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Genetic deficiency of adiponectin protects against acute kidney injury

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Adiponectin is a multifunctional cytokine that has a role in regulating inflammation. Here we determined whether adiponectin modulates ischemic acute kidney injury. Compared with wild-type mice, adiponectin-knockout mice were found to have lower serum creatinine and less tubular damage or apoptosis following ischemia/reperfusion injury. This latter process was associated with decreased Bax and reduced activation of p53 and caspase-3. Targeted disruption of adiponectin was also found to inhibit the infiltration of neutrophils, macrophages, and T cells into the injured kidneys. This was associated with inhibition of NF-κB activation and reduced expression of the proinflammatory molecules IL-6, TNF-α, MCP-1, and MIP-2 in the kidney after ischemia/reperfusion injury. Wild-type mice engrafted with adiponectin-null bone marrow had less kidney dysfunction and tubular damage than adiponectin-null mice engrafted with wild-type bone marrow. Conversely, adiponectin-null mice engrafted with wild-type bone marrow had similar renal dysfunction and tubular damage compared with wild-type mice engrafted with wild-type bone marrow. In cultured macrophages, adiponectin directly promoted macrophage migration: a process blocked by the PI3 kinase inhibitor, LY294002. Thus, our results show that adiponectin has a pivotal role in the pathogenesis of acute renal ischemia/reperfusion injury and may be a potential therapeutic target.

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Acute kidney injury (AKI) is a common clinical condition that is associated with high morbidity and mortality.[1,2] Ischemia–reperfusion injury (IRI) is a common cause of AKI.[3,4] The pathogenesis of IRI is complex and incompletely understood, which involves a number of pathogenic mechanisms that result in acute tubular necrosis/apoptosis and renal dysfunction.[5,6] These include ATP depletion, generation of reactive species, leukocyte infiltration, production of proinflammatory mediators, and induction of tubular apoptosis.[5–7] There is no effective therapy for this devastating clinical condition except renal replacement. Therefore, a better understanding of the pathogenic mechanisms underlying IRI is essential for ultimately developing an effective therapy.

Adiponectin is a multifunctional cytokine that has an important role in the regulation of energy metabolism and inflammation.[8,9] Adiponectin was initially reported to be synthesized exclusively by adipocytes.[10] However, recent studies have shown that it is also produced by other cell types such as endothelial cells,[11] macrophages and lymphocytes,[11,12] and epithelial cells.[13,14] Circulating adiponectin levels are elevated in patients with chronic kidney disease, and high levels of adiponectin predict increased cardiovascular and all-cause mortality as well as CKD progression.[15,16] However, its role in AKI is unknown.

In this study, we have found that adiponectin is upregulated in the kidney in response to IRI. Therefore, we examined the role of adiponectin in experimental renal IRI using adiponectin-knockout (APN-KO) mice. Our results revealed that targeted disruption of adiponectin protects the kidney from IRI by suppressing apoptosis and inflammation.

RESULTS

Adiponectin is induced in a mouse model of kidney IRI

We first characterized the induction of adiponectin in the kidney in a mouse model of renal IRI. Using real-time reverse transcriptase PCR, we found that the messenger RNA level of adiponectin was upregulated in injured kidneys compared with sham-operated controls after 30 min of ischemia, followed by 24 h of reperfusion (Figure 1a). To identify the cell types that are responsible for the induction of adiponectin in the kidney, serial sections of kidneys were stained with an anti-adiponectin antibody. Our results

