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The cyclic GMP–dependent protein kinase Iα suppresses kidney fibrosis

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Cyclic guanosine monophosphate (cGMP) is synthesized by nitric oxide or natriuretic peptide–stimulated guanylyl cyclases and exhibits pleiotropic regulatory functions in the kidney. Hence, integration of cGMP signaling by cGMP-dependent protein kinases (cGKs) might play a critical role in renal physiology; however, detailed renal localization of cGKs is still lacking. Here, we performed an immunohistochemical analysis of cGKIα and cGKIβ isozymes in the mouse kidney and found both in arterioles, the mesangium, and within the cortical interstitium. In contrast to cGKIα, the β-isoform was not detected in the juxtaglomerular apparatus or medullary fibroblasts. Since interstitial fibroblasts play a prominent role in interstitial fibrosis, we focused our study on cGKI function in the interstitium, emphasizing a functional differentiation of both isoforms, and determined whether cGKIs influence renal fibrosis induced by unilateral ureter obstruction. Treatment with the guanylyl cyclase activators YC1 or isosorbide dinitrate showed stronger antifibrotic effects in wild-type than in cGKI-knockout or in smooth muscle-cGKIα-rescue mice, which are cGKI deficient in the kidney except in the renal vasculature. Moreover, fibrosis influenced the mRNA and protein expression levels of cGKIα more strongly than cGKIβ. Thus, our results indicate that cGMP, acting primarily through cGKIα, is an important suppressor of kidney fibrosis.

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The signaling molecules nitric oxide (NO) and natriuretic peptides (NPs) modulate a variety of physiological functions in diverse organ systems. NO can activate soluble guanylyl cyclases (sGCs), and NPs activate particulate guanylyl cyclases. Both guanylyl cyclases synthesize the second messenger cGMP (cyclic guanosine monophosphate), which then stimulates cGMP-dependent protein kinase (cGK). There are three different cGKs in mammals: cGKIα, cGKIβ, and cGKII. In this study, we examined cGKIα and cGKIβ. The isoforms differ only in the N-terminal leucine zipper region, which operates as the dimerization and targeting domain. cGKI is expressed in several tissues including smooth muscle, where it causes relaxation.[1] Transgenic mice with a selective, smooth muscle–specific expression of cGKIα or cGKIβ show cGMP-dependent relaxation.[2] Interestingly, in some cell types, only one cGKI isoform is expressed.[3]

Analysis of the kidney shows cGK localization in the contractile cells of the kidney vasculature, including intra- and extra-glomerular cells, in vascular smooth muscle cells, and in interstitial myofibroblasts.[4] The diversity of cGKI isozyme localization in various organs indicates their functional differences. However, a detailed analysis of the distribution of these cGKI isozymes in the kidney is still lacking. Furthermore, conflicting results have been reported regarding cGKI localization in interstitial fibroblasts.[4–6] To address this problem, cGKI-deficient mice and cGKI isozyme–specific antibodies, for immunohistochemical analyses, are necessary.[3] This analysis allows for the functional and physiological differentiation of these isozymes in the kidney. Under physiological conditions, the interstitium is characterized by a balance between the synthesis and deposition of the extracellular matrix (ECM). An unbalanced ECM accumulation leads to the development of fibrosis with organ dysfunction. The model of unilateral ureter obstruction (UUO) can be used to generate progressive kidney fibrosis. Fibroblasts proliferate and undergo myofibroblast differentiation with increasing deposition of ECM.[7] In the fibrotic kidney, the number of myofibroblasts increases with the expression of α-smooth muscle actin (SMA), which is not expressed in fibroblasts.[8,9] Therefore, SMA serves as a marker for the transition of fibroblasts to myofibroblasts, indicating a mesenchymal phenotype.[10]

Several mitogenic stimuli for fibroblasts have been identified. Transforming growth factor β1 (TGFβ1) and platelet-derived growth factor B[11] are both able to differentiate fibroblasts into myofibroblasts.[12] TGFβ stimulates the synthesis of ECM proteins, including collagens or fibronectin,[13–16] and induces the epithelial-to-mesenchymal transition.[17–19] Consequently, TGFβ has a crucial role in the pathogenesis of fibrotic disorders.[15] The signaling molecules NO and NP show an antifibrotic potential, resulting in a decreased ECM deposition in the perivascular or cardiac interstitial areas.[20,21] Recent reports have suggested a role of cGMP elevation (by the stimulation of the sGCs) or NP receptor-A activation in the prevention of kidney fibrosis.[22,23] However, the relevance of cGKI in renal fibrosis remains unknown. Furthermore, it is currently unknown which cGKI isoform and which pathways might counteract kidney fibrosis development.

RESULTS

cGKIα and cGKIβ localization in the kidney

Understanding the localization of cGMP-dependent protein kinases is essential to elucidate the function of these enzymes. In our approach, the expression of the isozymes cGKIα and cGKIβ was characterized in murine kidney sections from wild-type (WT) animals using isozyme-specific cGKI antibodies (Figure 1 and Supplementary Figure S1 online). The specificity of these antibodies was confirmed in kidney sections from cGKI-knockout (KO) animals (data not shown).

