Renal Fibrosis and Mitochondrial Damage

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The first observations of intracellular structures that probably represented mitochondria were published in the 1840s. The mitochondrion (plural mitochondria) is a double membrane-bound organism that consists of the outer mitochondrial membrane (OMM), intermembrane space, inner mitochondrial membrane, and matrix space,[1] which is prevalent in all mammalian eukaryotic cells other than mature erythrocytes. The main function of mitochondria is the metabolism of glycolipid in the body; moreover, it also serves many other physiological functions, such as signaling, cellular differentiation and cell death, as well as maintaining control of the cell cycle and cell growth.[2]

Mitochondria are ubiquitous organelles that are sensitive to the changes in environmental factors. Some alterations can lead to mitochondrial malfunction or damage. Mitochondria can also swell or rupture under the circumstance of toxic substances infiltration, virus invasion, and so on. Disruption of this energy supply can bring about devastating consequences to cells, tissues, organs and even to individuals. The damage of the mitochondria can occur at any age and due to the ubiquitous demanding of ATP, which could affect almost any tissue and organs. Clinicians tend to focus on the clinical presentations caused by mitochondrial damage while ignoring the deep-seated reasons, which makes the situations more stressful.

When cells tackle irreversible damages, the balance of permeability of the OMM increases and proteins located in the intermembrane space, such as cytochrome c, flow out, and initiate the apoptosis program. Lacking the protection of histone, mitochondria DNA are more likely to be exposed to reactive oxygen species (ROS), ultraviolet, ionizing irradiation, alkylating agents, base analogs, modifier-induced base-pair variations, and aging.[3,4]

Renal fibrosis is generally considered as the final pathological path of end-stage renal disease.[5] The main feature of renal fibrosis is the accumulation of myofibroblasts, and extracellular matrix (ECM) deposition mainly comes from interstitial fibroblasts, local mesenchymal stem cell, endothelium, and injured epithelium.[6] In recent years, with the incidence of diabetic nephropathy and hypertensive nephropathy, the incidence rate of renal fibrosis is getting higher. For most kind of renal disease, it is critical to decreasing of the renal function to stop the renal from getting into the stage of fibrosis in the course of clinical processes; it is critical to preserve renal function to stop the progression to renal fibrosis. The premise of a better understanding of the renal fibrosis should start with fibrogenesis, including an understanding of renal fibroblasts which responsible the most for fibrogenesis.[7] Interstitial fibroblasts are the cellular basis of renal fibrosis. First of all, with the chronic inflammation caused by clinicopathologic variables during the process of renal injury, the process of the renal fibrosis will be promoted, and the tubulointerstitial cells will be activated. Meanwhile, the expression of diverse cytokines that relevant to fibrosis remarkably increased, which will lead to the massive deposition of the ECM, eventually the cicatricial tissue will gradually replace the normal renal tissue.[8] Due to the complexity of the pathogenesis of renal fibrosis, effective treatment of renal fibrosis has not yet developed. For this reason, the most promising path is having a better understanding of the pathogenesis and explores the effective treatment of renal fibrosis actively for the prevention and control.

Oxidative stress (OS) plays a crucial role in the pathogenesis of renal fibrosis, by causing damage to mitochondria, subsequently, inducing renal injury.[9] Under physiological conditions, an adequate amount of ROS, such as superoxide

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Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.245272

Received: 24-04-2018 **Edited by:** Li-Shao Guo
How to cite this article: Qin J, Peng ZZ, Li Q, Wen R, Tao LJ. Renal Fibrosis and Mitochondrial Damage. Chin Med J 2018;131:2769-72.

anion, can be eliminated rapidly by the enzyme antioxidants, such as superoxide mutase (SOD) in renal tissues.[10] The excessive production of superoxide anion will give rise to mitochondrial dysfunction and even mitochondrial damage, resulting in the decrease of mitochondrial ATP synthesis and insulin secretion. Evidently decrease the sensitivity to insulin, lead to insulin resistance. The excessive amount of superoxide anion may activate quite a few pathways, such as the activation of polyol pathway and protein kinase c (PKC), the increase of advanced glycation end products, the glucose oxidative phosphorylation.[11] In addition, there are some newly discovered factors such as nicotinamide adenine dinucleotide phosphate (NADPH) and 8-hydroxy-deoxyguanosine which are directly connected to the excessive amount of ROS.[12] Thus, superoxide anion originates from oxidative and mitochondria damage forms a vicious circle which leads to renal fibrosis eventually. Therefore, it is critical to prevent OS from the beginning to stop the process of mitochondria damage so as to prevent renal fibrosis.

