Cellular response against cytosolic leakage of mitochondrial DNA: insights into the pathology of Parkinson's disease

Akiko Sakai, Hideaki Matsui*

Mitochondria are important organelles for cellular metabolism and homeostasis, and their abnormalities are implicated in various diseases. Since mitochondria originate from protobacterium, their components are easily recognized by pathogen sensors called pattern-recognition receptors as foreign substances in the cytoplasm. This is prominent for mitochondrial DNA (mtDNA) which has different properties from nuclear DNA, such as low, if any, methylation status, vulnerability to oxidation due to the proximity to the oxidative phosphorylation machinery, and its circular structure. Recent studies indicate that perturbations in mitochondrial function and homeostasis cause mtDNA to leak into the cytoplasm, where it triggers the innate immune response (West and Shadel, 2017). Although inflammatory response is a cellular strategy to resist viral infections, it can also lead to undesirable outcomes by damaging own cells and tissues. Recently, we found that mtDNA leak into the cytoplasm in Parkinson's disease (PD) models of human cells and zebrafish. Furthermore, we revealed that the leaked mtDNA is detected by a viral DNA sensor interferon gamma inducible protein 16 (IFI16), which has not been implicated in mtDNA recognition, and triggers inflammatory responses. Decreasing mtDNA sensor or overexpression of mtDNA-degrading enzymes in the PD model eliminated cytosolic mtDNA and suppressed the inflammatory response as well as neurodegeneration. Importantly, accumulation of mtDNA and IFI16 in the cytoplasm was also observed in autopsy brains of human PD patients, pointing to pathological relevance (Matsui et al., 2021). In this perspective, we will first overview the various cases and pathways by which mtDNA leakage triggers inflammatory responses, and then in particular look into how mtDNA-mediated inflammation can explain the pathogenesis of PD, discussing the ways to ameliorate the pathological conditions.

mtDNA leakage – when, how, the sensors and the downstream pathways leading to inflammatory response: Leakage of mtDNA into the cytoplasm is observed when mitochondrial metabolic balance is disrupted and mitochondrial function becomes defective upon infection by viruses or bacteria, or due to cellular stress caused by disease or environmental factors (West and Shadel, 2017). There are mainly three pathways reported to date for sensing of cytoplasmic mtDNA leading to innate immune activation, which are common with viral DNA sensing: the pathway sensed by Toll-like receptor 9, a pattern-recognition receptor of endosome; the pathway mediated by inflammasome including Nod-like receptor pyrin domain containing 3 and absence in melanoma 2 sensors; and the pathway that activates stimulator of interferon genes (STING) by cytosolic double-stranded (ds) DNA sensors including cyclic GMP/AMP synthase (cGAS; **Figure 1A**). Once the pathways, either alone or in combination, are initiated, the inflammatory signals including type I interferon, interferon-stimulated genes (ISG), and inflammatory cytokines are amplified through autocrine and/or paracrine activation of inflammatory responses of own or neighboring cells. Thus, in the tissue and individual levels, the complex interplay of the inflammatory cycle can lead to autoimmune and neurodegenerative diseases. Leakage of mtDNA can be part of such inflammatory chains: for instance, the inflammatory cytokine interleukin-1β induces mtDNA release and subsequent activation of the cGAS-STING pathway in bystander cells to further potentiate innate immune response (Aarreberg et al., 2019).

