AMP-activated protein kinase/myocardin-related transcription factor-A signaling regulates fibroblast activation and renal fibrosis

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Chronic kidney disease is a major cause of death, and renal fibrosis is a common pathway leading to the progression of this disease. Although activated fibroblasts are responsible for the production of the extracellular matrix and the development of renal fibrosis, the molecular mechanisms underlying fibroblast activation are not fully defined. Here we examined the functional role of AMP-activated protein kinase (AMPK) in the activation of fibroblasts and the development of renal fibrosis. AMPKα1 was induced in the kidney during the development of renal fibrosis. Mice with global or fibroblast-specific knockout of AMPKα1 exhibited fewer myofibroblasts, developed less fibrosis, and produced less extracellular matrix protein in the kidneys following unilateral ureteral obstruction or ischemia-reperfusion injury. Mechanistically, AMPKα1 directly phosphorylated cofilin leading to cytoskeleton remodeling and myocardin-related transcription factor-A nuclear translocation resulting in fibroblast activation and extracellular matrix protein production. Thus, AMPK may be a critical regulator of fibroblast activation through regulation of cytoskeleton dynamics and myocardin-related transcription factor-A nuclear translocation. Hence, AMPK signaling may represent a novel therapeutic target for fibrotic kidney disease.

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Disease-related injury in organs triggers cellular and molecular responses that can culminate in tissue fibrosis. The dynamic deposition and insufficient resorption of extracellular matrix (ECM) promotes fibrosis and chronic loss of organ function,[1] which account for an estimated one-third of natural deaths worldwide.[2] As a major fibrotic disorder, renal fibrosis is a hallmark of chronic kidney disease.[3,4] Renal interstitial fibrosis is characterized by fibroblast activation and excessive production and deposition of ECM, which leads to the destruction of renal parenchyma and progressive loss of kidney function to end-stage renal disease.[5,6]

Activated fibroblasts are the principal cells responsible for ECM production, and their activation is regarded as a key event in the pathogenesis of renal fibrosis.[6–8] These cells express α-smooth muscle actin (α-SMA), and are referred to as myofibroblasts. However, the molecular mechanisms underlying fibroblast activation are not fully understood.

Adenosine monophosphate-activated protein kinase (AMPK) is a conserved sensor of cellular energy status and environmental stress, and regulates the activities of a number of enzymes through phosphorylation. Mammalian AMPKs are heterotrimeric complexes containing a catalytic (α) subunit and 2 regulatory (β and γ) subunits.[9,10] In addition to regulating energy homeostasis and metabolism, AMPK plays important roles in protein synthesis, cell growth, apoptosis,[11] cell differentiation,[12] actin cytoskeleton reorganization,[13] and gene transcription.[14] Recent studies have shown that AMPKα1 participates in the development of renal fibrosis.[15,16] However, how AMPK regulates the development of renal fibrosis is unknown.

To examine the functional role of AMPKα1 in the activation of fibroblasts and the development of renal fibrosis, we generated mice expressing tamoxifen-inducible Cre-recombinase (Cre/ESR1) under the control of the chicken beta actin promoter/enhancer coupled with the cytomegalovirus immediate-early enhancer (*CAG-Cre*)[17] or proα2(I) collagen promoter (*Col1-Cre*),[18] thus permitting temporally controlled deletion of floxed AMPKα1 gene in a global or fibroblast-specific manner. These transgenic mice were subjected to unilateral ureteral obstruction (UUO) or ischemia-reperfusion injury (IRI) to induce renal fibrosis. We show that AMPKα1 is induced in the kidney during the

development of renal fibrosis, and targeted disruption of AMPKα1 inhibits fibroblast activation and attenuates the development of renal fibrosis. Furthermore, we demonstrate that AMPKα1 directly phosphorylates cofilin, which results in actin cytoskeleton reorganization and nuclear translocation of myocardin-related transcription factor-A (MRTF-A; also known as MKL1, MAL, BSAC) leading to fibroblast activation and fibrogenesis.

RESULTS

AMPKα1 is induced during the development of renal fibrosis

We first determined whether AMPKα1 is induced in the kidneys in response to obstructive injury. Western blot analysis showed that the protein levels of AMPKα1 were markedly increased in kidneys after 10 days of UUO compared with the contralateral kidneys (Supplementary Figure S1). To characterize the cell types responsible for AMPKα1 induction in the kidney, serial kidney sections were stained with an anti-AMPKα1 antibody by immunohistochemistry. The results revealed that AMPKα1-positive cells increased in the tubulointerstitium of the fibrotic kidneys (Supplementary Figure S2). To examine whether AMPKα is activated in myofibroblasts of the kidney, kidney sections were stained for p-AMPKα and α-SMA, a myofibroblast marker, by immunofluorescence (Supplementary Figure S3). The immunofluorescence results demonstrated that p-AMPKα-positive cells are positive for α-SMA, indicating that AMPKα is mainly activated in myofibroblasts of the kidney in response to obstructive injury.

