Mechanisms of renal interstitial fibrosis: cross-talk between mitophagy and NLRP3 inflammasome

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Abbreviations
CKD, chronic kidney disease; RIF, renal interstitial fibrosis; AKI, acute kidney injury; RTECs, renal tubular epithelial cells; UUO, unilateral ureteric obstruction; MMT, macrophage myofibroblast transformation; EMT, epithelial-mesenchymal transition; DN, diabetic nephropathy; ROS, reactive oxygen species; mtDNA, mitochondrial DNA.

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Abstract
Renal interstitial fibrosis (RIF) is the main pathological basis leading to end-stage renal disease, and is closely related to the prognosis of patients with kidney disease. Increasing evidence as shown that mitophagy and NLRP3 inflammasome play important roles in the pathogenesis of RIF. Studies suggest that inhibiting NLRP3 inflammasome by activating mitophagy can prevent and alleviate RIF. This review summarizes role played by cross-talk between mitophagy and NLRP3 inflammasome in promoting RIF, so as to offer new perspectives on more effective slow the progression of renal diseases and fibrosis prevention.

Keywords: renal interstitial fibrosis; mitophagy; NLRP3 inflammasome
Introduction

Chronic kidney disease (CKD) is a common and frequently occurring disease worldwide and affects half of adults above age 70 and 10% of the world’s population [1]. By 2040, CKD will become the main fatal disease in humans, apart from ischemic heart disease, stroke, lower respiratory tract infections, and COPD [2]. The pathological mechanism of CKD is not yet clear, but fibrosis is considered the main factor in the progression of almost all forms of kidney disease [1].

Renal interstitial fibrosis (RIF) is the main pathological basis leading to end-stage renal disease, and is closely related to the prognosis of CKD patients [3, 4]. Although no targeted therapy yet exists to slow RIF, some studies have elucidated the complex pathological processes involved in this disease [1]. The pathological process of RIF not only includes pathological mechanisms such as tissue inflammation damage, oxidative stress damage, and abnormal synthesis of fibrin, but is also closely related to cellular life activities such as mitochondrial damage, cell pyroptosis, and autophagy [5, 6]. Increasing evidence has shown that mitophagy and NLRP3 inflammasome play important roles in kidney disease and renal fibrosis [7–9]. Mitophagy is involved in the pathogenesis of RIF, which would be linked to processes such as inflammatory responses and macrophage reprogramming. Activation of mitophagy may be a way to inhibit RIF [10]. In renal injury of different etiologies, activation of NLRP3 inflammasome and autophagy involved in the development of RIF, and NLRP3 knockdown inhibited the progression of RIF by suppressing inflammation [11, 12].

Mitophagy and renal interstitial fibrosis

Mitochondrial damage in the kidneys is a common feature of various kidney diseases [13]. Recent studies indicate that mitophagy, which eliminates damaged mitochondria, plays a vital function in maintaining renal health and preventing renal fibrosis [14, 15].

Mitophagy

Mitophagy is a form of selective autophagy that can identify and eliminate defective and excess mitochondria [16]. Under stressful conditions, mitophagy is produced as a defense or adaptation process for preserving a population of healthy mitochondria, and consequently cell survival [17].

Currently, the mechanisms of mitophagy are divided into ubiquitin-dependent and non-ubiquitin-dependent [16]. The ubiquitin-dependent mechanism includes the PTEN-induced kinase 1/Parkin pathway and the Parkin-independent pathway [10]. The non-ubiquitin-dependent mechanism is regulated by three receptors, including outer mitochondrial membrane protein receptors, inner mitochondrial membrane protein receptors prohibitin 2, and mitochondrial membrane lipid receptor cardiolipin [18–21].

Mitophagy in acute kidney injury and kidney repair

Severe or repeated acute kidney injury (AKI) often leads to renal fibrosis, ultimately leading to CKD [17]. The function of mitophagy in AKI has been established in multiple AKI models. Mitophagy in the kidneys was activated in AKI models induced by renal ischemia-reperfusion injury, sepsis, and nephrotoxicity [22–24]. Meanwhile, several studies have shown that mitophagy has a renal protective function in AKI, as it preserves mitochondrial mass and the integrity of renal tubular cells [25–27].

Following kidney injury, renal tubular cells that survive undergo dedifferentiation, proliferation, migration, and redifferentiation to become mature renal tubular cells. This process is vital in restoring damaged renal tubules. However, when kidney repair is abnormal, it results in renal fibrosis, which may ultimately lead to CKD [28]. It has been found that mitochondria are reduced, and autophagosomes are increased during renal repair after AKI [29]. These abnormal conditions were corrected in the normally repaired renal tubular cells. This suggests that mitophagy may be involved in renal repair after AKI. Meanwhile, the effects of some Chinese medicine decoction to protect renal function and delay renal fibrosis have been shown to be possible through mitophagy. Jian-Pi-Yi-Shen Formula can significantly improve chronic kidney disease caused by 5/6 nephrectomies, which may be associated with mitophagy and modification of the mitochondrial quality control network [30]. Tongluo Yishen decoction can ameliorate mitochondrial dynamics by regulating mitophagy, thereby reducing renal fibrosis [31]. In summary, these findings suggest that mitophagy may play a beneficial role in kidney repair.

