Mitochondria in Kidney Injury: When the Power Plant Fails

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Mitochondrial damage or dysfunction contribute critically to the pathogenesis of various diseases, including AKI. Upon stress, mitochondrial dynamics are disrupted and membrane integrity is compromised, resulting in the release of apoptogenic factors, mitochondrial permeability transition (MPT), loss of membrane potential, energetic failure, and reactive oxygen species production to induce cell injury and death. In this issue of the *Journal of the American Society of Nephrology* (*JASN*), Tsuji *et al.*[1] unravel a pathogenic role for mitochondrial DNA (mtDNA) in septic AKI *via* Toll-like receptor 9 (TLR9), and Suzuki *et al.*[2] identify mitochonic acid 5 (MA-5) as a renoprotective agent in ischemic and cisplatin-nephrotoxic AKI through binding mitofilin to facilitate ATP synthesis. An in-depth investigation into mitochondrial biology and pathology is essential to the discovery of effective therapeutics to protect kidneys from injury and enhance kidney repair and recovery.

Mitochondria are classically described as energy-producing organelles or "power plants" of the cell. However, other functions of mitochondria, such as calcium ion homeostasis and intermediary metabolism, are similarly remarkable, and when mitochondria are damaged or dysfunctional, cell death ensues. This mechanism of cell death is often not a direct result of energetic failure, but because mitochondria can actively trigger the process of cell demise.[3] In one such mechanism, permeabilization of the mitochondrial outer membrane (MOM) causes the release of apoptogenic factors (*e.g.,* cytochrome c) from the intermembrane space, leading to apoptosis. In another mechanism, damage of the mitochondrial inner membrane (MIM) in the form of MPT immediately dissipates the mitochondrial membrane potential to inactivate energy production and can result in MIM swelling, disruption of the cristae structure, and rupture of the organelle. Even sublethal injury of mitochondria can lead to the production of excessive ROS from the respiratory chain, especially at complexes I and III. The toxicity of ROS to mitochondria and other cellular targets then forms a vicious cycle that ultimately induces irreversible injury and cell death.[3]

Mitochondrial damage and dysfunction are recognized as a major pathogenic events in a variety of diseases, including both chronic and acute kidney diseases.[4,5] In AKI, mitochondrial damage contributes critically to sublethal and lethal injury of kidney tubules, and the consequent loss of renal function. In various models of AKI, mitochondrial dynamics are disrupted, resulting in mitochondrial fragmentation, membrane permeabilization, mitochondrial dysfunction, energetic failure, and ROS production (Figure 1). Notably, preservation of mitochondrial dynamics, prevention of mitochondrial membrane permeabilization, and/or promotion of mitochondrial biogenesis can protect kidney tubular cells and tissues in AKI.[6–8] As such, targeting the mitochondria has been proposed as a therapeutic strategy. Two studies[1,2] in this issue of the *JASN* have significantly advanced our understanding of the mitochondrial contribution to AKI.

In the first study, Tsuji *et al.*[1] report a mechanism whereby mitochondria contribute to sepsis-associated AKI. Specifically, they demonstrated that mtDNA might play an important role in the activation of inflammation during sepsis-associated AKI *via* TLR9. Toll-like receptors on immune cells recognize danger-associated molecular patterns leading to the activation of a robust inflammatory response in diseases such as AKI (Figure 1). TLR9 has been implicated in the pathogenesis of polymicrobial septic AKI,[9] but the underlying mechanism remains elusive: How is TLR9 activated? And, upon activation, how does TLR9 regulate the immune response or inflammation? The study by Tsuji *et al.*[1] provides important insights that enables us to address these questions. TLR9 specifically recognizes single-stranded, unmethylated CpG-DNA, which is common to prokaryotes and mitochondria. By comparing the responses of TLR9-knockout (KO) and wild-type mice to cecal ligation puncture (CLP), Tsuji *et al.*[1] demonstrate compelling evidence for the involvement of mtDNA in TLR9-associated inflammation in septic AKI. First, mtDNA was detected in the plasma early following CLP. In addition, TLR9-KO mice showed lower levels of plasma mtDNA than wild-type mice following CLP, accompanied by lower inflammation. Finally, intravenous injection of mitochondrial debris containing mtDNA in wild-type mice induced a rapid inflammatory response followed by kidney injury. In further support, degradation of mtDNA in mitochondrial debris *via* DNase pretreatment seemed to reduce the ability of mitochondrial debris to induce inflammation and kidney injury. Taken together, these results support a critical role for mtDNA in stimulating systemic inflammation and associated

