Mitochondrial dysfunction in the pathophysiology of renal diseases

Ruochen Che,1,2 Yanggang Yuan,3 Songming Huang,1,2 and Aihua Zhang1,2

1Department of Nephrology, Nanjing Children’s Hospital, Affiliated with Nanjing Medical University, Nanjing, China; 2Institute of Pediatrics, Nanjing Medical University, Nanjing, China; and 3Department of Nephrology, First Affiliated Hospital of Nanjing Medical University, Nanjing, China

Submitted 25 November 2013; accepted in final form 29 November 2013

Che R, Yuan Y, Huang S, Zhang A. Mitochondrial dysfunction in the pathophysiology of renal diseases. Am J Physiol Renal Physiol 306: F367–F378, 2014. First published December 4, 2013; doi:10.1152/ajprenal.00571.2013.—Mitochondrial dysfunction has gained recognition as a contributing factor in many diseases. The kidney is a kind of organ with high energy demand, rich in mitochondria. As such, mitochondrial dysfunction in the kidney plays a critical role in the pathogenesis of kidney diseases. Despite the recognized importance mitochondria play in the pathogenesis of the diseases, there is limited understanding of various aspects of mitochondrial biology. This review examines the physiology and pathophysiology of mitochondria. It begins by discussing mitochondrial structure, mitochondrial DNA, mitochondrial reactive oxygen species production, mitochondrial dynamics, and mitophagy, before turning to inherited mitochondrial cytopathies in kidneys (inherited or sporadic mitochondrial DNA or nuclear DNA mutations in genes that affect mitochondrial function). Glomerular diseases, tubular defects, and other renal diseases are then discussed. Next, acquired mitochondrial dysfunction in kidney diseases is discussed, emphasizing the role of mitochondrial dysfunction in the pathogenesis of chronic kidney disease and acute kidney injury, as their prevalence is increasing. Finally, it summarizes the possible beneficial effects of mitochondrial-targeted therapeutic agents for treatment of mitochondrial dysfunction-mediated kidney injury-genetic therapies, antioxidants, thiazolidinediones, sirtuins, and resveratrol-as mitochondrial-based drugs may offer potential treatments for renal diseases.

acute kidney injury; chronic kidney disease; mitochondria

THE ROLE OF MITOCHONDRIAL dysfunction in various diseases has been gradually recognized and widely studied since the first description of mitochondrial disease manifested as severe hypermetabolism of nonthyroid origin in 1962 (86). The number of mitochondria distributed in cells varies greatly, ranging from ~16 in human germ cells to 100,000 in oocytes, depending on the distinct energy demands of different tissues and organs. The kidney is a highly energetic organ and rich in mitochondria. Thus mitochondrial dysfunction plays a critical role in the pathogenesis of kidney diseases. To illuminate the involvement of mitochondrial dysfunction in kidney diseases, we first review the basic physiology and pathophysiology of the mitochondrion. Then, mitochondrial cytopathies (MCs) with the kidney involved will be discussed. Subsequently, we mainly focus on the role of mitochondrial dysfunction in the pathogenesis of two common pathways of end-stage kidney disease, namely, chronic kidney disease (CKD) and acute kidney injury (AKI). Finally, we summarize the prospective role of mitochondria-targeting therapeutic agents for the treatment of renal diseases. Mitochondria-based drugs may offer potential alternatives for the treatment of various glomerular diseases.

Physiology and Pathophysiology of the Mitochondrion

Mitochondrial structure. The mitochondrion is a double-membrane organelle that exists in most eukaryotic cells except for mature erythrocytes. The double-membrane structure forms three separate regions and two compartments, termed the outer mitochondrial membrane (OMM), intermembrane space, cristae formed by inner mitochondrial membrane (IMM), and matrix. The OMM has pores that allow passive diffusion of molecules smaller than 5,000 Daltons. Larger molecules pass through the mitochondrion via translocases on the OMM. When irreparable damage to cells occurs, the permeability of the OMM increases and proteins located in the intermembrane space, such as cytochrome c, flow out and initiate the apoptosis program. Owing to the numerous folds of cristae with oxy-somes, the area of the IMM is about five times greater than that of the OMM. The IMM is embedded with abundant proteins that perform redox reactions, synthetize adenosine triphosphate (ATP), block ionic diffusion, and regulate mitochondrial dynamics. The IMM also encloses the matrix, where the oxidative phosphorylation (OXPHOS) enzyme and mitochondrial genetic material reside(92).

Mitochondrial DNA. Unlike nuclear DNA (nDNA), human mitochondrial DNA (mtDNA) is a circular molecule composed of two strands, termed the heavy (H) and light (L) strands. Human mtDNA contains 16,569 base pairs and 37 genes. Of these genes, 22 encode transfer RNAs (tRNAs), two encode...
ribosomal RNAs (rRNAs; 12S and 16S), and the remaining 13 encode polypeptides. The 13 polypeptides encoded by mtDNA belong to the subunits of OXPHOS enzyme complexes I–V. More specifically, the polypeptides comprise seven subunits (ND1–ND6, and ND4L) of complex I (NADH dehydrogenase), cytochrome b (Cyt b) of complex III (ubiquinone-cytochrome c oxidoreductase), three subunits [cytochrome oxidase (COX) I–III] of complex IV (cytochrome c oxidase), and two subunits (ATPase 6 and ATPase 8) of complex V (ATP synthase) (29). Since germ cells have few mitochondria and are selectively degraded, the hereditary mode of most mitochondria is maternal inheritance (35).

mtDNA is apt to be exposed to reactive oxygen species (ROS) stress without histone protection. Moreover, almost the entire coding regions lack repair mechanisms. As a result, it is highly susceptible to damage and mutations, with a 10- to 1,000-fold greater mutation rate than nDNA. The main external risk factors of mtDNA damage include ROS, ultraviolet light, ionizing irradiation, alkylating agents, base analogs, modifier-induced base-pair variations, and aging. Even with no external damage, mtDNA undergoes natural damage, such as base mismatches during replication, spontaneous base changes, single-strand breakage, double-strand breakage, and interstrand cross-linking. Additionally, mtDNA is equipped with inadequate and inefficient repair mechanisms. Consequently, mtDNA mutations cause mitochondrial dysfunction, including reduced ATP synthesis, elevated intracellular calcium levels (resulting from calcium pump inactivation), activated phospholipases, and decomposition of membrane phospholipids.