Mitophagy and renal interstitial fibrosis

Macrophages and renal tubular epithelial cells (RTECs) both play crucial roles in the progression of kidney diseases and RIF. The correlation between mitophagy and the two has been fully confirmed (Figure 1).

In a mouse model of unilateral ureteric obstruction (UUO), it has been demonstrated that macrophages convert into fibroblasts through macrophage myofibroblast transformation (MMT), thereby promoting the progression of renal fibrosis [32]. MMT has also been shown to contribute to Src mediated renal fibrosis after injury [33]. Similarly, RIF decreased significantly after the inhibition of macrophages in the UUO renal fibrosis model [34]. Macrophages contribute to renal fibrosis when they predominantly exhibit M1 phenotype [35]. While mitophagy suppression can encourage the transformation of macrophages to the M1 phenotype, mitophagy activation can encourage the transformation of M1 macrophages to the M2 phenotype [35, 36]. Recent studies have found that the beneficial effect of Empagliflozin on RIF is achieved by inhibiting the polarization of CD206+CD68+M2 macrophages by affecting the mitophagy and mTOR pathways [37]. Bhatia et al. found that mitochondrial fusion protein MFN2 prevents mitochondrial dysfunction and macrophage derived RIF by mitophagy [38].

Accumulating evidence suggests that RTECs are associated with RIF progression. Epithelial–mesenchymal transition (EMT) is an important pathway of RIF, and 30% of fibroblasts are derived from RTECs through EMT [39]. Animal and cell experiments have shown that salvianolic acid C and quercetin, which are derived from Chinese herbs, can alleviate RIF by inhibiting EMT [40, 41]. Defective mitophagy induces further mitochondrial damage, triggers multiple cell death mechanisms, leads to the loss of RTECs, and participates in the progression of RIF [10]. During diabetic nephropathy (DN), the ectopic ceramide in RTECs may exacerbate RIF by inhibiting mitophagy [42]. The mitophagy activator UMI-77 increases mitophagy levels in vivo and in vitro. The damage to RTECs is reduced, and EMT is inhibited with mitophagy activation, thereby attenuating RIF [43].

Figure 1 Mitophagy inhibition of fibroblasts. Macrophages can be converted to fibroblasts by MMT when they predominantly exhibit M1 phenotype. Mitophagy can inhibitory the transformation of M2 macrophages to the M1 phenotype. 30% of fibroblasts are derived from RTECs through EMT, and mitophagy can inhibit the occurrence of EMT.

MMT: macrophage myofibroblast transformation; RTECs: renal tubular epithelial cells; EMT: epithelial-mesenchymal transition.

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NLRP3 inflammasome and renal interstitial fibrosis

Inflammation is a response to kidney damage. Abnormal and persistent inflammation can affect kidney repair and lead to renal fibrosis [44]. In recent years, research on inflammasomes has been continuously deepening, among which NLRP3 inflammasome is currently the most studied and characterized inflammasome [45]. The NLRP3 inflammasome has been implicated in the development of kidney disease and renal fibrosis in a number of studies [46–48].

NLRP3 inflammasome

The NLRP3 Inflammasome is a multi-protein complex composed of a sensor protein (NLRP3), an adaptor protein ASC (apoptosis-associated Speck-like protein containing CARD), and an effector caspase-1 [49]. To date, the exact mechanism of activation of NLRP3 inflammasome has not been fully defined, but three mechanisms are generally recognized by scholars. 1) Generation of reactive oxygen species (ROS). 2) The decrease of intracellular potassium (K+) concentration. 3) Lysosomal rupture leads to histone B release [50].

Activation and regulation of NLRP3 inflammasome

In the fibrotic kidneys of the UUO mouse model and 5/6-nephrectomized mice, the level of NLRP3 inflammasome has been proven to have significant improvements [48, 51, 52]. Activation of NLRP3 inflammasome leads to RIF pyroptosis, which in turn promotes renal fibrosis [51, 53, 54]. NLRP3 inflammasome can also promote IL-1β Release and macrophage infiltration to induce early renal fibrosis [55]. The suppression of renal inflammation and fibrosis in NLRP3 knockout mice suggests that the NLRP3 inflammasome’s pro-inflammatory effect may contribute to renal fibrosis [11, 51, 56]. These evidences suggest that NLRP3 inflammasome may be activated and participate in the regulation of renal fibrosis.

Delaying renal interstitial fibrosis by interfering with NLRP3 inflammasome

Research has shown that multiple drugs can alleviate renal fibrosis by acting on NLRP3 inflammasome. Foresto-Neto et al. used allopenurin to reduce the activation of NLRP3 inflammasome, thereby delaying the progression of RIF [57]. Dihydroquercetin, a significant dihydroflavone in nature, demonstrates properties that combat renal fibrosis by inhibiting the NLRP3 inflammasome [58]. Researchers have found that some anti-diabetes drugs can inhibit the activity of NLRP3 inflammasome to alleviate DN-induced renal fibrosis, such as SGLT2 inhibitors and DPP-4 inhibitors [48, 59, 60].

