論文の内容の要旨

論文題目　Mitochondrial DNA leakage causes inflammation via cGAS-STING axis in cisplatin-mediated tubular damage
(シスプラチンによるミトコンドリア DNA 漏出は近位尿細管において cGAS-STING 経路を介した炎症反応を惹起する)

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Inflammation is promoted by activation of an innate immune response mediated by pattern recognition receptors (PRRs). Recognition of pathogen-associated molecular patterns or danger-associated molecular patterns (DAMPs) via PRRs is critical for host defense, triggering signaling cascades that lead to the productions of pro-inflammatory cytokines and type I interferons. Although inflammation associated with innate immunity has mostly been described in the context of microbial infection, some PRRs also can be aberrantly activated by self molecules. Stimulator of interferon genes (STING) is an endoplasmic reticulum (ER)-resident protein that relays DNA-triggered signals and activates innate immunity. Cyclic (c)GMP-AMP (cGAMP) synthase (cGAS) has recently been identified as a cytosolic sensor of DNA that activates STING. Activated cGAS induces the production of cGAMP, which binds to STING. STING then translocates from ER to perinuclear compartments including the Golgi apparatus where it forms a complex with TANK-binding kinase (TBK)1, which is delivered to the endolysosome. There TBK1 phosphorylates transcription factors including interferon regulatory factor 3 and nuclear factor (NF)-κB, which enter the nucleus to initiate transcription of innate immunity-related genes. Aberrant activation of STING by self DNA is associated with several autoimmune diseases. Moreover, cGAS–STING signaling has also been implicated in other pathogenic processes including lipotoxicity, alcohol intoxication, and senescence. In addition to pathogen-derived DNA from microorganisms and self DNA from the nucleus, the cGAS–STING pathway is also activated by cytosolic mitochondrial (mt)DNA. While nuclear DNA is packaged by histones, this is not the case for mtDNA, making the latter more sensitive to damaging factors such as reactive oxygen species (ROS). In some autoinflammatory disorders, mitochondrial stress and ROS levels are elevated and mtDNA packaging is perturbed, resulting in mtDNA leakage into the cytosol.
Acute kidney injury (AKI) is characterized by a rapid decline in renal function; it can progress to chronic kidney disease (CKD) and is associated high morbidity and mortality rates. AKI, which is characterized by excessive inflammation and programmed cell death of

tubular epithelial cells, is caused by ischemia–reperfusion injury, sepsis, and nephrotoxins; 7%–25% of cases are attributable to adverse drug effects.
Cisplatin is a platinum-containing agent that is used to treat various malignancies. However, it is associated with dose-dependent nephrotoxicity in the renal proximal tubule in nearly one-third of patients, which is linked to DNA damage, mitochondrial dysfunction, apoptosis, oxidative stress, and inflammation. The molecular basis for the induction of inflammation and nephrotoxicity by cisplatin remains unclear. To address this issue, I investigated the role of cGAS–STING signaling in cisplatin-induced AKI.

To determine whether STING contributes to the pathogenesis of cisplatin nephrotoxicity in vivo, AKI was induced in wild-type (WT) and STING knockout (KO) mice by administration of 25 mg/kg cisplatin. On day 3, cisplatin-treated WT mice developed acute tubular necrosis accompanied by elevated plasma urea. STING expression was increased in the damaged tubules along with phosphorylation of TBK1 and NF-κB p65 and inductions of downstream pro-inflammatory molecules in the cortex of kidneys from cisplatin-treated WT mice. In contrast, cisplatin-induced tubular damage was attenuated in STING KO based on histological and functional analyses; this was associated with a decrease in phosphorylation of NF-κB p65 and downregulation of STING target gene expression. These in vivo data demonstrate that cGAS–STING signaling contributes to cisplatin-induced AKI. Moreover, cisplatin treatment increased the infiltration of neutrophils into the renal cortex, as determined by esterase staining; however, the degree of infiltration was lower in STING KO mice. Thus, STING mediates the inflammatory response leading to leukocyte infiltration in cisplatin nephropathy.