Respiratory chain and OXPHOS. Mitochondria are responsible for >90% of energy production by OXPHOS in the human body. The coordination between the tricarboxylic acid (TCA) cycle and the electron transport chain is the main process for ATP production. First, pyruvate molecules generated by glycolysis pass through the mitochondrial membranes and are converted into acetyl-coenzyme A (acetyl-CoA) with catalysis by the pyruvate dehydrogenase complex. Acetyl-CoA then enters the TCA cycle and produces the reducing substrates

carrier ubiquinone and then transferred to complex IV (ubiquinol-ferricytochrome c oxidoreductase) by the electron carrier ubiquinone and then transferred to complex IV (cytochrome c oxidase) by cytochrome c, which accumulates sufficient energy to motivate complexes I, III, and IV to pump the protons from the matrix to the intermembrane space. Owing to the electrochemical gradient, the protons influx back to the matrix through complex V (ATP synthase), which changes the configuration of complex V to generate ATP from the condensation of inorganic phosphate and adenosine diphosphate (109).

Mitochondrial ROS production. During the respiratory process, 0.4–4% of the total consumed oxygen is converted into superoxide radicals via electron leakage from the respiratory chain (109), which also participates in cellular signaling pathways as second messengers (99). Superoxide radicals released into the matrix or intermembrane space are dismutated into hydrogen peroxide (H_2O_2) and O_2 by Mn-superoxide dismutase (SOD) and Cu/Zn-SOD, respectively. During the Fenton reaction, H_2O_2 combines with superoxide radicals to generate hydroxyl radicals. Therefore, ROS comprise superoxide radicals, H_2O_2, and hydroxyl radicals (102). In biological situations, mitochondria have a well-established antioxidation system. H_2O_2 is scavenged effectively by the thioredoxin reductase/thioredoxin/peroxiredoxin-3,5 system, glutathione peroxidase (GPx), and glutathione (GSH) (32). ROS stimulate mild uncoupling of mitochondria, and the resulting increase in proton conductance can have a negative feedback effect on ROS production (102). Furthermore, the mitochondrial permeability transition pore (MPTP) is opened to reduce the electrochemical gradient and accelerate oxygen consumption, which decreases ROS production. Cytochrome c is also a powerful ROS scavenger. The presence of low ROS concentrations is physiologically normal, and ROS function as important second messengers. However, excessive ROS generation is injurious to mtDNA and potentially leads to impaired electron transport chain functions, reduced ATP synthesis, mitochondrial dysfunction, cell injury, and even apoptosis. ROS play key roles in the initiation and modulation of cell apoptosis (91).

Mitochondrial dynamics and mitophagy. Mitochondrial fusion and fission are necessary not only for mitochondrial morphology maintenance but also for maintaining mtDNA integrity, regulating cellular survival and death, transmitting redox-sensitive signals, and participating in metabolic processes. Mitochondrial fusion involves fusion of both the outer membrane (OMM) and inner membrane (IMM), depending on Mfn1, Mfn2, and the dynamin family GTPase OPA1 (21). Dynamin-related protein Drp1 and its downstream protein fission protein 1 (Fis1) are responsible for mitochondrial fission, which is involved in mitochondrial recruitment and segregation (98, 128). Drp1 overexpression induces mitochondrial fission, cytochrome c release, and the caspase cascade, but not ROS production, indicating that Drp1-dependent fission is a downstream event related to permeability of the OMM (149). Excessive mitochondrial fission or decreased fusion may be detrimental to mitochondrial functions and cellular survival. For example, Drp1 and Fis1 are increased in cyclosporine A and rhodomyolysis-induced tubular apoptosis, and blockade of these proteins can restore tubular functions (27, 134).

Mitophagy is a kind of macroautophagy specifically targeted toward mitochondrial degradation that removes damaged mitochondria and recycles and reallocates useful components. Mitochondrial fission initiates mitophagy, which is triggered by opening of MPTP pores and a decrease in the mitochondrial membrane potential. Subsequently, PTEN-induced putative kinase (PINK1) accumulates on the mitochondrial surface, instead of undergoing rapid degradation under normal conditions. PINK1 recruits Parkin, which ubiquitinates OMM proteins like Mfn1, Mfn2, and voltage-dependent anion channels that, in turn, interact with p62/sequestosome 1 and Ambra 1 and participate in recruiting autophagosomal membranes and new phagophores (43, 140). In addition, damaged mitochondria can induce the expression of FUN14 domain-containing 1, and apoptotic proteins BNIP3 and NIX, which interact with LC3/Atg8 and recruit autophagosomes to mitochondria, independently of PINK1/Parkin (82, 159). The relationship between mitophagy and apoptosis remains controversial. What
can be confirmed is that mitophagy aims to eliminate damaged mitochondria and maintain normal cellular function. However, excessive mitophagy can cause cell death, specifically type II programmed cell death.

