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DNA sensing in the pathological process of ischemic stroke

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Abstract

The innate immune response plays an important role in the pathological process of ischemic stroke. Increasing evidence suggests that the inflammatory response triggered by the innate immune system hinders neurological and behavioral recovery after stroke. The perception of abnormal DNA and its downstream effects are an essential part of the innate immune system. The abnormal DNA is the major inducing factor for innate immune response and is sensed by a series of DNA sensors. In this review, we discussed the multiple roles of DNA sensing in the pathological process of ischemic stroke, with a special focus on DNA sensors Toll-like receptor 9 (TLR9), absent in melanoma 2 (AIM2) and cyclic GMP-AMP synthase (cGAS).

Introduction

Ischemic stroke is mainly caused by the occlusion of the middle cerebral artery. Insufficient blood supply to the brain leads to acute ischemia and hypoxia, resulting in brain tissue necrosis. In the core area of cerebral ischemia, the dramatic reduction of cerebral blood flow (CBF) leads to massive cell death. In the ischemic penumbra near the infarcted core, the damage of low CBF is relatively less severe and reversible. The activity of neurons in the penumbra is suppressed, and their original functions are impaired(Baron, 2018). Glutamate excitotoxicity, oxidative stress, and calcium overload are major factors for ischemic cell death in the early stage of stroke(Dirnagl, Iadecola, & Moskowitz, 1999). The initial damage was followed by immune cell activation and inflammation. Within hours or days of cerebral ischemia, the secondary damage gradually expands from the ischemic core to the penumbra, aggravating the brain damage caused by ischemic stroke and hindering recovery after stroke(Baron, 2018; Moskowitz, Lo, & Iadecola, 2010). Preventing secondary brain damage is the key to the treatment of ischemic stroke.

The innate immune system is the first line of defense against invading pathogens and is initiated by pattern recognition receptors (PRRs). The known ligands of PRRs can be classified into two categories according to sources(Carty, Guy, & Bowie, 2021; Shimosato et al., 2006; Zindel & Kubes, 2020). The pathogen-associated molecular patterns (PAMPs) refer to ‘non-self’ molecules derived from pathogens such as lipopolysaccharides (LPS), nucleic acids, etc. The damage-associated molecular patterns (DAMPs) refer to endogenous danger signals released by distressed or damaged cells, which can initiate immune responses(T. Gong, Liu, Jiang, & Zhou, 2020; Zindel & Kubes, 2020). After binding with DAMPs or PAMPs, PRRs are activated, initiating diverse downstream signals to resist pathogen invasion and resolve endogenous Stimuli. Transcriptional and non-transcriptional responses are two major components of PRR-induced innate immune response. Transcriptional response leads to the production of proinflammatory cytokines and interferons (IFN)(Triantafilou, 2021). Nontranscriptional responses lead to the induction of phagocytosis, autophagy, cell death, and cytokine processing(Brubaker, Bonham, Zanoni, & Kagan, 2015).

Nucleic acids are essential PAMPs in the antiviral innate immune response

because the virus does not have a conserved, characteristic lipoprotein. The innate immune has DNA sensors that can sense and discriminate abnormal DNA signals. DNA sensors such as AIM2, TLR9, Interferon-inducible 16 (IFI16), and cGAS can receive abnormal DNA signals and initiate innate immune responses (Briard, Place, & Kanneganti, 2020). In many central nervous system diseases, such as Alzheimer's disease (AD), stroke, amyotrophic lateral sclerosis (ALS), and Parkinson's disease (PD), the DNA sensors are stimulated by cytoplasmic DNA, which is released from damaged nucleus and mitochondria (Kieroń, Żekanowski, Falk, & Wężyk, 2019; Q. Li et al., 2020). This cytoplasmic DNA is different from normal DNA in its tertiary structure, molecular modification, and subcellular localization, which prevents DNA sensors from overactivation under physiological conditions. Once activated, the DNA sensor could initiate the propagation of neuroinflammation and contribute to disease onset and progress. In ischemic stroke, DAMPs and cytokines produced by brain damage tissues enter the circulatory system and activate immune responses (Duan et al., 2010; Kong et al., 2022; Q. Li et al., 2020). Activated could mediate type I interferon production and inflammasome activation (Briard et al., 2020). This exacerbates the degree of brain damage and harms neurological recovery after stroke. This review focuses on DNA sensors that play essential roles in ischemic stroke. The signal, downstream effects, and mechanism played by these DNA sensors in ischemic stroke are described. We provide future research directions to find effective targets for recovering ischemic stroke.

