring the necessity for a multifaceted strategy that encompasses the preservation of mtDNA homeostasis, prevention of membrane leakage, reestablishment of mitochondrial dynamics, and inhibition the activation of cytosolic DNA sensors. Advancing our understanding of the complex interplay between mtDNA leakage and neuroinflammation is imperative for developing precision therapeutic interventions for neurological disorders.

Keywords mtDNA leakage, Neuroinflammation, DNA sensors, Innate immunity, Neurological disorders

Introduction

Mitochondria are pivotal organelles within cells that are primarily responsible for energy production and also play a critical role in regulating immune responses and cell death pathways [1]. Their multifaceted functions have established mitochondria as a central player in the pathogenesis of various diseases, with a particular influence on inflammatory conditions [2]. Inflammatory responses are initiated by innate immune cells that detect pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) through pattern recognition receptors (PRRs). Mitochondrial components, due to their evolutionary bacterial origin, can act as DAMPs, with mitochondrial DNA (mtDNA) being a notable example. Under normal conditions, mtDNA is confined within the mitochondrial matrix, shielded from cytosolic PRRs by the inner mitochondrial membrane (IMM) and the outer mitochondrial membrane (OMM).

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Upon mitochondrial stress or damage, mtDNA can leak into the cytosol through channels in the mitochondrial membrane, such as the open mitochondrial permeability transition pore (mPTP) or BAX/BAK macropores composed of bcl-2-associated x protein (BAX) and bcl-2 homologous antagonist/killer (BAK), this process is also known as mtDNA escape, mtDNA release, mtDNA efflux, and mtDNA extrusion [3]. The escaped mtDNA activates cytosolic DNA sensors, triggering innate immune responses and contributing to the immunological cascade [4, 5]. Consequently, mtDNA leakage emerges as a pivotal trigger in the inflammation induced by mitochondrial stress [6].

Given the high energy demands of neuronal function, cells within the nervous system are particularly enriched with mitochondria, rendering them acutely vulnerable to mitochondrial dysregulation. Therefore, the escape of mtDNA can have disastrous consequences for neurons [7, 8]. Emerging research robustly links mitochondrial damage to many inflammatory neurological disorders, suggesting that mtDNA leakage is a common phenomenon in these conditions [9]. Notably, the role of mtDNA escape in neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD) is significant, and inhibiting this process has been shown to mitigate neuroinflammation [10–12]. Moreover, the contribution of mtDNA escape and the subsequent activation of PRRs are gaining recognition in conditions such as ischemic stroke, intracerebral hemorrhage, brain injury, and neuropathic pain, highlighting their immunomodulatory roles in these pathologies [13, 14]. Therefore, a thorough synthesis of the mechanisms underlying mtDNA leakage is essential for advancing our understanding and therapeutic strategies for neurological diseases.

This review provides a comprehensive examination of the mechanisms of mtDNA leakage and its implications in neurological diseases. We focus on the significant impact and therapeutic potential of mtDNA leakage in neurodegenerative diseases, strokes, traumatic brain injury, and its role in cellular senescence. The aim is to elucidate the critical role of mtDNA leakage as a bridge between mitochondrial damage and subsequent inflammatory responses, offering a theoretical framework and insights for the development of therapeutic strategies.

Complex mechanisms underlying mtDNA release

MtDNA, a circular double-stranded molecule (~ 16.6 kb), typically resides within the mitochondrial matrix in the form of mitochondrial nucleoids, and plays a crucial role in cellular energy production through the oxidative phosphorylation system [15]. Neurons, with their high energy demands, possess an abundance of mtDNA copies [16]. The mtDNA molecule undergoes continuous cycles of repair and renewal, maintaining its integrity through a delicate balance of DNA polymerases, repair enzymes, and mitochondrial transcription factor a (TFAM). Dysregulation of this molecular network compromises the stability of mtDNA, thereby Leading to mtDNA escape [17, 18]. Furthermore, aberrations in mitochondrial dynamics, including fission and fusion, are also implicated in the extrusion of mtDNA [19]. mitochondrial inner membrane permeabilization (MIMP) and mitochondrial outer membrane permeabilization (MOMP), primarily involving mPTP pore opening, BAX/BAK macropore formation, and voltage-dependent anion channel (VDAC) oligomer pore, are critical for mtDNA release [20]. MtDNA, once released, functions as a DAMP to activate cytosolic DNA PRRs and initiate innate immune responses.

Mitochondrial membrane permeabilization

Mitochondrial membrane permeabilization is defined by MIMP due to mPTP opening and disturbance in mitochondrial cristae structure, and MOMP caused by the formation of BAX/BAK macropores on the OMM, coupled with VDAC oligomerization and the activation of GSDMD pore (Fig. 1). Mitochondrial membrane permeabilization typically serves as the primary pathway for mtDNA release from the mitochondrial membranes to the cytoplasm [21].

MIMP

The opening of mPTP The opening of the mPTP is a pivotal indicator of mitochondrial stress, commonly functioning as a pathway in the IMM for the release of cytochrome c and other apoptotic factors. Moreover, mPTP opening has been linked to mtDNA leakage [22], a finding supported by multiple studies [23, 24]. For instance, the pharmacological blockade of mPTP with cyclosporin significantly prevents mtDNA release in the nucleus pulpous cells, indicating its decisive role in mtDNA leakage [25]. Normally sequestered near the inner membrane, mtDNA may rely on mPTP as a primary barrier against leakage. However, as mPTP opening often marks the onset of mitochondrial apoptosis, it is crucial to determine if mtDNA release is coincident with the apoptotic process. Previous research suggests that apoptotic caspases may mitigate inflammation from mtDNA leaks by inhibiting the cyclic gmp-amp synthase (cGAS) signaling and stimulator of interferon genes (STING) signaling [26]. This implies that the inflammatory cascade initiated by mtDNA release through mPTP may be modulated indirectly by caspase activity, albeit the precise mechanism linking caspases to mtDNA leakage remains to be elucidated.

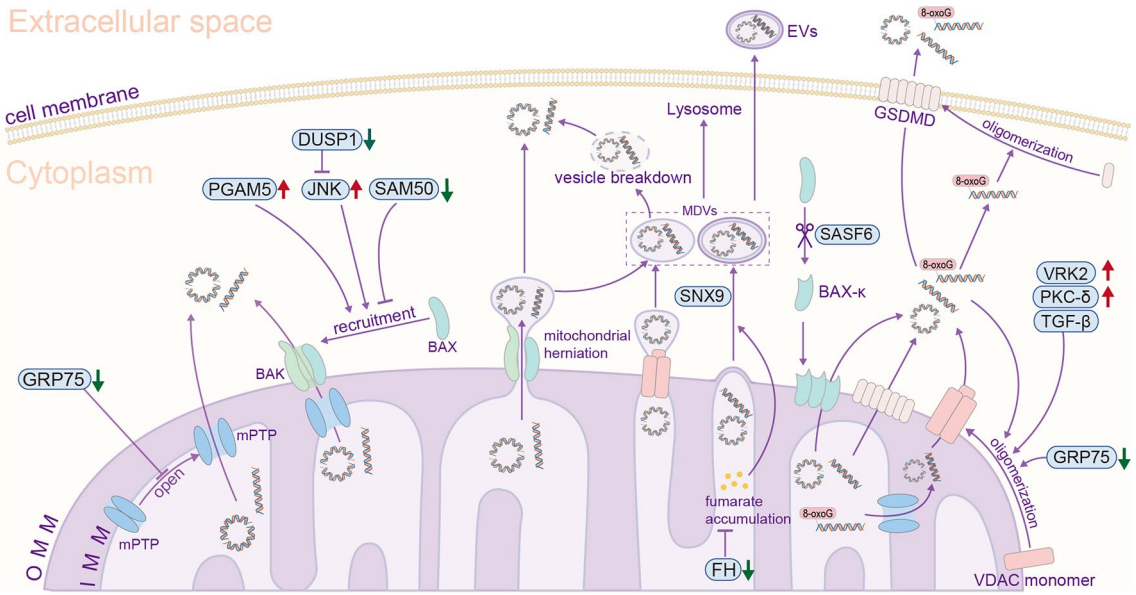

Fig.1 Mitochondrial membrane permeabilization results in mtDNA leakage. The opening of the mPTP induces MIMP, leading to mtDNA release, a process that is further intensified by GRP75 deficiency. The formation of BAX/BAK macropores initiates MOMP, allowing greater amounts of mtDNA to be released into the cytosol, while PGAM5, the DUSP1/JNK pathway, and the reduction of SAM50 facilitate the recruitment of BAX to the mitochondrial membrane. BAX/BAK macropores can also cause the extrusion of the IMM, leading to mitochondrial herniation, which may directly result in IMM rupture and the release of mtDNA. Alternatively, this process can lead to the formation of MDV that transport mtDNA to lysosomes or the extracellular space. The rupture of MDV during this process can also lead to the release of mtDNA into the cytosol. MDV can also bud directly from the IMM, a process that depends on the SNX9 protein. This process is further enhanced by fumarate accumulation resulting from FH deficiency. Following the selective splicing of SASF6, BAX forms the BAX-κ splice variant, whose accumulation on the membrane also contributes to mtDNA leakage. VDAC oligomeric pores mediate mtDNA leakage by inducing MOMP. The presence of mtDNA in the intermembrane space and ox-mtDNA in the cytosol promotes VDAC oligomerization. Similarly, VRK2, PKC-δ, and TGF-β contribute to this process, while GRP75 deficiency indirectly facilitates VDAC aggregation. GSDMD forms pores in the mitochondrial or cellular membranes, allowing the release of mtDNA into the cytosol or extracellular space, with ox-mtDNA also promoting the formation of GSDMD pores

Disturbance in Mitochondrial Cristae Structure Mitochondrial cristae, formed by the invagination of the IMM, serve as the primary site for oxidative phosphorylation and are known as the "Dynamic Biochemical Reactors" in mitochondrial bioenergetics [27]. The proper folding and maintenance of mitochondrial cristae rely on the functional integrity of various regulatory, including optic atrophy 1 (OPA1), sorting and assembly machinery component 50 (SAM50), and other IMM dynamics-related proteins, as well as structural proteins such as the MICOS complex and prohibitin. These regulatory proteins are essential for maintaining the integrity of the IMM, while the structural proteins contribute to the assembly of cardiolipin, a crucial component of the IMM [28]. Mitochondrial cristae act as "sentinels" against mtDNA leakage, with damage to critical regulators of the cristae structure, such as OPA1 and SAM50, persistently triggers mtDNA release and cGAS pathway activation, linking cristae disruption to mtDNA escape in neurodegenerative and age-related diseases [29].

In AD models, reduced mitochondrial OPA1 expression contributes to pathology [30], with OPA1 overexpression shown to reduce mtDNA leakage in neurons, implying its therapeutic potential in suppressing mtDNA-induced inflammation [31]. Recent research also attests to the intricate role of the regulatory protein SAM50 in mtDNA leakage. For example, depletion of SAM50 leads to cardiolipin exposure, which not only disrupts the mitochondrial cristae membrane, facilitating mtDNA clustering (a harbinger of mtDNA leakage) but also enables the formation of BAX/BAK macropores, allowing mtDNA to escape into the cytoplasm [32]. Thus, preserving the stable expression of SAM50 is essential for maintaining cristae integrity and inhibiting mtDNA leakage, positioning it as a potential therapeutic target [33]. Similarly, the depletion of prohibitin 1 (PHB1), part of the inner membrane protein complex, can perturb mitochondrial homeostasis in macrophages and promote mtDNA cytosolic leakage via mPTP and VDAC-dependent channels, subsequently triggering inflammatory responses [34]. Furthermore, the knockout of the inner membrane protein Mitofilin results in cristae damage and mPTP opening, causing mtDNA leakage [35]. In general, the stability of mitochondrial cristae is

critical for preventing mtDNA leakage, and cristae disarray promotes mtDNA leakage through various pathways by inducing mtDNA clustering and enhancing mitochondrial membrane permeability (Fig. 2).

MOMP

BAX/BAK macropores The anti-apoptotic Bcl-2 family proteins BAX is found in the cytoplasm of quiescent cells as monomers. When abnormal signals, such as apoptotic or mitochondrial damage signals, are received, they undergo conformational changes, translocation to the OMM and binding with BAK on the membrane, and oligomerize to form BAX/BAK macropores, which increase the permeability of the OMM [36]. Unlike mPTP pores, BAX/BAK macropores permit passage of larger molecules, possibly resulting in the extrusion of IMMs and the formation of mitochondrial hernias, leading to the loss of IMM integrity in the cytoplasm and the release of mtDNA [37]. The finding indicates that the opening of mPTP does not fully determine mtDNA escape. Further research confirms that BAX/BAK-induced mitochondrial hernias can also trigger MIMP independently of mPTP opening, leading to the extrusion of the mitochondrial nucleoid into the cytoplasm through BAX/BAK macropores [20]. Therefore, BAX/BAK macropores facilitate the release of not just mtDNA fragments, but the entire mitochondrial nucleoid into the cytoplasm independently of mPTP. In addition, the injection of mtDNA into the vitreous body can promote the transcription of BAX and BAK, suggesting that mtDNA escape may facilitate the opening of BAX/BAK macropores [38].

Moreover, BAX/BAK macropores also influence the dynamics of mtDNA escape. BAK forms smaller pores more rapidly than BAX, leading to a quick release of mtDNA, while simultaneously promoting BAX oligomerization to assemble larger pores, accelerating the sustained escape of mtDNA [39]. It has been shown that BAX/BAK-dependent MOMP is a critical step for cellular

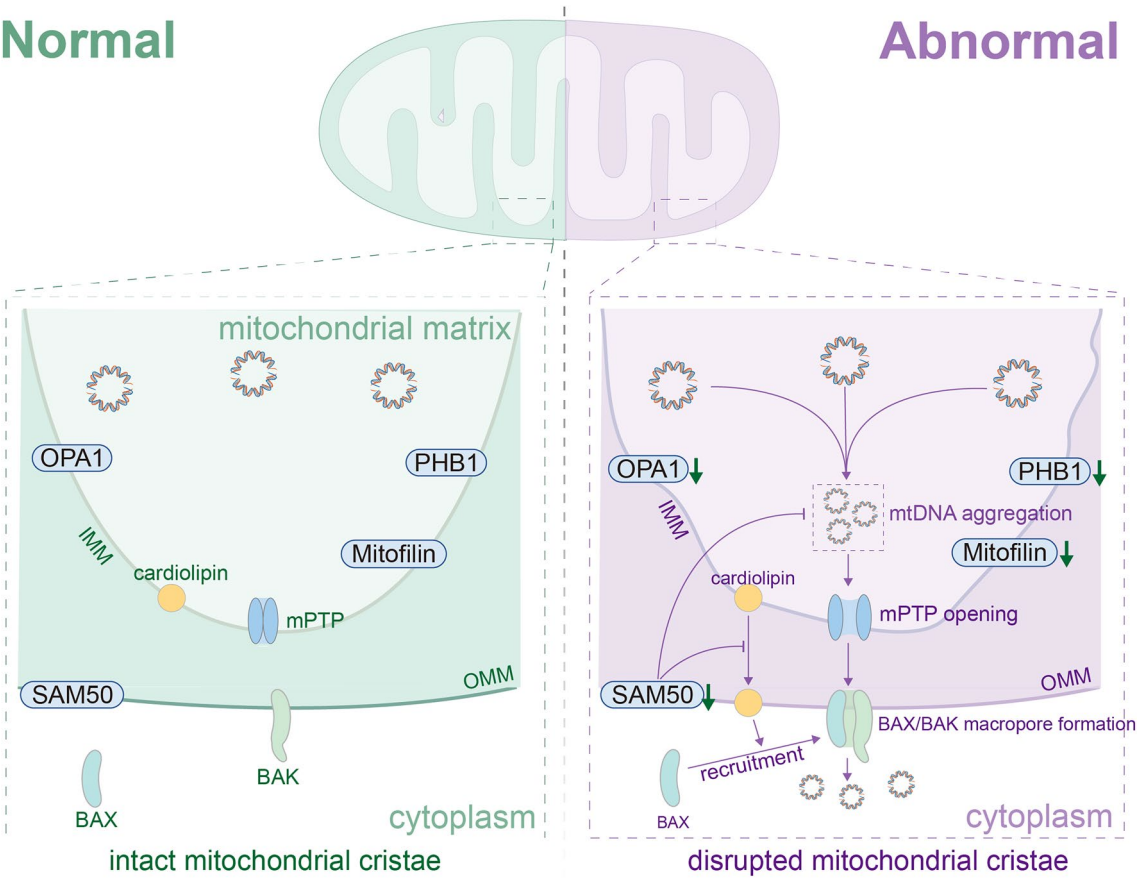

Fig.2 Disruption of mitochondrial cristae structure leads to mtDNA leakage. Under normal conditions, the IMM folds inward to form mitochondrial cristae. However, under abnormal conditions, dysfunction in structural maintenance proteins such as OPA1, SAM50, Mitofilin, and PHB1 causes disorganization of the cristae structure. This disorganization is accompanied by the opening of the mPTP and the formation of BAX/BAK macropores. SAM50 deficiency, in particular, leads to the accumulation of mtDNA near the IMM and triggers cardiolipin externalization, which the latter promotes the recruitment of BAX to the mitochondrial membrane

senescence, allowing mtDNA to escape and release senescence-associated secretory phenotype [40]. Given the pivotal role of BAX/BAK macropores in mtDNA release, it is probable that factors modulating BAX/BAK activation indirectly facilitate mtDNA escape. For example, SAM50 controls the distribution of BAK in the OMM, and its knockout results in BAK aggregation and activation, thereby enhancing mtDNA release [41]. In a model of acute kidney injury, phosphoglycerate mutase family member 5 (PGAM5), a protein phosphatase, initiates MOMP and mtDNA leakage by dephosphorylating BAX, thereby facilitating its recruitment to the mitochondrial membrane [42]. Moreover, it has also been suggested that the DUSP1/JNK pathway may play a similar role [43]. Specifically, dual specificity phosphatase 1 (DUSP1) deficiency results in abnormal c-jun n-terminal kinase (JNK) phosphorylation, mediating the translocation of BAX to the OMM, and triggering mtDNA leakage and inflammatory responses [43]. Beyond changes in subcellular localization and phosphorylation levels, the alternative splicing of BAX yields the BAX-κ variant, which can form pores by binding to BAX or BAK, or assemble into homologous oligomeric pores that preferentially release mtDNA [44]. Recent studies show that serine/arginine-rich splicing factor 6 (SRSF6) maintains mitochondrial homeostasis by regulating the alternative splicing of BAX, preventing excessive cell death, whereas the knockout of SRSF6 leads to the accumulation of the BAX-κ variant and induces mtDNA escape [45]. Therefore, the subcellular localization and modification of BAX profoundly affect the extent of mtDNA escape. Opposite results, however, have also been reported that tBID, a Bcl-2 family protein, can trigger mtDNA escape and mitochondrial cristae remodeling even without BAX/BAK [46].