Global deletion of AMPKα1 reduces renal fibrosis

To examine whether AMPKα1 regulates renal fibrosis *in vivo*, mice with loxP-flanked alleles of *AMPKα1* were bred with mice harboring a hemizygous *CAG-Cre-ER* transgene.[17] *CAG-Cre*$^{+}$*AMPKα1*$^{flox/flox}$ mice were referred to as GAKO mice, and littermate *CAG-Cre*$^{-}$*AMPKα1*$^{flox/flox}$ mice served as controls (Supplementary Figure S4). These mice were treated with tamoxifen and then subjected to UUO for 10 days. Western blot analysis showed that protein levels of AMPKα1 and p-AMPKα were markedly increased in the obstructed kidneys of control mice, and significantly reduced in the kidneys of GAKO mice with UUO (Figure 1a). GAKO mice displayed much less collagen deposition in the obstructed kidneys compared with those of their littermate controls (Figure 1b). We next investigated the effect of AMPKα1 deficiency on the expression of α-SMA, a myofibroblast marker, and collagen I and fibronectin, 2 major ECM proteins. GAKO mice expressed considerably less α-SMA, collagen I, and fibronectin in the obstructed kidneys compared with their littermate controls (Figure 1c–f). These data indicate that AMPKα1 is indispensable for fibroblast activation and development of interstitial fibrosis induced by UUO.

To further confirm the role of the AMPKα1 in the development of renal fibrosis, we extended our studies to an additional model of renal fibrosis, IRI-induced nephropathy. Western blot analysis showed that protein levels of AMPKα1 and p-AMPKα were significantly increased in the kidneys of control mice with IRI, and substantially reduced in the kidneys of GAKO mice with IRI (Figure 2a). Global deletion of AMPKα1 markedly reduced interstitial collagen deposition in the IRI-injured kidneys (Figure 2b). Furthermore, global deletion of AMPKα1 significantly inhibited the expression of α-SMA, collagen I, and fibronectin in kidneys following IRI (Figure 2c–f). These data indicate that global deletion of AMPKα1 attenuates IRI-induced fibroblast activation and interstitial fibrosis in the kidney.

Fibroblast-specific deletion of AMPKα1 reduces renal fibrosis

To determine whether the observed effect of AMPKα1 on renal fibrosis was mediated by AMPKα1 in fibroblasts, we generated mice with fibroblast-specific deletion of AMPKα1 by crossing *AMPKα1*$^{flox/flox}$ mice with tamoxifen-inducible collagen type I promoter/enhancer-driven Cre-ER recombinase transgenic mice (*Col1-Cre*$^{+}$).[18] *AMPKα1*$^{flox/flox}$ littermate controls and *Col1-Cre*$^{+}$*AMPKα1*$^{flox/flox}$ mice (referred to as FAKO) were treated with tamoxifen to induce fibroblast-specific deletion of AMPKα1 (Supplementary Figure S4). These mice were then subjected to UUO or IRI to induce renal fibrosis. Western blot analysis showed that AMPKα1 protein levels were markedly reduced in the kidneys of FAKO mice compared with those of littermate controls (Figures 3a and 4a). Immunofluorescence staining revealed that AMPKα1 abundance was considerably reduced in the PDGFRβ-positive fibroblasts (Figure 3c). We then examined the effect of AMPKα1 deficiency in fibroblasts on the development of renal fibrosis. Total collagen accumulation and deposition were significantly attenuated in the injured kidneys of FAKO mice compared with their littermate controls (Figures 3d and 4b). Furthermore, both Western blot analysis and immunofluorescence staining demonstrated that fibroblast-specific AMPKα1 deficiency attenuated the upregulation of α-SMA, collagen I, and fibronectin in the kidneys with UUO (Figure 3e–h) or IRI (Figure 4c–f). Collectively, these data indicate that fibroblast-specific deletion of AMPKα1 attenuates renal fibrosis in UUO and IRI models by inhibiting fibroblast activation and ECM protein production.

AMPKα1 is required for fibroblast activation

To examine the role of AMPKα1 in activating kidney fibroblasts, the normal rat kidney fibroblasts (NRK-49F), primary kidney fibroblasts, and bone marrow-derived monocytes were treated with a direct activator of AMPK, A-769662,[10] for 24 hours. A-769662 induced fibroblast activation identified as increased production of α-SMA, fibronectin, and collagen I (Figure 5a–c). Compound C, a selective AMPK inhibitor,[19] blocked A-769662-induced fibronectin, collagen I, and α-SMA production in NRK-49F cells (Figure 5a). Furthermore, AMPKα1 deficiency abolished A-769662-induced fibronectin, collagen I, and α-SMA protein production in primary kidney fibroblasts and bone marrow–derived monocytes (Figure 5b and c, and Supplementary Figure S5). Bone marrow–derived monocytes were also treated with