After ischemic stroke, cell necrosis releases dsDNA fragments (Duan et al., 2010). In the penumbra region of stroke, dsDNA gradually increases, disintegrates from the nucleus, and diffuses into the cytoplasm and internal environment, where it is captured by microglia (Q. Li et al., 2020). Necrotic and apoptotic cells can increase the content of cell-free DNA (cf-DNA) in an internal environment. Some scholars believe cell-free DNA can be used as a potential biomarker of stroke to characterize the severity and prognosis of stroke (Glebova, Veiko, Nikonov, Porokhovnik, & Kostuyk, 2018). Under normal physiological conditions, mitochondrial DNA (mtDNA) mainly exists in mitochondria. The dysfunction of mitochondria after stroke can lead to the oxidation and abnormal release of mtDNA, and the accumulation of mtDNA in the cytoplasm will eventually activate DNA sensors and lead to the occurrence of inflammatory response stroke. (Kong et al., 2022; Peng et al., 2020). Correspondingly, the abnormality of mtDNA also affects the energy metabolism of mitochondria (Z. Zhang et al., 2022). When stroke occurs, the innate immune system responds rapidly and can recognize multiple DAMPs. As a potential DAMP during stroke, DNA can be recognized by various DNA sensors to initiate downstream immune responses.

The role of Toll-like receptor 9 in ischemic stroke

TLR9 is the only member of the Toll-like receptor family that can recognize DNA signals. TLR9 is mainly located in intracellular compartments such as endosomes and endoplasmic reticulum (Akira, Uematsu, & Takeuchi, 2006). TLR9 consists of N-

terminal leucine-rich repeats (LRRs), transmembrane domains, and cytoplasmic Toll/IL-1R homology domains (TIRs). LRRs are located inside the compartment membrane, and TIR is located on the cytoplasmic side. The classic ligand of TLR9 is an oligonucleotide with a CpG motif (CpG-ODA), often found in bacteria and DNA viruses (Kumagai, Takeuchi, & Akira, 2008). Some studies have also reported that non-CpG motif DNA and malaria pigment hemozoin could also activate TLR9 (Coban et al., 2005; Shimosato et al., 2006; Yasuda et al., 2006). After binding with its ligand, TLR9 recruits myeloid differentiation protein 88 (MyD88) (Häcker et al., 2000). MyD88 subsequently interacts with IL-1 receptor-associated kinases 1 (IRAK-1) and IL-1 receptor-associated kinases 4 (IRAK-4) to recruit tumor necrosis factor-related factor 6 (TRAF6) (Kawagoe et al., 2007), which promotes the expression of pro-inflammatory factors. Interestingly, when type I interferon expression is induced, tumor necrosis factor-related factor 3 (TRAF3) is recruited together with TRAF6 (Häcker et al., 2006; Oganessian et al., 2006).

After ischemic stroke, TLR9 is upregulated and peaks for a considerable period (Y. Ji et al., 2016). In the central nervous system, microglia are the primary resident immune cells. After cerebral ischemia injury, TLR9 is mainly expressed in microglia and activates the inflammatory response by the persistent activation of the pro-inflammatory NF- κ B pathway (Z. Gong et al., 2018; Y. Ji et al., 2016). Inhibiting the activation of TLR9 after stroke can suppress neuroinflammation, reduce infarcted volume and promote neurological function recovery (Z. Gong et al., 2018; Stevens et al., 2008; Zhou et al., 2017). Some studies have also shown that TLR9 can reduce brain injury after ischemia-reperfusion by activating the PI3K/Akt signaling pathway, including reducing the activation of microglia and apoptosis in the brain (C. Lu et al., 2014). Pretreatment of mice with TLR9 ligand cytosine-guanine (CpG) oligodeoxynucleotides can reduce the accumulation of hemolytic phosphatidylcholine in brain tissue after stroke, resulting in neuroprotection against cerebral ischemia (Mavrouidakis et al., 2021). These contradictory results suggested that the pathological roles of TLR9 are complex at the early stage of stroke. On the one hand, the occurrence of inflammatory response changes the microenvironment in the brain. The release of many inflammatory factors breaks homeostasis and exacerbates Brain injury after stroke. On the other hand, early activation of TLR9 appears to reduce brain injury and promote recovery after stroke through several other pathways. TLR9 may play different roles in different periods of ischemia stroke.