VDAC oligomer pore VDAC located on the OMM regulates the release of mtDNA by forming a release pore through oligomerization [47]. There is a debate regarding whether VDAC is an essential component of the mPTP [48]. However, evidence largely supports that mPTP opening is accompanied by VDAC oligomer pore, a prerequisite for mPTP opening [47, 49]. In macrophages, the blockade of mPTP opening using cyclosporine A resulted in a decrease of approximately 30–40% mtDNA in the cytosolic pool. In contrast, the suppression of VDAC oligomer pores by VBIT-4 led to a more substantial reduction of 50–60%. This discrepancy indicates distinct pathways or kinetics in the mtDNA release process involving the mPTP opening and VDAC oligomer pores [50]. VBIT-4 did not disrupt mPTP opening but instead resulted in the accumulation of oxidized mitochondrial DNA (ox-mtDNA) fragments between the IMM and the OMM. This observation offers preliminary evidence for a sequential mtDNA escape route, proceeding through the open mPTP and VDAC oligomer pore, into the cytoplasm [50]. Emerging evidence indicates that calcium signaling from the endoplasmic reticulum stimulates the oligomerization of VDAC, thereby permitting the leakage of ox-mtDNA, VDAC oligomer pores are indispensable for the activation of the nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome by ox-mtDNA [51]. Consequently, calcium signaling may serve as a pivotal activator of the VDAC channel. Additionally, mtDNA located in the intermembrane space can interact with the N-terminus of VDAC, promoting the formation of VDAC1 oligomers, which may be one of the reasons for VDAC oligomerization following the opening of the mPTP [47]. Recent evidence indicates that, following oxidative stress, the damaged mitochondrial inner membrane undergoes herniation through VDAC1 oligomeric pores, subsequently transporting mtDNA to lysosomes for degradation in the form of mitochondria-derived vesicles [52].

Moreover, certain mitochondrial proteins indirectly affect mtDNA escape by regulating VDAC oligomer pore. For instance, the mitochondrial-associated kinase vaccinia-related kinase 2 (VRK2), activated by calcium overload, promotes mtDNA efflux by influencing the oligomerization of VDAC1. In contrast, VRK2 knockout results in a defective oligomerization of VDAC1, thereby highlighting VRK2's pivotal role in this process [53]. Similarly, protein kinase PKC-δ was observed to induce oligomerization of VDAC1 through direct binding, subsequently triggering mtDNA leakage [54]. The mitochondrial chaperone protein glucose-regulated protein 75 (GRP75) also affects mtDNA escape via the VDAC channel. In cells deficient for GRP75, there is a marked increase in the interaction between mtDNA and VDAC1, accompanied by enhanced mtDNA escape [55]. GRP75 interacts with VDAC1 to maintain Ca^{2+} homeostasis between the endoplasmic reticulum and mitochondria. Loss of GRP75 disrupts this balance, potentially leading to mitochondrial calcium overload, which in turn initiates mPTP opening and induces VDAC oligomer pore, leading to mtDNA escape through mPTP pore and VDAC oligomeric pore [56]. Recent findings suggest that TGF-β promotes the VDAC1-dependent efflux of mtDNA in normal cellular contexts, yet the precise pathway by which this occurs has not been fully delineated [57]. Additionally, ubiquitination of the VDAC1 protein inhibits its oligomerization, thereby reducing mtDNA escape [58].

GSDMD pore Gasdermin D (GSDMD), a member of the gasdermin protein family, frequently seen in pyroptosis and immune responses. Upon activation, the N-terminal domain of GSDMD promotes the release of intracellular contents by oligomerizing and forming

pores in the membrane [59]. Previous studies primarily suggested that GSDMD promotes the leakage of intracellular DAMPs by forming transmembrane pores on the cell membrane, thereby triggering a strong immune response. However, a recent series of studies has provided compelling evidence for the critical role of GSDMD in mtDNA leakage. Specifically, during pyroptosis or immune responses, GSDMD is recruited to the surface of the OMM and forms GSDMD pores. These pores work in conjunction with BAX/BAK macropores and promote the leakage of mtDNA and activate various cytosolic PRRs [60–64]. More importantly, mitochondrial damage caused by GSDMD pores may also be a triggering factor for the opening of BAX/BAK macropores and the oligomerization of VDAC [65]. The ox-mtDNA released into the cytoplasm directly interacts with GSDMD and promotes the oligomerization of GSDMD's N-terminus on the cell membrane, leading to the formation of large GSDMD pores and the extracellular release of mtDNA, thereby triggering inflammatory responses in neighboring cells [66, 67]. It remains unclear whether GSDMD pores simultaneously span both the IMM and the OMM, hich determines whether it relies on MIMP to release mtDNA into the mitochondrial intermembrane space.

Vesicular transport of mtDNA

Mitochondrial-derived vesicle (MDV) [68, 69] and Extracellular vehicle (EV) [70, 71] are also involved in transporting mtDNA in the cytoplasm and extracellular environment. Experimental evidence suggests that vesicles harboring mtDNA are predominantly derived from the IMM and are expelled from the mitochondrion via the expansive BAX/BAK macropores in the OMM, a process reminiscent of mitochondrial herniation [72]. This implies that following extrusion through these BAX/BAK channels, the IMM might either undergo herniation or give rise to MDVs, both pathways potentially resulting in the leakage of mtDNA (Fig. 1). A recent study revealed that MDV-mediated mtDNA escape depends on the endocytic accessory protein sorting nexin 9 (SNX9), and this process is triggered by the accumulation of fumarate due to fumarate hydratase deficiency [73]. Fumarate is an intermediate of the mitochondrial tricarboxylic acid (TCA) cycle, and its levels reflect the metabolic state of mitochondria, suggesting that mitochondrial metabolism may influence MDV-dependent mtDNA escape through fumarate. Additionally, monocytes, in response to harmful stimuli and caspase-1 activation, encapsulate mtDNA within intraluminal vesicles and escape to the extracellular space in the form of exosomes through Gasdermin-D pores formed during pyroptosis, triggering inflammatory responses in additional cells [70]. Free mtDNA has been detected in the blood and cerebrospinal fluid of patients with neurodegenerative diseases, which may primarily derive from mtDNA-carrying vesicles that have escaped to the extracellular environment [74].

Dysregulation of mtDNA homeostasis

MtDNA homeostasis is preserved by a sophisticated, finely tuned system encompassing replication, packaging, repair, and transport, which maintains nucleotide metabolic balance [17]. MtDNA replication in the mitochondrial matrix is succeeded by TFAM-mediated nucleoid formation, crucial for mtDNA copy and functional integrity [75]. Studies have demonstrated that dysregulation and low expression of TFAM are key factors contributing to mtDNA packaging defects, resulting in mtDNA leakage [76]. Furthermore, 8-oxoguanine dna glycosylase (OGG1) and other excision repair enzymes play a crucial role in repairing oxidatively damaged bases by base excision, a process essential for forestalling the production and release of ox-mtDNA [17, 77]. In pathological conditions or cellular stress responses, abnormal synthesis and transport of mtDNA also contribute to the escape of mtDNA. Experimental evidence confirmed that inhibiting enzymes like cytidine monophosphate kinase 2 (CMPK2) effectively promote the maintenance of mtDNA homeostasis, thereby reducing the incidence of mtDNA leakage events [78]. In summary, the conservation of mtDNA homeostasis is of paramount importance for the prevention of mtDNA leakage (Fig. 3).

Synthesis of mtDNA

The stability of the mitochondrial genome relies on tightly regulated replication and proper packaging of mtDNA. Abnormalities in these processes contribute to mtDNA leakage, which is a key driver of cytosolic inflammatory signaling. In mitochondrial damage scenarios, mtDNA overreplication contributes to persistent leakage, which can be mitigated by inhibiting specific synthase. CMPK2, a nucleotide kinase that facilitates mtDNA synthesis, is indispensable for preserving mitochondrial function under normal physiological conditions. Nevertheless, evidence indicates that CMPK2-dependent mtDNA synthesis is a prerequisite for the leakage of ox-mtDNA and the subsequent activation of cytosolic inflammatory signaling [79]. Specifically, in immune-responsive macrophages, mtDNA synthesized by CMPK2 is subject to modification by mtROS, resulting in cytosolic accumulation of ox-mtDNA [79]. The facilitative role of CMPK2 in mtDNA leakage has been corroborated across diverse pathological contexts [78, 80]. The nuclease miotic recombination 11 (MRE11) has been identified as a crucial molecule necessary for mtDNA leakage in the cells of patients with certain hereditary diseases, a process that is likely connected to defects in

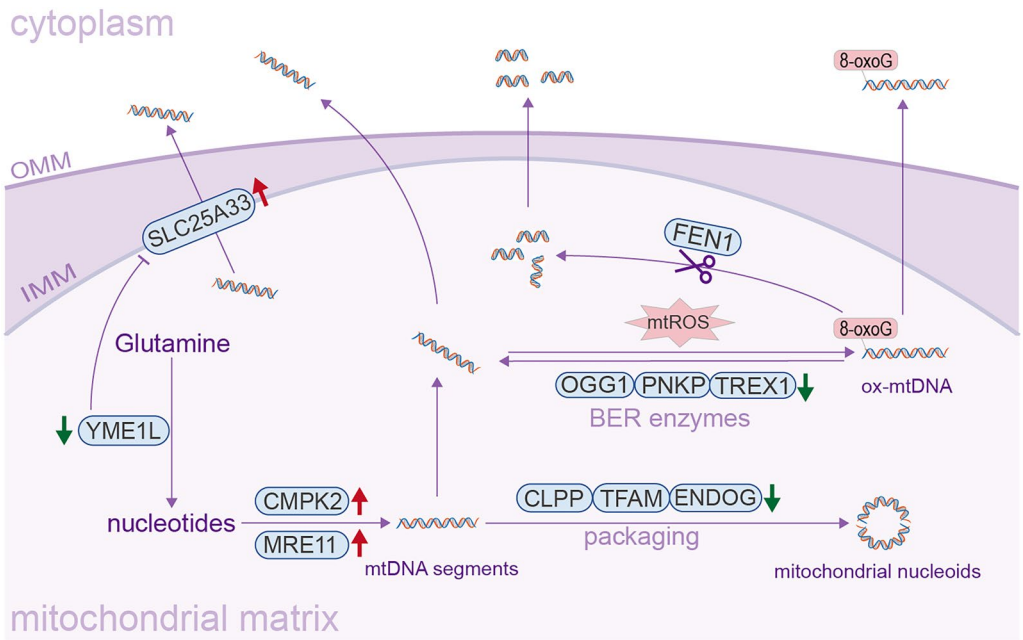

Fig.3 Abnormal increases in the DNA synthesis enzymes CMPK2 and MRE11 can lead to the leakage of mtDNA fragments into the cytosol. CLPP, TFAM, and ENDOG are responsible for folding mtDNA into mitochondrial nucleoid; a reduction in these proteins results in the improper folding of newly synthesized mtDNA, ultimately causing mtDNA leakage. A decrease in BER enzymes such as OGG1, PNKP, and TREX1 prevents the repair of oxidative damage to mtDNA, promoting the accumulation and leakage of ox-mtDNA. YME1L, while facilitating de novo nucleotide synthesis, also suppresses the levels of the pyrimidine carrier SLC25A33 on the IMM. A reduction in YME1L leads to the accumulation of SLC25A33, which in turn facilitates the transport of mtDNA into the cytosol

mtDNA fork protection during replication, leading to damage in newly synthesized mtDNA [81]. Inhibiting MRE11 has been shown to significantly suppress mtDNA leakage and mitigate the subsequent activation of downstream PRR responses [81].

TFAM is a critical protein that maintains the function of mtDNA by initiating its replication and transcription, and by ensuring the proper folding and packaging of the strands into mitochondrial nucleoids [82]. Early studies have shown that the depletion of TFAM leads to erroneous mtDNA packaging and abnormal accumulation, which can enter the cytosol, indicating that TFAM directly participates in regulating mtDNA leakage [83]. Recent findings suggest that inhibiting TFAM gene expression triggers mtDNA instability, thereby promoting its release [18]. Beyond disrupting mtDNA homeostasis, the deficiency of TFAM may further enhance mtDNA release by modulating mitochondrial membrane potential and altering the oligomerization status of VDAC channels. As a consequence, inhibiting mPTP opening or VDAC oligomer pore prevents the accumulation of cytosolic mtDNA induced by TFAM depletion [84]. Recent studies suggest that TFAM depletion leads to mtDNA replication stress characterized by enlarged mitochondrial nucleoids. These nucleoids are transported to lysosomes for degradation, but a failure in this process results in the release of mtDNA into the cytosol [85]. Consequently, TFAM insufficiency is a critical determinant in mtDNA leakage.

Similarly, the mitochondrial protease caseinolytic mitochondrial matrix peptidase proteolytic subunit (CLPP) can also trigger mtDNA stress, leading to mtDNA leakage. CLPP-deficient cells exhibit disrupted nucleoid architecture and TFAM aggregation, which leads to mtDNA release into the cytoplasm [86]. Another nuclease, Endonuclease G (ENDOG), is closely linked to mtDNA release, with its deficiency increasing mitochondrial oxidative stress and triggering mtDNA release, though the precise mechanisms are still unclear [47].

Repair of mtDNA

DNA base excision repair (BER) enzymes are essential for protecting mtDNA from oxidative insults, thereby preventing the accumulation of ox-mtDNA by specifically targeting and removing damaged segments [87]. The proneness of mtDNA to mtROS-induced harm underscores the relentless requirement for BER enzyme function to preserve genomic integrity [88]. Impairment of BER enzymes can precipitate mtDNA leakage, a process initiated by oxidative DNA damage. OGG1, a kind of DNA repair enzyme, is capable of excising 8-oxodG lesions in mtDNA [89]. There is evidence that ox-mtDNA

is either repaired by OGG1 or cleaved into 500–650 bp fragments by FEN1 that are more likely to escape via mPTP and VDAC [50]. Thus, OGG1, a DNA repair enzyme, inhibits mtDNA leakage by protecting it from oxidative damage. With the rise in ROS and DNA damage during cellular aging, ox-mtDNA becomes prevalent in senescent neuron [90]. The age-related decline in OGG1 expression could potentiate the escape of ox-mtDNA and the associated inflammatory responses in aging-related pathologies [91]. In contrast, overexpression of mitochondrial OGG1 in microglia has been shown to dampen inflammation by inhibiting mtDNA (most likely ox-mtDNA) escape, suggesting its therapeutic potential in neurological disorders such as AD [92]. Recently, another BER enzyme, polynucleotide kinase/phosphatase (PNKP), has been identified as pivotal in preventing mtDNA leakage. PNKP deficiency, in concert with increased mtROS, exacerbates mtDNA damage and promotes its translocation to the cytosol, triggering downstream signaling cascades [93]. Furthermore, the degradation of the exonuclease TREX1 leads to mtDNA leakage and its build-up in the cytosol, a phenomenon that can be reversed by TREX1 overexpression [94].