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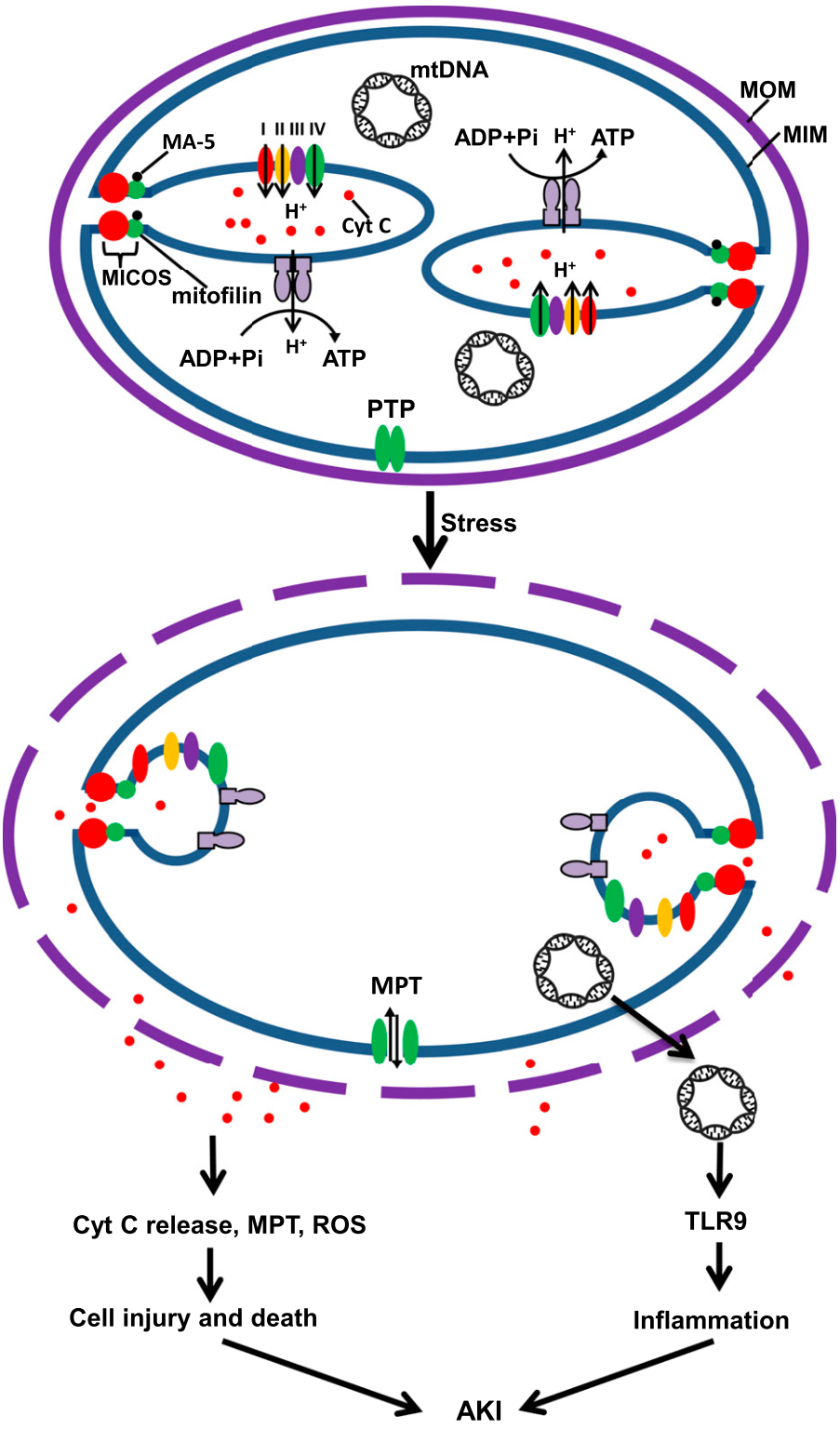

