MITOCHONDRIA AS A THERAPEUTIC TARGET IN ACUTE KIDNEY INJURY

Dr. Sangeetha R , MD

ABSTRACT

Acute kidney injury (AKI) is a common clinical entity that is associated with high mortality and morbidity. It is a risk factor for the development and progression of chronic kidney disease. Presently, no effective treatment for AKI is available, and novel therapeutic approaches are desperately needed. Accumulating evidence highlights mitochondrial dysfunction as an important factor in the pathogenesis of AKI. Recent advances in our understanding of the molecules involved in mitochondrial biogenesis, fusion/fission, mitophagy and their pathophysiological roles will lead to the development of drugs that target mitochondria for the treatment of various diseases, including AKI.

In this review, current knowledge of the contribution of mitochondria-related pathophysiology in AKI and the prospective benefits of mitochondria-targeting therapeutic approaches against AKI are discussed.

Keywords: acute kidney disease, mitiphagy, mitochondrial biogenesis, mitochondrial fusion and fission, tubular damage

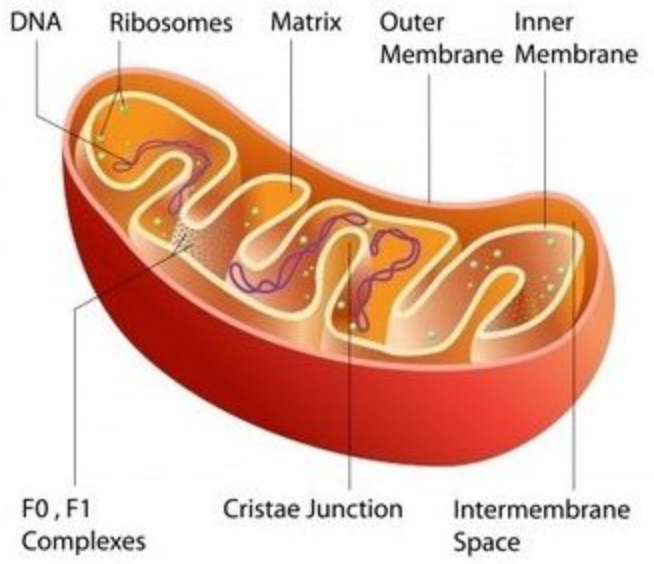
INTRODUCTION

The cytoplasms of nearly all eukaryotic cells contain mitochondria. They are especially abundant in cells and parts of cells that are associated with active processes. For example, in flagellated protozoa or in mammalian sperm, mitochondria are concentrated around the base of the flagellum. In cardiac muscle, mitochondria surround the contractile elements. Hummingbird flight muscle is one of the richest sources of mitochondria. Through oxidative phosphoryation mitochondria make efficient use of nutrient molecules. The

mitochondria are important since these organelles supply all the necessary biological energy of the cell, and they obtain this energy by oxidizing the substrates of the Krebs cycle. Hence, the mitochondria are referred to as the 'power houses' of the cell.

In 1890, mitochondria was first described by Richard Altmann and he called them as bioblasts. Benda in the year 1897 coined the term mitochondrion. In the 1920s, a biochemist Warburg found that oxidative reactions takes place in most tissues in small parts of the cell. It is becoming increasingly clear that disorders in mitochondrial dynamics, recycling, and biogenesis can all cause or worsen disease states in humans , leading to the concept that these processes could represent targets for therapeutic interventions.

STRUCTURE OF MITOCHONDRIA



MITOCHONDRIA AND KIDNEY

Acute kidney injury (AKI) is a common clinical entity that is associated with high mortality and morbidity. It is a risk factor for the development and progression

of chronic kidney disease. Accumulating evidence highlights mitochondrial dysfunction as an important factor in the pathogenesis of AKI. Kidneys are highly vulnerable to ischemic or nephrotoxic insults. The renal tubule, and in particular the proximal tubule, is densely packed with mitochondria, which exist primarily to generate sufficient quantities of ATP, via oxidative phosphorylation to power the huge amounts of solute transport performed every day in the kidney. Mitochondria not only produce cellular energy but also modulate several cellular functions, including proliferation and intracellular calcium homeostasis. Mitochondrial dysfunction induces apoptosis and the generation of reactive oxygen species (ROS), both of which contribute to the development and progression of various diseases. Although mitochondria have their own genome, most of the proteins and enzymes that reside in mitochondrial membranes are nuclear gene products.