The cGAS-STING pathway has been attracting much attention these years as it induces innate immune responses by sensing not only foreign DNA but also its own cytosolic DNA in a wide range of cell types. The role of cytosolic mtDNA in activating the cGAS-STING pathway was first reported in apoptotic cells. Notably, the apoptotic caspase inhibits induction of pro-inflammatory cytokines, ensuring that cells remain immunologically silent during apoptotic cell death (Rongvaux et al., 2014; White et al., 2014). Meanwhile, mtDNA leakage-mediated cGAS-STING activation is also induced by moderate stress derived from mtDNA instability that does not lead to cell death, as shown in mice with decreased amount of the mtDNA packaging protein transcription factor A mitochondrial (TFAM). Intriguingly, herpesvirus infection induces loss of TFAM and leakage of mtDNA, which was necessary for induction of ISGs and anti-virus priming. These observations highlight the role of the monitoring system of mtDNA homeostasis via sensing cytosolic leakage as an effective anti-viral response mechanism in addition to intrinsic virus DNA-sensing system (West et al., 2015). In this context, infection by RNA viruses such as influenza virus was also reported to cause mtDNA leakage to induce anti-viral response via cGAS and DDX41 sensors (Moriyama et al., 2019). In addition to viral infection, mtDNA leakage is also closely related to pathological conditions such as metabolic disorders. For example, mtDNA leakage is involved in inflammation and metabolic abnormalities caused by obesity. In the adipocytes lacking the chaperone protein DsbA-L, unregulated leakage of mtDNA into cytosol results in increased insulin resistance (Bai et al., 2017). Not only leaked mtDNA *in vivo* but also direct external administration of mtDNA has been shown to induce inflammatory responses in animals. Intraperitoneal administration of mtDNA in mice causes lung damage and systemic inflammation, which was ineffective with nuclear DNA, suggesting the direct and specific roles of mtDNA to activate inflammatory responses (Zhang et al., 2016).

How mtDNA leakage through mitochondrial membrane occurs has only recently begun to be clarified. Detailed microscopic observations of mtDNA release upon activation of apoptosis demonstrated that following macropore formation by BAX/BAK in mitochondrial outer membrane (MOM), inner membrane herniation occurs allowing exit of mtDNA (McArthur et al., 2018). On the other hand, under oxidative stress, mtDNA promotes oligomerization of voltage-dependent anion channel to form a pore on MOM from which it is released. Inhibiting voltage-dependent anion channel oligomerization by VBIT-4 suppresses leakage of mtDNA and associated interferon signaling, and also ameliorates disease severity of the mouse model of systemic lupus erythematosus, a representative autoimmune disease (Kim et al., 2019). Regarding the passage of the inner membrane of mtDNA, the permeability transition pore seems to be involved, but the details are unknown. Taken together, mtDNA leakage can be seen in

many situations where cells are stressed, although the detailed mechanisms and the use of downstream pathways are not fully understood.

Accumulation of cytosolic mtDNA is a key to the pathology of PD: Since neurons are highly energy-demanding, functions and homeostasis of mitochondria are particularly important in these cells. Thus, dysfunction of mitophagy, the quality control system that degrades damaged mitochondria, is common in neurodegenerative diseases. PD is a neurodegenerative disease that exhibits movement and non-movement disorders with age partly due to progressive loss of dopaminergic (DA) neurons. As the genes related to mitophagy and lysosome, that are involved in degradation systems, are commonly mutated in familial PD, degradation of damaged mitochondria has been suggested to be important. However, it remained unclear which component of damaged mitochondria caused a problem when persisted, and why it led to neural loss. On the other hand, chronic and age-related inflammation is a hallmark of neurodegenerative diseases, and it has been known that pro-inflammatory cytokines are enriched in the blood of PD patients, but whether this is a cause or a consequence of neurological loss had been unclear. Recently, using PD models, two studies including ours have revealed that mtDNA becomes a key inflammatory signal when the degradation system is impaired and damaged mitochondria are not removed (Sliter et al., 2018; Matsui et al., 2021). Importantly, inhibition of an mtDNA sensor and the inflammatory pathway suppressed loss of DA neurons, indicating that inflammation is a cause rather than a consequence of neuronal loss.