**Inherited MCs in Kidney Diseases**

MCs refer to inherited or sporadic mtDNA or nDNA mutations in genes that affect mitochondrial functions. Different from nDNA, there are hundreds of mtDNA copies in a cell. This means that mutant mtDNA can coexist with normal mtDNA, which is called heteroplasmy. Cell dysfunction only occurs when the proportion of mutant mtDNA exceeds a threshold level, which is determined by the cell OXPHOS rate (30). In addition, because of the existence of mitochondrial fission and fusion, mitochondria with different mutational loads may mix with one another. The fluctuations of heteroplasmy among various daughter cells are dampened (21). Therefore, almost all organs can be implicated in mitochondrial-related genetic defects, but the clinical outcomes are varied. MCs in kidneys mainly manifest as focal segmental glomerular sclerosis (FSGS), tubular defects, and cystic kidney disease (31, 40, 104). The etiology can be classified into two glomerular sclerosis (FSGS), tubular defects, and cystic kidney disease (31, 40, 104). The etiology can be classified into two categories: the genetic background and tubular cell derivation of PCKD, among which mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS) syndrome accounts for a large proportion. MELAS syndrome is mainly caused by point mutations in the MTTL1 gene, encoding mitochondrial tRNA^LEU^ (46). The point mutations are distributed as follows: 80% of patients have an A3243G mutation, 7.5–10% have A3253G, and 7.5% have T3271C (67). Although the mechanism of MELAS-associated FSGS remains unclear, the renal biopsies from patients with coexistence of MELAS and FSGS often manifest with numerous dysmorphic mitochondria in podocytes and effacement of foot processes (49, 150). Podocytes, which will be described below, have limited potency for regeneration. In addition, in these cases arteriolar hyalinosis is commonly seen (124). Therefore, it is reasonable to believe that MCs induce irreversible podocyte damage and microvascular lesions, which comprise a fatal strike to the kidney. Steroid-resistant nephrotic syndrome (SRNS) does not specifically occur with the A3243G mutation. Patients with other mtDNA mutations and CoQ10 deficiencies may also suffer from the disease (112, 119, 122), indicating the involvement of mitochondrial dysfunction in the mechanisms of SRNS.

**Tubular defects and MCs.** Renal tubules comprise one of the major victims of MCs, of which the most frequently reported is proximal tubular defects. Proximal tubular cells are relatively vulnerable to oxidative stress and are therefore apt to suffer from respiratory chain defects. Renal tubule defects mainly manifest as loss of electrolytes and low-molecular-weight proteins, which are frequently characterized as Fanconi syndrome and Bartter-like syndrome. Patients with mitochondrial tubulopathy are usually accompanied by myoclonic epilepsy and ragged red muscle fibers (MERRF), and Pearson’s, Kearns-Sayre, and Leigh syndromes. The majority of genetic mutations detected in these diseases are fragment deletions of mtDNA.

**Other renal diseases and MCs.** Cystic renal diseases have been associated with MCs in a number of reports (50, 51), mimicking polycystic kidney disease (PCKD). The mechanism of cystic formation remains unknown. In view of the genetic background and tubular cell derivation of PCKD, there may be some correlations between cystic renal dis-
eases induced by MCs and PCKD. In addition, some signaling pathways for cystogenesis, such as mammalian target of rapamycin (mTOR), are also involved in mitochondrial dysfunction (24). Similarly, there are a few reports about the coexistence of renal cell carcinoma (RCC) and MCs (108, 120). Sangkhathat et al. (120) reported a 41-year-old man with full-blown MELAS syndrome suffering translocation of the transcription factor TFE3 as well as an A3243G mtDNA transition. Piccoli (108) reported a 2-year-old boy with RCC associated with a tumor-specific mutation involving increasingly clear that podocyte injury leads to proteinuria and occurs in many glomerular diseases that finally progress to CKD. Studies have shown significantly increased ROS production, upregulation of COX I and IV expressions, and inactivation of complex IV in peripheral blood mononuclear cells of patients with phase IV–V CKD, thereby demonstrating the close association between mitochondrial dysfunction and CKD progression (47, 126).

The 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines particularly classify proteinuria into risk categories for the prognosis of CKD (1). Recently, it is becoming increasingly clear that podocyte injury leads to proteinuria and occurs in many glomerular diseases that finally progress to CKD. Podocytes (or visceral epithelial cells) wrap around the