Mitochondrial biogenesis requires coordination of the nuclear and mitochondrial genome. Nuclear respiratory factors 1 and 2 (NRF1 and NRF2) are nuclear-encoded transcription factors that act on the nuclear genes coding for constituent subunits of the OXPHOS system. In addition, NRF1 and NRF2 also regulate the expression of many other genes involved in mtDNA replication.

Peroxisome proliferator-activated receptor gamma (PPAR γ) coactivator-1alpha (PGC-1α) is a nuclear-encoded transcriptional coactivator that regulates the expression of nuclear encoded mitochondrial proteins, including NRF1 and NRF2. The overexpression of PGC-1α produces a robust increase in mitochondrial number, respiratory capacity and intracellular ATP concentration in cultured proximal tubular cells and may promote repair and recovery after oxidative damage.

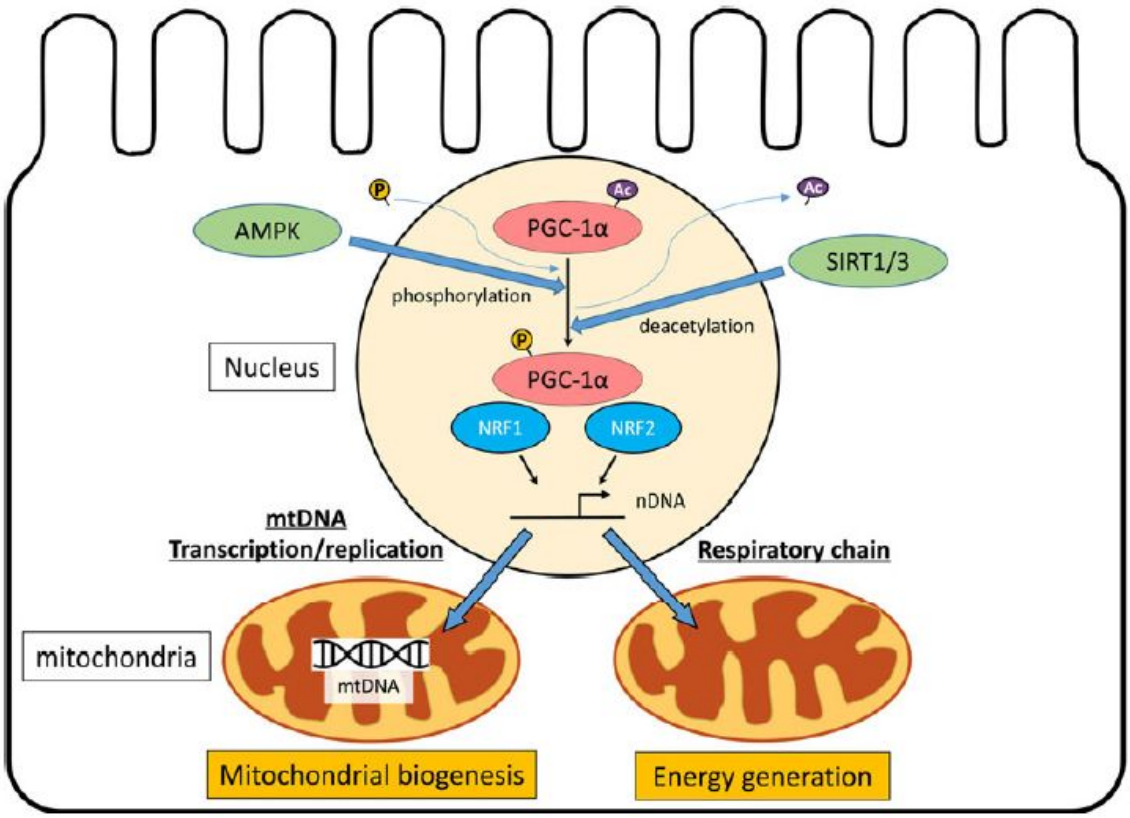
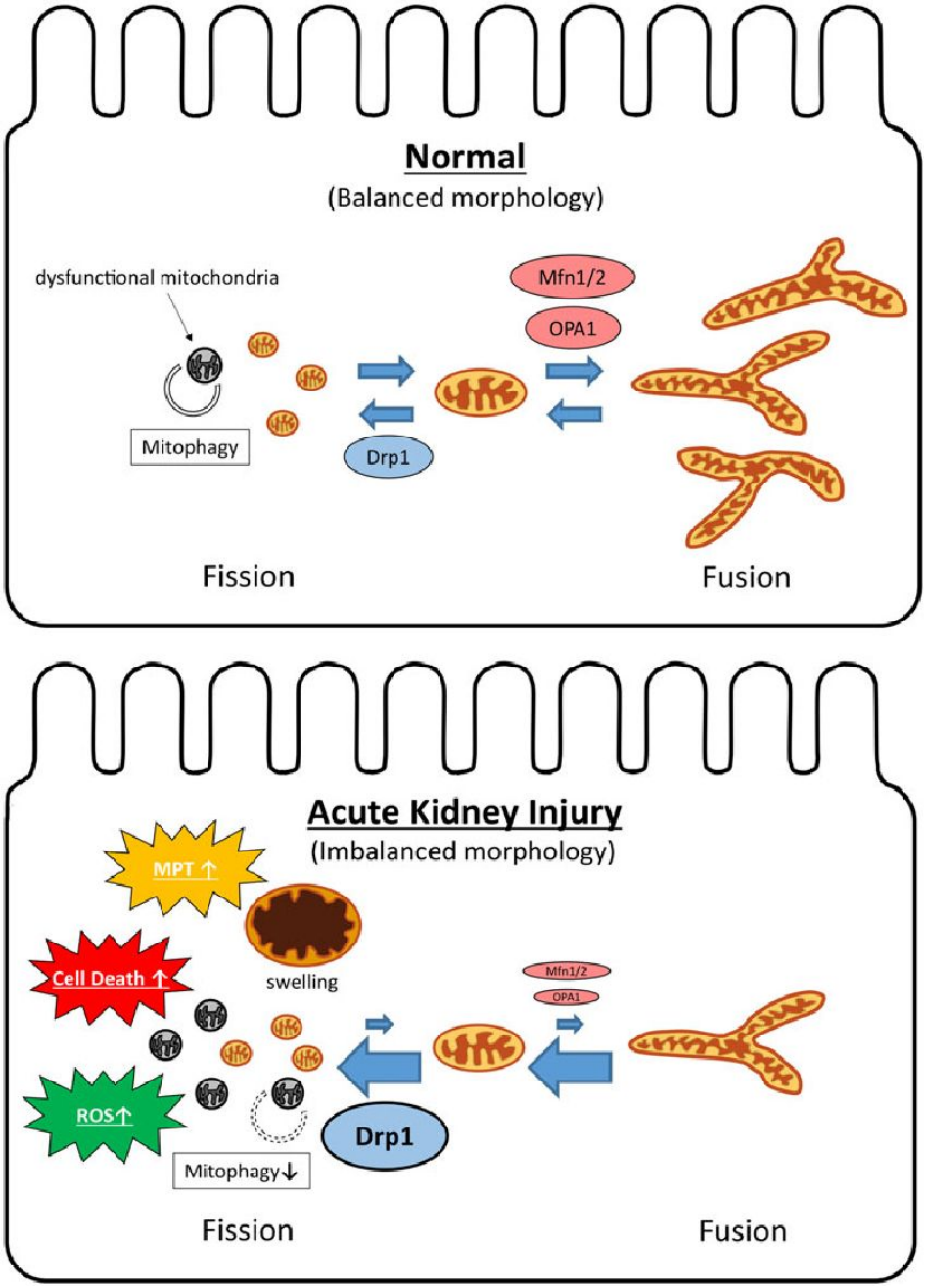


FIGURE 1: PGC-1α-NRF1/NRF2-related pathways for mitochondrial biogenesis and energy generation. Phosphorylation by AMPK and deacetylation by SIRT1 or SIRT3 are required for PGC-1α activation. NRF1 and NRF2 are key transcription factors and are two targets of PGC-1α. Activated PGC-1α cooperates with NRF1/NRF2 and promotes the expression of multiple nuclear-encoded genes that regulate mtDNA transcription/replication and respiratory chain. PGC-1α, peroxisome proliferator-activated receptor gamma (PPARγ) coactivator-1α; AMPK, AMP-activated protein kinase; SIRT1/3, sirtuin 1/3; NRF1, nuclear respiratory factor 1; NRF2, nuclear respiratory factor 2; nDNA, nuclear DNA; mtDNA, mitochondrial DNA; P, phosphate group; Ac, acetyl group.