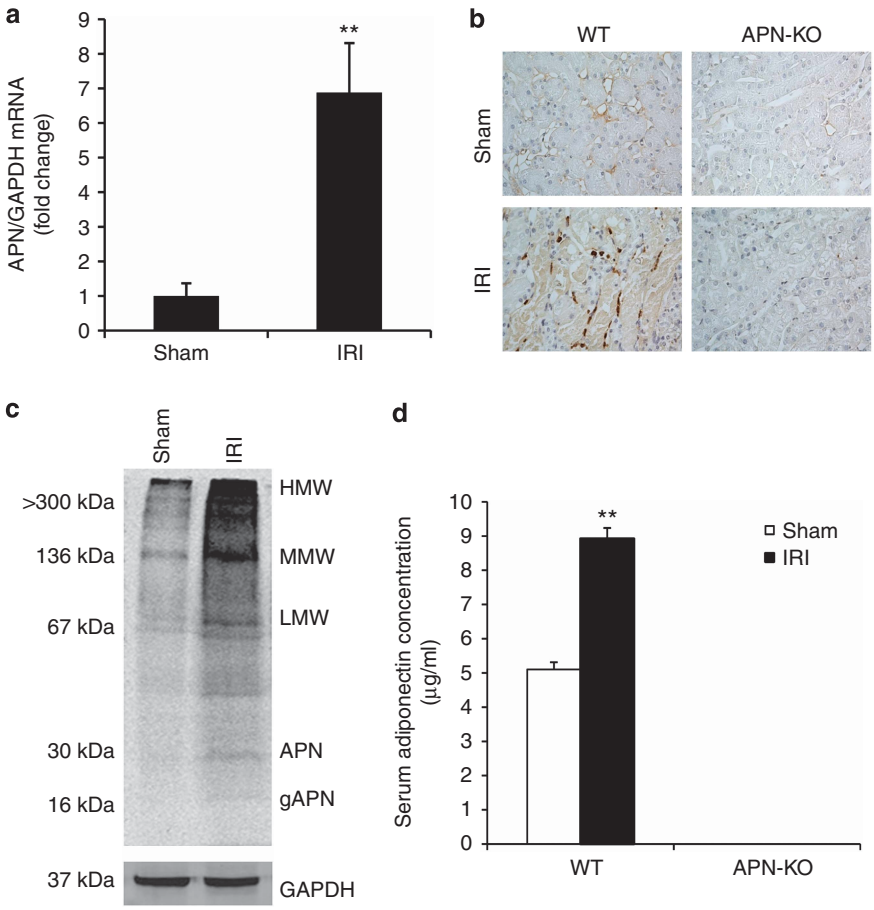

Figure 1 | Adiponectin is upregulated in the kidney after ischemia–reperfusion injury. (**a**) Adiponectin (APN) messenger RNA (mRNA) is induced significantly in kidneys of wild-type (WT) mice after ischemia–reperfusion injury (IRI) compared with sham-operated mice. $^{**}P<0.01$ vs. WT sham. $n=4$ in each group. (**b**) Representative photomicrographs of kidney sections stained for adiponectin (brown) and counterstained with hematoxylin (blue) (original magnification ×400). (**c**) Representative western blot under nonreducing conditions shows that all isoforms of adiponectin protein is induced in IRI kidneys of WT mice. (**d**) Quantification of serum adiponectin levels in sham-operated mice and mice with IRI. $^{**}P<0.01$ vs. WT sham. $n=5$–6 in each group. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HMW, high-molecular-weight; KO, knockout.

revealed that adiponectin protein was mainly induced in the infiltrated inflammatory cells of injured kidneys of wild-type (WT) mice. No positive staining for adiponectin was detected in KO mice (Figure 1b). To further characterize the induction of adiponectin in the inflammatory cells, kidney sections were stained for adiponectin and either F4/80—a macrophage marker, CD11c—a dentritic cell marker, CD3—a T-cell marker, or MPO—a neutrophil marker. Our results showed that macrophages, dentritic cells, and T cells express adiponectin but not neutrophils (Supplementary Figure S1 online). A western blot analysis was performed to determine which isoforms of adiponectin is induced in the kidney after IRI. Under nonreducing conditions, all isoforms of adiponectin were induced in the kidney following IRI, especially high-molecular-weight form (Figure 1c). Under reducing conditions, a major band of 30 KDa was detected in the WT mice, consistent with a full-length adiponectin (Supplementary Figure S2 online). It is noteworthy that adiponectin was not detected in the kidney of KO mice (Supplementary Figure S2 online). We determined whether IRI affects serum levels of adiponectin. Our results showed that the serum levels of adiponetin increased significantly after IRI, whereas adiponectin was not detected in the serum of KO mice (Figure 1d), confirming the disruption of adiponectin gene.

APN-KO mice are protected from kidney IRI

To determine the role of adiponectin in the pathogenesis of kidney IRI, WT and APN-KO mice were subjected to 30 min of ischemia, followed by 24 h of reperfusion injury. IRI caused kidney dysfunction in WT mice as reflected by significant elevation of serum creatinine at 24 h after IRI. Kidney function was preserved in APN-KO mice with serum creatinine and blood urea nitrogen markedly lower compared with that of WT mice at 24 h after IRI (Figure 2a and b). Consistent with the preservation of renal function in APN-KO mice following IRI, there was substantial reduction in histological injury of the kidney as reflected by less tubular