Transport of mtDNA

Transport proteins embedded within the mitochondrial membrane, such as SLC25A33, play a pivotal role in shuttling pyrimidine nucleotides across the mitochondrial membrane, providing the essential substrates for mtDNA synthesis [95]. The i-AAA protease YME1L, anchored to the IMM, curtails the flux of nucleotides by targeting SLC25A33 for degradation, thereby preserving the nucleotide equilibrium between mitochondrial and cytosolic compartments. YME1L deficiency has been shown to cause SLC25A33 buildup, facilitating mtDNA cytosolic leakage, and triggering cGAS-STING pathway [96]. Thus boosting YME1L expression may inhibit mtDNA efflux, offering a therapeutic strategy.

In summary, the homeostasis of mtDNA is subject to a variety of factors, including the absence or functional impairment of proteins such as TFAM and OGG1, which can lead to abnormalities in mtDNA synthesis, cleavage, and repair. Moreover, dysregulation of mtDNA transport contributes to the release of mtDNA. However, the escape of mtDNA is not solely determined by its own homeostasis; factors such as mitochondrial membrane permeabilization are essential for enabling mtDNA to leak into the cytosol.

Dysfunction of mitochondrial dynamics

Mitochondrial dynamics, including fission and fusion, regulate mtDNA turnover and distribution, with disruptions leading to cytosolic mtDNA release and activation of PRRs. (Fig. 4) [97]. Mitochondrial fission, driven by dynamin-related protein 1 (DRP1), is critical for cellular homeostasis but can also induce mtDNA leakage and activates cytosolic PRRs [98, 99]. Overexpression of DRP1 not only results in mitochondrial fission but also causes enlargement of the mitochondrial nucleoid and mtDNA release [100]. This suggests that excessive

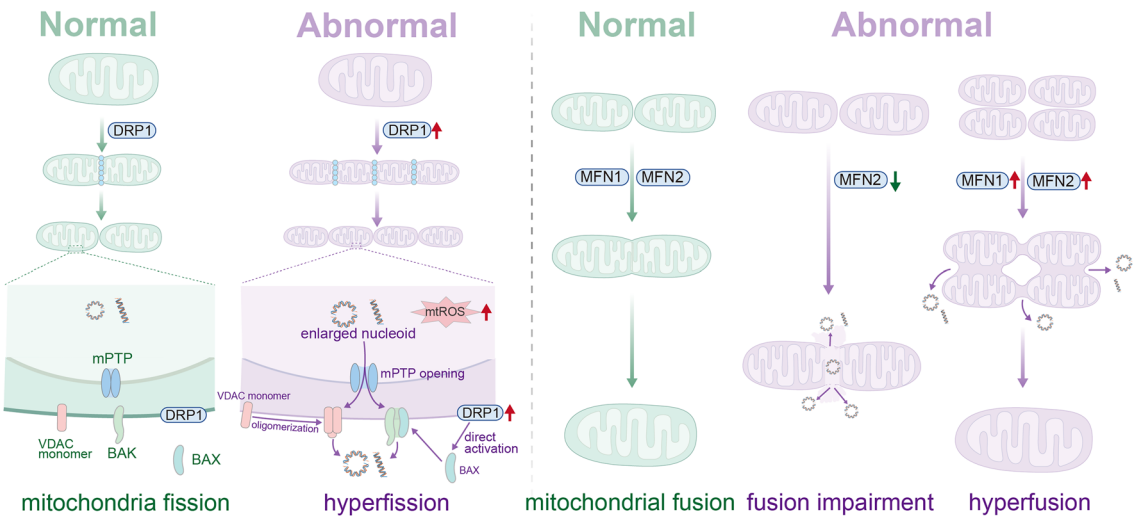

Fig.4 Mitochondrial dynamics dysfunction is closely associated with mtDNA leakage. Elevated levels of DRP1 mediate excessive mitochondrial fission, leading to increased mtROS production and the formation of BAX/BAK macropores and VDAC oligomeric pores on the OMM. This process also results in the enlargement of mitochondrial nucleoids, all of which contribute to mtDNA leakage. MFN1 and MFN2 mediate mitochondrial fusion, and a deficiency in MFN2 can cause fusion defects, resulting in mtDNA leakage. However, in certain situations, overexpression of MFN1 or MFN2 can also trigger mtDNA leakage

mitochondrial fission induced by elevated DRP1 levels may lead to impaired mtDNA replication, mitochondrial fission is responsible for segregating these abnormal nucleoids for clearance, yet this process may inadvertently lead to the release of these nucleoids into the cytosol [85]. Besides, the potential mechanism by which DRP1 induces mtDNA escape may involve the opening of mPTP and the promotion of VDAC1 oligomerization [99], enhancing mtROS production [98], and BAX activation [101]. In pathological conditions, DRP1 is often abundantly expressed or hyperphosphorylated, a response potentially initiated by OPA1 depletion [102]. This implies that the disrupted cristae structure may influence fission sites, although its impact on mtDNA leakage remains unclear. Additionally, post-translational modifications that regulate DRP1 activity have been confirmed to affect mtDNA leakage [103]. Mitochondrial fusion is predominantly regulated by mitofusin 1 (MFN1) and mitofusin 2 (MFN2) on the outer mitochondrial membrane [104]. MFN1 expression and mtDNA efflux were markedly increased in lung injury mouse models, but mtDNA efflux was inhibited by prior CsA administration [105]. In aged astrocytes, the upregulation of MFN2 led to mtDNA release, which may be a consequence of the excessive mitochondrial fusion driven by elevated MFN2 levels [106]. Similar phenomena and neuroinflammatory responses were also observed in microglia exposed to organic dust [107]. Conversely, in a spinal cord injury model, reduced MFN2 in microglia induced mtDNA escape [108]. This paradox illustrates the multifaceted function of MFN2 across various disease models and cellular settings. While increased MFN2 levels can avert mtDNA release by dampening mitochondrial fission and lowering mtROS generation, both abnormal mitochondrial fusion mediated by MFN2 reduction and the hyperfusion resulting from excessive MFN2 can precipitate mtDNA efflux [108]. Hence, it is crucial to consider the distinct potential of MFN2-mediated mtDNA release in diverse pathological contexts, especially within the framework of aging. Furthermore, MFN2 activity is modulated by its phosphorylation status. MFN2 activity is influenced by phosphorylation, with PGAM5 acting as a phosphatase that stabilizes MFN2 and potentially modulates mtDNA release independently of BAX [109].

Dysfunction of mitophagy

Mitophagy is a critical process that ensures mitochondrial quality control via the lysosomal degradation pathway. In this process, mitochondria are enveloped by double-membrane phagophores to form mitophagosomes, which then fuse with lysosomes for degradation [110]. Deficiencies in mitophagy lead to the suboptimal clearance of dysfunctional mitochondria, such as those undergoing MIMP and MOMP, potentially resulting in the cytosolic release of mtDNA. On the other hand, mitophagy helps to clear the accumulated mtDNA in the cytosol, preventing its activation of PRRs [19]. Studies suggest that aging is frequently associated with reduced mitophagy, leading to mtDNA release-mediated inflammation in diverse organs and species. The pharmacological enhancement of mitophagy has demonstrated efficacy in attenuating mtDNA leakage and the resultant inflammatory response [111].

The PINK1/Parkin pathway is a key pathway for triggering mitophagy by recruiting LC3 on the surface of damaged mitochondria, thereby promoting the formation of mitophagosomes [112]. In senescent macrophages, deficits in autophagy facilitate the extrusion of mtDNA; however, overexpression of pten-induced putative kinase 1 (PINK1) can restore mitophagy flux through the PINK1/Parkin pathway, thereby diminishing mtDNA efflux [113]. Tumor necrosis factor (TNF), a pro-inflammatory cytokine, has been demonstrated to suppress PINK1-dependent mitophagy, thereby potentiating mtDNA leakage and inflammation. This finding implies that the inhibition of autophagy may promote mtDNA release [114]. Similarly, the depletion of immunity-related gtpase family m member 1 (IRGM1) [115] or immunity-related gtpase family m member 1 (XBP1) [67] impairs mitophagy by inhibiting the PINK1/Parkin pathway, leading to mtDNA leakage and the activation of PRRs.

Furthermore, Parkin prevents the oligomerization of VDAC1 through ubiquitination at specific sites. The absence of Parkin or its pharmacological inhibition allows VDAC1 oligomerization, leading mtDNA escape and exacerbating tissue inflammation [58]. SAM50 directly binds to PINK1, and its depletion enhances PINK1/Parkin-mediated mitophagy by promoting the recruitment of PINK1 and stabilizing Parkin. Nevertheless, SAM50 depletion induces a transition to large spherical mitochondria, which protects mtDNA from degradation [116]. Under conditions of disrupted mitochondrial dynamics or oxidative mtDNA damage, this protective mechanism could impede the autophagic clearance of damaged mtDNA. This suggests that mitophagy is not always effective in clearing cytosolic mtDNA. While most studies suggest that mitophagy clears cytosolic mtDNA, some research indicates that mitophagy may exacerbate the activation of the mtDNA–cGAS–STING pathway [117]. In addition to SAM50's protective role over mtDNA, this contradiction possibly due to incomplete degradation of mitochondrial contents by lysosomes, a phenomenon referred to as "incomplete mitophagy" [118].

Recent research has revealed alternative mitophagy pathways that operate independently of the PINK1/Parkin pathway. Specifically, during mitochondrial herniation, the exposure of the IMM triggers a non-canonical autophagic process, facilitating the the capture of damaged mitochondria by mitophagosomes, which are then transported to lysosomes for degradation [111]. In addition, the latest research suggests that that TFAM, which accompanies nucleoid escape into the cytoplasm, can function as an autophagy receptor by recruiting LC3, initiating mitophagy, and promoting the clearance of cytosolic mtDNA [119]. The outer mitochondrial membrane protein fun14 domain containing 1 (FUNDC1) initiates mitophagy by recruiting LC3, thereby facilitating the clearance of cytosolic mtDNA and suppressing inflammatory responses. Ablation of FUNDC1 increases the levels of cytosolic mtDNA and enhances inflammatory reactions [120]. The aforementioned mitophagy pathways all rely on LC3-mediated mitophagosome synthesis.

Moreover, endosomal vesicles or MDV participate in the mitophagy process by transporting abnormal mtDNA to the lysosome for degradation [121]. This is crucial for maintaining mtDNA quality control, especially when the PINK1/Parkin pathway is inactive or when the impaired formation of mitophagosomes [122]. In cases of TFAM deficiency leading to enlarged mitochondrial nucleoids, mitochondrial fission is impaired, defective mitochondrial fission segregates these incompletely replicated nucleoids into endosomes, facilitating their transport to the lysosome for degradation [85]. Under the mediation of ras-related protein rab-5 (RAB5), early endosomes internalize mtDNA from the mitochondrial membrane and then transfer it to late endosomes through a RAB7-dependent pathway, eventually fusing with lysosomes [123]. In the absence of RAB7, the maturation of early endosomes is compromised, precluding their fusion with lysosomes, the vesicles rupture and potentially lead to the release of mtDNA into the cytoplasm [85]. The deacetylase SIRT1 has been shown to interact with the critical late endosomal protein RAB7, thereby enhancing mitophagy and constraining cytosolic accumulation of mtDNA [124]. In mitochondria with BAX/BAK macropore open, the vacuolar membrane protein vacuolar protein sorting 35 (VPS35) facilitates the targeting of endosomes to the proximity of BAK, and enhancing the maturation of late endosomes and promoting their fusion with lysosomes. This process is crucial for the autophagic degradation of mtDNA [41]. Similarly, vacuole membrane protein 1 (VMP1), another vacuolar membrane protein, modulates autophagosome formation and autophagic flux, VMP1 knockout cells exhibit defective autophagic flux and an increased release of mtDNA into the cytoplasm and extracellular environment [125]. Even when endosomes and lysosomes fuse appropriately, lysosomal dysfunction can preclude the completion of autophagy, leading to the accumulation of lysosomal substrates and impaired lysosomal function—a hallmark of many neurodegenerative diseases, such as PD [126]. Dysfunctional lysosomes can also precipitate cytosolic accumulation of mtDNA in neurons, culminating in neurodegeneration and innate immune activation [127].

Thus, the impact of mitophagy on mtDNA leakage is multifaceted. On one hand, reduced autophagic flux leads to the inability to clear damaged mitochondria in a timely manner, thereby leading cytosolic accumulation of mtDNA, while increasing autophagic flux serves as an effective approach to suppress the accumulation of mtDNA in the cytosol. On the other hand, rupture of endosomal vesicles preventing the transport of mtDNA to lysosomes, or lysosomal dysfunction hindering the complete degradation of mtDNA instead exacerbates mtDNA leakage. Under these circumstances, inhibiting mitophagy might be a strategy to prevent mtDNA leakage (Fig. 5).

Downstream PRRs of escaped mtDNA

Upon mtDNA leakage into the cytoplasm, it can be recognized by various DNA sensors, such as the cGAS-STING pathway, inflammasomes, the toll-like receptor 9 (TLR9) signaling pathway, and the z-dna binding protein 1 (ZBP1) protein. In response to this recognition, these PRRs initiate a cascade of inflammatory reactions, which aim to recognize and respond to cell damage or pathogen infection (Fig. 6). Furthermore, proteins such as IFI16 [128] and TLR7 [115] function as cytosolic DNA sensors, but details are not discussed in detail herein due to space limitations.

The cGAS-STING pathway

The cGAS-STING pathway, a focal point of recent research DNA sensor, has been implicated in various neurological disorders [106, 129–131]. Upon sensing mtDNA, cGAS facilitates the activation of the endoplasmic reticulum-associated protein STING, prompting its translocation to the Golgi apparatus. Subsequently, STING activates tank-binding kinase 1 (TBK1), initiating a signaling cascade (Fig. 6), that culminates in the robust induction of a type I IFN response, thereby promoting the secretion of pro-inflammatory cytokines [132]. This pathway is a pivotal connection between mtDNA escape and the onset of innate immunity; inhibiting it efficiently alleviates inflammation triggered by mtDNA [133]. Moreover, most Caspase family proteins potently inhibit cGAS signaling by cleaving cGAS, thus dampening the inflammatory response elicited by mtDNA [134]. There is evidence of crosstalk between the cGAS-STING