The role of AIM2 in ischemic stroke

AIM2 inflammasome is a member of the ALR family, mainly composed of the N-terminal HIN domain and the C-terminal Pyrin domain (PYD). AIM2 mainly exists in the cytoplasm and can recognize cytoplasmic dsDNA signals (Fernandes-Alnemri, Yu, Datta, Wu, & Alnemri, 2009; Hornung et al., 2009). Unlike TLR9, the dsDNA is recognized by AIM2 without sequence specificity. The length of dsDNA determines whether it can activate AIM2 and how fast the activation reaction occurs. At least 70

base pairs (bp) of dsDNA are required to activate AIM2. 200bp dsDNA is the optimal length to activate (Jin et al., 2012). When abnormal dsDNA appears in the cytoplasm, the HIN domain of AIM2 is responsible for binding dsDNA (Jin et al., 2012), and the PYD domain is responsible for binding ASC (Hornung et al., 2009). ASC is an important adaptor factor for inflammatory complexes, which can recruit pro-caspase-1 to form a ternary complex (B. Wang, Tian, & Yin, 2019). The inflammatory complex can activate pro-caspase-1 by auto-cleavage. Activated caspase-1 cleaves pro-IL-1 β and pro-IL-18 into mature IL-1 β and IL-18 (Hornung et al., 2009; Miao, Rajan, & Aderem, 2011). In addition, caspase-1 can also cleave gasdermin family member GSDMD after activation to induce cell pyroptosis (J. Shi et al., 2015).

AIM2 can recognize its abnormal dsDNA sequence. The dsDNA released during the pathological process of ischemic stroke becomes its potential ligand. The expression of AIM2 is up-regulated on the first day after cerebral ischemia and peak on the seventh day (Kim et al., 2020). Microglia and endothelial cells are the main cell types that express AIM2 in the brain (Kim et al., 2020; Xu et al., 2021). The role of AIM2 in stroke is diverse. It can promote the release of pro-inflammatory factors IL-1 β , IL-18 and trigger cell pyroptosis, and result in brain damage after stroke (Liang, Wang, Li, Guo, & Yu, 2020). Studies have shown that the expression of AIM2 in the hippocampus can impair spatial memory and synaptic plasticity in mice (Chen et al., 2019). This phenomenon has also been confirmed in stroke. The activation of AIM2 inflammasome in the hippocampus after stroke aggravates cognitive impairment, and the knockout of AIM2 can improve the cognitive function of mice (Kim et al., 2020). AIM2 can also damage the integrity of the blood-brain barrier (BBB) after stroke in mice through the STAT3 signaling pathway, aggravating the degree of brain damage (Xu et al., 2021). After stroke, cf-dsDNA in the blood can activate the AIM2 inflammasome in peripheral monocytes, leading to the apoptosis of T cells and increasing the risk of bacterial infection in the body (Roth et al., 2021). The activation mechanism of AIM2 in the brain after stroke is still unclear, but it has been found that histone deacetylase 3 (HDAC3) can regulate the activation of AIM2 (Kim et al., 2020). In addition, AIM2 can also participate in the cGAS-STING signaling pathway after stroke. STING activates AIM2 and mediates pyroptosis and inflammatory responses (Q. Li et al., 2020). The above studies have all proved that the activation of AIM2 in ischemic stroke can aggravate brain damage. Targeting AIM2 for treatment can improve the prognosis and promote recovery after stroke. However, whether AIM2 can play a more significant role in clinical research as a potential therapeutic target is still unclear. The issues still need to be resolved in the future.