Mitochondria are involved in multiple cellar processes, such as homeostasis, proliferation, motility, senescence, and cell death. Many researchers suggested that mitochondria might be the target for metal stressors like cadmium, as the thiol groups of proteins appeared to be the preferential target of cadmium binding.[13] It is thought that the ROS may cause the oxidation of thiol protein selectively, which plays a pivotal role of the cellular antioxidant defenses and redox signaling. Cadmium could penetrate through the cell membrane by binding into metallothionein complexes, which could cause the endocytosis of brush-border transporters in rat renal proximal tubules.[13] Nevertheless, the penetrations of the combined metallothionein complexes may compromise cell membrane function, eventually lead to nephrotoxicity and renal disease induced by cadmium. It has been identified that the cytosol of renal proximal tubular epithelial cells contains some of the cadmium-metallothionein complexes, which confirmed that mitochondria are the target being attacked.[14] Cadmium penetration through the membrane into the mitochondria is considered as the cause significant inhibition to the function of mitochondria, increases the production of ROS, leading to apoptotic cell death, would possibly develop into renal fibrosis eventually. The pathways and mechanisms between cadmium and cell death have been discussed in many studies, but there still need deep reach for an affirmative conclusion.

The kidney is an organ with a high energy requirement with low-glycolytic capacity; it depends on the synthetic action of mitochondria to synthesize ATP to meet the energy requirement for the transportation of sodium ions through the renal tubular. Since the restoration of the inner cellular ATP plays a significant role in the recovery of the cell, mitochondrial damage is involved in many nephropathies such as acute kidney injury and renal fibrosis.[15] Uncoupling protein 2 (UCP2) belongs to mitochondrial anion-carrier proteins protein, which locates on the mitochondrial inner membrane and exists in many organs.[16] In the study of Jiang *et al*.,[17] UCP2 could be remarkably induced by transforming growth factor-β1 (TGF-β1) and targeted by miR-30e, which belongs to the family of endogenous noncoding RNA, and the downregulation of miR-30e could trigger the upregulation of UPC2. With the proton-leak activity of the UPC2, the excess production of UPC2 could break the transmembrane proton gradient between inner-membrane and outer-membrane; subsequently, the function of the mitochondria was disrupted or even damaged, the production of ATP can be decreased, furthermore lead to tubular cell epithelial-mesenchymal transition (EMT) and promote tubular cell phenotype changes. Besides, researches showed that under disease conditions, the deletion of UPC2 may contribute to the ameliorate of kidney fibrosis by inhibiting macrophage infiltration.[18-20] In conclusion, the miR-30e/UCP2 axis could play crucial roles in mediating TGF-β1-induced mitochondria dysfunction or damage and finally leads to EMT and renal fibrosis.

The damage of mitochondria and NADPH oxidase is involved in the excessive production of ROS in the course of diabetes process in the OS reaction and with an effect of signal amplification. Stimulate the production of cytokines such as PKC, TGF-β1 and angiotensin II (Ang II).[21] In addition, the production of these cytokines can react to ROS, eventually promoting the development of renal fibrosis.[22] Both endogenous and exogenous antioxidant enzyme system has the ability to scavenging active oxygen and renal protection. Including antioxidant enzyme system, chain block antioxidant, and transporting metal-binding proteins.

Drugs such as angiotensin II type 1 receptor antagonist (Ang II) and angiotensin-converting enzyme inhibitors (ACEI) play an important role in renal anti-OS and kidney injury. ACEI and Ang drugs acting on the renin-angiotensin system now exhibit good anti-fibrotic activity, part of which is achieved through antioxidant stress.[23] These drugs can reduce the lipid peroxidation products in diabetic nephropathy rats, increase the activity of SOD and other antioxidant enzymes and inhibit the expression of NADPH oxidase and the production of collagen in unilateral ureteral ligation model.[24,25]