### Table 2. Renal involvement in nDNA-related MCs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Model/Case</th>
<th>Function</th>
<th>Renal Involvement</th>
<th>Other Clinical Features</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtDNA</td>
<td>Maintenance</td>
<td>Mitochondrial DNA polymerase</td>
<td>Proximal tubular damage</td>
<td>Aging-associated phenotypes</td>
<td>41</td>
</tr>
<tr>
<td>POLG</td>
<td>Mutation</td>
<td>Mitochondrial DNA helicase, Twinkle</td>
<td>Renal tubulopathy</td>
<td>Liver disease</td>
<td>111</td>
</tr>
<tr>
<td>C10orf2</td>
<td>Mutation</td>
<td>Mitochondrial inner membrane protein</td>
<td>FSFG</td>
<td>Hearing loss</td>
<td>143</td>
</tr>
<tr>
<td>MPV17</td>
<td>Mpv17−/− mice</td>
<td>Mitochondrial ribosomal protein</td>
<td>Tubulopathy</td>
<td>Antenatal skin edema, hypotonia, cardiomyopathy</td>
<td>117</td>
</tr>
<tr>
<td>MRPS22</td>
<td>Mutation</td>
<td>Mitochondrial translation elongation factor</td>
<td>Tubulopathy</td>
<td>Hepatic insufficiency, hypotonia</td>
<td>61</td>
</tr>
<tr>
<td>TF5M</td>
<td>Mutation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OXPHOS</td>
<td>Subunits</td>
<td>Subunits of succinate dehydrogenase</td>
<td>Renal cell carcinoma</td>
<td>Paraganglioma syndromes</td>
<td>20</td>
</tr>
<tr>
<td>SDHB/SDHD</td>
<td>Mutation</td>
<td>Cytochrome c oxidase subunit</td>
<td>de Toni-Fanconi-Debre renal syndrome</td>
<td>Lactic acidosis</td>
<td>63</td>
</tr>
<tr>
<td>COX6B1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OXPHOS</td>
<td>Biogenesis/Regulation</td>
<td>Complex I assembly</td>
<td>Tubular acidosis</td>
<td>Muscular hypotonia, motor delay</td>
<td>60</td>
</tr>
<tr>
<td>NDUFAB2</td>
<td>Mutation</td>
<td>Complex III assembly factor</td>
<td>Neonatal renal tubulopathy</td>
<td>Encephalopathy, liver failure</td>
<td>9</td>
</tr>
<tr>
<td>BCS1L</td>
<td></td>
<td></td>
<td></td>
<td>LS</td>
<td>60</td>
</tr>
<tr>
<td>SURF1</td>
<td>Mutation</td>
<td>Cytochrome c oxidase biogenesis</td>
<td>Renal tubular acidosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COX10</td>
<td>Mutation</td>
<td>Cytochrome c oxidase assembly protein</td>
<td>Neonatal tubulopathy</td>
<td>Encephalopathy, LS, cardiomyopathy</td>
<td>4</td>
</tr>
<tr>
<td>ETHE1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMEM70</td>
<td>Mutation</td>
<td>Complex V assembly</td>
<td>Tubulopathy</td>
<td>Encephalocardiomypathy</td>
<td>63</td>
</tr>
<tr>
<td>DGU1/OK</td>
<td>Mutation</td>
<td>Deoxyguanosine kinase</td>
<td>Tubulopathy</td>
<td>Early-onset hepatocerebral disease</td>
<td>53</td>
</tr>
<tr>
<td>TK2</td>
<td>Mutation</td>
<td>Thymidine kinase 2</td>
<td>Tubulopathy</td>
<td>CNS and skeletal muscle involvement</td>
<td>19</td>
</tr>
<tr>
<td>SUCLA2</td>
<td>Mutation</td>
<td>β-Subunit, uccinate-CoA ligase</td>
<td>Tubulopathy</td>
<td>Methylmalonic aciduria, Leigh-like</td>
<td>139</td>
</tr>
<tr>
<td>RRM2B</td>
<td>Mutation</td>
<td>Subunit of p53-inducible ribonucleotide reductase</td>
<td>tubulopathy</td>
<td>Encephalomyopathy, central hypomyelination</td>
<td>13</td>
</tr>
<tr>
<td>Membrane</td>
<td>Dynamics/Composition</td>
<td>Coenzyme Q10 biosynthesis</td>
<td>Steroid-resistant nephritic syndrome</td>
<td>Encephalomyopathy, multiorgan failure</td>
<td>115</td>
</tr>
<tr>
<td>COQ2</td>
<td>Mutation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COQ6</td>
<td>Mutation</td>
<td>Coenzyme Q10 biosynthesis</td>
<td>Steroid-resistant nephritic syndrome</td>
<td>Sensorineural deafness</td>
<td>56</td>
</tr>
<tr>
<td>COQ9</td>
<td>Mutation</td>
<td>Coenzyme Q10 biosynthesis</td>
<td>Renal tubular dysfunction</td>
<td>Lactic acidosis, hypertrophic cardiomyopathy</td>
<td>33</td>
</tr>
<tr>
<td>PDSS2</td>
<td>Mutation</td>
<td>Coenzyme Q10 biosynthesis</td>
<td>Steroid-resistant nephritic syndrome</td>
<td>Leigh syndrome</td>
<td>84</td>
</tr>
<tr>
<td>PDSS2</td>
<td>Pdox2−/− mice</td>
<td>Coenzyme Q10 biosynthesis</td>
<td>Interstitial nephritis</td>
<td>None</td>
<td>118</td>
</tr>
</tbody>
</table>

See text for definitions.
capillaries of the glomerulus and establish the last guard for selective permeability of the glomerular filtration barrier by the slit diaphragm between their interdigitated foot processes. Therefore, disturbances of podocytes result in proteinuria. As terminally differentiated, high-energy-requiring cells, podocytes contain abundant mitochondria. Cucer et al. (49) reported two children with FSGS associated with mtDNA mutations in podocytes, providing clinical evidence that podocytes with abnormal mitochondria result in glomerular diseases. We described that mitochondrial dysfunction is an early event in aldosterone-induced podocyte injury. In an aldosterone-infused mouse model, before fusion of podocyte processes and proteinuria, the mitochondrial membrane potential, copy number of mtDNA, and ATP production start to decrease with the increase in ROS production (130, 160). In addition, mitochondrial dynamics and quality control processes are involved in podocyte injury. Mitochondrial fission promoted by high-glucose conditions causes podocyte foot process effacement, probably through phosphorylation of Drp-1 by Rho-associated coiled coil-containing protein kinase 1 (ROCK1). Podocyte-specific silencing of ROCK1 can reverse the phenomenon (146). Furthermore, mitophagy is responsible for scavenging abnormal mitochondria with debility. Podocytes exhibit high levels of autophagy, but an inability to regenerate. Parietal cells in Bowman’s capsule compensate for the loss of podocytes and gradually develop FSGS (66). However, suppressed autophagy by inhibition of mTOR maintains damaged mitochondria, also leading to proteinuria (22), which is highly suggestive of the double-edged sword effect of autophagy.