The cGAS-STING signaling pathway in ischemic stroke

The cGAS-STING signaling axis contains cyclic GMP-AMP synthase (cGAS) and stimulator of interferon genes (STING), which can receive abnormal DNA signals to initiate type I interferon responses. cGAS consists of a catalytic domain and an extended amino-terminal (N-terminal) domain. It mainly exists in the cytoplasm, which

may facilitate its recognition of cytoplasmic DNA and avoid the misrecognition of nuclear and mitochondrial DNA. Recently, some studies have also found that cGAS exists in a quiescent state in the nucleus, separated from DNA by tight nuclear binding (Volkman, Cambier, Gray, & Stetson, 2019). The DNA recognized by cGAS is mainly dsDNA, including DNA from microorganisms, extracellular self-DNA, intracellular mitochondrial DNA, and nuclear DNA (Hopfner & Hornung, 2020). Some single-stranded DNA with special structures can also activate cGAS (Herzner et al., 2015). These abnormal DNAs bind to the cGAS catalytic domain and induce cGAS to form dimers (X. Li et al., 2013; X. Zhang et al., 2014). Once cGAS is activated, it catalyzes the formation of the second messenger cGAMP, which activates STING and downstream signaling pathways (Sun, Wu, Du, Chen, & Chen, 2013).

STING is a transmembrane protein located on the endoplasmic reticulum. Its N-terminus transmembrane four times, and its C-terminus faces the cytoplasmic side, consisting of a ligand-binding domain (LBD) and a C-terminal tail (CTT). STING is the core molecule of DNA recognition, which can receive signals from various DNA sensors to initiate downstream IRF3-mediated type I interferon transcriptional activity (Cao, 2016). When STING binds to cGAMP, the dimer undergoes a conformational transition, and TBK1 undergoes trans-phosphorylation (C. Zhang et al., 2019). Subsequently, TBK1 catalyzes the phosphorylation of IRF3, phosphorylated IRF3 dimerized itself, enters the nucleus, initiates transcriptional activity, and promotes the expression of type I interferons (Liu et al., 2015; Zhao et al., 2016).

In recent years, more and more scholars have recognized the critical role of the cGAS-STING signaling pathway in stroke. After mouse tMCAO modeling, the expressions of cGAS and STING were gradually up-regulated and mainly localized in microglia (Kong et al., 2022). The mechanism of cGAS-STING activation in ischemic stroke is still unclear. After stroke, mtDNA and dsDNA in the nucleus will be released into the cytoplasm. It is speculated that these DNA signals may be recognized by cGAS as potential ligands, although there is no direct evidence to prove this process (Kong et al., 2022; Q. Li et al., 2020). HDAC3 is an important histone regulator and is essential for controlling DNA damage (H. Ji et al., 2019). Some studies have found that HDAC3 can promote the expression of cGAS in microglia and play an essential role in activating the cGAS-STING signaling pathway (Liao et al., 2020). Researchers generally believe that the cGAS-STING signaling pathway plays a critical role in the inflammatory response after stroke. It can promote the release of IL-1 β , IL-6, and TNF- α and initiate the inflammatory cascade. (Kong et al., 2022; Q. Li et al., 2020; Liao et al., 2020). On the other hand, in different stroke models, cGAS-STING has been shown to activate multiple inflammasomes, such as AIM2 and NLRP3, and subsequently lead to pyroptosis (Ding et al., 2022; Q. Li et al., 2020). The polarization direction of microglia has an important influence on rehabilitation after stroke. Microglia with the M2 phenotype can reduce brain damage after stroke and exert neuroprotective effects, which is beneficial to the rehabilitation after stroke (J. Wang et al., 2018). Activation of STING can promote the polarization of microglia to the M1 phenotype and limit the generation of M2 phenotype microglia (Kong et al., 2022). Knockdown of cGAS can promote the polarization of microglia towards the M2 phenotype and reduce brain

damage after stroke(Jiang et al., 2021). Current studies generally believe that the cGAS-STING signaling pathway will aggravate brain damage after ischemic stroke and is not conducive to post-stroke rehabilitation. However, these studies mainly focus on inflammation. The cGAS-STING signaling pathway, as an essential part of the innate immune system, may play a more extensive role in the process of ischemic stroke.