Both cGKI isozymes were highly expressed in the glomerular mesangium and in the kidney vasculature including the arterioles, the vas afferens, and vas efferens (Supplementary Figure S1 online). In contrast to cGKIβ, the α-isoform was detected in the juxtaglomerular apparatus, using renin as the marker (Supplementary Figure S1 online). Moreover, cGKIα was expressed in the cortical (Figure 1a and Supplementary Figure S2 online) and medullary interstitium (Figure 2a), whereas cGKIβ was limited to the cortical interstitium (Figures 1b and 2b and Supplementary Figure S2 online) and in vascular bundles extending from the outer medulla to the inner medulla (Figure 2c).

Therefore, the lack of cGKIβ expression in the medullary interstitium could indicate a specific function of the α-isoform in the kidney medullary structures.

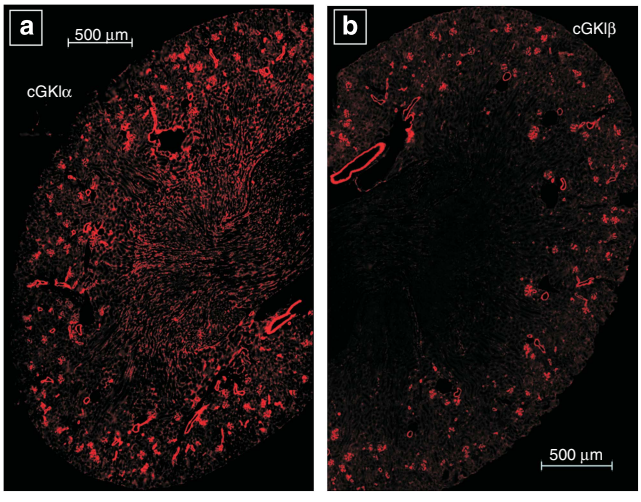

Figure 1 | The distribution of cGMP (cyclic guanosine monophosphate)-dependent protein kinase I (cGKI) and its isoforms in the kidney. (**a**) The overview shows the renal distribution of cGKIα (shown in red, Alexa 647 labeled) in wild-type (WT) mice. (**b**) The overview shows the renal distribution of cGKIβ (shown in red, Alexa 647 labeled) in WT mice. For an overview of kidney analysis, the MosaiX module was used.

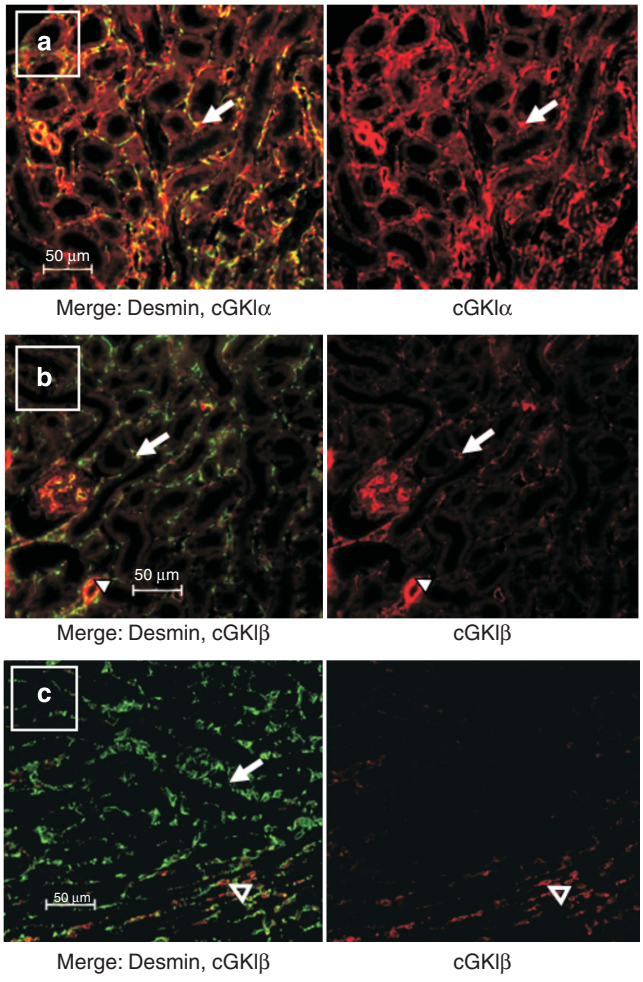

Figure 2 | Analysis of cGMP (cyclic guanosine monophosphate)-dependent protein kinase Iα (cGKIα) and cGKIβ expression in the renal interstitium. (**a**) cGKIα (shown in red, Alexa 647 labeled) is highly expressed in the interstitium of the inner and outer medulla (indicated with arrow), colocalizing with the fibroblast marker desmin (shown in green, Cy2 labeled). Moreover, cGKIα is expressed in the cortical interstitium (Supplementary Figure S2a online). (**b**) cGKIβ (shown in red, Alexa 647 labeled) is only weakly expressed in the cortical interstitium (indicated with arrow) and is very highly expressed in vessels (indicated with arrowhead). (**c**) No cGKIβ (shown in red) staining was observed in the interstitium of the inner and outer medulla (indicated with arrow). The vascular bundles (indicated with arrowhead) extending from the outer medulla to the inner medulla show specific cGKIβ staining.