Both animal models and human renal biopsy specimens have shown that proteinuria induces tubular epithelial cell apoptosis and epithelial-mesenchymal transition (EMT) (101). Albumin is usually bound to free fatty acids (FFAs). After endocytosis, albumin is degraded into amino acids by lysosomes, while the FFAs are transferred to mitochondria, where they are utilized for ATP production. However, excessive proteinuria brings abundant FFAs into tubular mitochondria, thus accelerating ROS production (105). In proximal tubular cells, albumin activates PKCδ to induce Bax activation, resulting in mitochondrial outer membrane leakage of apoptogenic factors such as cytochrome, following by apoptosis (81). Furthermore, reabsorption of albumin activates the GTPase Rac1 in proximal tubule cells, resulting in ROS production (147). However, the time sequence of ROS production and tubular cell apoptosis induced by albumin remains controversial. EMT refers to phenotypic conversion with loss of epithelial features and acquisition of a fibroblastic phenotype, which is a major mechanism of renal tubulointerstitial fibrosis (83). Our previous studies showed that mitochondrial dysfunction is responsible for causing EMT (152, 158). EMT and overwhelming oxidative stress induced by aldosterone were abolished by the mitochondrial respiratory chain complex I inhibitor rotenone, but not by the NADPH oxidase inhibitor apocynin (158). HK-2 cells cultured in vitro exhibited EMT after mitochondrial dysfunction with a decrease in the mtDNA copy number induced by the reversible inhibitor ethidium bromide (EtBr). The morphology of HK-2 cells was restored after removal of EtBr (152).

Besides proteinuria, uremic toxin retention is another risk factor for CKD progression. An in vitro study showed that a variety of uremic toxins, including indole-3-acetic acid, in-doxyl sulfate, phenylacetic acid, and kynurenic acid, impair the electron transport system of renal proximal tubule epithelial cells (100). The serum uric acid level often precedes the deterioration of CKD, with about half of patients developing hyperuricemia at the initiation of dialysis (131). Although there are some controversies regarding the relationship between hyperuricemia and the prognosis of established CKD patients, multiple studies have shown that hyperuricemia is an independent risk factor for the development and progression in CKD patients with normal renal function (69), the mechanisms of which may involve induction of EMT, activation of the rennin-angiotensin (RAS) system, endothelium defects, and so on. Given the underlying antioxidant-prooxidant urate redox shuttle, the antioxidant uric acid at the normal plasma concentration becomes a prooxidant induced by multiple injurious stimuli (55). Hyperuricemia induces endothelial dysfunction by increasing mitochondrial superoxide generation triggered by mitochondrial calcium overload (62), which may be caused by calpain 1 cleavage of Na+/Ca²⁺ exchangers. Calpains are Ca²⁺-activated non-lysosomal cysteine proteases, and the subunit members, such as calpain 1, 2, and 10, induce necrosis and apoptosis of endothelial cells in the kidney, thereby increasing the plasma membrane permeability (129).

Transforming growth factor (TGF)-β is considered a major culprit for renal cell injury in progressive CKD (20) and contributes to both renal cell apoptosis and renal fibrosis. On the one hand, TGF-β overexpression is associated with mitochondrial dysfunction in multiple renal cells. TGF-β-transgenic mice exhibit progressive glomerulosclerosis with podocyte apoptosis (123). Enhanced TGF-β activity induces mesangial cell apoptosis, expansion as well as glomerular basement membrane (GBM) thickening by abnormal extracellular matrix accumulation, and impaired GBM degradation. TGF-β also induces mitochondrial fragmentation in proximal tubular epithelial cells (20). On the other hand, many factors are known to regulate the fibrogenic process after tissue injury, in which TGF-β is believed to play a central role. The TGF-β/Smad pathway is critical for EMT and endothelial-mesenchymal transition (EndoMT) (12, 110). Oxidative stress and abnormal mitochondrial biogenesis contribute to the profibrogenic process. Through downregulation of mitochondrial antioxidant systems with concomitant stimulation of prooxidant NADPH oxidase (Nox4), TGF-β increases oxidative stress and stimulates Smad3 expression in a positive feedback loop (20). In addition, disruption of mitochondria-derived ROS attenuates TGF-β-induced profibrotic gene expression (68). Of note, mitochondrial biogenesis is not inhibited in the program of fibrosis. Hickey et al. (58) observed that renal biopsies from patients with diabetic nephropathy showed higher expression of genes encoding key mitochondrial proteins in a gene expression database, thus demonstrating the existence of mitochondrial biogenesis in proliferation and fibrosis. Mitochondrial protein induced in high glucose 1 (IGH1) suppresses the TGF-β1 inhibitor Smad7, amplifies TGF-β1 signaling, and maintains PGC-1α expression, thereby promoting mitochondrial biogenesis, which further contributes to renal fibrosis (23, 58).

Mitochondrial dysfunction and AKI. AKI is a common clinical complication characterized by an abrupt decrease in the glomerular filtration rate (GFR). It is increasingly recognized that AKI is frequently superimposed on CKD and may be an important precipitant for progression to end-stage renal dis-
Mitochondrial Dysfunction in Renal Diseases

Despite supportive care including renal replacement therapy, the 5-yr mortality after AKI remains high (54). Previous studies have demonstrated the critical roles of apoptosis, oxidant- and iron-mediated injury, endothelial changes, regeneration and repair, and inflammatory responses in the pathogenesis of AKI (28). Increasing findings suggest that mitochondrial dysfunction is a major contributor to AKI (137).

Renal tubular epithelial cells (RTECs) are one of the major targets of AKI. RTECs account for reabsorption of useful substances filtered by the glomerulus and regulation of the water-electrolyte and acid-base balance. Immersion in abundant cytotoxins makes RTECs the major victims of various renal diseases, which will be discussed in detail below. In addition, the metabolic demands differ among tubule segments. Proximal tubules mainly rely on aerobic metabolism (6) and have mitochondria with a more oxidized state (52) than distal tubule segments, which can utilize glycolysis (38). Even worse, proximal tubules lack the ability to synthesize glutathione, while other segments of the nephron have this ability (142). So proximal tubules have to take up glutathione from the bloodstream (79). All the above-mentioned aspects make proximal tubules especially vulnerable to mitochondrial dysfunction, which leads to RTEC apoptosis/necrosis, EMT, and renal fibrosis. A case report showed that mtDNA deletion can lead to extremely dysmorphic mitochondria with defects in respiratory chain enzymes encoded by mtDNA in tubular cells, which clinically manifests as tubular atrophy and interstitial fibrosis (132).