We performed depletion of three PD-related genes (PINK1, a mitophagy regulator, and two lysosomal factors ATP13A2 and acid beta-glucosidase) in human neuroblastoma cells and observed inflammatory responses (increased expression of Type I IFN and inflammatory cytokines such as IL-1 and IL-6) as well as cell death. Careful cytological observation of these cells revealed cytosolic dots of dsDNA (which are only detected by anti-dsDNA staining), and *in situ* hybridization showed that it was indeed mtDNA. Also in the newly generated acid beta-glucosidase knockout zebrafish as a PD model, cytoplasmic dsDNA dots were found in the brain, and loss of DA neuron and movement disorder were observed (Matsui et al., 2021). Sliter et al. (2018) found that in mice, although PINK1$^{-/-}$ or Prkn$^{-/-}$ alone does not show PD-related phenotypes, when mitochondria are stressed by exhaustive exercise or genetic induction of mtDNA mutations, pro-inflammatory cytokines increase in the serum along with an increase in circulating mtDNA. Prkn$^{-/-}$; mutator mice further showed DA neuron dropout and motor defects. Since this was STING dependent, it was suggested that cytosolic mtDNA was not degraded and activated the cGAS-STING pathway, triggering an inflammatory response leading to neuronal loss (Sliter et al., 2018). We have shown that in fact, DNaseII, a DNA-degrading enzyme in lysosomes, plays a crucial role in the removal of cytosolic mtDNA and is thus important in preventing inflammation (Matsui et al., 2021). In DNaseII knock-out cultured cells and zebrafish, cytosolic mtDNA accumulates and increases the inflammatory response. Conversely, overexpression of DNase II in the PD model cells eliminates cytosolic mtDNA and suppresses the inflammatory response. Moreover, in acid beta-glucosidase knockout zebrafish, introduction of DNase II can restore DA neuron loss and movement disorder. These results strongly suggest that the pathogenesis of PD is due to the inflammatory response induced by mtDNA accumulated in cytosol, which could be controlled by manipulating DNA degrading enzymes (Matsui et al., 2021). In this regard, degradation of mtDNA is considered to be an active mechanism toward mtDNA damage. In response to genetic induction of double-strand breaks in mtDNA, rapid degradation of mtDNA occurs instead of repairing the damage (Moretton et al., 2017). Whether the mitochondrial enzymes involved in this process such as exonuclease also have roles in inhibiting the leakage of mtDNA into cytosol, and whether the manipulation of them can also suppress cytosolic accumulation would be of interest.

Cytosolic dsDNA can be detected by multiple immune response sensors in addition to cGAS. In our study, we have shown for the first time that IFI16, a known sensor of viral DNA, is indeed a cytosolic mtDNA sensor (Matsui et al., 2021). IFI16 co-localized well with cytoplasmic mtDNA dots in the PD model cells, whereby cytoplasmic IFI16 co-immunoprecipitated with transfected mtDNA. Downregulation of IFI16 suppresses inflammatory responses and cell death in the PD model cells, suggesting that IFI16 is necessary for mediating inflammatory responses upon mtDNA leakage into cytosol. Of note, investigation of postmortem brain of PD patients revealed that cytosolic mtDNA and IFI16 accumulated in medulla oblongata. Strikingly, mtDNA and IFI16 co-localized with Lewy bodies (abnormal aggregates of α-synuclein) characteristic of PD. Although it remains elusive whether formation of Lewy body and mtDNA accumulation affect each other, our results strongly suggest that mtDNA deposits and IFI16 are indeed involved in the pathogenesis of PD (Matsui et al., 2021). The relationship between IFI16 and other dsDNA sensors such as cGAS is not fully understood. It is reported that IFI16 cooperatively activates STING with cGAS (Almine, 2017), but the detailed molecular mechanism and cell-type dependency remain to be determined. In our PD models, downregulation of IFI16 alone suppresses the inflammatory response by ectopic mtDNA. It could be speculated that synergistic action of each sensor is required in order to fully activate the immune response. In other words, inhibition of a single sensor (possibly with preference for mtDNA) may be sufficient for a therapeutic strategy aimed at blocking the inflammatory response. From this point of view, IFI16 may be a high potential drug target.

In summary, we have shown that the accumulation of mtDNA into the cytoplasm is a key trigger of inflammatory responses in PD, which ultimately leads to neuronal loss and movement disorders. Inhibition of the mtDNA sensor and promotion of cytosolic mtDNA degradation are potential targets to improve inflammatory responses and the PD pathology (**Figure 1B**). Given the accumulating evidence of cytosolic mtDNA as being toxic, it will be interesting to see if these strategies are also effective in the treatment of other diseases that involve inflammation. Future general questions that are interrelated include: 1) detailed mechanism of pore formation and the process of mtDNA leakage, 2) identification of the entire set of the sensors for mtDNA and the divisions of roles among them, 3) differences of mtDNA, nuclear DNA and viral DNA in regard to cytosolic sensors and downstream responses, 4) differences among cell types in all of the above issues. In a complex tissue like the brain, differences in the metabolic state and the aging process of various cell types would determine the degree of inflammation induced by cellular interactions (e.g., neurons and glia). Furthermore, the form in which mtDNA is released could be an important factor defining the sensors and the downstream reactions. Whereas entire mtDNA with TFAM seems to leak out from BAX/BAK macropore (McArthur et al., 2018), mtDNA released from voltage-dependent anion channel pore is estimated to be fragments around 110 bp (Kim, 2019). Although the forms of mtDNA