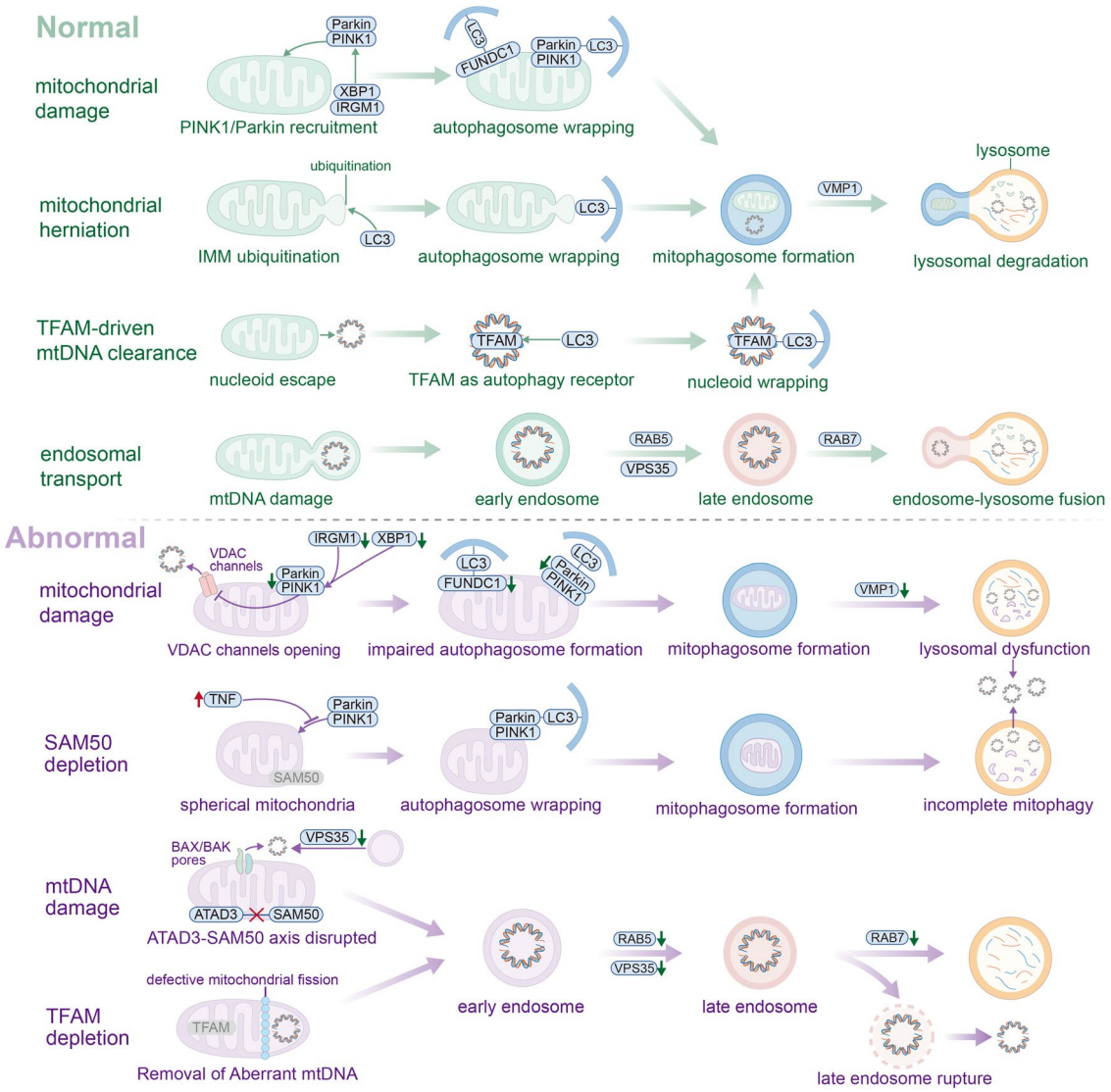

Fig.5 Dysfunction in mitophagy is closely related to mtDNA leakage. Under normal conditions, damaged mitochondria and abnormal mtDNA can be cleared through mitophagy, a process typically mediated by the PINK1/Parkin pathway. After IMM herniation, ubiquitination can recruit LC3 to form autophagosomes, and TFAM can also act as an autophagy receptor to recruit LC3. Damaged mtDNA can also be transported to lysosomes for degradation via endosomal pathways. However, under abnormal conditions, defects in the PINK1/Parkin pathway can hinder LC3 recruitment, and indirectly caused the VDAC channel to open, while SAM50 deficiency causes changes in mitochondrial morphology that prevent mtDNA from being degraded by lysosomes. Additionally, a decrease in endosome-related proteins such as VPS35, RAB5, and RAB7 can impair the transport of mtDNA to lysosomes, ultimately leading to the accumulation of mtDNA in the cytosol

pathway and caspases, underscoring the intricate role of apoptosis in governing mtDNA release and subsequent inflammation during MOMP, potentially culminating in immunological silence [135]. Similarly, nuclear or exogenous DNA can also activate cGAS, eliciting comparable responses [136, 137]. Additionally, the cGAS-STING pathway components, including STING, are targeted to lysosomes through direct interactions with LC3 following STING activation. This lysosome-dependent negative feedback mechanism prevents prolonged IFN signaling [138–140]. The STING-mediated autophagy likely contributes to the clearance of cytosolic DAMPs, including escaped mtDNA [141]. However, given the pervasive activation of the cGAS pathway and the ensuing inflammation in numerous pathological contexts, the negative feedback mechanism is often compromised by issues like lysosomal dysfunction, facilitating the accumulation of mtDNA in the cytoplasm following its leakage [141]. Recent findings have revealed a complex interaction between STING and PINK, modulating mitophagy

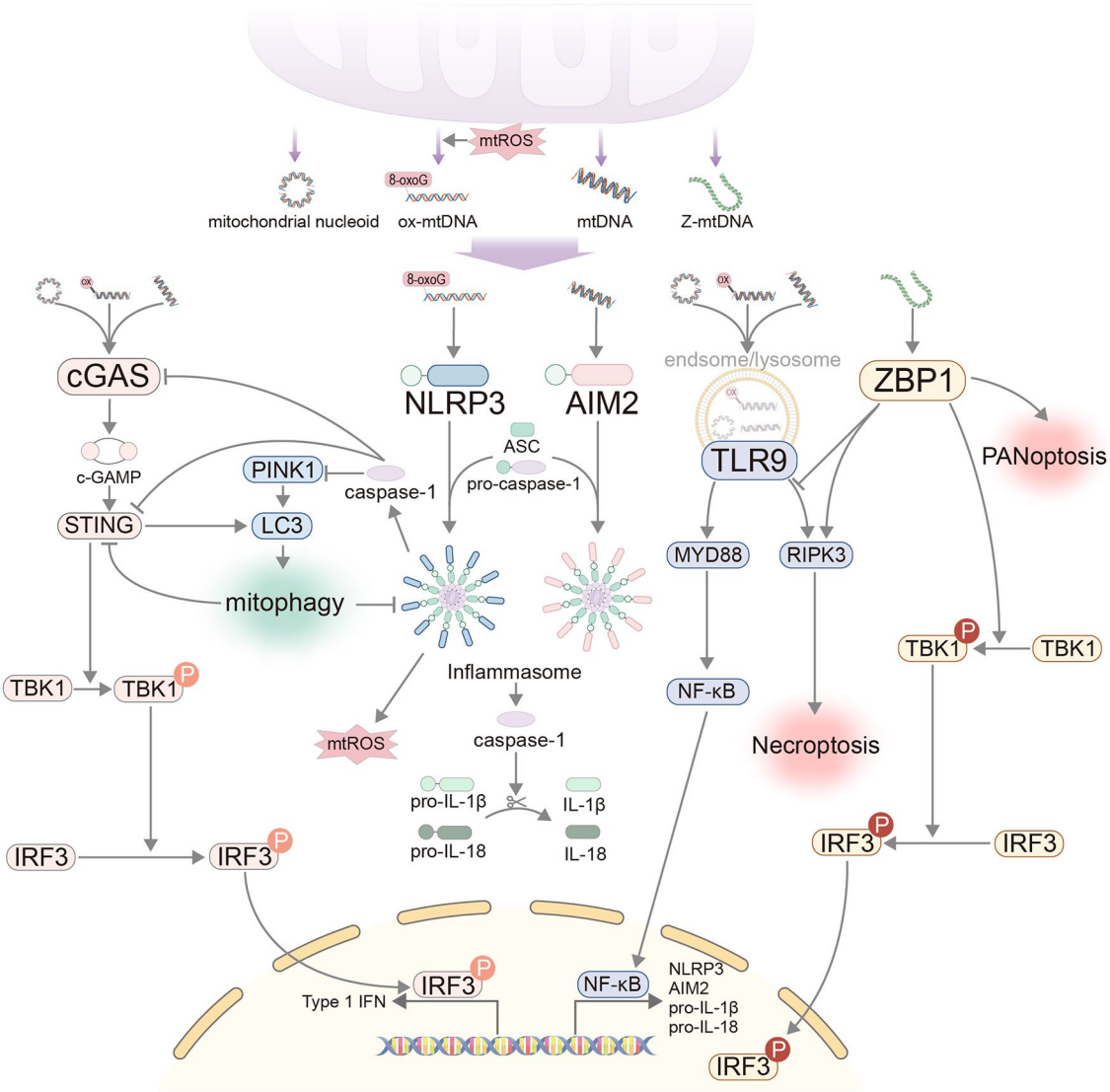

Fig.6 The primary cytoplasmic DNA sensors activated by cytosolic mtDNA. Upon mtDNA leakage into the cytosol, various forms of mtDNA activate DNA sensors including cGAS, NLRP3, AIM2, TLR9, and ZBP1, thereby triggering inflammatory responses, necroptosis, and PANoptosis. The cGAS-STING signaling pathway and NLRP3 inflammasome exhibit varying degrees of crosstalk with mitochondrial autophagy, with the NLRP3 inflammasome further promoting ox-mtDNA leakage by enhancing mtROS production

[142]. Notably, the cGAS pathway is not exclusively detrimental; its activation following peripheral nerve injury is linked to enhanced neuronal axonal regeneration, indicating potential neuroprotective or restorative functions [143].

Inflammasome

NLRP3 inflammasome

The NLRP3 inflammasome is a prominently researched component within the inflammasome family, renowned for its role as an innate immune sensor in the cytoplasm. It responds to a spectrum of DAMP stimuli, leading to the assembly of inflammasomes and the initiation of a pro-inflammatory signaling cascade. In numerous neurological disorders, the NLRP3 inflammasome has been identified as a critical mediator within the inflammatory pathway [144]. Recent research confirms a crucial connection between mtDNA, particularly ox-mtDNA released into cytosolic, and NLRP3 inflammasome activation [50, 79], underscoring the key role of mtDNA in inflammatory signaling. Comparative studies demonstrate that ox-mtDNA binds more avidly to NLRP3 than mtDNA, facilitating direct interaction with NLRP3's pyrin domain, a critical step in inflammasome activation

[145]. Blocking this ox-mtDNA-NLRP3 interaction markedly reduces NLRP3-dependent inflammation [146]. Ox-mtDNA formation, predominantly due to mtROS buildup, is a key initiator of NLRP3 inflammasome activation, potentially serving as a linker between mtROS and this inflammatory pathway [147]. Furthermore, emerging evidence points to a reciprocal relationship between mtROS and the NLRP3 inflammasome, implying that the inflammasome might enhance mtROS production, thereby exacerbating ox-mtDNA release [148].

Parkin-dependent mitophagy regulates the NLRP3 inflammasome by degrading mtDNA to avert hyperactivation [149]. However, Caspase-1, activated by the NLRP3 inflammasome, can inhibit this by cleaving Parkin, elevating ox-mtDNA, and enhancing inflammasome activity [150]. This points to a positive feedback loop where the NLRP3 inflammasome may facilitate ox-mtDNA release via mitophagy suppression or ROS enhancement [149, 150]. Additionally, further exploration is required to unravel the intricate relationship between the NLRP3 inflammasome, mtDNA leakage, and cGAS signaling pathway interactions [151]. The consequences of NLRP3 inflammasome activation by escaped mtDNA are not limited to inflammation but may also impact mtDNA release control and the activity of other downstream sensors.

AIM2 inflammasome

The absent in melanoma 2 (AIM2) inflammasome functions as a critical intracellular innate immune sensor, essential for recognizing and responding to DNA damage and viral pathogens. Detection of cytosolic dsDNA, including mtDNA, triggers inflammasome assembly, caspase-1 activation, and the proteolytic maturation of IL-1β and IL-18, thereby propagating the inflammatory cascade [152]. Notably, perfluorooctane sulfonate, an industrial chemical, promotes BAX/BAK-mediated release of unoxidized mtDNA, preferentially activating the AIM2 inflammasome over the NLRP3 inflammasome [153], implying differential sensitivities of AIM2 and NLRP3 to unoxidized versus ox-mtDNA, which could account for variations observed in mtDNA leakage research. Heavy metals [154] and nanoplastics [63] are recognized inducers of mtDNA release and AIM2 inflammasome activation, with their distinctive characteristics potentially explaining their preference for inflammasome activation over traditional triggers such as MMP. Additionally, AIM2 inflammasome activation parallels cGAS stimulation, being triggered by TFAM depletion [155] or BAK activation-induced mtDNA release [156]. In the absence of cGAS, the AIM2 inflammasome emerges as a pivotal compensatory pathway for mounting an immune response to cytosolic mtDNA [157].

TLR9 signaling

The TLR9 is an innate immune receptor predominantly expressed in immune cells, known for its ability to detect a spectrum of DAMPs, with a particular affinity for mtDNA among various DNA species [158]. TLR9 is predominantly sequestered within endosomal and lysosomal membranes and is poised to be activated during the autophagic trafficking and vesicular shuttling of mtDNA, with its dysregulation leading to amplified inflammatory responses and tissue damage [159]. mtDNA-induced TLR9 activation initiates the NF-κB pathway, priming the NLRP3 inflammasome for assembly and triggering the inflammatory cascade [160, 161]. In the activation of the NLRP3 inflammasome by ox-mtDNA, TLR9 colocalization with ox-mtDNA is noted, and TLR9 deletion or inhibition markedly reduces ox-mtDNA-triggered NLRP3 activation, underscoring the essential role of TLR9 in this process [162]. Additionally, post-NLRP3 inflammasome pyroptosis, ox-mtDNA is extruded, activating TLR9 in neighboring cells and amplifying inflammation to naive cells [162]. Considering TLR9's main localization in endosomal compartments, mtDNA transport to lysosomes can trigger TLR9 signaling, particularly during lysosomal dysfunction from DNase II deficiency [163] or compromised mitophagy due to PINK1 deficiency [164]. In fact, the early contact between endosomes and mitochondria is essential for the activation of TLR9 [97]. As previously noted, RAB7 deficiency induces late endosome rupture, leading to the release of mtDNA into the cytosol. This may simultaneously release activated TLR9, allowing it to interact with the NLRP3 inflammasome. Moreover, TLR9 activation by mtDNA released during mitophagy influences the regulation of long-term depression in neurons, potentially linking to memory impairments observed in neurodegenerative disorders [165]. Following sciatic nerve injury, TLR9 activation by mtDNA fosters a pro-regenerative phenotype in both the injured and distant, unaffected neurons, emphasizing the receptor's multifaceted roles [166].

ZBP1

mtDNA predominantly adopts a right-handed B-DNA structure but can switch to A-, C-, or Z-conformations due to folding or damage. ZBP1, a mammalian cell-expressed DNA sensor, specifically targets Z-conformation mtDNA [167]. This recognition of Z-conformed mtDNA by ZBP1, likely following structural changes, triggers immune responses to mtDNA disturbances, thus maintaining cellular homeostasis and resilience to stress. Cytoplasmically anchored ZBP1, upon activation, migrates between the cytoplasm and nucleus, sensing Z-DNA in the cytoplasm. Persistent low-grade oxidative stress, causing mtDNA leakage, elicits inflammation

through the TBK1/IFN3 pathway, dependent on ZBP1 activation [168, 169]. Furthermore, mtDNA-ZBP1 engagement triggers PANoptosis [170] and necroptosis [171], indicating that mtDNA leakage not only incites inflammation but also directly activates programmed cell death pathways [172]. Research indicates that instability of the mitochondrial genome can lead to an accumulation of Z-DNA, and ZBP1, in concert with cGAS, facilitates a type I IFN response [173]. Conversely, Alternatively, ZBP1's detection of Z-DNA also mitigates the mtDNA-TLR9-induced inflammation triggered by inhibiting the RIPK3/NF-κB/NLRP3 axis, thus protecting the heart from inflammation and remodeling post-ischemia. ZBP1 deletion eliminates this protective effect, enhancing IL-1β and IL-6 secretion [174]. As proposed by Nobuyuki et al., a complex regulatory network involving STING, RIPK1, and the ZBP1-RIPK3 pathway is established.

In essence, the cytosolic DNA sensors discussed expose various routes for mtDNA leakage to elicit innate immunity, where the involvement of specific PRRs is governed by intricate elements such as cellular environment and activation conditions. Illustratively, alterations in cellular bioenergetics may determine the activation of the cGAS pathway versus the NLRP3 inflammasome by mtDNA leakage [175], where the NLRP3 and AIM2 inflammasomes show selective responsiveness to ox-mtDNA and mtDNA, respectively. Given that AIM2 inflammasome activation necessitates a minimum of 80 base pairs of double-stranded DNA [176], Ambika et al. suggest that cGAS may be more sensitive to DNA than AIM2 [177]. Although certain PRRs possess repair functions, no beneficial effects have been linked to their activation by mtDNA leakage, possibly due to the inhibition of repair mechanisms under pathophysiological states. This results in the overactivation of cytosolic PRRs by continuous mtDNA leakage and the ensuing inflammatory cascade.

The impact of mtDNA release on central nervous system pathologies

mtDNA release is extensively reported across various neural cell types, such as neurons [165, 178], astrocytes [179], and microglia [107, 180], where it activities innate immunity and drives neuroinflammation. This process is likely a significant contributor to the neuroinflammatory cascade observed in numerous neurological conditions (Fig. 7). Of particular interest, the occurrence of mtDNA release appears to be augmented in aging neural cells, suggesting it may serve as a pivotal pathogenic factor in the onset and progression of age-related neurological diseases [92]. Consequently, this could underlie the pronounced variability in pathological manifestations and clinical symptoms observed across different age groups of patients.