The major contributors to AKI are ischemia and hypoxia (78), which are common in patients suffering from sepsis or undergoing major surgery. Although acute tubular necrosis is characteristic of most intrinsic AKI, surprisingly limited histological evidence of injury was found despite severe organ failure (11). Zorov et al. (161) believe that it is the immune system involved in mitochondrial signaling, and not kidney failure itself, that leads to an organism’s senescence and death. Recent reviews have highlighted the critical role of sublethal hypoxic tubular cell injury and microcirculation disturbance in the pathogenesis of AKI (2, 57).

When hypoxia occurs, the renal medullary oxygen insufficiency reduces tubular transport and stimulates adenosine release by ATP breakdown, which triggers tubuloglomerular feedback, resulting in afferent arteriole vasoconstriction and decreased GFR. This represents a renoprotective mechanism that decreases oxygen expenditure and maintains the medullary blood flow (57). Therefore, tubular cells undergo a sublethal state, with characteristics of brush border loss, mitochondrial swelling, and nuclear pyknosis (14). The sublethal cellular hypoxia engenders adaptational responses through hypoxia-inducible factors (HIFs) (114). HIFs are a family of oxygen-sensitive basic helix-loop-helix proteins that transactivate genes promoting hypoxia responses. HIFs form transcription complexes with signal transducer and activator of transcription (STAT)-3 and p300. STAT-3 can translocate into mitochondria and affect the electron transport chain (133), indicating the potential role of mitochondria in hypoxia adaptation. If a hypoxic state cannot be corrected in time, cellular hypoxia leads to excessive ROS production, which in turn enhances tubular transport with increasing oxygen consumption and ROS formation (71), thereby inducing cell autophagy or apoptosis. In addition, HIF1 can directly induce apoptosis in response to severe hypoxia (48, 53). Overwhelming oxidative stress and inflammation induce compromise of the endothelial barrier, which results in intensified hypoxia.

Recent studies have shed light on the relationship between tubular oxidative stress and microcirculation in AKI. Endotoxic insults selectively suppress OXPHOS genes and down-regulate PGC-1α, a direct regulator of mtDNA replication, thus inducing mitochondrial dysfunction and excessive oxidants in tubular cells, which can be reversed by excess PGC-1α (137). Furthermore, inducible nitric oxide synthase (iNOS) increases and nitrotyrosine protein adducts are formed in tubules in septic AKI (148). Holthoff et al. (61) demonstrated that tubular oxidative stress is closely coupled with sluggish blood flow in adjacent capillaries. Further studies are needed to explore the time sequence and relationship between tubular oxidative stress and vasoconstriction and the impact of this reflex on the whole hemodynamic abnormality during septic AKI.

Furthermore, an important new development in this area is the recognition of the disruption of mitochondrial dynamics resulting in mitochondrial fragmentation in renal tubular cells. Mitochondrial disruption occurs early during hypoxia, ATP depletion, and nephrotoxic injury of proximal tubular cells in vitro and ischemic and cisplatin-induced AKI in vivo (16). Importantly, preservation of mitochondrial dynamics and therefore maintaining filamentous mitochondrial morphology protect the cells and kidneys against AKI, supporting a critical role of mitochondrial fragmentation in tubular cell death and kidney tissue damage in these pathological conditions (16). Mechanistically, mitochondrial fragmentation is a combined result of fusion arrest and fission activation (157). Fusion arrest is caused by the interaction of the mitochondrial proapoptotic protein Bak with mitofusins, while fission is activated by Drp-1 following dephosphorylation (15). Fragmented mitochondria are sensitized to Bax insertion, oligomerization, and permeabilization pore formation on the outer membrane, leading to cytochrome c release and cell death by apoptosis (15). Consistently, knockdown of mitofusins results in heightened apoptosis following ATP depletion in renal tubular cells (42). Together, these studies have demonstrated a central role of disruption of mitochondrial dynamics in tubular cell injury and death in AKI. Approaches to preserving mitochondrial dynamics may offer effective therapies for this devastating disease.

Despite the pivotal role of mitochondria in AKI and an abundance of studies on biomarkers for AKI, mitochondrial biomarkers are still a new aspect in AKI. The focus on exploring the potential role of cytochrome c in AKI strongly suggests a promising future for mitochondria-targeted diagnosis and therapeutics in AKI treatments, although the difficulty in detection of cytochrome c transient increases and nonspecificity for kidney injury may prevent it from becoming a biomarker for AKI (127, 155).

Treatment of Mitochondrial Dysfunction-Induced Kidney Injury

The mitochondria-targeted therapies in nephropathy are inadequate. For mitochondrial dysfunction induced by genetic defects, causal therapies such as correcting mitochondrial mutations with targeted RNA import are largely limited at the laboratory stage (145). Clinically, allogeneic hematopoietic stem cell transplantation has been verified as a promising...
therapy for mitochondrial neurogastrointestinal encephalomyopathy, one of the MCs (59), but it specifically focuses on eliminating the toxicity that induces mtDNA mutations and is therefore hard to utilize for other MCs. Given the current situation, it is presently more critical for physicians to be aware of and diagnose patients with nephropathy induced by MCs. It is pivotal to avoid administration of unnecessary, ineffective, but deleterious drugs (e.g., barbiturates, bignuanides, glucocorticoids) to patients with MCs, although single cases have reported benefits for high doses of corticosteroids (40). Meanwhile, the adverse effects of therapies that can alleviate extrarenal symptoms on renal function should be considered in patients with MCs, since their renal functions are especially vulnerable to attack (10). Here, we conclude with some therapies or potential future treatments that may be beneficial for kidney diseases induced by mitochondrial dysfunction.