MITOCHONDRIA IN ACUTE KIDNEY INJURY

The proximal tubule lacks the capacity to perform anaerobic glycolysis . As such, it is exquisitely sensitive to aerobic insults, such as Ischemia Reperfusion Injury and cellular ATP levels plummet within seconds of oxygen deprivation. Increasing evidence suggests that mitochondria are also damaged in septic AKI, but it remains unclear whether this is secondary to a lack of oxygen delivery (the classical paradigm) or rather direct toxicity induced by the inflammatory milieu, although opinion seems to coalescing more around the latter concept. (1)Mitochondria in the proximal tubule are also damaged by therapeutic drugs

that frequently cause AKI, such as ifosfamide, cisplatin, tenofovir, and gentamicin [2]. Moreover, a very recent study suggests that elevated urinary mitochondrial DNA (mtDNA) predicts the development of AKI in humans undergoing cardiac surgery.The realization that mitochondria play a central role in AKI has stimulated the search for new therapies that can target these complex and fascinating organelles.



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MITOCHONDRIAL DYNAMICS

Mitochondria are highly dynamic organelles that shift between tubular network-like and fragmented morphologies by means of coordinated fusion and fission. This structural remodeling has been recognized only recently. Under physiological conditions, the frequency of fusion and fission events are finely tuned and balanced to maintain mitochondrial homeostasis.

These mitochondrial dynamics also play an important role in the quality control of mitochondria . The molecular machinery governing mitochondrial dynamics is determined by fission mediators, such as dynamin-related protein 1 (Drp1), and fusion proteins, such as mitofusin 1 (Mfn1), mitofusin 2 (Mfn2) and optic atropy 1 (OPA1). Defects in these proteins lead to both severely altered mitochondrial morphology and impaired mitochondrial function, and cause human diseases.

At the protein level, mitochondria contain a number of proteases that preferentially degrade particular protein and the mitochondrial unfolded protein response, a specific stress response, which together regulate the level of mitochondrial proteases and molecular chaperones to accommodate the unfolded protein load. However, these approaches are not sufficient for the rapid removal of whole dysfunctional mitochondria or a large number of damaged mitochondria. Organelle level control is believed to be accomplished by mitophagy, a particular type of macroautophagy in which a single mitochondrion or cluster of mitochondria is sequestered by the isolation membrane to form an autophagosome (mitophagosome). The autophagosome eventually fuses with a lysosome, which leads to the complete degradation of autophagosomal contents. Injured mitochondria induce harm through the induction of mitochondrial ROS production or MPT, or both, and are thus selectively removed by mitophagy. This process may prevent cell death from mitochondrial oxidant stress and the release of pro-apoptotic proteins.

MECHANISMS OF MITOCHONDRIAL INJURY IN AKI

Genetic inheritance of mtDNA causes a subset of renal diseases often characterized by tubulopathy . These findings strongly indicate that mitochondrial deficiencies are causal factors in the development and progression of renal dysfunction.

1. PGC-1α-related mitochondrial biogenesis

PGC-1α is a master regulator of mitochondrial biogenesis. In the kidney, PGC-1α is predominantly expressed in proximal tubules, indicating the effectiveness of PGC-1α in proximal tubular homeostasis , and enforced overexpression of PGC-1α in cultured proximal tubular cells increased mitochondrial number, respiratory capacity and mitochondrial proteins. Tran et al. showed that PGC-1α expression in tubular cells is proportionally suppressed with the degree of renal impairment in septic AKI models, and that PGC-1α knockout mice exhibited exacerbation of increases in BUN and serum creatinine in these models .(3) These findings in turn suggest that PGC-1α may play an important role in recovery from septic AKI via the maintenance of tubular mitochondrial biogenesis.