Neurodegenerative diseases

Neurodegenerative disorders, including PD, AD, Amyotrophic Lateral Sclerosis (ALS), and huntington's disease (HD), ZBP1 hallmarked by the gradual depletion of neurons, frequently accompanied by significant neuroinflammation and cellular senescence [181, 182]. Evidence indicates that mtDNA release is a prevalent feature in neurons affected by these conditions, activating downstream DNA sensors and precipitating neuroinflammatory responses [183]. The reduced expression of TFAM may contribute to the mtDNA release observed in these cells[76]. Furthermore, mtDNA efflux through the VDAC oligomer pathway may be a pivotal route underlying the inflammatory phenotype in aging microglia [184]. Collectively, mtDNA efflux from senescent cells, promotes inflammatory cascades across various neurodegenerative pathologies through downstream sensors, likely constituting a pathogenic pathway underlying neuronal depletion.

Parkinson's disease

PD, a chronic neurodegenerative condition, is marked by motor impairment, dopaminergic neuron loss in the substantia nigra, and α-synuclein-rich Lewy body formation [185]. Post-mortem PD brain studies and animal model research reveal cytosolic mtDNA accumulation in brain cells, colocalizing with Lewy bodies, suggesting mtDNA release contributes to PD pathogenesis [128]. By activating the cGAS-STING pathway and releasing mtDNA, Rotenone and MPTP, well-established toxins used to model PD in vitro and in vivo, induce pro-inflammatory responses in microglia and astrocyte senescence, respectively, contributing to the degeneration of dopaminergic neurons [106, 186]. Furthermore, mtDNA escape may also induce necroptosis in dopaminergic neurons through the activation of cGAS signaling and NLRP3-mediated neuroinflammation [187]. In patients with PD who harbor mutations in PINK1 or Parkin, circulating cell-free mtDNA (ccf-mtDNA) has been proposed as a potential biomarker for monitoring disease progression [188].

Dysfunction of mitophagy is a pivotal factor contributing to the escape of mtDNA in PD. Mutations in the Parkin and PINK1 genes, common in early-onset PD [189], result in a substantial elevation of cytosolic and circulating mtDNA in murine models, initiating a cGAS-STING pathway-dependent neuroinflammatory response [190]. PINK1 deficiency impairs autophagy, enabling mtDNA to circumvent lysosomal degradation and accumulates in the cytoplasm, where it activates the DNA sensor IFI16. Overexpression of lysosomal DNase II or the depletion of IFI16 significantly mitigates the type I interferon response [128]. Additionally, Parkin deficiency is

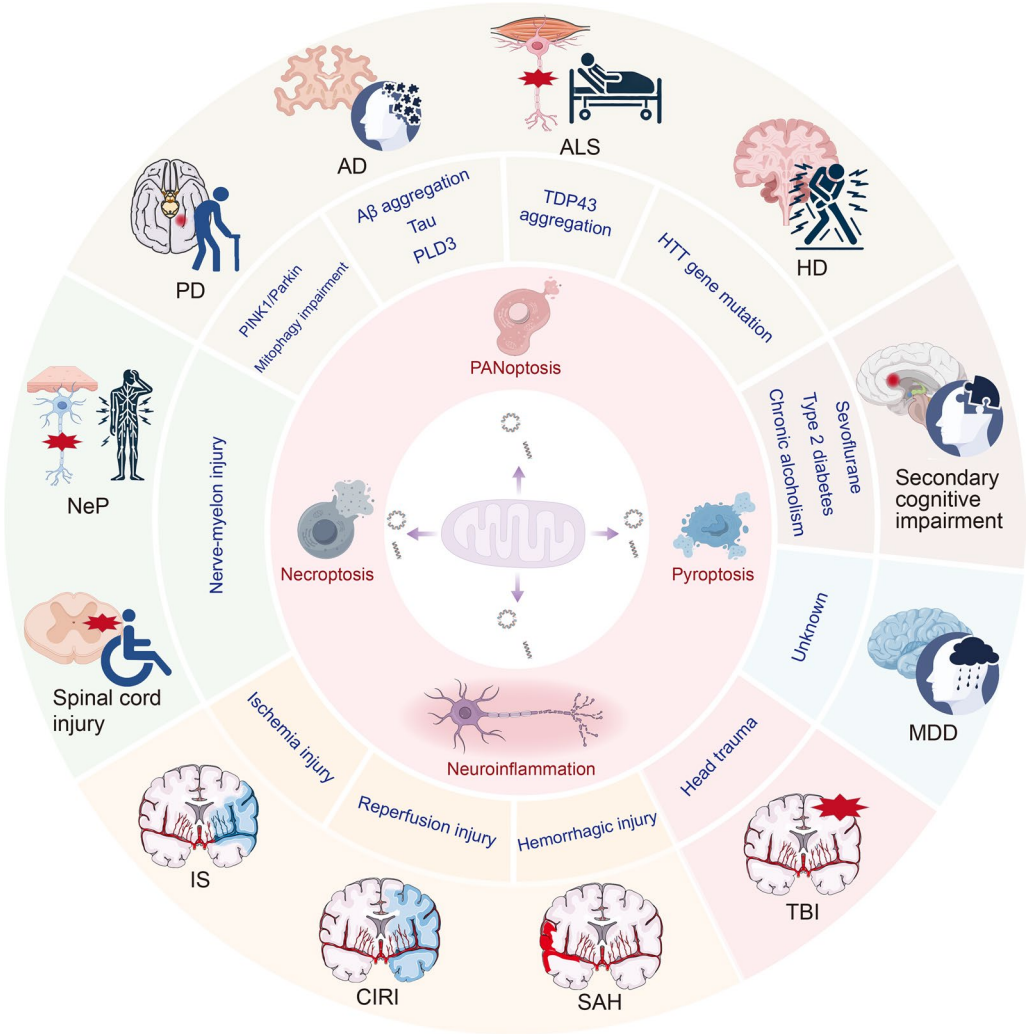

Fig.7 The association between mtDNA leakage and various neurological disorders. In different neurological diseases, multiple pathogenic factors directly or indirectly induce mtDNA leakage, resulting in cytosolic mtDNA accumulation. This accumulation subsequently triggers neuroinflammation as well as programmed cell death, including PANoptosis, necroptosis, and panoptosis, ultimately exacerbating neuronal damage

associated with disrupted mitochondrial biogenesis, as evidenced by altered levels of proteins such as TFAM, leading to mtDNA escape, a phenomenon corroborated in post-mortem PD brain tissue [191]. Deficits in the lysosomal lipid regulator VPS13C or the accumulation of glucosylceramide due to glucocerebrosidase deficiency also impair mitophagy and precipitate mtDNA escape [192, 193]. Conversely, enhancing autophagy with rapamycin diminishes cGAS activation, suggesting that mtDNA escape elucidates the role of lysosomal dysfunction in PD pathogenesis.

Moreover, excessive mitochondrial fission during PD progression is another determinant of mtDNA escape. Leucine-rich repeat kinase 2 (LRRK2) mutations, prevalent in both familial and sporadic PD, are linked to increased ccf-mtDNA levels in the cerebrospinal fluid of affected individuals [194]. Subsequent studies have observed mtDNA escape and the activation of type I interferon responses in LRRK2-deficient macrophages, potentially through the facilitation of DRP1-dependent mitochondrial fission [195]. This finding implies that LRRK2 mutation-induced mtDNA escape may be a pathogenic mechanism in PD.

Alzheimer's disease

AD, a prevalent neurodegenerative condition, is marked by cognitive decline, pathognomonic Aβ plaques, and hyperphosphorylated tau tangles [196]. Cytosolic

accumulation of ox-mtDNA has been observed in hippocampal and cortical neurons of preclinical AD model and actual AD patients [197, 198]. Released mtDNA triggers the cGAS-STING pathway in microglia, inducing neuroinflammation and Aβ deposition. The deletion of cGAS or the inhibition of STING in 5×FAD AD mice, an AD model, mitigates these pathologies and improves cognitive function [199]. Subsequent research reveals that mtDNA escape, by activating type I IFN responses via cGAS in microglia, disrupts the MEF2C expression network—a gene associated with cognitive resilience in neurons—thus impairing cognitive recovery in tauopathy mouse models [200]. Pharmacological inhibition of cGAS can restore synaptic integrity, plasticity, and memory in these mice [200]. Notably, tau fibrils localized to lysosomes and mitochondria can induce mtDNA escape, which, in turn, accelerates tau fibrils accumulation, suggesting a potential positive feedback loop between tau pathology and mtDNA escape [200]. Furthermore, APP/PS1 mutations, prevalent genetic determinants of AD, enhance mtDNA escape [201], with mtDNA release altering APP metabolism through cGAS pathway activation [202]. These data imply that mtDNA escape may exacerbate pathological progression in the early stages of AD. As in PD, mtDNA escape in AD is linked to defects in mitophagy [198]. Phospholipase d family member 3 (PLD3), a risk gene for late-onset AD, encodes a critical lysosomal $5'-3'$ exonuclease necessary for mtDNA degradation. PLD3 deletion or mutation leads to mtDNA aggregation within lysosomes, activates cGAS signaling, and enhances APP accumulation, thus exacerbating AD-related pathologies [202]. Of note, both acute and chronic mtDNA depletion in SH-SY5Y neurons increases tau oligomers [203], underscoring the intricacy of targeting mtDNA escape in AD therapy and the necessity to evaluate its impact on tau oligomerization.

Amyotrophic lateral sclerosis

ALS, a motor neuron disease, features progressive neurodegeneration, muscle wasting, and respiratory compromise, hallmarked by TAR DNA-binding protein 43 (TDP-43) protein aggregation [204]. Emerging evidence suggests a link between ALS and the escape of mtDNA into the cytoplasm. For example, TDP-43 can translocate to mitochondria, triggering the opening of the mPTP, leading to mtDNA release and activating a cGAS-STING-dependent neuroinflammatory cascade that accelerates ALS. The pharmacological inhibition or genetic knockout of STING delays the onset of ALS-related pathologies in TDP-43 mutant mice [205]. Proteomic analyses reveal that the TDP-43 A382T mutation induces fragmentation of the mitochondrial network in fibroblasts from ALS patients, resulting in mtDNA release [206]. Additionally, SOD1 gene mutations, which are strongly implicated in ALS [207], cause mitochondrial damage in microglia and astrocytes, leading to mPTP-independent mtDNA escape. The released mtDNA spreads intercellularly via gap junctions, amplifying the inflammatory response through cGAS-STING signaling activation [208]. However, factors secreted from ALS patient-derived induced pluripotent stem cells, but not the mPTP inhibitor CsA, reduce inflammatory gene expression by stabilizing mitochondria, mitigate motor neuron loss, enhance muscle health, and slow ALS progression in mice, suggesting that solely inhibiting mPTP may be inadequate to prevent mtDNA release and treat ALS [209].

Others

Huntington's disease (HD), a chromosome 4-linked autosomal dominant disorder, selectively deteriorates the striatum and cortex via CAG triplet expansion in HTT, presenting with chorea, dementia, and cognitive impairment [210]. In HD mouse models, neuronal melatonin depletion is associated with elevated mtDNA efflux and cGAS pathway activation; exogenous melatonin treatment mitigates neuroinflammation and damage by repressing mtDNA escape [211]. Studies show that neural stem cells and fibroblasts from HD patients exhibit reduced mitochondrial metabolism and a buildup of mitophagy-related proteins, alongside EV-mediated mtDNA export into the bloodstream [212]. These results imply that elucidating the role of mtDNA escape in HD pathogenesis could aid in identifying diagnostic biomarkers and therapeutic targets. Moreover, mtDNA leakage has been detected in preclinical models and patients with glaucoma-induced neurodegeneration, underscoring the potential relevance of this phenomenon across various neurodegenerative conditions [213].

Stroke

Ischemic stroke

Ischemic stroke (IS), a common subtype of stroke, results from vascular obstruction that disrupts cerebral blood flow. Recent studies confirm that ox-mtDNA escape follows IS, with CMPK2 overexpression as a key factor. NDGA, the inhibitor of CMPK2 shows promise in IS mouse model and stroke patient monocytes, indicating clinical potential [78]. In the acute phase of IS, 7–14 days post-onset, ZBP1 upregulation in microglia adjacent to the infarct contributes to neuroinflammation triggered by mtDNA escape [214]. Excessive phagocytosis by microglia post-IS leads to synaptic depletion, which can impair recovery. MtDNA escape triggers the STING pathway, boosting microglial synaptic phagocytosis, which can be reversed with STING inhibitors to protect synapses and support recovery, highlighting the diverse impacts of

mtDNA escape beyond inflammation [215]. Neuronally expressed chemokine-like factor 1 (CKLF1) is linked to IS pathology by activating NLRP3 inflammasome, microglial activation, and pyroptosis, suggesting it is a therapeutic target for IS [216–218]. Recently, treatment with CKLF1 active peptide has been shown to disrupt microglial mitophagy, leading to elevated cytosolic mtDNA and cGAS signaling activation, suggesting a mechanism by which CKLF1 may trigger mtDNA escape and pyroptosis induced by inflammasome activation [219]. Single-cell RNA-sequencing analyses have highlighted the IFN response as a hallmark of post-IS microglia [220], indicating that mtDNA-induced cGAS signaling is a prevalent and pivotal route in microglial activation following IS. Given the crucial role of microglia in IS, mtDNA leakage profoundly affects the regulation of microglial activity, thereby significantly influencing the pathological progression of IS [221]. Activation of the AIM2 inflammasome after mtDNA escape results in inflammation in atherosclerotic mice and promotes stroke [222], suggesting that AIM2 activation could increase the risk of IS recurrence after mtDNA escape.

Hig1 hypoxia inducible domain family member 1a (HIGD1A), a protein located in the mitochondrial inner membrane, is essential for regulating mitochondrial metabolism and respiration under hypoxic conditions [223]. HIGD1A deficiency has been associated with increased ox-mtDNA and its subsequent escape to the cytoplasm, activating the NLRP3 inflammasome. Overexpression of HIGD1A, however, can effectively reduce ox-mtDNA escape [224], positioning HIGD1A as a potential target for controlling mtDNA escape in the context of IS and other hypoxia-related neurological disorders [225].

Cerebral ischemia–reperfusion injury (CIRI) is a common complication of reperfusion in IS, which exacerbates ischemic damage [226]. Elevations in mtDNA levels have been observed within the infarct zone of CIRI mouse models [227]. Evidence indicates that mtDNA escape occurs during CIRI, promoting microglial pro-inflammation via cGAS signaling, which is dampened by the STING inhibitor C-176, thereby enhancing neurological recovery [228]. Neuronal ox-mtDNA escape has also been shown to activate cGAS signaling and inflammation, a response mitigated by CsA treatment, showing therapeutic promise in CIRI model both in vitro and in vivo [229]. Additionally, oxidative stress associated with CIRI potentiates the interaction between mtDNA and cGAS, amplifying the inflammatory response triggered by mtDNA escape [230]. The NLRP3 inflammasome, a canonical sensor of ox-mtDNA, is activated by cytosolic ox-mtDNA accumulation, and its activation can be inhibited by the mitochondrial protectant Edaravone, reducing ox-mtDNA-induced neurological impairments [231]. Furthermore, cGAS signaling is implicated in autophagy, ferroptosis, pyroptosis, calcium dyshomeostasis, and blood–brain barrier disruption, underscoring the multifaceted role of mtDNA escape in CIRI pathogenesis [232].

In summary, mtDNA escape offers critical insights into the interplay between oxidative stress, mitochondrial dysfunction, and inflammatory injury in IS and CIRI, and it may represent a promising target for therapeutic intervention.

Subarachnoid hemorrhage

Subarachnoid Hemorrhage (SAH) is a catastrophic form of hemorrhagic stroke that occurs when a cerebral blood vessel bursts, leading to blood entry into the subarachnoid space [233]. Clinical evidence is emerging that ccf-mtDNA may act as a prognostic biomarker for SAH, influencing the management of associated complications and clinical outcomes [234]. Following SAH, the accumulation of mtDNA in microglial cytoplasm triggers cGAS signaling, eliciting a pro-inflammatory response that exacerbates neurotrauma [235]. The fibroblast growth factor FGF21 has recently been shown to mitigate mtDNA release and the subsequent cGAS signaling activation, and inflammation, by enhancing mitophagy, markedly improving neurological outcomes in a murine SAH model [130]. In a rat model of cerebral hemorrhagic shock with reperfusion injury, mtDNA release was detected, and its inhibition was found to reduce pyroptosis via inhibiting NLRP3 inflammasome activation, affording hippocampal neuroprotection and improving motor function [236]. This is consistent with findings in a murine cerebral hemorrhage model, where elevated cytosolic mtDNA in microglia adjacent to the hematoma induced AIM2-dependent neuroinflammation. The use of the mitochondrial fission inhibitor Mdivi-1 decreased mtDNA release, thereby reducing inflammation and neurological damage [237].