Glutathione (GSH) is a highly active tripeptide widely found in various animal tissues and is the most important protective agent in the cell. It could prevent the cells from being damaged by oxygen-free radicals and various cytotoxic substances.[26] N-acetylcysteine (NAC) deacetylation as a precursor of GSH synthesis, can promote the synthesis of GSH, increase the content of GSH in the organization, which helps to protect the cells from the cytotoxic damage caused by the low level of GSH in the body. Its capability of anti-OS may be associated with blocking the low-density lipoprotein oxidation from being oxidized into low-density lipoprotein.[27-29]

Fluorofenidone (AKF-PD) is a novel nonpeptide small molecule compound, which could delay the fibrosis process of various organs such as kidney, liver, and heart, inhibit the renal fibrosis factor such as TGF-β1, besides, can significantly reduce the proliferation and activity of

fibroblasts.[30,31] The mechanism of anti-fibrosis of AKF-PD is also associated with the inhibition of OS, by inhibiting the production of ROS in NADPH-dependent renal tubular epithelial cells.[30,32]

Apocynin is NADPH oxidase inhibitor that inhibits the aggregation of NADPH oxidase subunits thereby specifically inhibit the activity of NADPH oxidation enzyme.[33] Since NADPH oxidase is the key enzyme in the process of Ang-II-induced OS;[34] moreover, OS plays a significant role in the development of renal interstitial fibrosis; we can come to a conclusion that the apocynin could be used as a protective agent to protect the mitochondrial form being damaged by OS.

As the miR-30e/UCP2 axis plays significant roles in the course of mitochondria dysfunction or damage and renal fibrosis, thus UCP2 blockers could be used in this pathway to be new therapies for renal fibrosis. Genipin is a chemical compound extracted from the gardenia plant,[35] which could suppress UCP2 lead renal fibrosis properties by inhibiting the expression and activity of UCP2 and decrease reactive oxygen with the advantage that UCP1, 3, 4, and 5 unaffected.[36]

In renal cells, heavy metals like cadmium may induce OS, and the change of metal homeostasis may activate the defense mechanisms involving heme oxygenase-1 (HO-1), which could be induced by OS, as well as metallothionein.[37] The kidney cytoprotection mainly derives from the increase of HO-1 and its biological characteristics of reducing the heme moiety part of destabilized heme proteins and generate biliverdin and bilirubin, which can be seen as products of HO-1 that can potentially function as antioxidant protection.[38] As cadmium penetrates through mitochondrial membrane of the renal cells and gets accumulated in the proximal tubular epithelium of the kidney, which may induce host response mediated by HO-1 to protect the renal cells from being damaged by heavy-metal-induced OS, the chronic kidney disease and even the renal fibrosis the end stage of renal disease. Thus, the application of HO inhibitor could be a potential benefit pathway to prevent OA and then prevent renal fibrosis.

Ginkgo biloba extract (EGb) mainly contains physiologically active substances such as flavonoid glycosides and terpene lactones.[39] Flavonoids have functional groups reductive hydroxyl and could terminate the free radical chain reaction by acting as a hydrogen atom donor for free radicals, directly clear the O_2-, OH-, H_2O_2, alkane radicals, and lipid-free radicals. By inhibiting free radical reaction and lipid peroxidation to inhibit serum lipid peroxidation and its metabolites malondialdehyde, conjugated diene and other active toxic substances.[40] Flavonoids are also involved in regulating and enhancing the activity of antioxidant enzymes such as SOD, catalase, and glutathione peroxidase. Therefore, EGb can be regarded as the broad-spectrum oxygen-free radical scavenger.

In conclusion, renal fibrosis is a final stage of a variety of chronic kidney disease progression. Most researchers found that OS plays a significant role in the pathogenesis of renal fibrosis, by giving rise to damage to the mitochondria directly and indirectly. There are many treatments for OS aiming to prevent or reduce the mitochondria from getting damaged so as to prevent renal fibrosis. Nonetheless, some mechanisms of drug action are still not clear. Therefore, more researches are need to determine the specific mechanisms of the relationship between mitochondria damage and renal fibrosis and the pharmacological effects of the drugs, in order to provide theoretical support.

Financial support and sponsorship

This study was supported by grants from the National Natural Science Foundation of China (No. 81400749), ZuoLi cup of kidney disease research foundation in human (No. 0442016002) and Changsha Central Hospital Subject (No.YNKY201802).

Conflicts of interest

There are no conflicts of interest.

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