Figure 1. Critical Role of mitochondria in AKI. (A) In unstressed, healthy mitochondria, the integrity of both MOM and MIM is maintained to retain cytochrome c (Cyt C) in cristae and mtDNA in matrix. MICOS, including mitofilin, preserves the cristae structure and regulates the activity of ATP synthase. The newly identified MA-5 binds to mitofilin to facilitate ATP generation *via* ATP synthase (Suzuki *et al.*[8]). (B) Upon stress, mitochondria become permeable at both MOM and MIM, leading to the release of cytochrome c, MPT, loss of membrane potential, inactivation of ATP synthase, and production of ROS for cell injury and death. Dying or dead cells release mtDNA to activate systemic inflammation *via* TLR9 to further induce AKI (Tsuji *et al.*[7]). H+, proton; Pi, phosphate; PTP, permeability transition pore.

kidney injury in polymicrobial septic AKI. The source of circulating mtDNA, however, remains unclear. One possibility is that mtDNA transitions from the peritoneal cavity. However, this possibility is not supported by the observation that TLR9-KO mice had high levels of mtDNA in the peritoneal cavity, but low levels of mtDNA in the plasma following CLP. Dying or dead kidney tubular cells can release mtDNA, but tubular cell death is not extensive in septic AKI, and often occurs at late time points. The other possible sources of mtDNA include the plasma, following immune cell death during bacteria clearance, and the spleen, following activation of an immune response. Obviously, further investigations are required to clarify these possibilities. It is intriguing to note that TLR9-KO mice have high mtDNA in the peritoneal cavity, but low circulating mtDNA: Does TLR9 modulate mtDNA transition from the peritoneal cavity? The results of Tsuji *et al.*[1] support an interesting mechanism for septic AKI. Following CLP, bacteria are released into the peritoneal cavity, leading to the infiltration of leukocytes, which fight against bacteria in the peritoneal cavity and many die, releasing mtDNA. In TLR9-KO mice, leukocyte infiltration in the peritoneal cavity increases, resulting in more effective clearance of bacteria; however, this is associated with more leukocyte death and the release of more mtDNA in the peritoneal cavity. As the bacteria in the peritoneal cavity are cleared quicker and the level of circulating mtDNA is lower in TLR9-KO mice, systemic inflammation is reduced and, as a result, kidney injury is reduced in these mice. Taken together, this study unravels a novel mechanism of septic AKI whereby mtDNA induces systemic inflammation and kidney injury *via* TLR9 (Figure 1). As a consequence of this study, it is suggested that mitochondrial protection and/or timely removal of damaged mitochondria might be effective therapeutic strategies for septic AKI.[10]