Traumatic brain injury

Acute traumatic brain injury (TBI) is compounded by a cascade of secondary injury processes subsequent to the primary insult, culminating in exacerbated brain tissue damage and pronounced neurological impairment [238]. Studies in both clinical TBI cohorts and murine experimental models have revealed a significant activation of the cGAS-STING pathway, which is likely attributed to the leakage of mtDNA into the cytoplasm [239]. Subsequent research has validated that the released mtDNA following TBI acts as a stimulant for cGAS, and that blocking cGAS signaling ameliorates inflammation and secondary brain injury [131, 240]. Furthermore, the levels of EV-carried ccf-mtDNA in peripheral blood surge

during the acute phase of TBI. The potential of EV-carried ccf-mtDNA as a prognostic biomarker for TBI, however, warrants further investigation [241].

Neuropathic pain

Neuropathic pain (NeP) is a chronic condition arising from neuronal injury or dysfunction, characterized by spontaneous pain and hyperalgesia [242]. Evidence suggests that mtDNA leakage occurs in the central nervous system during NeP pathogenesis. In particular, mtDNA leakage and subsequent activation of the cGAS pathway were observed in the hippocampal tissues of mice with NeP, induced by sciatic nerve injury [243]. This mtDNA leakage triggers microglial activation, which is associated with the emergence of anxiety- and depression-like behaviors, as well as increased pain sensitivity. Inhibition of mtDNA leakage or the type I IFN pathway markedly mitigates these pathological changes [243]. In NeP mice, neuronally released mtDNA activates cGAS in microglia, exacerbating neuroinflammation and pain, a process ameliorated by the cGAS inhibitor RU.521 [244]. In the spinal cord, mtDNA leakage-induced cGAS activation promotes the IFN response and the differentiation of A1 reactive astrocytes, contributing to the progression of chronic postoperative pain [245]. This cascade can be interrupted by CsA to prevent mtDNA leakage or C-176 to inhibit cGAS activation, thereby alleviating mechanical allodynia in the mice [245]. Similarly, inhibiting cGAS signaling in the dorsal root ganglia of mice provides analgesic effects in the setting of acute postoperative pain [246]. These findings suggest that targeting mtDNA leakage may represent a promising therapeutic approach for acute and chronic postoperative pain. However, mechanical allodynia in NeP mice is independent of spinal microglial STING expression, yet intrathecal administration of a STING agonist can induce a sex-dependent reduction in hyperalgesia [247]. This indicates that STING activation by moderate cytosolic mtDNA may have therapeutic potential for pain management. The variability in outcomes highlights the complex and context-dependent roles of cGAS signaling in NeP, necessitating comprehensive exploration of mtDNA leakage and cGAS activation in microglia under NeP conditions.

Major depressive disorder

Major depressive disorder (MDD), a prevalent mental health disorder, may involve mtDNA escape. Clinical studies indicate that MDD patients with suicide attempts have significantly higher levels of ccf-mtDNA than healthy controls, which may correlate with hypothalamic–pituitary–adrenal (HPA) axis dysfunction [248]. Ccf-mtDNA levels in MDD patients mirror aspects of pathophysiological alterations and antidepressant responsiveness, suggesting its potential as a clinical biomarker [249]. Exosome-transported ccf-mtDNA is integral to MDD neuroinflammation and may serve as diagnostic and therapeutic targets [250]. Conversely, certain studies show reduced ccf-mtDNA in MDD patients versus controls, with potential declines during depressive episodes [251]. Therefore, elucidating the role of mtDNA escape in MDD necessitates extensive research and preclinical model validation.

Secondary cognitive impairment

Postoperative cognitive decline (POCD) refers to the impairment in cognitive abilities that may arise after surgery, associated with perioperative stress and anesthetic exposure. In an elderly murine POCD model, PHB2-mediated mitophagy in microglia suppressed mtDNA release. The mitochondrial peptide SS-31 (Elamipretide) enhances this autophagy, reducing mtDNA-triggered cGAS inflammation and improving cognitive impairments [252]. Sevoflurane-induced cognitive impairment links to mitochondrial fission, mPTP-VDAC complex opening, and mtDNA release, triggering cGAS and NLRP3 inflammasome pathways. Inhibition of fission by Mdivi-1, mPTP by CsA, or VDAC by VBIT-4 blocks mtDNA release and alleviates cognitive decline [253]. Additionally, sevoflurane-induced mtDNA release triggers the ZBP1, RIPK3/MLKL pathway, culminating in neuronal necroptosis and associated cognitive impairment [254]. Furthermore, mtDNA release contributes to neuroinflammation and cell death linked to cognitive deficits in type 2 diabetes and chronic alcoholism [255, 256].

Other diseases

In a spinal cord injury mouse model, mtDNA release in spinal microglia mediated by MFN2 reduction was reversed with MFN2 agonist MASM7 delivered by biomimetic nanoparticles, markedly diminishing mtDNA-cGAS-STING neuroinflammation and tissue damage [108]. MtDNA-induced neuronal necroptosis is observed in spinal cord injury resulting from acrylamide exposure or a high-fat dietary regimen [257]. Atrazine, an environmental pollutant, induces neuronal pyroptosis and cerebellar harm via the mtDNA-cGAS-STING pathway, a condition rectifiable by melatonin therapy [258]. Ccf-mtDNA has been detected in the cerebrospinal fluid of HIV patients, potentially serving as a biomarker for iron metabolism dysregulation and neuroinflammation during HIV infection [259]. Similar evidence from hippocampal tissue and blood of mesial temporal lobe epilepsy patients, including mitochondrial cristae disarray, points to mtDNA release in epilepsy pathogenesis [260]. Moreover, intermittent hypoxia induces mPTP-mediated mtDNA release in hippocampal neurons in vitro,

triggering cGAS signaling-driven PANoptosis and ER stress [261].

Therapeutic potential in neurological diseases

Cytosolic leakage of mtDNA in neurological pathologies triggers inflammatory cascades and potentiates cell death. Consequently, the regulation of mtDNA release and subsequent PRR engagement is emerging as a strategic approach to mitigate disease exacerbation. The release of mtDNA is governed by factors including mtDNA homeostasis, mitochondrial membrane integrity, and the regulation of mitochondrial dynamics, which are critical for activating cytosolic PRRs. This complexity affords a rich tapestry of therapeutic targets for neurological disorders. Numerous pharmacological entities and natural derivatives have shown promise in preserving mtDNA stability, securing the mitochondrial barrier, enhancing mitochondrial homeostasis, and suppressing PRR activation, highlighting their therapeutic potential. This section summarizes the currently identified drugs that target mtDNA leakage for the treatment of neurological disorders (Table 1). Several potential pharmacological agents have also been included in the scope of discussion, aiming to provide broader prospects for the development of therapeutic strategies targeting mtDNA leakage.

Inhibition of mitochondrial membrane permeabilization

mPTP

CsA, a potent mPTP inhibitor, has demonstrated efficacy in various preclinical models of neurological disorders, including CIRI [229], chronic pain [245], secondary cognitive dysfunction [253], and neuronal hypoxia [261], reducing mtDNA release and neuroinflammation. Clinical trials for acute TBI patients treated with CsA revealed dose-dependent benefits and a favorable safety profile [262]. However, prior acute anterior circulation stroke trials did not observe infarct size reduction with CsA [263], potentially due to factors like dosage, route, and

Table 1 A summary table of therapeutic approaches to inhibit mtDNA leakage for the treatment of neurological diseases

Drug	Therapeutic mechanism	Preclinical evidence	Potential clinical applications
CsA	mPTP inhibitor	Suppress mtDNA release and various PRRs activation [229, 245, 253, 261]	Ischemic stroke [229], chronic pain [245], secondary cognitive dysfunction [253], nerve hypoxia damage [261]
VBIT-4	Inhibitor of VDAC oligomer pore	Suppress mtDNA release and various PRRs activation [47, 253, 284]	Alzheimer's disease [286], amyotrophic lateral sclerosis [287], postoperative cognitive dysfunction [253]
Resveratrol	Suppress VDAC1 expression	Inhibit mPTP opening and mtDNA release [289]	Parkinson's disease [289]
MitoQ	Promote TFAM expression, suppress mtROS production	Suppress ox-mtDNA production and release, suppress various PRRs activation [292, 301–303]	Ischemic stroke [292], Alzheimer's disease [304], hepatic encephalopathy [300]
NDGA	CMPK2 inhibitor	Suppress ox-mtDNA release and NLRP3 inflammasome activation [78]	Ischemic stroke [78]
hydroxytyrosol butyrate	Inhibit mtROS production	Suppress mtDNA oxidation and leakage [306]	Behavioral impairment induced by sleep deprivation[306]
Mdivi-1	Mitochondrial fission inhibitor	Blocks mtDNA release and suppress activation of cGAS and TLR9 [98, 160]	Subarachnoid hemorrhage [307], ischemic stroke [308], amyotrophic lateral sclerosis [309], diabetic cognitive impairment [310], postoperative cognitive impairment [253]
Metformin	Suppress MFN2 expression	Suppress mtDNA release and cGAS activation [106]	Parkinson's disease [106]
Urolithin A	Activator of mitophagy	suppress mtDNA release and cGAS activation [111]	Neurodegenerative diseases [327, 328, 330], secondary cognitive impairment [331]
SS-31	Activator of mitophagy	Blocks mtDNA release and various PRRs activation [252, 334, 335]	Neurodegenerative diseases [341], secondary cognitive dysfunction [337], spinal cord injury [338]
Rapamycin	Autophagy inducer	Blocks mtDNA release and various PRRs activation [41, 193, 342, 343]	Spinal cord injury [344], cognitive dysfunction [417], depression [418], Sturge-Weber syndrome [345]
Hydroxychloroquine	Activator of mitophagy	Diminish mtDNA accumulation in TLR9 endosomes and suppress activation of cGAS and TLR9 [346, 347]	Alzheimer's disease [350]

cGAS: cyclic GMP-AMP synthase; VDAC: voltage-dependent anion channel; mPTP: mitochondrial permeability transition pore; TFAM: mitochondrial transcription factor a; NLRP3: nod-like receptor family pyrin domain containing 3; PRR: Pattern recognition receptor; TLR9: Toll-like receptor 9; AIM2: absent in melanoma 2; CMPK2: cytidine monophosphate kinase 2

timing of treatment [264]. Novel mPTP inhibitors, such as Icariin and Etifoxine, have shown promise in animal models of IS and TBI, potentially through the inhibition of mtDNA release [265, 266]. Moreover, indirect approaches with GSK-3β and JAK/STAT pathway inhibitors have been reported to inhibit mPTP opening [264], yet their efficacy in suppressing mtDNA release and enhancing neurological outcomes needs further study.

OPA1

The strategy of augmenting OPA1 expression is a method of preventing mtDNA leakage by maintaining mitochondrial cristae integrity. According to research, SIRT3 upregulates OPA1 expression and its deacetylation, thereby enhancing its function. Stimulation of the SIRT3/OPA1 pathway has been shown to reduce brain injury in CIRI and TBI models [267, 268], hinting at its therapeutic promise. Natural compounds like Celastrol [269], Pectolinarigenin [270], and Rhodiola crenulata [271] restore mitochondrial health and protect against hemorrhage- or ischemia-induced brain damage by modulating the SIRT3/OPA1 axis. Furthermore, Empagliflozin, an antidiabetic agent, suppresses LPS-induced NLRP3 inflammasome activation and neuroinflammation by elevating OPA1, potentially through preventing mtDNA release [272].

BAX/BAK macropores

BAI1, a BAX inhibitor, effectively prevents BAX's mitochondrial translocation and oligomerization, thus inhibiting mtDNA release in diverse cell types [40, 43]. In spite of its efficacy in cardiomyopathy, its potential in neurological disorders remains unexplored [273]. In MOMP-induced mtDNA escape studies, inhibitors like BAI1 and BAI2 are being tested for direct effects and safety. These inhibitors serve as valuable tools for studying MOMP-dependent mtDNA escape [274, 275]. Moreover, signaling molecules such as PGAM5 [42], the ATAD3-SAM50 axis [41], the DUSP/JNK pathway [43], and SRSF6 [45] are known to inhibit BAX/BAK macropores and prevent mtDNA escape. Plasma PGAM5 is a potential AD biomarker [276], with preclinical evidence suggesting its knockdown alleviates neuronal damage and neuroinflammation in epilepsy [277], spinal cord injury [278], and TBI [279]. Furthermore, PGAM5 depletion suppresses mitophagy and fission, potentially preventing mtDNA escape to improve TBI recovery. LFHP-1c, a novel PGAM5 inhibitor, demonstrates potential in mitigating ischemic injury and preserving the BBB [280]. However, the deletion of PGAM5 can lead to depression-like behavior by affecting dendritic spine density and neuronal ATP production [281]. The complex interplay of PGAM5 in mtDNA escape, energy metabolism, and mitochondrial dynamics calls for strategic inhibition in therapy. The role of SRSF6 in neurodegenerative diseases like AD and PD remains an area of ongoing research [282, 283].

VDAC oligomers

VBIT-4, a known inhibitor of VDAC oligomer pore, curbs mtDNA release and subsequent PRR activation by targeting VDAC expression and oligomerization [47, 253, 284]. A similar effect has been observed with other oligomerization inhibitors of VDAC1, including VBIT-12 [54] and DIDS [285]. For instance, VBIT-4 ameliorates neuroinflammation, AD pathology, and cognition in 5×FAD mice by inhibiting VDAC1 in Aβ-adjacent neurons [286]. VBIT-4 and VBIT-12 have also shown positive results in ALS mouse model [287] and retinal ischemia–reperfusion models [288]. In the PD model, VDAC1 has been shown to positively correlate with α-synuclein expression [289]. Resveratrol mitigates dopaminergic neuron degeneration by reducing mtDNA release and inhibiting mPTP opening through the suppression of VDAC1 protein expression [289].

Maintenance of mtDNA homeostasis

TFAM is vital for stabilizing mtDNA and inhibiting its escape, emerging as a potent therapeutic target for neurological disorders. Preclinical studies in models of PD [290], spinal cord injury [291], and IS [292] have shown beneficial outcomes following the upregulation of TFAM expression. Drug interventions primarily boost TFAM levels by stimulating the AMPK/SIRT1/PGC-1α axis, leading to PGC-1α nuclear translocation and subsequent induction of TFAM gene expression [293, 294]. Compounds like the AMPK activator ezetimibe show promise in treating neurodegenerative disorders, stroke, and spinal injuries by enhancing TFAM and curbing mtDNA leakage [295, 296]. Natural products like Tetramethylpyrazine and Gastrodin have been shown to ameliorate CIRI and vascular dementia by bolstering TFAM function [297, 298]. Yet, research on TFAM enhancement to prevent mtDNA leakage predominantly employs overexpression methods.

MitoQ, a mitochondria-targeted antioxidant, has been shown to mitigate neural damage by reducing mtROS production and restoring mitochondrial integrity [299, 300]. It effectively curbs mtROS, lessening oxidative mtDNA damage and blocking its dissemination into the cytoplasm and circulation, thus inhibiting cGAS and NLRP3 inflammasome activation [301–303]. Additional studies imply that MitoQ might enhance TFAM expression through SIRT6 regulation, concomitantly lowering ox-mtDNA levels to ameliorate CIRI [292]. This indicates that TFAM regulation could be a mechanism by which

MitoQ inhibits ox-mtDNA escape, although its broader effects on mitochondrial function and mtROS inhibition are also significant. Although MitoQ has shown good therapeutic effects in a variety of neurological diseases [292, 300, 304], critical to our understanding is discerning whether the therapeutic efficacy is rooted in the augmentation of energy metabolism, the suppression of mtDNA leakage, or a synergistic interplay of these mechanisms. Assessing the immunomodulatory effects of these targets may delineate the pharmacological mechanisms of these agents.

Molecules like resveratrol and NDGA show promise as mtDNA homeostasis regulators for therapeutic applications. In an Aβ-induced AD model, SIRT1 inhibition reduced OGG1, counteracted by resveratrol via SIRT1 activation, indicating its potential to inhibit mtDNA escape [305]. As noted, NDGA, an inhibitor of CMPK2, holds clinical promise for reduces neuroinflammation and ameliorates ischemic injury by curbing ox-mtDNA release and suppressing NLRP3 inflammasome activation [78]. Moreover, the mitochondrial nutrient hydroxytyrosol butyrate has demonstrated efficacy in inhibiting mtDNA oxidation and leakage in microglia, thus repressing neuroinflammation [306].

