INTEGRIN LINKED KINASE DELETION PROTECTS FROM INFLAMMATION AND FIBROSIS PROGRESSION UNDERLIE ADENINE-INDUCED CKD

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OBJECTIVES

Chronic kidney diseases (CKDs) are progressive scarring conditions characterized by a decrease in renal function. A common final pathway of CKD is tubule-interstitial fibrosis, caused by excessive extracellular matrix (ECM) deposition after chronic insults. In addition, an inflammatory process is usually present in kidney diseases (1,2). Such an inflammatory process affects tubular cells and may lead to apoptosis, necrosis or activation of interstitial fibroblasts, which produce collagens leading to a fibrotic scar. Integrin-linked kinase (ILK) is an intracellular serine/threonine protein kinase that plays a fundamental role in the regulation of cell adhesion, survival, proliferation, and ECM deposition. In adittion, ILK is a key intracellular mediator of tubularepithelial-to-mesenchymal transition (EMT) and ILK expression is upregulated in a wide variety of chronic kidney diseases (3). Therefore, the objective of this work was to study the consequences of the deletion of ILK in renal functional and structural alterations in CKD.

METHODS

Adult conditional ILK knock-down (cKD-ILK) mice were fed a diet containing 0.2% adenine (which longterm ingestion results in 2,8-dihydroxyadenine precipitation inside the renal tubules), ad libitum for 2, 4 or 6 weeks and were compared with their corresponding littermates (control) fed the standard diet. Blood, urine and kidney samples were collected for analysis. Serum creatinine, blood ureic nitrite and urine osmolality was measured by commercially purchased kits. Tubular damage, inflammatory cellular infiltration and interstitial ECM deposition was analyzed in hematoxilin-eosin, PAS and Sirius red stained sections. Moreover, protein expression levels were measured by Western Blot and RT-qPCR.

RESULTS

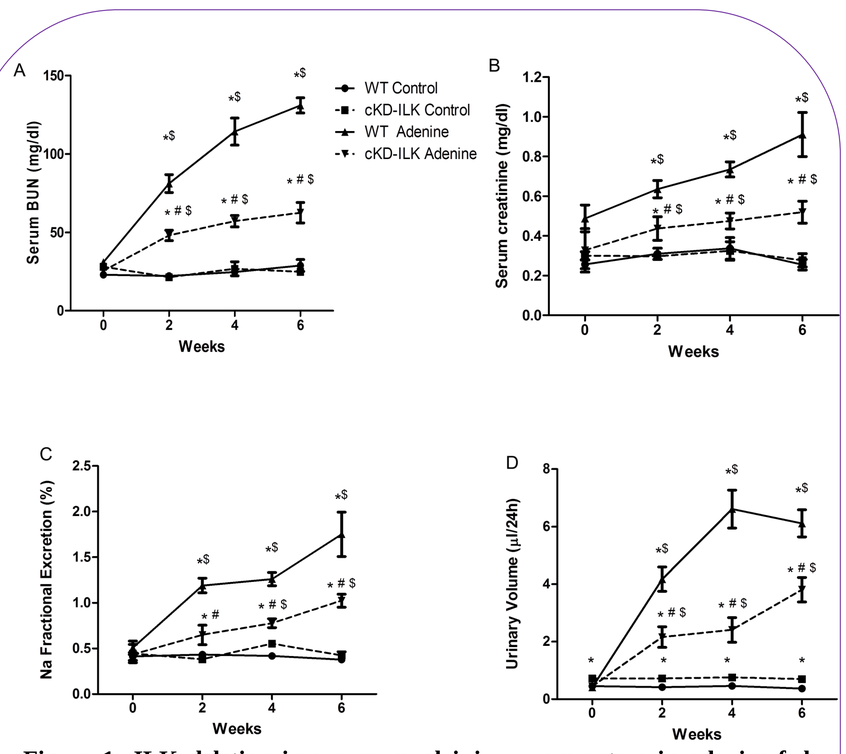
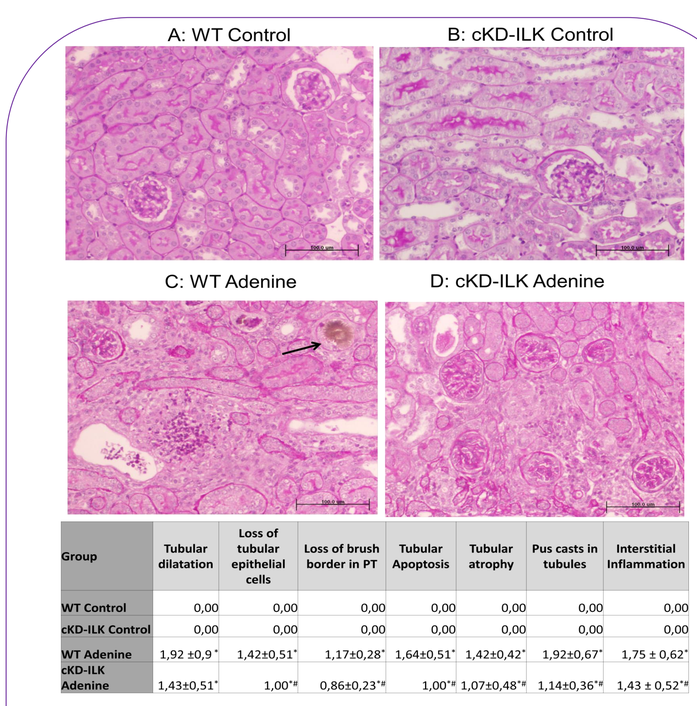