2. Drp1- and Mfn2-related mitochondrial fragmentation

During cell injury or stress dynamics, the balance between mitochondrial fusion and fission shifts to mitochondrial fission. This results in mitochondrial fragmentation and subsequent alterations in mitochondrial structure . Emerging evidence suggests that the mitochondrial fragmentation caused by changes in the activity of mitochondrial fusion and fission related Drp1,(4) a mitochondrial fission mediator, is rapidly activated following AKI, such as in ischemia reperfusion and cisplatin induced nephrotoxicity, and induces mitochondrial fragmentation and subsequent renal tubular cell apoptosis. Primary cultured proximal tubular cells from mice with conditional knockout of Mfn2, a mitochondrial fusion mediator, in the kidney were shown to be highly sensitive to Bax activation and cytochrome c release, which lead to apoptosis following ATP depletion . These findings indicate that either or both increased Drp1 and

decreased Mfn2 (6)exacerbate tubular damage via an imbalance in mitochondrial fission and fusion, with subsequent enhancement of mitochondrial fragmentation, and might thereby contribute to kidney disease, including AKI.

3. Alteration of mitophagy

Mitophagy has been shown to have indispensable protective roles in several disease models, such as ischemia reperfusion injury. Alteration of mitophagic state significantly exacerbated AKI in a cisplatin-induced AKI model in mice. For example, inhibition of autophagy with chloroquine or knockout of a mitophagy-related molecule, autophagy gene-related 7 (Atg7), in proximal tubules exacerbated renal function, tissue damage and apoptosis compared with wild type. In contrast, rapamycin treatment, which activates the mitophagic pathway, attenuated tubular damage in cisplatin-induced AKI .(8) Further, in kidney tissues of patients with septicemia, renal tubular cells reveal increased autophagosomes, juxtaposed with hydropic mitochondria showing cristae damage. This pattern indicates the clearance of damaged mitochondria by mitophagy. Recently, it was revealed that p53 binds to Parkin and disturbs its translocation to damaged mitochondria in mouse heart and inhibits mitophagy . Molecular mechanisms of mitophagy in kidney are not well known, but activation of p53 has been reported in several models of AKI, and p53-mediated inhibition of mitophagy contributes to AKI pathophysiology. (9)

Taken together, these findings show that tubular cell homeostasis is closely related to mitochondrial homeostasis. On this basis, the various phenotypic changes in mitochondrial structure and function contribute to the phenotypic changes in tubular cell damage in AKI. The molecules related to mitochondrial biogenesis, fragmentation and mitophagy, both of which play important roles in AKI progression, as well as to recovery are important to understanding the pathophysiology of AKI, and to the mechanism linking AKI to the progression of chronic kidney disease.

Novel therapeutic approaches against AKI targeting mitochondria

Aims of mitochondrial-targeted therapies in AKI

- to limit harm to mitochondria and minimize the downstream consequences for the cell;
- to enhance recycling of damaged mitochondria
- to accelerate recovery of normal mitochondrial mass and function post insult

More specifically, they can currently be classified as targeting one of four distinct processes: mitochondrial ROS generation and oxidative stress; mitochondrial fission and activation of cell death pathways; mitochondrial breakdown via autophagy/mitophagy; and mitochondrial biogenesis.

MITOCHONDRIAL-TARGETED ANTIOXIDANTS

Three major classes of mitochondrial-targeted antioxidants have been developed in recent years – SSpeptides, MitoQ, and plastoquinone analogues

(SkQ1/SkQR1) – all of which work on a similar principle. As lipophilic cations, they selectively accumulate into the mitochondrial matrix at very high concentrations, utilizing the voltage gradient across the mitochondrial inner membrane created by theOXPHOS complexes (the SS peptides may also interact with cardiolipin, a major constituent of the mitochondrial inner membrane). All three classes of these agents have shown evidence of benefit in preclinical models of AKI and appear to have satisfactory safety profiles.

The SS peptides arguably show the greatest promise for AKI at present. In a series of studies in rodent models by Szeto et al. , it has been shown that two

agents in this class (SS-20 and SS-31) offer significant protection in AKI because of IRI, with striking positive effects on mitochondrial morphology and function,

cell polarity, and overall kidney function.(2,11) SS-31 (marketed as Bendavia) has also shown evidence of benefit in a larger animal model (renovascular disease in pigs) , and the effects in human AKI are now being investigated. Meanwhile,MitoQ, an analogue of the OXPHOS component ubiquinone (CoQ10), has been studied in a multitude of different disease processes in different organs, including in humans . Regarding the kidney, it has recently been shown to have beneficial effects in AKI induced by IRI and cisplatin . The plastoquinone analogues also seem to be protective in preclinical AKI models, including IRI, rhabdomyolysis, and gentamicin toxicity .(11,12)

One potential limitation of all of these agents is that they have to be given before the insult, so their clinical usage would effectively be confined to scenarios where AKI is predictable. However, this is also the case for many other nonmitochondrial therapies currently in development. Moreover, the fact that such structurally heterogeneous compounds have all shown benefit in various models in different research groups suggests that targeting mitochondrial ROS production is a strategy worth pursuing.