In the second study, Suzuki *et al.*[2] demonstrate the protective effect of MA-5 against kidney and cardiac injury. MA-5 is a synthesized indole acetic acid derivative that was recently identified for its effect on increasing cellular ATP and survival in fibroblasts from patients with mitochondrial disease.[11] Suzuki *et al.*[2] have now further verified the protective effect of MA-5 in both ischemic and cisplatin-nephrotoxic AKI. Moreover, MA-5 was protective in the novel model of "mitomice", which have mutations in mtDNA. Remarkably, MA-5 increases ATP production without significantly affecting the activity of respiratory complexes. So, what is the mechanism of action? A series of biochemical studies suggested that MA-5 binds specifically to mitofilin (Figure 1), a core component of the so-called mitochondrial contact site and cristae organizing system (MICOS).[12] Structurally, mitochondria consist of four compartments: MOM, MIM, intermembrane space, and matrix. Although MOM forms a barrier to the cytosol, it is permeable to small molecules, including water and ions. The permeability of the MIM, however, is much more restricted, resulting in the maintenance of the proton gradient that creates the membrane potential for ATP synthesis *via* F1Fo-ATP synthase (sometimes called complex V). Notably, MIM consists of two morphologically and functionally distinct domains: the inner boundary membrane and the cristae membrane. The inner boundary membrane and the cristae membrane are connected by the crista junction, which forms a barrier that limits the movement of proteins and metabolites between the intracrista and the intermembrane space, as well as between the inter-boundary membrane and cristae. Functionally, the crista junction has been implicated in the modulation of the proton gradient across the MIM to regulate oxidative phosphorylation. A recent, important development in our understanding of mitochondrial biology is the discovery of MICOS, a conserved hetero-oligomeric protein complex at the crista junction that functions as the organizing center for the crista junction, the cristae, and the overall architecture of the MIM.[12] At the core of MICOS is mitofilin (or MIC60 according to recently agreed nomenclature[12]), which provides the docking site for the association of several MICOS proteins and regulates mitochondrial dynamics *via* OPA1, as well as ATP production *via* F1Fo–ATP synthase. Suzuki *et al.*[2] showed that MA-5 accumulates in the mitochondria and binds to mitofilin, but intriguingly it does not affect the activity of respiration complexes I –IV. On the basis of these findings, Suzuki *et al.*[2] postulate that MA-5 promotes the oligomerization of F1Fo–ATP synthase to increase ATP generation. In support of this theory, previous work suggested that Fcj1 (a yeast homolog of mitofilin) prevents F1Fo–ATP synthase assembly.[13] Although this is a logical possibility, MA-5 might also affect mitochondrial activities through mitofilin and MICOS. In particular, it might prevent the disruption of mitochondrial dynamics and consequent mitochondrial fragmentation, which is known to contribute to cell injury and death in ischemic and nephrotoxic AKI.[6] With this in mind, future studies should examine the effect of MA-5 on the ultrastructure and dynamics of mitochondria pertaining to cell death under pathogenic conditions to gain further insights.

The studies by Tsuji *et al.*[1] and Suzuki *et al.*[2] provide further support to the concept that mitochondrial protection is an effective therapeutic strategy for diseases such as AKI. The genetic and pharmacologic inhibition of the mitochondrial pathway of apoptosis rescues tubular cells to protect against AKI.[3] Additional mitochondria-targeting therapeutic strategies include the prevention of MPT, preserving mitochondrial dynamics, antagonizing mitochondrial oxidants, and promoting mitochondrial biogenesis, to name just a few, and mitochondria-targeted antioxidants (*e.g.,* MitoQ, CoQ10, SkQ1, SkQR1, and Mito-CP), the peptide bendavia (SS-31), and mitochondrial fission inhibitors, confer beneficial effects in various experiment models of AKI.[3–5] Despite the expanding list of mitochondria-protecting agents, their translation into clinical use remains a big challenge, largely because of their potential side effects and unclear mechanism of action. For example, cyclosporin A exerts protective effects by blocking MPT, but its nephrotoxicity limits its use in kidneys. Rapamycin, an mTOR inhibitor, protects against AKI by promoting autophagy and mitophagy[14,15], but might also suppress kidney repair and recovery following AKI. Thus, more specific and efficacious approaches are needed to target mitochondria for renoprotection. Future in-depth investigations into mitochondrial

biology and pathology are essential to the discovery of novel therapeutics for disorders caused by mitochondrial damage and dysfunction, including both chronic and acute kidney diseases.

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DISCLOSURES

None.

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See related articles, "Mitochonic Acid 5 Binds Mitochondria and Ameliorates Renal Tubular and Cardiac Myocyte Damage," and "Role of Mitochondrial DNA in Septic AKI *via* Toll-Like Receptor 9," on pages 1925–1932 and 2009–2020, respectively.

Water, Water Everywhere: A New Cause and a New Treatment for Nephrogenic Diabetes Insipidus

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Diabetes insipidus is a rare condition in which patients produce very large quantities of dilute urine. In the most severe forms, patients can produce 1 L urine every 1 hour 24 h/d, 7 d/wk, and 365 d/yr and must drink a comparable amount of water to avoid severe dehydration. Diabetes insipidus can be either central, resulting from failure of the posterior pituitary to make or secrete vasopressin (also called antidiuretic hormone), or nephrogenic, resulting from failure of the kidney to respond to vasopressin (reviewed in ref. 1). There are good therapies available for

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