Figure 1: ILK deletion improves renal injury parameters in adenine-fed mice. Both groups of mice receiving an adenine-containing diet presented a progressive reduced renal function, with maximal decline observed at 6 weeks of excessive adenine intake. Renal function was assessed by serum BUN levels (A), serum creatinine levels (B) and Na fractional excretion (%) (C) and urinary volume (µl/24 h) was measured (D). All values are represented as mean ± SEM. *P<0.05 vs. control WT, #P<0.05 vs. adenine-fed WT, $P<0.05 vs. 0 week. n=10–15 animals/group.



Group	Tubular dilatation	Loss of tubular epithelial cells	Loss of brush border in PT	Tubular Apoptosis	Tubular atrophy	Pus casts in tubules	Interstitial Inflammation
WT Control	0,00	0,00	0,00	0,00	0,00	0,00	0,00
cKD-ILK Control	0,00	0,00	0,00	0,00	0,00	0,00	0,00
WT Adenine	1,92 ±0,9*	1,42±0,51*	1,17±0,28*	1,64±0,51*	1,42±0,42*	1,92±0,67*	1,75 ± 0,62*
cKD-ILK Adenine	1,43±0,51*	1,00*#	0,86±0,23*#	1,00*#	1,07±0,48*#	1,14±0,36*#	1,43 ± 0,52*#

Figure 2: ILK deletion protects against tubulointerstitial injury in mice with adenine-induced CKD. The images shows morphological changes in the kidneys of the mice after 6 weeks of feeding with adenine. The analyses of the tubulointerstitial compartment revealed that the cKD-ILK group exhibited a marked decrease in renal damage after 6 weeks compared with the WT group and a significant reduction of interstitial inflammation, tubular damage and pus cast in tubules (Table). All values are represented as mean ± SEM. *P<0.05 vs. control WT, #P<0.05 vs. adenine-fed WT. n=10–15 animals/group.

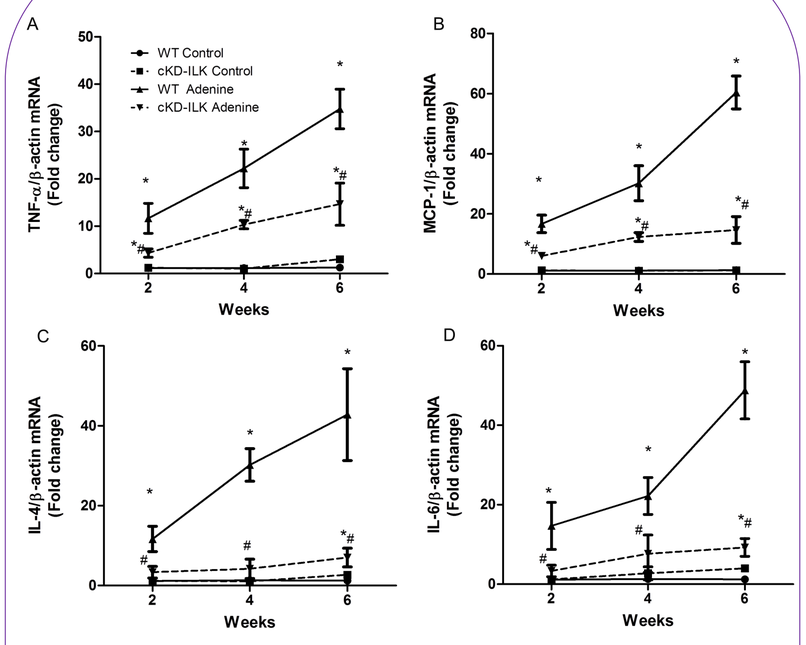


Figure 3: ILK deletion prevents inflammatory genes overexpression in mice with adenine-induced CKD. mRNA levels of inflammatory cytokines, such as TNF-α, IL-4, IL-6 and MCP-1, were analyzed in the renal tissue by real-time RT–PCR. All values are represented as mean ± SEM vs. control WT. *P<0.05 vs. control WT, #P<0.05 vs. adenine-fed WT. n=10–15 animals/group.