Improvement of mitochondrial dynamics

Regulation of mtochondrial fission

Curbing excessive mitochondrial fission and abnormal fusion is pivotal in preventing mtDNA escape, making it a strategic approach to minimize leakage. Mdivi-1, a fission inhibitor, blocks mtDNA release via DRP1 suppression, halting cGAS [98] or TLR9 signaling [160], and reducing microglial-driven neuroinflammation [253]. Additionally, Mdivi-1 mitigates mitochondrial overfission and neuronal damage across various preclinical models, including SAH [307], CIRI [308], ALS [309], and diabetic cognitive impairment [310]. Past research has primarily emphasized the ability of Mdivi-1 to effectively inhibit mtROS production, which is closely linked to the excessive generation and leakage of ox-mtDNA [311]. Particular attention should be given to the inhibitory effects of drugs like Mdivi-1 on ox-mtDNA leakage. Notably, Mdivi-1 also inhibits mitophagy, indicating its multifaceted impact on mtDNA escape via various pathways [312, 313].

Peptide P110, by targeting DRP1's GTPase activity and Fis1 interaction, markedly lowers plasma ccf-mtDNA in HD mice [314]. It shows therapeutic promise in neurological conditions including HD [315], AD [316], ALS [317], septic encephalopathy [318], Friedreich ataxia [319], and hereditary spastic paraplegia [320]. It remains unclear whether the peptide P110 is as effective as Mdivi-1 in inhibiting mtDNA leakage. In addition to these known mitochondrial fission inhibitors, regulating the post-translational modifications of DRP1 represents a potential therapeutic strategy, which has been shown to significantly suppress mtDNA leakage and alleviate symptoms in AD models [103].

Another crucial consideration is the multifaceted role of mitochondrial fission in various neurological disorders and at different stages of disease progression. Earlier studies have reported adverse effects of DRP1 gene knockout in mice, including embryonic lethality, impaired synaptogenesis, and female infertility [321]. For instance, during the recovery phase of IS, which involves extensive neural repair and synaptic regeneration, the use of mitochondrial fission inhibitors may negatively impact these processes [322]. These factors collectively determine the clinical applicability and limitations of mitochondrial fission inhibitors.

Regulation of mtochondrial fusion

Metformin, an antidiabetic, reverses PD-linked behavioral deficits and dopaminergic neuron loss in mice by normalizing mitochondrial function through MFN2 reduction, inhibiting mtDNA leakage and cGAS activation [106]. MFN2 modulators like Phelligridimer A [323] and cannabidiol [324] demonstrate efficacy in cerebral ischemia models, highlighting their potential in neuroprotective strategies.

Similar to the challenges faced by mitochondrial fission inhibitors, it is premature to draw definitive conclusions about the effects of MFN2 modulators due to the complex role of mitochondrial fusion in neurological disorders, especially considering that these inhibitors or agonists have shown promising efficacy in various diseases [324]. The current conflicting evidence suggests that careful consideration should be given to the effects of MFN2 modulators under aging and normal conditions, with future research emphasizing the need to distinguish between these two states [106, 108]. Indeed, rather than exclusively inhibiting either mitochondrial fission or fusion, maintaining a balanced interplay between the two processes may represent a more prudent and promising approach [325].

Enhancement of mitochondrial autophagy

Enhanced mitophagy is pivotal for the removal of damaged mitochondria and the prevention of mtDNA release, thereby suppressing inflammation. Urolithin A, an activator of mitophagy derived from plants or produced by the microbiota, is recognized for its neuroprotective effects in AD, PD, and brain injury through the induction of mitophagy [326]. In senescent cells, Urolithin A induces mitophagy to maintain mtDNA integrity, inhibit leakage, and downregulate cGAS signaling,

thus alleviating neuroinflammation associated with aging [111]. Additionally, it counteracts inflammation and microglial senescence resulting from autophagy defects [327] and reduces NLRP3 inflammasome activation [328], potentially via the inhibition of mtDNA leakage. Notably, Urolithin A has shown promising efficacy in preclinical studies of neurodegenerative diseases [329, 330], secondary cognitive impairment [328, 331], and other conditions. More importantly, clinical trials have revealed a favorable pharmacokinetic and safety profile for Urolithin A [332, 333].

SS-31, a peptide capable of penetrating the mitochondrial membrane, promotes mitophagy by stabilizing PINK1 through its interaction with PHB2. This action has been shown to reduce oxidative damage and prevent the leakage of mtDNA significantly, consequently alleviating cognitive deficits associated with POCD [252]. Moreover, SS-31 has been found to inhibit the activation of cGAS and TLR9 signaling pathways by escaped mtDNA, further mitigating inflammatory responses [334, 335]. Research also reveals that SS-31 binds to various mitochondrial proteins, influencing mitochondrial ATP metabolism and cardiolipin remodeling associated with the integrity of the IMM [336]. Collectively, these findings indicate that SS-31 acts through multiple mechanisms to prevent mtDNA escape. In both preclinical studies [337, 338] and clinical trials [339], SS-31 has demonstrated efficacy in reducing neuroinflammation across various neurological conditions [340, 341].

Rapamycin, also known as Sirolimus, is a well-known autophagy inducer that enhances the autophagic process by activating the mTOR signaling pathway. Studies have shown that Rapamycin can inhibit mtDNA escape induced by mtROS [193, 342] or LPS [343]. Recent findings reveal that Rapamycin specifically eliminates oxidatively damaged mtDNA by promoting mitophagy, thereby preventing its extramitochondrial leakage [41]. Additionally, Rapamycin has been found to improve spinal cord injury rat prognosis by inhibiting AIM2 inflammasome via enhanced mitophagy, suppressing mtDNA release [344]. In trials for Sturge-Weber syndrome [345], mild cognitive impairment (NCT04200911), and depression (NCT02487485), Rapamycin has shown a favorable safety profile and promising therapeutic potential.

Hydroxychloroquine, an antimalarial, diminishes mtDNA accumulation in TLR9 endosomes, thus preventing mtDNA escape-induced TLR9 signaling activation [346] and cytosolic DNA-cGAS binding [347]. Enhanced autophagic flux may be the intrinsic mechanism by which hydroxychloroquine suppresses mtDNA escape [348, 349]. Evidence has shown that Hydroxychloroquine treatment in APP/PS1 mice ameliorated neuroinflammation and synaptic deficits, lowering AD risk [350], yet its role in neurodegenerative disease pathogenesis is debated [351]. Recent research confirms that SIRT1 fosters late endosome maturation through RAB7 upregulation, facilitating mtDNA transport to lysosomes for degradation. SIRT1 activator SRT1720 or RAB7 overexpression can rescue mitophagy, suppressing cGAS and NLRP3 inflammasome activity to diminish tissue injury [124]. There is growing scientific interest in the therapeutic potential of mitophagy activators for neurodegenerative diseases, which may become a central focus of future drug discovery efforts [352]. The role of diverse and innovative mitophagy activators in mitigating mtDNA leakage warrants particular attention.

Inhibition of downstream PRRs in mtDNA escape

Beyond halting mtDNA leakage, cytosolic PRR inhibition is key to preventing immune reactions from free mtDNA. Recent years have seen significant advances in the development of cGAS and STING inhibitors for addressing neurological disorders [13]. Inhibiting NLRP3 and AIM2 inflammasomes prevents mtDNA-provoked inflammation; some inhibitors demonstrate neuroprotection in neurological diseases and are in clinical trials [353, 354].

Inhibition of cGAS signaling

Significant advances in cGAS-STING inhibitors, including drugs, synthetics, and naturals, show neuroprotective potential in disease models (Table 2), though most await clinical trials [355]. First, blocking the binding of mtDNA to cGAS can indirectly inhibit cGAS activation. Quinacrine, a lipophilic antimalarial, effectively crosses the BBB and can be used to mitigate AD [356] and viral neuroinflammation [357]. Studies reveal that Quinacrine binds to dsDNA like mtDNA, dissociates the DNA-cGAS complex, and inhibits cGAS signaling by escaped mtDNA [358]. Derivative compounds like X6 exhibit similar efficacy with reduced toxicity in suppressing cGAS activity [347]. A151, an inhibitory oligodeoxynucleotide, competitively binds to the DNA-binding domain of cGAS to inhibit its activity [347]. It can promote the polarization of microglia to an anti-inflammatory phenotype, thereby reducing brain inflammation in IS or CIRI model [359, 360].

Furthermore, regulators directly targeting the inhibition of cGAS activity have shown promising prospects in the treatment of neurological diseases. RU.521, a prototypical cGAS inhibitor, effectively blocks catalytic activity by binding its pocket, eliciting anti-inflammatory effects in neurological disorder models [129, 244]. Simultaneously, it fosters the polarization of microglia to an anti-inflammatory phenotype [361], diminishes pyroptosis [129, 362], ferritin autophagy [363], and other derivative effects as detailed in Table 2. Notably, the small-molecule

Table 2 cGAS inhibitor used to treat neurological disorders

Drug	Mechanism	Diease	Model	Therapeutic effect	Refs.
Quinacrine	Blocking the cGAS–dsDNA	AD	5 × FAD mice	↓: Aβ aggregates, Aβ deposition, synaptic abnormality	[356]
A151	Blocking the cGAS–dsDNA	IS	MCAO rat	↑: microglial M2 phenotype	[360]
A151		– CIRI	MCAO/R mice	↓: AIM2 inflammasome activation, microglial pyroptosis, neutrophil infiltration, production of microglia pro-inflammatory factors, neurodeficits, apoptosis ↑: neuromotor function	[359]
RU.521		– CIRI	OGD/R Induction of HT22 Cells	↓: ferritinophagy	[363]
RU.521		– SAH	In vivo: rat after endovascular in vitro: perforation OxyHb-treated BV2 microglia and HT22 hippocampal neurons	↓: brain edema, microglia activation, activation of NF-κB pathways ↑: microglial M2 phenotype, neuromotor function, recovery of neurological function, dendritic spine densities	[361]
RU.521		– NeP	CCI mice model	↓: allodynia and hyperalgesia, microglia activation	[244]
RU.521		– POCD	Mice after laparotomy	↓: activation of NF-κB pathways, caspase-3/GSDME-dependent pyroptosis ↓: cognitive function	[129]
RU.521		– POCD	In vivo: mice after sevoflurane anesthesia In vitro: sevoflurane treated BV2 microglia	↓: hippocampal neuronal injury, p-Tau, APP, NLRP3 inflammasome activation ↑: cognitive function	[253]
RU.521		– CVST	Mice with superior sagittal sinus thrombosis	↓: apoptpsis, neurodegeneration, oxidative stress injury, infiltration of monocyte/macrophages, NLRP3 inflammasome activation, pyroptosis, neurological dysfunction	[362]

AD: Alzheimer's Disease; IS: ischemic stroke; CIRI: cerebral ischemia–reperfusion injury; SAH: subarachnoid hemorrhage; NeP: neuropathic pain; POCD: postoperative cognitive dysfunction; CVST: cerebral venous sinus thrombosis; MCAO: middle cerebral artery occlusion; MCAO/R: middle cerebral artery occlusion/reperfusion; OGD/R: oxygen–glucose deprivation/reperfusion; CCI: chronic constriction injury

inhibitor TDI-6570, identified through high-throughput screening, demonstrates potent inhibition of cGAS signaling [364]. It exhibits excellent brain penetrability while effectively improving cognitive function in a mouse model of tauopathy, indicating its enhanced pharmacokinetic properties [200].

Due to space constraints, several macromolecular pharmacophores and other allosteric cGAS inhibitors were not included in the discussion [13]. Although their application in neurological disorders has not yet been explored, their development potential warrants further attention.

Similarly, the STING-binding inhibitor C-176 demonstrates robust pharmacodynamic effects in neurological disease models, including suppression of inflammatory factors [365], inflammasome activation [256, 366, 367], glial activation, and promotion of an anti-inflammatory microglial phenotype [228, 245, 255, 368–371], mitigating BBB damage [240], fostering neuroregeneration [368], and enhancing autophagy [372] (Table 3). Nanoparticles loaded with C-176 further enhance their efficacy by extending drug retention time and rescuing ischemic neuronal injury [368]. Another highly selective covalent STING small molecule antagonist, H-151, also exerts neuroprotective effects in IS [215], neurodegenerative diseases [199, 373], and secondary anxiety disorder [374] through similar mechanisms (Table 3). A series of recent studies have confirmed the potential effects of STING on autophagy, lipid metabolism, and glucose metabolism, highlighting our limited understanding of non-inflammatory functions of STING [375]. Comprehensive investigation into the cellular heterogeneity of STING inhibitors and their effects across different disease contexts is essential, suggesting that the clinical translation of STING inhibitors should proceed with caution.

Additionally, inhibiting the phosphorylation of the direct downstream TBK1 of STING can also block cGAS signaling, and the recently developed TBK1 inhibitor BX795 improves PD pathological phenotypes by inhibiting TBK1 activation [376]. In summary, selecting drugs that inhibit cGAS signaling can effectively block the inflammatory response caused by escaped mtDNA and improve neurological diseases. Given the remarkable performance of cGAS signaling inhibitors in preclinical studies, comparing the safety, metabolic activity, and pharmacokinetic profiles of various cGAS inhibitors will be a key focus for future research.

Table 3 STING inhibitor used to treat neurological disorders

Drug	Mechanism	Diease	model	Therapeutic effect	Refs.
C-176	Inhibit STING activation	PD	In vivo: MPTP-induced mice model In vitro: MPTP-treated BV2 microglia	↓: NLRP3 inflammasome activation, dopaminergic neurodegeneration	[367]
C-176	–	TBI	Mice after dura mater impact	↓: blood–brain barrier damage, brain edema	[240]
C-176	–	TBI	Controlled cortical impact mouse model	↓: cortical damage area, proinflammatory cytokine, gait disturbances	[365]
C-176	–	TBI	Controlled cortical impact mice model	↓: microglial M1 phenotype, number of FJC-positive neurons and TUNEL-positive neurons, neurological dysfunction ↑: microglial M2 phenotype	[369]
C-176	–	Severe TBI	Weight-drop plus blood loss rat reinfusion model	↓: cognitive dysfunction, emotional impairments, neuronal loss, NLRP3 inflammasome activation, pyroptosis	[366]
C-176 loaded Ce DNase nanoparticles	–	IS	MCAO mice model	↓: infarction area, microglia activation, neurological dysfunction ↑: neurogenesis	[368]
C-176	–	CIRI	In vivo: MCAO mice model in vitro: BV2 microglia OGD/R model	↓: neurodegeneration, brain infarction, edema and neuronal injury ↑: microglial M2 phenotype	[228]
C-176	–	SAH	Rat after intravascular perforation	↓: autophagic flux injury, brain injury	[372]
C-176	–	SAH	Mice after intravascular perforation	↓: brain edema, neuronal injury ↑: microglial M2 phenotype	[370]
C-176	–	AUD-related cognitive impairment	Chonic alcohol exposure mice	↓: NLRP3 inflammasome activation, apoptosis ↑: cognitive function	[256]
C-176	–	T2DM-related cognitive impairment	HFD fed mice	↓: plasma inflammatory, NF-κB activation, microglia activation ↑: cognitive function	[255]
C-176	–	CPSP	Skin/muscle incision and retraction mice	↓: A1 reactive astrocytes, mechanical allodynia	[245]
C-176	–	SNI	Spared nerve injury-induced mice	↓: pain hypersensitivity, microglia activation, proinflammatory factors, activation of JAK2/STAT3 pathways	[371]
H-151	Block STING conformational changes	Ethanol-induced anxiety symptoms	Chronic alcohol exposure of mice	↓: anxiety-like behavior, microglia activation	[374]
H-151	–	IS	MCAO mice	↓: microglia overactivation, Microglia-mediated synapse phagocytosis, the nucleus translocation of phosphorylated STAT1 ↑: motor function recovery after stroke	[215]
H-151	–	Sporadic ALS	Macrophage cultures from ALS patients	↓: TNF-α, IL-1β, NF-κB signaling	[373]
H-151	–	AD	5 × FAD mice	↓: Aβ42, microglia activation, satrocyte activation, inflammatory cytokines, complement factors	[199]

Table 3 (continued)

PD: Parkinson's disease; TBI: traumatic brain injury; IS: ischemic stroke; CIRI: cerebral ischemia–reperfusion injury; SAH: subarachnoid hemorrhage; AUD: alcohol use disorder; T2DM: type 2 diabetes mellitus; CPSP: chronic post-surgical pain; SNI: spared nerve injury; ALS: amyotrophic lateral sclerosis; AD: Alzheimer's Disease

Inhibition of inflammasome activation

Several small molecule inhibitors targeting the NLRP3 inflammasome have been developed and are in clinical trial phases [377]. Nevertheless, it remains to be determined whether inhibiting the NLRP3 inflammasome can entirely prevent mtDNA-induced inflammation, highlighting the need for further research on the activation of downstream PRRs by escaped mtDNA.