In addition, the role of effective ingredients in traditional Chinese medicine in alleviating RIF has also received increasing attention. The anti-malaria drug artemisinin can downregulate the NF-κB/NLRP3 signaling pathway weakens renal tubulointerstitial inflammation and RIF [53]. Panax Notoginseng saponins extracted from traditional Chinese medicine Panax notoginseng alleviates renal fibrosis by inhibiting the activation of NLRP3 inflammasome and pyroptosis [61].

Cross-talk between mitophagy and NLRP3 inflammasome in renal interstitial fibrosis

Damaged mitochondria release high levels of ROS, inducing oxidative stress, and further mitochondrial damage leading to cytoplasmic leakage of mitochondrial DNA (mtDNA) and triggering inflammation [10, 62]. Damaged mitochondria can cause inflammation by releasing mtDNA that activates the cGAS-STING pathway. If the mtDNA is oxidized, it binds to cytosolic NLRP3, triggering the activation of the NLRP3 inflammasome [63]. Mitochondrial dysfunction is considered a key factor in triggering NLRP3 inflammasome, while mitophagy removes damaged mitochondria, avoiding the direct release of a large amount of ROS, effectively inhibiting the activation of NLRP3 inflammasome. The studies found that the herbal ingredients have been shown to inhibit the activation of NLRP3 inflammasome by promoting mitophagy, such as resveratrol extracted from Reynoutria japonica and divanilil sulfone extracted from Gastrodia elata [64, 65].

Figure 2 Cross-talk between mitophagy and NLRP3 inflammasome. Damaged mitochondria release mtDNA and ROS to promote activation of the NLRP3 inflammasome, which drives RIF progression. Mitophagy eliminates damaged mitochondria and inhibits the NLRP3 inflammasome, which in turn alleviates RIF. Also mitophagy alleviates RIF by inhibiting macrophage myofibroblast transformation and epithelial-mesenchymal transition.

Mitophagy negatively regulates NLRP3 inflammasome, but the regulatory effect of NLRP3 inflammasome activation on mitophagy is not clear. Cell experiments have shown that NLRP3 inflammasome can activate and promote autophagy in macrophages [66]. However, NLRP3 inflammasomes have also been found to bind to mitochondrial antiviral signaling proteins under hypoxia, thereby inhibiting mitophagy [10]. In the UUO mouse model with NLRP3 gene knockout, autophagy function was enhanced, and the degree of renal fibrosis decreased, indicating that the interaction between autophagy and NLRP3 inflammasome may not be an upstream-downstream relationship [49]. The mutual regulatory effect between the two is called cross-talk between mitophagy and NLRP3 inflammasome and may be an important regulatory mechanism for RIF.

In recent years, an increasing number of scholars have been investigating the interaction between mitophagy and NLRP3 inflammasome in the management of RIF. Szeto et al. found that enhancing mitochondrial autophagy to protect mitochondrial integrity can effectively inhibit the assembly and activation of NLRP3, as well as inhibit the synthesis of inflammasome to alleviate ischemia-induced RIF [67]. The protective effect of herbs on renal function has also been shown to be realized through this pathway. Gao et al.’s research on the treatment of sepsis-induced AKI with polydatin has shown that inhibiting NLRP3 inflammasome activation is an important mechanism for Parkin-mediated mitophagy to protect the kidneys and delay RIF [68]. Pterostilbene is an autophagy inducer that inducing autophagy helps prevent RIF by weakening NLRP3 inflammasome activation and EMT [69]. The above suggests that cross-talk between mitophagy and NLRP3 inflammasome has great potential in delaying RIF.

Conclusion and perspectives

Numerous recent studies have shown that cross-talk between mitophagy and NLRP3 inflammasome is a significant factor in the pathogenesis of RIF. The role of mitophagy on the NLRP3 inflammasome is complex, and there is a bidirectional relationship between the two. The prevailing view is that inhibiting NLRP3 inflammasome by activating mitophagy can prevent and alleviate RIF (Figure 2). Various drugs targeting the cross-talk between mitophagy and NLRP3 inflammasome, especially Chinese medicines, have the potential to be translated into drugs for kidney diseases in the future. Despite the encouraging evidence so far, understanding of aspects of cross-talk between mitophagy and NLRP3 inflammasome is still lacking. For example, the interaction between mitophagy and the
NRIP3 inflammasome is not yet fully characterized. Moreover, though studies have shown that cross-talk between mitochondry and NRIP3 inflammasome affects the progress of RIF, and preclinical studies suggest that treatments targeting this cross-talk have the potential for prevention and management of RIF, there is currently a lack of translational studies demonstrating the clinical significance and practical applications of these mechanisms in human subjects. Thus, further exploration of this area is necessary. We anticipate that these findings may have implications for future therapeutic options to ameliorate kidney injury and delay the progression of RIF.

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