Genetic therapies. Mitochondria-targeted genetic therapies in nephropathy, which are aimed at correcting gene defects by direct gene replacement or mutation repair, are still very limited. Importation of mitochondrial tRNAs into mitochondria may be a valuable way to relieve a variety of clinical disorders with kidney involvement. Kolesnikova (77) imported yeast tRNA^Lys^ into mitochondrial cybrid cells and patient-derived fibroblasts containing an A8344G MERRF mutation, which partially restored the mitochondrial functions. Karicheva et al. (73) demonstrated significant rescue of respiration chain after importing yeast tRNA^Leu^ into MELAS cybrid cells with an A3243G mutation.

MicroRNAs (miRNAs) are a class of endogenous small noncoding RNAs that interfere with the translation or stability of target transcripts to regulate gene expression (3). In recent years, some miRNAs have been shown to be localized in mitochondria and associated with mitochondrial metabolism, morphology, and biogenesis and have been termed MitomiRs (8). Bai et al. (7) reported that aging mesangial cells exhibit significant upregulation of miR-335 and miR-34a, which inhibit mitochondrial antioxidant enzymes with a concomitant increase in ROS, indicating the potential therapeutic value of targeted mitomiRs.

Antioxidants. There are some antioxidants that show some benefits in kidney diseases, such as omega-3 polyunsaturated fatty acids, N-acetylcysteine (NAC), and allopurinol (126). Long-chain omega-3 polyunsaturated fatty acids have been investigated for their enhancement of endogenous antioxidant systems like γ-glutamylcysteinyl ligase and glutathione reductase (5). In vivo studies have demonstrated that omega-3 polyunsaturated fatty acids can reduce inflammation, fibrosis, and oxidative damage in nephrotoxicity induced by a variety of causes (75, 106). Multicenter trials are underway. NAC is the precursor to many endogenous antioxidants and attenuates oxidative stress by restoring intracellular glutathione stores. Allopurinol is a xanthine oxidoreductase inhibitor that lowers serum uric acid levels and is used to treat gout and some inherited diseases like Lesch-Nyhan syndrome. Both NAC and allopurinol can reduce endothelial dysfunction by inhibiting uremic toxins and oxidative stress (72, 138). In addition, NAC and allopurinol can confer synergistic cardioprotection against ischemia-reperfusion injury by stabilizing HIF-1α/heme oxygenase 1 signaling (90), which can be further investigated in the field of nephropathy.

Supplemental therapies. To date, one example of the few treatable genetic disorders is primary CoQ10 deficiency. Oral CoQ10 supplements show improvements in neurological symptoms, and early initiation of the treatment may have benefits for a renal prognosis, although their effects on the kidney remain controversial. Based on the research about CoQ10 and idebenone, EPL-743, a para-benzoquinone analog, was designed and shown to bring about clinical improvement in other inherited mitochondrial diseases, including polymerase γ deficiency, Leigh syndrome, MELAS, Friedreich ataxia, and mtDNA deletion syndrome (37). Prospective controlled studies will be performed to further verify their efficacy and safety. In addition, it has been proven that supplementation with artificial electron acceptors and cofactors, like vitamin C and carnitine, is partially effective, although the benefits are minimal (40).

Thiazolidinediones. In patients with acquired mitochondrial dysfunction, scavenging of oxidative stress mediators and restoration of mitochondrial function are the aims of treatment. Thiazolidinediones (TZDs), the ligands of PPARγ, are classic medicines for type 2 diabetes mellitus. In recent years, the benefits of TZDs in kidney diseases have also been reported. For example, they were able to reduce proteinuria and fibrotic responses and prevent podocyte as well as vascular injury, in both animal and clinical trials (87, 121). Rosiglitazone, one of the TZDs, can effectively inhibit podocyte injury and MC proliferation through reduced ROS production and recovery of mitochondrial electron transport function in vivo and in vitro (65, 154, 160). TZDs also decrease TGF-β production in glomeruli, thus attenuating renal interstitial fibrosis (74). A meta-analysis in 2010 showed that TZDs effectively decreased proteinuria in diabetic patients, potentially through direct re-
nal-protective effects (121). However, sufficient evidence is still lacking for TZD effectiveness in patients with nephropathy without diabetes. The side effects and potential risks of TZDs should also be of concern. A phase I study of rosiglitazone in patients with primary FSGS showed that it was safe and well tolerated (70). The mean 18-mo follow-up in another phase I trial of rosiglitazone suggested that nearly 50% of patients had a legacy effect with delayed deterioration in kidney function (107). The ongoing phase II/III trial will further ascertain the efficacy of TZDs in nephropathy.

Sirtuins and resveratrol. Calorie restriction can slow aging and promote longevity, but it is difficult to apply and has various disadvantages. Consequently, the sirtuin family, which promotes chromatin silencing and transcriptional repression, has emerged as an alternative target to mimic calorie restriction (64) and plays a critical role in the pathogenesis of kidney diseases. SIRT1 deacetylates not only histones but also various transcriptional regulators, including HIF-2α, COX2, PGC-1α, Smad3, Smad7, tumor suppressor p53, FOXO3, FOXO4, NF-κB, and endothelial NOS, which are closely related to mitochondrial biogenesis and function. The upregulation of SIRT1 reduces apoptosis of renal cells, like podocytes, mesangial, endothelial, and tubular epithelial cells. In addition, its suppression induces oxidative stress, apoptosis, and fibrosis (76). The SIRT1 activator resveratrol, which is present in red grapes and red wine, improves mitochondrial function and lipid levels (136) and promotes and maintains PGC-1α expression, consequently protecting podocyte integrity (153). Besides resveratrol, other novel SIRT1 agonists have also been reported (88, 94, 144). NAD+, the enzymatic cofactor, is critical for SIRT1 signaling. Dietary supplementation with the NAD+ precursor nicotinamide mononucleotide or riboside enhances oxidative metabolism (18, 151). Thus sirtuin family members and their activators can be promising therapeutic targets.