In addition to the canonical type I interferon signaling pathway, scientists have discovered many non-canonical STING downstream pathways in recent years. Recent studies show that the activation of the NF- κ B signaling pathway by cGAS-STING has broadened the understanding of its role in mediating inflammation. NF- κ B is a fast-reacting molecule, and recent studies have shown that NF- κ B plays an essential role in the process of nerve damage and synaptic plasticity disruption(Shih, Wang, & Yang, 2015; Q. Zhang, Lenardo, & Baltimore, 2017). NF- κ B is activated during ischemic stroke, aggravating the inflammatory microenvironment in the brain. Inhibition of NF- κ B activation can reduce microglia-mediated ischemic brain injury(Y. Lu et al., 2017; Zang, Zhou, Wang, Li, & Huang, 2018). Although it is not clear how cGAS-STING activates NF- κ B, it is well established that STING exacerbates brain damage via NF- κ B after ischemic stroke. In treating ischemic stroke, inhibition of cGAS-STING, the upstream signal of NF- κ B, may achieve a better therapeutic effect.

Autophagy plays a double-edged role in ischemic stroke(Q. Shi, Cheng, & Chen, 2021) and is thought to be the original function of STING(Martin, Hiroyasu, Guzman, Roberts, & Goodman, 2018). Although the mechanism of autophagy in ischemic stroke remains unclear, researchers agree that moderate activation of autophagy has neuroprotective effects, while excessive activation of autophagy is not conducive to neurological recovery after stroke(Mo, Sun, & Liu, 2020). In the early stage of stroke, autophagy can degrade damaged organelles and misfolded proteins to reduce inflammatory responses, maintain the intracellular environment and play a neuroprotective role(W. Wang et al., 2013). However, persistent, excessive autophagy can cause damage. In addition to the time factor, the protein type of autophagy also impacts the pathological process of stroke. Studies have shown that STING can activate the autophagy of ferritin in the early stage of ischemic stroke and aggravate post-stroke brain damage(B. Li et al., 2022). STING reaches a high level 3 days after ischemic stroke and is maintained until seven days after ischemic stroke(Kong et al., 2022). During this period, STING may enhance the activation of autophagy by inducing autophagy, resulting in nerve damage. Although there has been no research on this aspect, post-ischemic stroke STING may have a time and degree amplification effect on autophagy, thus impacting post-stroke functional rehabilitation.

Conclusion and future directions

As mentioned above, a large amount of research evidence shows that ischemic stroke can lead to the release of abnormal DNA to the cytosol and circulatory system. Different types of DNA receptors will be upregulated after ischemic stroke, and through a series of signal transduction, trigger downstream immune responses, aggravate the brain injury after stroke, and hinder the rehabilitation after stroke. However, there are

still many questions worth discussing. What dsDNA signals directly activate a wide variety of DNA receptors? After ischemic stroke, a large number of cells die, and nuclear dsDNA and mitochondrial mtDNA are released into the cytoplasm and then released into the internal environment into the blood circulation(Glebova et al., 2018). The abnormal DNA causes the activation of DNA receptors(Kong et al., 2022; Q. Li et al., 2020). However, most DNA receptors are predominantly expressed in microglia. Microglia produce abnormal DNA itself could activate their DNA sensors. After stroke, microglia can phagocyte fragments and abnormal stress state of neurons(Cai et al., 2019; Neher et al., 2013). The cell fragments in the abnormal DNA in the microglia may also activate microglia DNA sensors, leading to downstream immune response.

Microglia are probably not the only cell type in the brain that mediates DNA signaling. After ischemic stroke, peripheral cells infiltrate the brain due to the destruction of the blood-brain barrier(L. Shi et al., 2021). T cells, macrophages, and neutrophils accumulate at the infarct site. The activation of the peripheral immune system will aggravate the inflammatory response after ischemic stroke, increase the infarct size, and hinder the rehabilitation of neurological function after stroke. DNA receptors are widely expressed in immune cells such as neutrophils and macrophages(Kwon & Bakhoun, 2020). DNA signal will stimulate peripheral immune cells to produce an inflammatory response, and the release of inflammatory factors will further aggravate cell necrosis in the brain, resulting in the release of abnormal DNA, forming positive feedback of inflammatory response, and hindering functional recovery after stroke. In terms of DNA signaling, microglia and infiltrating peripheral immune cells may promote each other after ischemic stroke.

