In vivo evidence for a limited role of proximal tubular Klotho in renal phosphate handling

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Klotho is a transmembrane protein expressed in the renal tubules where it acts as a permissive coreceptor for fibroblast growth factor 23 (FGF23). FGF23 signaling reduces the abundance of CYP27b1 and phosphate cotransporters NPT2a and NPT2c, leading to a decrease in $1,25(OH)_2D_3$ synthesis and a rise in urinary phosphate excretion, respectively. Systemic or whole-nephron deletion of Klotho in mice results in renal FGF23 resistance characterized by high $1,25(OH)_2D_3$ and phosphate levels and premature aging. Expression of Klotho is highest in the distal tubules, whereas 25OH vitamin D 1α hydroxylation and phosphate reabsorption predominantly occur in the proximal tubules. Currently, the segment-specific roles of Klotho in renal tubules are not fully understood. Here we have generated mice with Klotho specifically ablated from the proximal tubules using 3 different Cre mouse strains. All 3 models displayed impaired urinary phosphate excretion and increased abundance of NPT2a in the brush border membrane. Notably, hyperphosphatemia in knockout mice was mild or nonexistent under basal conditions but occurred upon high phosphate loading, indicating the presence of compensatory mechanisms. Effects on $1,25(OH)_2D_3$ varied between mouse strains but were modest overall. Thus, Klotho expressed in the proximal tubules has a defined but limited role in renal phosphate handling *in vivo*.

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Type I membrane-bound alpha-Klotho (Klotho) is predominantly expressed in the renal tubules,[1] where it acts as an obligate coreceptor for the phosphaturic hormone fibroblast growth factor 23 (FGF23).[2–4] FGF23 binds to a Klotho-FGF-receptor complex[4] and inhibits renal phosphate reabsorption by internalizing the sodium-dependent phosphate cotransporters NPT2a and NPT2c.[5–7] FGF23 signaling also suppresses 1,25 dihydroxyvitamin D ($1,25(OH)_2D_3$) synthesis by decreasing the activating enzyme CYP27b1, and by increasing the catabolic enzyme CYP24a1.[8,9] Systemic deletion of Klotho causes severe disturbances in mineral metabolism and an accelerated aging phenotype, involving growth retardation, organ dysfunction, vascular and soft tissue calcification, and premature death.[1] An almost identical phenotype is observed in mice with whole-nephron Klotho deletion, underlining the functional significance of renal Klotho.[10] Because genetic or dietary correction of the hyperphosphatemia and high $1,25(OH)_2D_3$ ameliorates the phenotype in Klotho-deficient mice,[11–15] the phenotype is at least in part related to the role of Klotho in renal phosphate and vitamin D handling. In the kidney, Klotho is expressed primarily in the distal tubules, and to a much lower extent in the proximal tubules.[16] However, renal phosphate is reabsorbed mainly in the proximal tubules[17–19] and activation of vitamin D occurs primarily in the proximal tubules.[20] It is currently unknown whether Klotho expressed in the proximal tubule is sufficient to mediate the phosphaturic and anti–vitamin D actions of FGF23, or whether cross talk between the distal and proximal tubules is needed for such regulation. Whereas one study[21] reported activation of the mitogen-activated protein kinase/extracellular signal–regulated kinase (MAPK/ERK) pathway exclusively in the distal tubules upon administration of FGF23, a subsequent study reported direct effects of FGF23 in microdissected proximal tubules.[22] We have previously shown that ablation of Klotho specifically from the distal tubules results in a hyperphosphatemic phenotype, although not as pronounced as in the systemic or whole-nephron knockouts.[23] Also, mice with a distal tubule-specific deletion of Klotho have unchanged serum levels of $1,25(OH)_2D_3$. This proposes a functional role for proximal tubular Klotho in regulating phosphate reabsorption and controlling $1,25(OH)_2D_3$ synthesis.

To explore the functional role of Klotho in proximal tubules, we generated mice with Klotho specifically ablated from the proximal tubules using Cre-Lox recombination. Because no single Cre strain provides efficient recombination in all proximal tubular segments, 3 different models were established. Floxed Klotho mice (*Klotho$^{fl/fl}$*) were crossed with mice expressing Cre recombinase under the kidney androgen-regulated protein (*Kap^{Cre}*), the phosphoenolpyruvate carboxykinase (*PEPCK^{Cre}*), and the type II sodium phosphate cotransporter, member 1 (*Slc34a1^{Cre}*) promoters, respectively. Using this approach, we demonstrate a key role for proximal tubular Klotho in renal phosphate handling and vitamin D metabolism. Importantly, while global and whole-nephron deletion of Klotho result in a severe phenotype, deletion in either the distal or the proximal renal tubules alone results only in minor changes in mineral homeostasis and a normal gross phenotype. These results are the first to suggest an integrated regulatory function of Klotho in the distal and proximal tubules on mineral metabolism.