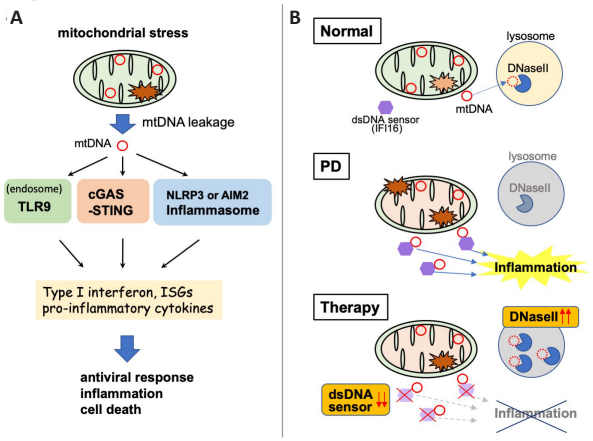

Figure 1 | Inflammatory pathways activated by cytosolic mitochondrial DNA (mtDNA) (A) and implications in the pathology and therapeutic potential in Parkinson's disease (PD) (B).
(A) Three pathways for sensing cytosolic mtDNA. (B) Normally, mtDNA leaked from old or stressed mitochondria is rapidly degraded. In PD, mitochondrial dysfunction and defective degradation cause accumulation of cytosolic mtDNA, which triggers an inflammatory response. As therapeutic strategies such as pharmacological targeting, blocking of the dsDNA sensor or promoting degradation of mtDNA by DNaseII would suppress inflammatory response and prevent cell death. AIM2: Absence in melanoma 2; cGAS: cyclic GMP/AMP synthase; dsDNA: double-stranded DNA; IFI16: interferon gamma inducible protein 16; NLRP3: Nod-like receptor pyrin domain containing 3; STING: stimulator of interferon genes; TLR9: Toll-like receptor 9.

that pass through these pores are unknown, it is expected that the length, oxidation state, and TFAM binding of released mtDNA would bring about the difference in sensor recognition and subsequent reactions, presumably depending on the cell-type specific availability of the components of the pathway. Detailed studies of the causes and processes that lead to mtDNA leakage, as well as the cellular responses to ectopic mtDNA, in different cells and tissues will enhance our understanding of aging and disease.

This work was supported by grants from AMED (Grant Numbers JP19gm6110028 and JP19dm0107154 (to HM)), the Takeda Science Foundation (to HM), JSPS KAKENHI (Grant Numbers JP 14516799 (to HM), JP 16690735 (to HM), and JP 17925674 (to HM)) and JST [Moonshot R&D][Grant Number JPMJMS2024] (to HM).

We acknowledge Ms. Noriko Matsui and Ms. Shinano Kobayashi for participating in helpful discussions and providing continuous support.

Akiko Sakai, Hideaki Matsui*
Department of Neuroscience of Disease, Brain Research Institute, Niigata University, Niigata, Japan
***Correspondence to:** Hideaki Matsui, MD, PhD, hide0729@bri.niigata-u.ac.jp.
https://orcid.org/0000-0003-4908-1035 (Hideaki Matsui)

Date of submission: October 13, 2021
Date of decision: December 2, 2021
Date of acceptance: December 10, 2021
Date of web publication: April 29, 2022

https://doi.org/10.4103/1673-5374.335816
How to cite this article: *Sakai A, Matsui H (2022) Cellular response against cytosolic leakage of mitochondrial DNA: insights into the pathology of Parkinson's disease. Neural Regen Res 17(12): 2682-2684.*

References

Aarreberg LD, Esser-Nobis K, Driscoll C, Shuvarikov A, Roby JA, Gale M (2019) Interleukin-1β induces mtDNA release to activate innate immune signaling via cGAS-STING. Mol Cell 74:801-815.