Then, I investigated inflammation-mediated tubular damage caused by cisplatin using immortalized HK-2 human proximal tubular cells. Exposure to cisplatin (20 μM) increased the secretion of the inflammatory cytokines interleukin (IL)-6 and IL-8 as well as other inflammation-related molecules into the culture supernatant. Similar results were obtained by real-time quantitative PCR. The induction of tubular inflammation by cisplatin was associated with mitochondrial damage, as evidenced by a decrease in cell viability followed by apoptosis, upregulation of pro-apoptotic genes including B cell lymphoma (BCL)-2-associated X protein (BAX) and phorbol-12-myristate-13-acetate-induced protein 1 (also known as NOXA), and downregulation of the anti-apoptotic gene BCL-2. Based on the observed inflammation and mitochondrial damage in tubular cells caused by cisplatin, I speculated that cGAS–STING signaling is activated in the presence of cytosolic mtDNA, leading to tubular inflammation. To test this hypothesis, I assessed the change in cGAS–

STING activation in HK-2 cells with or without cisplatin treatment. cGAS and STING was increased by cisplatin at mRNA and protein levels. Moreover, cGAS–STING activation—as estimated by phosphorylation of the downstream factors TBK1 and NF-κB p65—was also enhanced by cisplatin treatment. This was further confirmed by the translocation of STING to the Golgi apparatus: in the absence of cisplatin, there was no colocalization of STING with cis-Golgi matrix protein (GM)130, but this was enhanced by cisplatin treatment. These results demonstrate that cGAS–STING signaling is activated in cisplatin-induced renal proximal tubular inflammation.
To clarify the relationship between cGAS–STING pathway and cisplatin-induced inflammation, I examined the secretion of inflammatory cytokines in STING-deficient HK-2 cells treated with cisplatin. Small interfering (si)RNA-mediated knockdown of STING reversed the cisplatin-induced phosphorylation of TBK1 and NF-κB p65 and the expression and secretion of IL-6 and IL-8 as well as other inflammation-related factors. Notably, expression of the cisplatin-induced C-X-C motif chemokine (CXCL)10 gene—which encodes a chemokine that acts downstream of STING—was reduced by STING knockdown. Inflammation-related molecules induced by cisplatin have been shown to enhance the recruitment, differentiation, transcellular migration, and adhesion of neutrophils. Moreover, neutrophils are involved in the development of cisplatin-induced AKI. I therefore investigated whether STING contributes to cisplatin-induced neutrophil chemotaxis and found that the culture supernatant from HK-2 cells treated with cisplatin stimulated neutrophil migration and the effect was reduced by STING knockdown. These results suggest that activation of cGAS–STING signaling by cisplatin leads to the production of inflammation-related molecules, neutrophil recruitment.

Mitochondrial damage is one cause of cisplatin-associated nephrotoxicity.
Thus, I speculated that mtDNA leakage activates the cGAS-STING signaling in cisplatin-induced tubular inflammation. To visualize this damage, I doublestained cytoplasmic double-stranded DNA (dsDNA) and mitochondria in HK-2 cells with or without cisplatin treatment. Cisplatin treatment revealed significantly enhanced the cytoplasmic dsDNA signal. Because the dsDNA signal was well colocalized with mitochondria when enhanced the mitochondrial channel, damaged mitochondria may release their DNA onto their surface by cisplatin treatment. I further carried out mtDNA-specific PCR of the cytosolic DNA after the cisplatin treatment that revealed significant increase of cytosolic mtDNA, which further suggested that the dsDNA was of mitochondrial origin. To determine whether the leaked cytosolic mtDNA is a ligand of the cGAS–STING pathway, mtDNA was depleted by ethidium bromide (EtBr) treatment, which blocks the replication of mtDNA but not

nuclear DNA at low concentrations (0.1–2 μg/ml). Application of 1.0 μg/ml EtBr to the culture medium for 2 days reduced mtDNA copy number by around 90%. Cisplatin-induced phosphorylation of TBK1, and NF-κB p65 was abolished in these mtDNA-depleted HK-2 cells, whereas cisplatin-induced expression of inflammation-related molecules was markedly attenuated. To confirm cGAS–STING activation by cytosolic mtDNA, HK-2 cells were transfected with isolated mtDNA and activation of the cGAS–STING pathway was assessed. The transfected cytosolic mtDNA induced the phosphorylation of TBK-1 and NF-κB p65. The observed activation of cGAS–STING signaling upon mtDNA transfection was abrogated by STING knockdown. Additionally, mtDNA is released through BAX oligomeric pores in the mitochondrial outer membrane that allow the passage of mitochondrial matrix components including mtDNA into the cytosol. Indeed, cisplatin treatment enhanced BAX expression, suggesting that mtDNA leaks into the cytosol through such pores. Although downregulation of the mtDNA-binding protein transcription factor A, mitochondrial (TFAM)—which regulates nucleoid architecture, abundance, and segregation—was shown to promote the escape of mtDNA into the cytosol, in this study TFAM level was unaltered by cisplatin treatment. Together, leaked cytosolic mtDNA acts as a ligand for the cGAS–STING pathway in cisplatin-induced tubular inflammation.

The results of the present study reveal a novel renal inflammatory pathway induced by cisplatin. Notably, mtDNA leakage into the cytosol was a key mechanism activating cGAS–STING-p65 signaling, leading to tubular inflammation and AKI progression.