Blocking the activation of AIM2 inflammasome is beneficial in treating a range of neurological conditions, including stroke, brain injury, psychiatric illnesses, and neurodegenerative diseases [378]. P202, a specific inhibitor of AIM2 inflammasome, blocks the activation of this inflammasome in microglia by preventing the binding of escaped mtDNA to AIM2, thereby improving brain damage caused by cerebral hemorrhage [237]. Some small molecule compounds have been identified that reduce AIM2 inflammasome priming by inhibiting the NF-κB pathway, thus improving neuroinflammatory damage in stroke model [379, 380]. Additionally, certain natural products have been found to directly or indirectly inhibit AIM2 inflammasome activation, thereby ameliorating neurological disorders [381–386] (Table 4). Currently, there are no specific inhibitors of the AIM2 inflammasome available for clinical use. However, A151, a small molecule inhibitor of cGAS, has been shown to inhibit AIM2 inflammasome activation and improve inflammatory brain damage after ischemia [359]. Caspase-1 inhibitors like Ac-YVAD-cmk [387] and VX765 [388], which target a shared step in the activation of both NLRP3 and AIM2 inflammasome, have been shown to effectively suppress these pathways, mitigating microglial activation, pyroptosis, and BBB disruption in TBI mouse model (Table 4). Therefore, some NLRP3 inflammasome inhibitors, such as the ASC recruitment inhibitor J114, may also be applicable for inhibiting AIM2 inflammasome activation [389]. Furthermore, indirect modulators such as RO27-3225 have been validated to potently inhibit AIM2 inflammasome activation, offering therapeutic potential for neurological conditions [390, 391]. Although the development of inflammasome inhibitors has reached a relatively advanced stage, a comprehensive understanding of the role of mtDNA leakage in inflammasome activation may identify new inhibition sites, paving the way for the development of more effective therapeutics. Importantly, whether these inhibitors can block the interaction between mtDNA and inflammasomes warrants further investigation, offering a novel perspective for secondary screening of existing candidate drugs.

Inhibition of TLR9 signaling

TLR9 is capable of selectively identifying a range of DNA types, including mtDNA, and activating downstream inflammatory signals, making it one of the important participants in mediating neuroinflammation [392]. TLR9 inhibitors efficiently prevent NF-κB pathway and NLRP3 inflammasome activation by escaped mtDNA or ox-mtDNA [161, 162]. It has been shown that ODN 2088, an oligodeoxynucleotide and a mature TLR9 antagonist, effectively blocks the activation of TLR9 by mtDNA [393]. ODN 2088 intrathecal injection curbs astrocyte proliferation, triggers the release of chemokines such as CCL1, and fosters M2 macrophage polarization, ameliorating spinal cord injury histopathology and function [394, 395]. Notably, the therapeutic effects of ODN 2088 on neuropathy exhibit gender-specific outcomes, indicating sex-dependent roles of TLR9 signaling in nerve injury [396]. COV08-0064, another TLR9 antagonist, inhibits the loss of dopaminergic neurons in PD by blocking peripheral TLR9 activation [397]. Studies reveal that the TLR9 agonist CpG ODN boosts amyloid-β clearance via transient recruitment of peripheral macrophages, improving behavior and reducing amyloid pathology in aged squirrel monkeys, without causing microhemorrhages or encephalitis [398]. Further research is essential to reassess the role of TLR9 signaling modulation in various neuropathologies. Early-stage mtDNA-induced TLR9 activation may be advantageous while mitigating excessive TLR9 activation is key to neuroinflammation management. The timing of therapeutic interventions is also crucial.

Melatonin and its derivatives

Melatonin, an endogenous hormone from the brain's pineal gland, mainly regulates the sleep–wake cycle. Recent research highlights melatonin as a potent antioxidant for enhancing outcomes in neurological conditions such as neurodegeneration [399], TBI [400], depression [401], and sleep disorders [402]. Melatonin can effectively inhibit mtDNA escape and reduce cytosolic mtDNA-induced cGAS signaling activation [201, 403]. This effect may involve multiple actions of melatonin, including the improvement of mtDNA oxidative damage [404], enhancement of PINK1/Parkin-mediated mitophagy [201, 405], and upregulation of the expression of mtDNA

Table 4 AIM2 inhibitor used to treat neurological disorders

Drug	Mechanism	Diease	Model	Therapeutic effect	Refs.
Inhibit priming					
Ozanimod	Regulate SIRT3/NF-κB/AIM2 pathways	ICH	Intracranial injection induced ICH mice model	↓: inflammatory cytokines, microglia activation, hematoma size	[379]
Nicorandil	Regulate NF-κB/AIM2 pathways	CIRI	MCAO/R mice	↓: inflammatory cytokines, microglia activation, infarction area, apoptosis, pyroptosis	[380]
Inhibit activation					
Ac-YVAD-cmk	Inhibit caspase-1 and ASC oligomerization	TBI	CCI mice	↓: pyroptosis, brain edema, BBB damage, neurological dysfunction	[387]
VX765	Inhibit caspase-1	TBI	CCI mice	↓: apoptosis, microglia activation, BBB damage, BBB damage, neurological dysfunction	[388]
Natural products					
Crocin	Inhibit the expression of AIM2, ASC, caspase-1	PD	Mice model induced by the stereotaxic injection of LPS	↓: inflammatory cytokines	[381]
Curcumin	Inhibit the expression of AIM2, ASC, caspase-1	PD	MPTP-induced mice model	↓: pyroptosis, dopaminergic neuronal degeneration, motor impairment	[382]
Ginsenoside Rg1	Inhibiting AIM2 inflammasome activation	LPS-related cognitive impairments	Mice model induced by the stereotaxic injection of LPS	↓: cognitive dysfunction, neuronal ferroptosis	[383]
Forsythoside A	Inhibit the expression of AIM2, ASC, caspase-1	Acute pancreatitis- induced brain injury	Mice model induced by sodium taurocholate	↓: hippocampal brain tissue water content, Hippocampal lesions, neurological dysfunction	[384]
Tangeretin	Inhibit the expression of AIM2, ASC	CIRI	In vivo: MCAO/R mice in vitro: hippocampal HT22 cells with OGD/R injury	↓: neuronal pyroptosis, neuronal mitochondrial damage, infarction area, neurological dysfunction	[385]
Wedelolactone	Inhibit the expression of AIM2, caspase-1	Retinal neurodegeneration	NMU-induced mice model, NMU-treated 661W photoreceptor cells	↓: photoreceptor degeneration, photoreceptor cell death	[386]
Indirect regulator					
RO27-3225	Activate Melanocortin receptor 4	Spinal cord injury	Crush spinal cord injury mice model	↓: AIM2 inflammasome activation	[390]
Probenecid	Blocked pannexin-1 channel	SAH	Rat after intravascular perforation	↓: AIM2 inflammasome activation, brain edema, neuronal death, neurological dysfunction	[391]

PD: Parkinson's disease; CIRI: cerebral ischemia–reperfusion injury; TBI: traumatic brain injury; SAH: subarachnoid hemorrhage; ICH: Intracerebral Hemorrhage; CIRI: cerebral ischemia–reperfusion injury

escape-related proteins such as TFAM and SAM50. Melatonin deficiency contributes to mtDNA escape and cGAS activation, but exogenous supplementation in HD mice inhibits this, reducing neuroinflammation and neuronal aging [211]. Similarly, melatonin improves cerebellar damage caused by neurotoxicity by inhibiting the cGAS-STING-NLRP3 axis induced by mtDNA escape [258]. Therefore, melatonin holds great therapeutic promise in mtDNA escape-related neurological diseases. The melatonin receptor agonist ramelteon, a derivative of melatonin, has shown neuroprotective effects in treating IS, but its relevance to mtDNA escape requires further study.

In essence, targeting mtDNA escape from various perspectives effectively reduces neuroinflammation, with compounds like resveratrol and melatonin showing potential as multi-stage inhibitors for related therapies. Single-target PRR inhibition may induce compensatory

activation, reducing efficacy [157], suggesting that combined or broad-spectrum inhibitors could be more effective strategies. Targeting damaged mitochondria with drugs to inhibit mtDNA escape and PRRs is challenging, but combining drugs with nanocarriers offers a potential strategy [108, 368]. Exogenous mitochondrial transplantation or modulating intercellular transfer, as novel therapies, enhance energy metabolism and dynamics, replace damaged mitochondria, and show promise in preventing mtDNA escape [406].

Techniques for mtDNA detection

Given the complexity of mtDNA leakage as a dynamic event, the methodologies used for its detection significantly impact research outcomes. One key factor is mitochondrial rupture during detergent-based cell fractionation, which can artificially increase cytosolic mtDNA content [407]. Bryant et al. have outlined several optimized protocols to minimize such artifacts and ensure more accurate quantification of cytosolic mtDNA [407].

Capturing the process of mtDNA release from mitochondria into the cytosol or extracellular space remains a significant challenge. Quantitative Polymerase Chain Reaction (PCR) and digital PCR are the primary methods employed in most preclinical studies. As the more traditional approach, qPCR is favored for its simplicity and cost-effectiveness [79, 106, 125]. In contrast, digital PCR offers the advantage of gene copy analysis without requiring a reference, amplifying DNA in nanoliter droplets and quantifying the total droplet count [408]. Puigròs et al. employed an improved Multiplex Digital PCR combined with Long-Range PCR to validate mtDNA fragments. This approach allows the assessment of ccf-mtDNA integrity and deletion sites, surpassing the capabilities of standard digital PCR by enabling targeted detection of EV-encapsulated ccf-mtDNA [409]. Due to the highly fragmented nature of cytosolic mtDNA, techniques such as fluorescence in situ hybridization or conventional microscopy are challenging for observing mtDNA leakage. Notably, advanced imaging techniques like 3D super-resolution Airyscan confocal microscopy and immunogold labeling transmission electron microscopy provide precise visualization of cytosolic mtDNA [40]. Additionally, cytosolic mtDNA in nucleoid-like forms can be detected using TFAM as a marker [52], which can be labeled with fluorescence and tracked via live-cell imaging to monitor mtDNA positional changes [20]. However, this approach may overlook fragmented mtDNA. Similarly, 8-oxoG serves as a marker for quantifying ox-mtDNA [79]. Liu et al. developed a Fluorescent Platinum Complex, which may facilitate real-time monitoring of mtDNA translocation in living cells [410]. In summary, the cross-validation of multiple detection methods may provide a more comprehensive and accurate approach.

In clinical research, due to the challenges of detecting mtDNA leakage in neuronal cells, most studies focus on measuring ccf-mtDNA in serum and cerebrospinal fluid. Owing to cost constraints, digital PCR is the most commonly used detection method [74, 409, 411]. A novel single microfluidic device has been developed to rapidly collect, purify, and amplify circulating free DNA from minimal plasma volumes, offering significant advantages for the rapid analysis of ccf-mtDNA [412]. Furthermore, sequencing of free DNA provides a detailed landscape of various forms of ccf-mtDNA. This approach has been extensively studied in cancer research, it holds similar potential for application in neurological diseases, enabling rapid and precise clinical diagnostics [413, 414].

Conclusion and outlook

This review encapsulates the pivotal role of mtDNA leakage in neurological disorders at the crossroads of mitochondrial impairment and inflammation. Research on mitochondrial diseases has historically centered on energy metabolism, acknowledging mtDNA's crucial role in maintaining function. However, the mislocalization of mtDNA following its escape transforms it into a DAMP, triggering immune activation. The causes of this transformation are multifaceted, including mtDNA homeostasis, mitochondrial membrane permeability, and dynamics. This review identifies mtDNA leakage triggers, the current status of research on this event in various neurological diseases is summarized, followed by a discussion of the development of relevant treatment strategies.

Although the role of mtDNA leakage in immunology has been extensively evaluated, several questions remain unanswered. For instance, what causes the dysfunction or abnormal expression of the associated molecules? Given that most of these molecules are mitochondrial proteins, it is yet to be determined whether mtDNA damage or mutations at the initial stages contribute to these abnormalities. Clarifying the logical relationship between mtDNA dysfunction, protein abnormalities, and mtDNA leakage is essential. Moreover, research on mtDNA leakage in neurological disorders remains relatively limited. Comprehensive investigations into mtDNA leakage across different cell types are needed, including the roles of non-neuronal cells within the brain and peripheral immune cells, which should not be overlooked. Considering the pathophysiological differences among diseases and their varying stages of progression, the development of related therapeutics should be both comprehensive and cautious. Determining the applicability and potential adverse effects of these therapies poses a significant

challenge. Additionally, it is worth noting that the scope of this review is confined to sterile inflammation. The roles of mtDNA leakage and downstream PRRs in infectious neurological diseases require further reevaluation.

mtDNA leakage is a continuous phenomenon, with current studies primarily focused on cytosolic mtDNA quantification. Advanced techniques now allow in-cell visualization of mtDNA dynamics, facilitating real-time tracking of mtDNA escape and the measurement of kinetic factors like escape rate [415, 416]. In summary, despite numerous unresolved issues, probing mtDNA leakage deepens insights into mitochondrial involvement in innate immunity and neurologic disease pathobiology, driving the translation of these findings into clinical treatment strategies.

Abbreviations

PRRs	Pattern recognition receptors
DAMPs	Damage-associated molecular patterns
IMM	Inner mitochondrial membrane
OMM	Outer mitochondrial membrane
mPTP	Mitochondrial permeability transition pore
BAX	Bcl-2-associated X protein
BAK	Bcl-2 homologous antagonist/killer
VDAC	Voltage-dependent anion channel
cGAS	Cyclic GMP-AMP synthase
STING	Stimulator of interferon genes
TFAM	Mitochondrial transcription factor A
OGG1	8-Oxoguanine DNA glycosylase
PNKP	Polynucleotide kinase/phosphatase
CMPK2	Cytidine monophosphate kinase 2
HIGD1A	HIG1 hypoxia inducible domain family member 1A
MOMP	Mitochondrial outer membrane permeabilization
OPA1	Optic atrophy 1
SAM50	Sorting and assembly machinery component 50
PHB1	Prohibitin 1
PGAM5	Phosphoglycerate mutase family member 5
DUSP1	Dual specificity phosphatase 1
JNK	C-Jun N-terminal kinase
SRSF6	Serine/arginine-rich splicing factor 6
VRK2	Vaccinia-related kinase 2
GRP75	Glucose-regulated protein 75
MDV	Mitochondria-derived vesicles
EV	Extracellular vesicles
SNX9	Sorting nexin 9
DRP1	Dynamin-related protein 1
MFN	Mitofusin 1
PINK1	PTEN-induced putative kinase 1
IRGM1	Immunity-related GTPase family M member 1
XBP1	X-box binding protein 1
FUNDC1	FUN14 domain containing 1
RAB7	Ras-related protein Rab-7
VPS35	Vacuolar protein sorting 35
IFN	Interferon
TBK1	TANK-binding kinase 1
NLRP3	NOD-like receptor family pyrin domain containing 3
AIM2	Absent in melanoma 2
TLR9	Toll-like receptor 9
ZBP1	Z-DNA binding protein 1
PD	Parkinson's disease
ccf-mtDNA	Circulating cell-free mitochondrial DNA
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
HD	Huntington's disease
VMP1	Vacuole membrane protein 1
LRRK2	Leucine-rich repeat kinase 2
PLD3	Phospholipase D family member 3
TDP-43	TAR DNA-binding protein 43
IS	Ischemic stroke
CIRI	Cerebral infarction with reperfusion injury
CKLF1	Chemokine-like factor 1
SAH	Subarachnoid hemorrhage
TBI	Traumatic brain injury
NEP	Neuropathic pain
MDD	Major depressive disorder
POCD	Postoperative cognitive dysfunction
AMPK	AMP-activated protein kinase
SIRT	Sirtuins
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
CLPP	Caseinolytic mitochondrial matrix peptidase proteolytic subunit
ENDOG	Endonuclease G
MRE11	Meiotic recombination 11
YME1L	YME1 Like 1 ATPase
IRF3	Interferon regulatory factor 3
MYD88	Myeloid differentiation primary response 88
ox-mtDNA	Oxidized mitochondrial DNA
PCR	Polymerase chain reaction

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Author contributions
Investigation was conducted by G.Z., H.W., X.L., and J.G. The original draft was written by G.Z. and H.W. Review and editing were performed by A.Z. and X.Y. Software development was carried out by X.Z. Visualization was performed by M.G. Conceptualization was provided by J.W. Supervision was managed by F.Z. and Y.J. Project administration was handled by Z.Y. Funding acquisition was completed by X.J. The study conception and design were contributed by all authors.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

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The authors declare no competing interests.

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