Conclusions

The research discussed throughout this review indicates that mitochondrial dysfunction is important in the pathophysiology of renal diseases (Fig. 1). Although the existing findings are encouraging, our understanding is still quite immature regarding various aspects of mitochondrial biology. Moreover, the clinical manifestations of MCs vary dramatically in terms of symptoms, severity, and age of onset. Therefore, it is impossible to predict the kidney involvement in mitochondrial diseases based on genetic defects. In addition, it remains unclear how mtDNA interacts with nDNA and how their abnormalities are relevant to kidney injury. Although numerous studies have shown that mitochondrial dysfunction contributes to different types of kidney diseases, only a relatively small number of translational studies have shown the clinical relevance of these mechanisms in humans. The presented studies suggest that mitochondria-directed therapies have the potential for prevention and management of renal diseases. However, the use of these agents has only been studied to very limited extents and as yet has no clinical applications. Given that the underlying pathophysiology is not understood in any depth, important challenges for the future involve understanding the mechanisms of upstream regulators and downstream effects of mitochondrial dysfunction in renal cells and kidney diseases.

GRANTS

This work was supported by grants from the National Basic Research Program of China 973 Program (no. 2012CB517602), the National Natural Science Foundation of China (nos. 30872803 and 81270797), and the Natural Science Foundation of Jiangsu Province (no. BK2012001).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

7. Bai XY, Ma Y, Ding R, Fu B, Shi S, Chen XM. miR-335 and miR-34a: novel SIRT1 agonists have also been reported (88, 94, 144). NAD+, the enzymatic cofactor, is critical for SIRT1 signaling. Dietary supplementation with the NAD+ precursor nicotinamide mononucleotide or riboside enhances oxidative metabolism (18, 151). Thus sirtuin family members and their activators can be promising therapeutic targets.

Conclusions

The research discussed throughout this review indicates that mitochondrial dysfunction is important in the pathophysiology of renal diseases (Fig. 1). Although the existing findings are encouraging, our understanding is still quite immature regarding various aspects of mitochondrial biology. Moreover, the clinical manifestations of MCs vary dramatically in terms of symptoms, severity, and age of onset. Therefore, it is impossible to predict the kidney involvement in mitochondrial diseases based on genetic defects. In addition, it remains unclear how mtDNA interacts with nDNA and how their abnormalities are relevant to kidney injury. Although numerous studies have shown that mitochondrial dysfunction contributes to different types of kidney diseases, only a relatively small number of translational studies have shown the clinical relevance of these mechanisms in humans. The presented studies suggest that mitochondria-directed therapies have the potential for prevention and management of renal diseases. However, the use of these agents has only been studied to very limited extents and as yet has no clinical applications. Given that the underlying pathophysiology is not understood in any depth, important challenges for the future involve understanding the mechanisms of upstream regulators and downstream effects of mitochondrial dysfunction in renal cells and kidney diseases.

GRANTS

This work was supported by grants from the National Basic Research Program of China 973 Program (no. 2012CB517602), the National Natural Science Foundation of China (nos. 30872803 and 81270797), and the Natural Science Foundation of Jiangsu Province (no. BK2012001).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

7. Bai XY, Ma Y, Ding R, Fu B, Shi S, Chen XM. miR-335 and miR-34a: novel SIRT1 agonists have also been reported (88, 94, 144). NAD+, the enzymatic cofactor, is critical for SIRT1 signaling. Dietary supplementation with the NAD+ precursor nicotinamide mononucleotide or riboside enhances oxidative metabolism (18, 151). Thus sirtuin family members and their activators can be promising therapeutic targets.

Conclusions

The research discussed throughout this review indicates that mitochondrial dysfunction is important in the pathophysiology of renal diseases (Fig. 1). Although the existing findings are encouraging, our understanding is still quite immature regarding various aspects of mitochondrial biology. Moreover, the clinical manifestations of MCs vary dramatically in terms of symptoms, severity, and age of onset. Therefore, it is impossible to predict the kidney involvement in mitochondrial diseases based on genetic defects. In addition, it remains unclear how mtDNA interacts with nDNA and how their abnormalities are relevant to kidney injury. Although numerous studies have shown that mitochondrial dysfunction contributes to different types of kidney diseases, only a relatively small number of translational studies have shown the clinical relevance of these mechanisms in humans. The presented studies suggest that mitochondria-directed therapies have the potential for prevention and management of renal diseases. However, the use of these agents has only been studied to very limited extents and as yet has no clinical applications. Given that the underlying pathophysiology is not understood in any depth, important challenges for the future involve understanding the mechanisms of upstream regulators and downstream effects of mitochondrial dysfunction in renal cells and kidney diseases.
MITOCHONDRIAL DYSFUNCTION IN RENAL DISEASES


108. Nangaku M.


Review

F378  MITOCHONDRIAL DYSFUNCTION IN RENAL DISEASES


Zorov DB, Plotnikov EY, Jankauskas SS, Isaev NK, Silachev DN, Zorova LD, Plotnikov EY, Jankauskas SS, Isaev NK, Silachev DN, Zeviani M, Nonaka I, Bonilla E, Okino E, Moggio M, Jones S, DiMauro S. Fatal infantile mitochondrial myopathy and renal dysfunc-

AJP-Renal Physiol • doi:10.1152/ajprenal.00571.2013 • www.ajprenal.org