RESULTS

Klotho is expressed in proximal tubules in the kidney

First, the renal expression pattern of Klotho was examined using immunohistochemistry. Klotho staining was detected in the renal cortex but not in the medulla of wild-type mice (Figure 1a, left panel). In the cortex, distal tubules had a strong positive staining, whereas proximal tubules stained substantially weaker (Figure 1a, right panel). Next, we

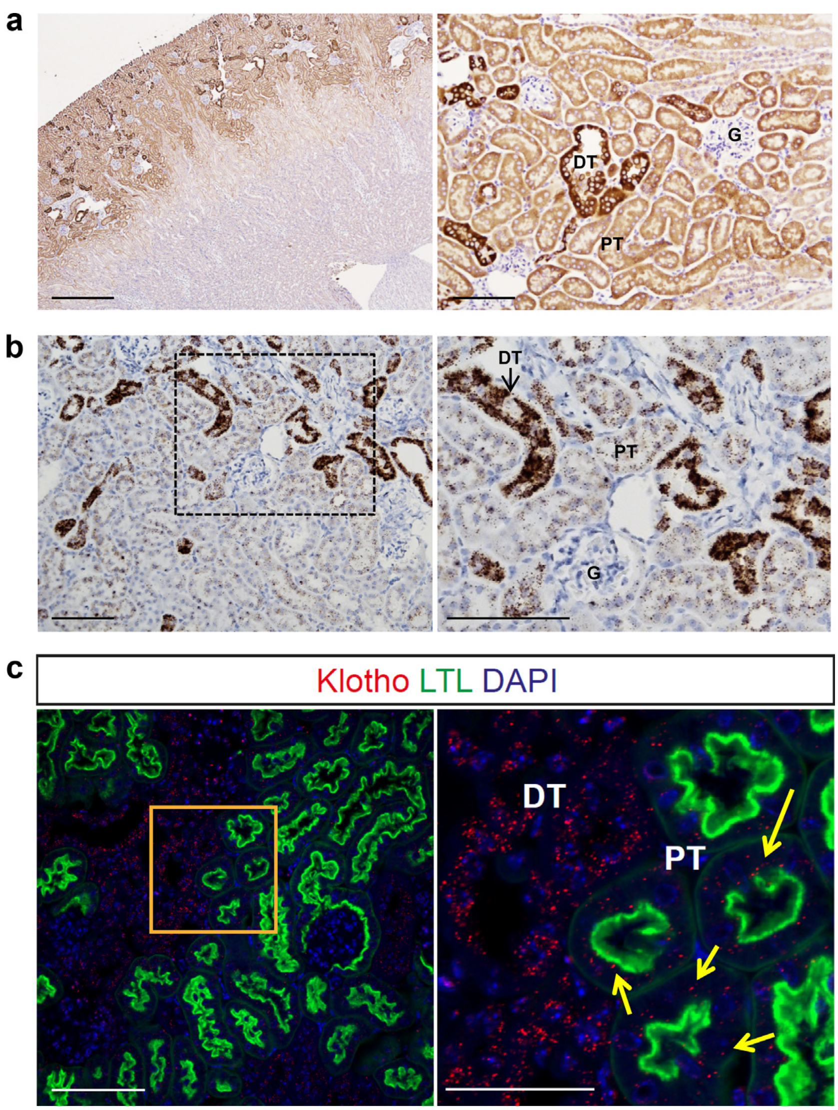

Figure 1 | Renal expression pattern of Klotho. (**a**) Immunohistochemistry showed cortical staining of Klotho. (Left panel, bar = 500 μm.) Distal tubules stained strongly positive for Klotho, whereas proximal tubules had weak positive staining. (Right panel, bar = 100 μm.) (**b,c**) *In situ* hybridization revealed high expression of Klotho in distal tubules, and substantially weaker, however distinct, expression in proximal tubules. Glomeruli and intrarenal arteries did not express Klotho. (Left panel, 20× magnification; right panel, enlarged view of indicated area in left panel, bar = 100 μm.) (**c**) Klotho expression (red) was colabeled with Lotus tetragonolobus lectin (LTL) (green) and 4′,6-diamidino-2-phenylindole (DAPI) (blue). (Left panel, bar = 100 μm; right panel, enlarged view of indicated area in left panel, bar = 50 μm.) Yellow arrows point to positive Klotho signals in PT. DT, distal tubules; G, glomerular; KL, Klotho; PT, proximal tubules.