After ischemic stroke, the main downstream effect caused by the activation of DNA sensors and STING is inflammation. Activation of DNA receptors can promote the release of inflammatory factors, form an inflammatory microenvironment, and promote the polarization of microglia to a proinflammatory phenotype, hindering recovery after stroke(Kong et al., 2022). Although there is a small amount of evidence that TLR9 activation after stroke can reduce the injury of cerebral infarction to a certain extent, the inflammatory response is still the main effect after TLR9 activation(Zhou et al., 2017). In addition, the activation of cGAS and AIM2 can lead to inflammasome-related downstream effects(Q. Li et al., 2020). In addition to releasing inflammatory factors, pyroptosis of cells is also important in aggravating brain tissue injury(B. Wang et al., 2019). In addition, phagocytosis is also a primitive function of the innate immune response. In some neurodegenerative diseases such as Alzheimer's disease, type I interferons promote synaptic phagocytosis by microglia(Roy et al., 2022; Roy et al., 2020). DNA sensors, as the upstream link of type I interferon initiation, may indirectly enhance the phagocytosis of microglia. Phagocytosis of different components by microglia at different stages after ischemic stroke may lead to different outcomes. Studies have shown that microglia phagocytose stressed but salvageable neurons in the acute phase after stroke, which is not conducive to functional recovery in mice for a long time after stroke(Neher et al., 2013). At the 14-day time point, microglia still had vigorous phagocytosis activity to synapses, which would impair synaptic plasticity after stroke and affect sensorimotor function in mice(X. Shi et al., 2021). Although there is

no clear evidence that DNA sensors are involved in the phagocytosis of microglia, there is likely some intrinsic relationship between the phagocytosis of microglia and the activation of DNA sensors.

There are many other DNA sensors, such as IFI16, DEAH- and DEAD-box helicases DHX9, DHX36, DDX41, and RNA polymerase III (POL III). Although these sensors activate a type I IFN response to signal to the immune system (Briard et al., 2020), there is less research to investigate their role in stroke. Whether these DNA sensors also influence the pathological process of ischemic stroke is still a question worth exploring. STING is a crucial link in DNA sensor signaling. Although it has not been confirmed in ischemic stroke, there is evidence that many DNA receptors, after receiving abnormal DNA signals, transmit the signal to STING, which mediates inflammation and type I interferon expression (Cao, 2016; Wu & Chen, 2014). Therefore, STING is essential for immune responses mediated by DNA sensor activation. In recent years, more and more researchers have recognized the importance of STING and gradually shifted their attention from DNA receptors to STING. At the same time, researchers have developed a variety of small-molecule STING inhibitors from different mechanisms of action. Although there are still many problems, with the deepening of research, STING is expected to become an effective target for rehabilitation after stroke.

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Competing interests

The authors declare that they have no competing interests.

Author contributions

SZ and HL conceived the manuscript, and SZ wrote the initial drafts. CW provided editorial advice. All authors contributed to discussing contents, reviewing, and approving the final version before submission.

Abbreviations

AD	Alzheimer's disease
AIM2	Absent in melanoma 2
ALS	Amyotrophic lateral sclerosis
BBB	Blood-brain barrier
CBF	Cerebral blood flow

cGAS	Cyclic GMP-AMP synthase
CTT	C-terminal tail
DAMPs	Damage associated molecular patterns
HDAC3	Histone deacetylase 3
IFI16	Interferon-inducible 16
IFN	Interferon
IRAK-1	IL-1 receptor-associated kinases 1
IRAK-4	IL-1 receptor-associated kinases 4
LBD	Ligand-binding domain
LPS	Lipopolysaccharides
LRRs	Leucine-rich repeats
mtDNA	Mitochondrial DNA
MyD88	Myeloid differentiation protein 88
PAMPs	Pathogen-associated molecular patterns
PD	Parkinson disease
PRRs	Pattern recognition receptor
PYD	Pyrin domain
TIRs	Toll/IL-1R homology domains
TLR9	Toll-like receptor 9
TRAF6	Tumor necrosis factor-related factor 6
STING	Stimulator of interferon genes