Another recently identified method to lower mitochondrial ROS production involves the signaling molecule stanniocalcin-1 (STC1), (14)which is highly expressed in the kidney. STC1 is a stress protein that responds to stimuli such as hypoxia, and traffics to the mitochondrial membrane, where it increases the expression of uncoupling proteins, which, in turn, may reduce the rate of ROS production by the mitochondrial OXPHOS.

Transgenic overexpression of STC1 in mice provides protection against IRI-induced

AKI , and a very recent follow up study has suggested that it may also activate AMP-activated kinase (AMPK) , a crucial metabolic sensor that regulates mitochondrial function . Meanwhile, another study has suggested that mitochonic acid, a derivative of the plant hormone indole-3-acetic acid, also lowers mitochondrial ROS production, and shows evidence of benefit in AKI

because of IRI. Mitochonic acid is thought to target the inner mitochondrial membrane protein mitofilin, and may also work by maintaining the normal structure of mitochondrial cristae and promoting ATP production.

MITOCHONDRIAL DYNAMICS

Mitochondria are highly dynamic organelles, capable of fusing and dividing with each other, to exchange genetic and other information. In the last few years, several key players in mitochondrial fusion/fission have been identified, including the profusion proteins mitofusin 1 and 2 (MFN 1 and 2) and OPA1, and the profission protein DRP1 . It has also become clear that genetic mutations in fission/fusion proteins can cause diseases in humans ,and that mitochondrial fission is probably an important step in the release of proapoptotic factors, such as cytochrome c, from mitochondria into the cytosol. There is, therefore, increasing interest in targeting mitochondrial dynamics pharmacologically in AKI. Brooks et al. demonstrated in both cell and mouse models that DRP1 is rapidly recruited to fragmented mitochondria in the proximal tubule. They reported that inhibition of DRP1 activity, using either genetic or pharmacological approaches, was protective against mitochondrial fragmentation, initiation of apoptosis, and overall kidney damage. The DRP1 inhibitor used (Mdivi-1) has also now been shown to have beneficial effects in various other organ systems and could represent a potential new therapy in human AKI.

AUTOPHAGY AND MITOPHAGY

Damaged mitochondria within cells are identified and removed by a process of selective autophagy termed mitophagy, whereby they are engulfed by auto-phagosomes, which then subsequently fuse with lysosomes, within which degradation of contents takes place. The turnover of mitochondria in the proximal tubule is thought to be quite high (estimated half-life of 2 weeks), and inhibition of autophagy leads to the accumulation of damaged mitochondria and proximal tubule dysfunction(13) . Previous studies have shown that autophagy is

activated in tubular cells in AKI, and pharmacological enhancement of this process could in theory minimize cellular stress and accelerate recovery. Support for this concept was provided by studies showing that genetic or pharmacological inhibition of autophagy worsened outcome in response to insults such as cisplatin and IRI, whereas activation with the mTOR inhibitor rapamycin was protective.(13,14) However, other studies have reported very different findings, and the role of autophagy in AKI thus remains hotly debated .

MITOCHONDRIAL BIOGENESIS

Much has been learnt in the last few years concerning the key molecules that drive mitochondrial biogenesis within cells, and pharmacological enhancement of this process might accelerate recovery in AKI.(17)

Peroxisome proliferator-activated receptor-g coactivator-1a (PGC-1a) is a transcriptional coactivator that has been identified as a master regulator of mitochondrial biogenesis. The expression of PGC-1a in the kidney cortex has been shown to decline in parallel with renal function in a model of septic AKI, and then increase in the recovery phase,when mitochondrial biogenesis should be occurring. Moreover, mice with a genetic defect of PGC- 1a in the proximal tubule were more susceptible to injury, whereas in-vitro cell experiments suggest that overexpression of PGC-1a postinsult can enhance recover. Peroxisome proliferator-activated receptor-g activators act upstream of PGC-1a and were previously used in the treatment of diabetes mellitus. Experimentally, these agents have shown beneficial effects in AKI because of insults including IRI, cisplatin and tenofovir .Another upstream activator of PGC-1a is AMPK, which acts as a crucial metabolic switch, effectively upregulating cellular metabolism in response to a fall in ATP level, and pharmacological activation of AMPK has been shown to be protective in AKI because of IRI. Moreover, as mentioned earlier, very recent work suggests that the extracellular signaling protein STC1 also acts via the AMPK pathway.