Almine JF, Hare CAJOR, Dunphy G, Haga IR, Naik RJ, Atrih A, Connolly DJ, Taylor J, Kelsall IR, Bowie AG, Beard PM, Unterholzner L (2019) IFI16 and cGAS cooperate in the activation of STING during DNA sensing in human keratinocytes. Nat Commun 8:14392.

Bai J, Cervantes C, Liu J, He S, Zhou H, Zhang B, Cai H, Yin D, Hu D, Li Z, Chen H, Gao X, Wang F, O'Connor JC, Xu Y, Liu M, Dong LQ, Liu F (2017) DsbA-L prevents obesity-induced inflammation and insulin resistance by suppressing the mtDNA release-activated cGAS-cGAMP-STING pathway. Proc Natl Acad Sci U S A 114:12196-12201.

Kim J, Gupta R, Blanco LP, Yang S, Shteinfer-Kuzmine A, Wang K, Zhu J, Yoon HE, Wang X, Kerkhofs M, Kang H, Brown AL, Park SJ, Xu X, Zandee van Rilland E, Kim MK, Cohen JI, Kaplan MJ, Shoshan-Barmatz V, Chung JH (2019) VDAC oligomers form mitochondrial pores to release mtDNA fragments and promote lupus-like disease. Science 366:1531-1536.

Matsui H, Ito J, Matsui N, Uechi T, Onodera O, Kakita A (2021) Cytosolic dsDNA of mitochondrial origin induces cytotoxicity and neurodegeneration in cellular and zebrafish models of Parkinson's disease. Nat Commun 12:3101.

McArthur K, Whitehead LW, Heddleston JM, Li L, Padman BS, Oorschot V, Geoghegan ND, Chappaz S, Davidson S, San Chin H, Lane RM, Dramicanin M, Saunders TL, Sugiana C, Lessene R, Osellame LD, Chew TL, Dewson G, Lazarou M, Ramm G, et al. (2018) BAK/BAX macropores facilitate mitochondrial herniation and mtDNA efflux during apoptosis. Science 359:eaao6047.

Moretton A, Morel F, Macao B, Lachaume P, Ishak L, Lefebvre M, Garreau-Balandier I, Vernet P, Falkenberg M, Farge G (2017) Selective mitochondrial DNA degradation following double-strand breaks. PLoS ONE 12:e0176795.

Moriyama M, Koshiba T, Ichinohe T (2019) Influenza A virus M2 protein triggers mitochondrial DNA-mediated antiviral immune responses. Nat Commun 10:4624.

Rongvaux A, Jackson R, Harman CC, Li T, West AP, de Zoete MR, Wu Y, Yordy B, Lakhani SA, Kuan CY, Taniguchi T, Shadel GS, Chen ZJ, Iwasaki A, Flavell RA (2014) Apoptotic caspases prevent the induction of type I interferons by mitochondrial DNA. Cell 159:1563-1577.

Sliter DA, Martinez J, Hao L, Chen X, Sun N, Fischer TD, Burman JL, Li Y, Zhang Z, Narendra DP, Cai H, Borsche M, Klein C, Youle RJ (2018) Parkin and PINK1 mitigate STING-induced inflammation. Nature 561:258-262.

West AP, Khoury-Hanold W, Staron M, Tal MC, Pineda CM, Lang SM, Bestwick M, Duguay BA, Raimundo N, MacDuff DA, Kaech SM, Smiley JR, Means RE, Iwasaki A, Shadel GS (2015) Mitochondrial DNA stress primes the antiviral innate immune response. Nature 520:553-557.

West AP, Shadel GS (2017) Mitochondrial DNA in innate immune responses and inflammatory pathology. Nat Rev Immunol 17:363-375.

White MJ, McArthur K, Metcalf D, Lane RM, Cambier JC, Herold MJ, van Delft MF, Bedoui S, Lessene G, Ritchie ME, Huang DC, Kile BT (2014) Apoptotic caspases suppress mtDNA-induced STING-mediated type I IFN production. Cell 159:1549-1562.

Zhang L, Deng S, Zhao S, Ai Y, Zhang L, Pan P, Su X, Tan H, Wu D (2016) Intra-peritoneal administration of mitochondrial DNA provokes acute lung injury and systemic inflammation via Toll-like receptor 9. Int J Mol Sci 17:1425.

C-Editors: Zhao M, Liu WJ, Wang Lu; T-Editor: Jia Y