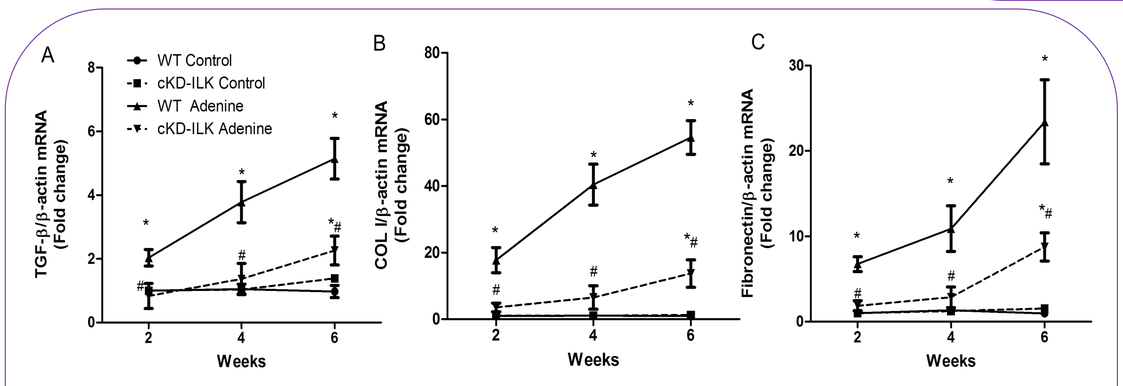


Figure 4: ILK deletion reduces TGF-β1 overexpression and prevents matrix genes expression in mice with adenine-induced CKD. The panels shows the mRNA expression levels, one of the most important cytokines which regulates the EMT and one of the cellular consequences: the excessive production of interstitial matrix components, implied in renal fibrosis. We also examined the mRNA expression of TGF-β1, collagen type I (COL I) and fibronectin in kidney. All values are represented as mean ± SEM vs. control WT. *P<0.05 vs. control WT, #P<0.05 vs. adenine-fed WT. n=10–15 animals/group.

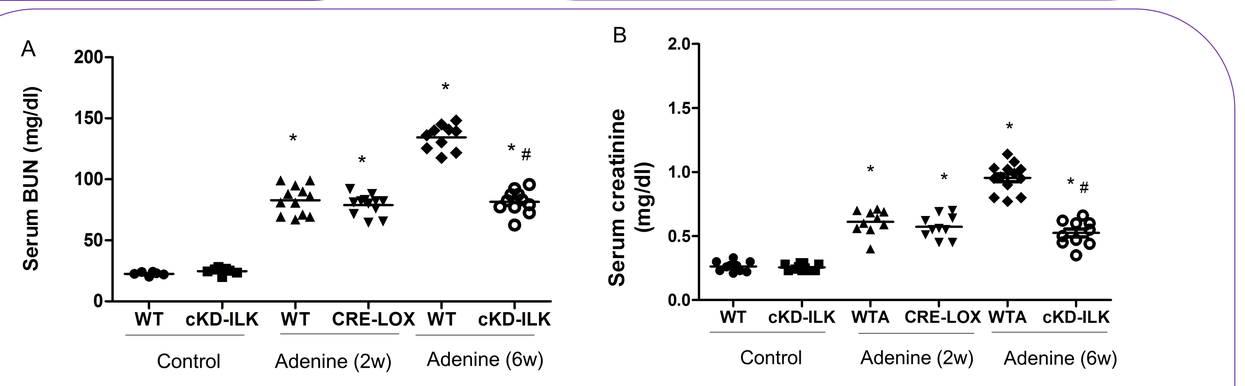


Figure 5: ILK deletion impairs CKD progression in mice with mild adenine-induced CKD. We next sought to test whether ILK deletion affects the progression of CKD in a more real clinical situation. In both groups of mice had significantly elevated serum BUN and creatinine levels after 2 weeks of diet, which progressed to terminal CKD values in the WT group after a further 4 weeks of adenine diet, whereas no progression was observed in the cKD-ILK group (Figure 5 A and B). All values are represented as mean ± SEM vs. control WT. *P<0.05 vs. control WT, #P<0.05 vs. adenine-fed WT. n=10–15 animals/group.

CONCLUSIONS

The decrease in the tissue content of ILK can prevent the progression of CKD, and its quantification can contribute to make a more accurate prognosis of the situation of the patients and to provide appropriate treatments

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