Attention in the mitochondrial biogenesis field has also focused recently on sirtuins, which are protein deacetylases that have key roles in regulating cellular

metabolism, mainly in response to changes in NADH/NADþ ratio. The sirtuin 1 (SIRT1) activator SRT1720 stimulates mitochondrial biogenesis via the PGC-1a pathway, and has been shown to enhance mitochondrial recovery and tubular function following IRI.(20) Finally, Schnellmann et al. have used unbiased high-throughput screening approaches to search for novel activators of mitochondrial biogenesis, and have identified the b2-adrenergic receptor agonist formoterol as a promising candidate. In their most recent work, they have demonstrated that formoterol enhances the restoration of mitochondrial proteins and function in the kidney post-IRI, and also completely restores kidney function, suggesting that it could represent a promising new treatment for AKI .(21)

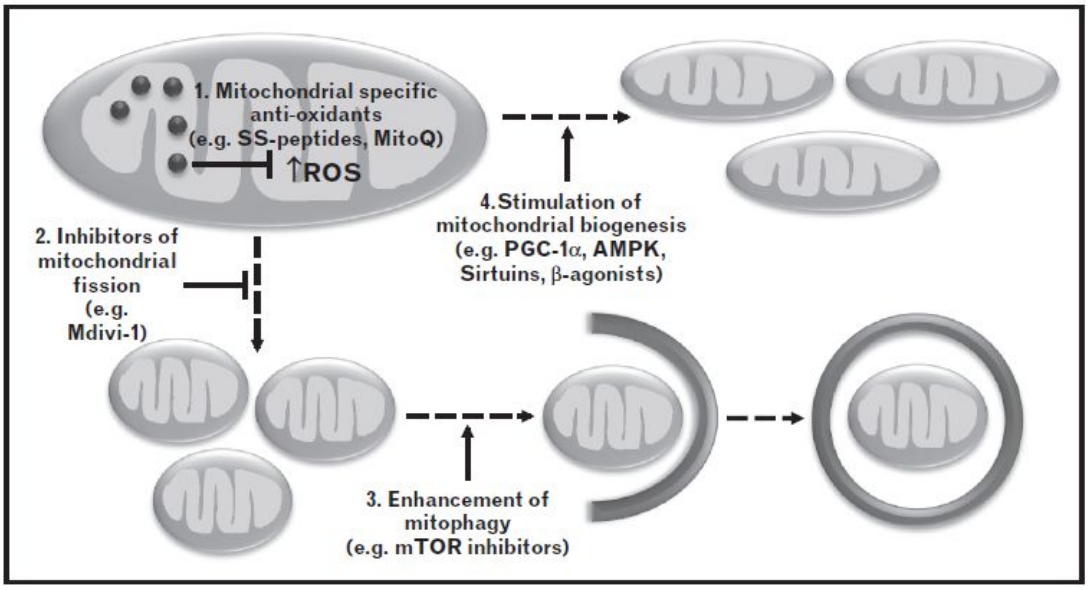

FIGURE 2. Summary diagram of current experimental strategies to target mitochondria in acute kidney injury. (1) Mitochondrial-specific antioxidants, such as SS-peptides and MitoQ, accumulate within the mitochondrial matrix and can limit the increase in reactive oxygen species (ROS) that is thought to occur in acute kidney injury (AKI), thus minimizing oxidative stress. (2) Inhibition of the pro-fission protein DRP-1 with Mdivi-1 can limit mitochondrial fragmentation and the subsequent activation of cell death pathways. (3) Damaged mitochondria are removed via mitophagy, whereby they are engulfed by autþ-phagosomes, and enhancement of this process might be beneficial in AKI, but this remains controversial. (4) Stimulation of mitochondrial biogenesis by various methods can accelerate recovery post AKI. AMPK, AMP-activated kinase; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator-1 alpha.

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CONFLICT OF INTEREST STATEMENT

None declared.