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Figure Legends

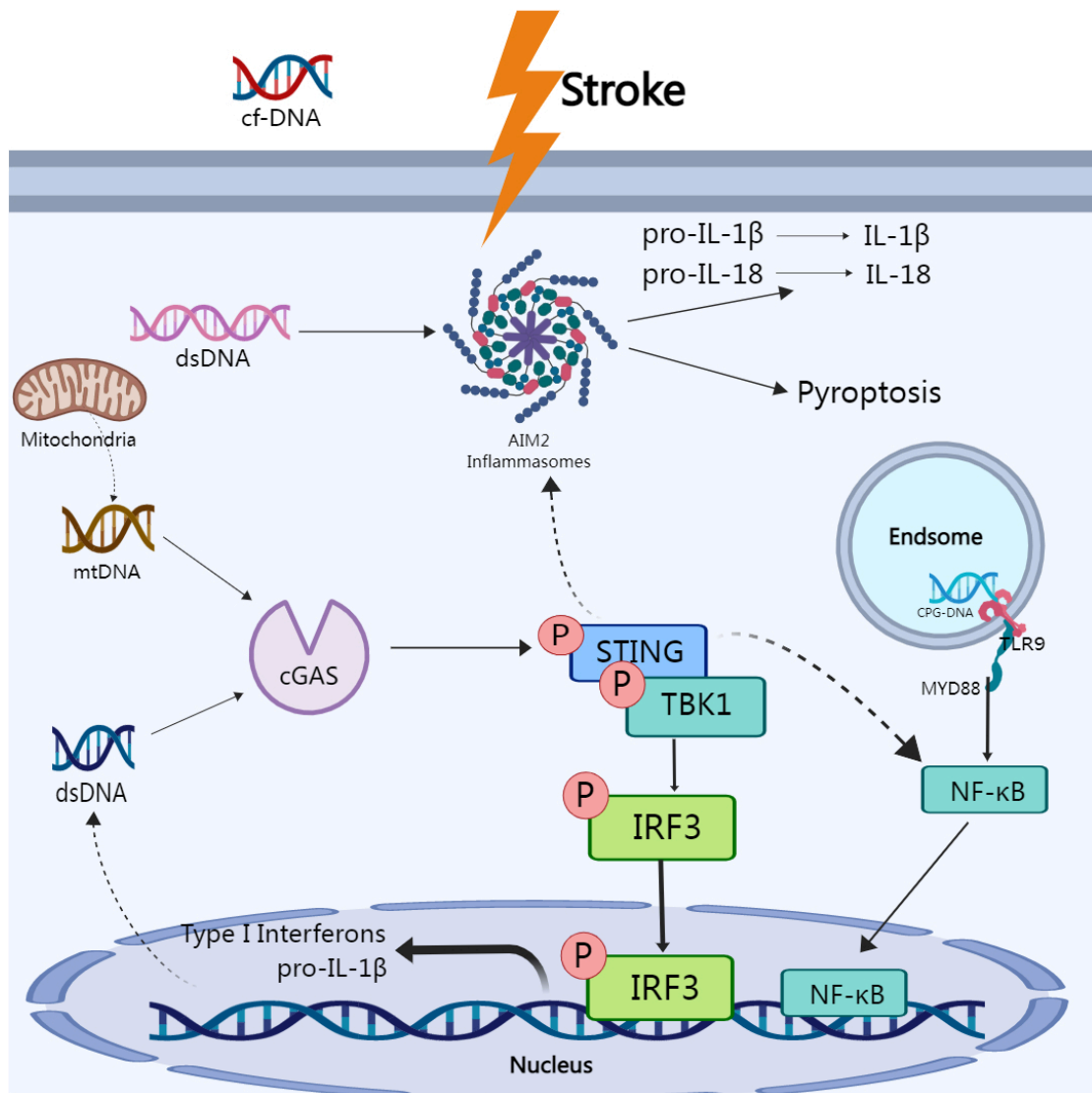


Figure1

Schematic illustrating various intracellular DNA sensing and signals pathways in the pathological process of ischemic stroke. After stroke, DNA sensors AIM2, TLR9, and cGAS receive abnormal DNA and innate immune response. Through a series of signal transduction, the transcriptional response leads to the production of proinflammatory cytokines and interferons (IFN).