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The role of klotho in mineral metabolism and inflammation

AKADEMISK AVHANDLING
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av
Risul Amin
Lic.

Huvudhandledare:
Docent Manuchehr Abedi-Valugerdi
Karolinska Institutet
Institutionen för laboratoriemedicin

Bihandledare:
Professor Moustapha Hassan
Karolinska Institutet
Institutionen för laboratoriemedicin

Docent Samir El Andaloussi
Karolinska Institutet
Institutionen för laboratoriemedicin

Fakultetsopponent:
Professor Bernd Stegmayr
Umeå Universitet
Institutionen för folkhälsa och klinisk medicin

Betygsnämnd:
Docent Gunnar Sterner
Lunds universitet Institutionen för kliniska vetenskaper

Docent Ylva Pernow
Karolinska Institutet
Institutionen för molekylär medicin och Kirurgi

Docent Dan Hauzenberger
Karolinska Institutet
Institutionen för klinisk vetenskap, intervention och teknik

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ABSTRACT

Accumulated data suggest that a disrupted FGF23-Klotho axis is a major contributor to the development of chronic kidney disease (CKD). This axis might have a broader role in the regulation of inflammation, yet the underlying mechanisms remain largely elusive. To understand the biological actions of FGF23, it is important to elucidate the function of the receptors that mediate the FGF23 signaling. Klotho, a type I membrane-bound protein directly interacts with the FGF-receptor 1c and functions as a specific FGF23-receptor. FGF23-induced activation of the FGF receptor/Klotho complex exerts a reduction in re-absorption of phosphate as well as in the down-regulation in the synthesis of vitamin-D in renal tubules. In addition to its potential role in phosphate metabolism, Klotho might have a wider role in the regulation of inflammation; i.e., it has been shown that its production is substantially reduced in different inflammatory diseases such as acute kidney injury (AKI), CKD, inflammatory bowel disease (IBD), and rheumatoid arthritis (RA). The overall aim of the present research project is to elucidate the pathogenic mechanisms of the FGF23-Klotho axis in the development of CKD and hematopoietic stem cell transplantation (HSCT)-associated AKI.

In order to understand the basic mechanisms underlying these conditions, we generated distal tubule-specific Klotho (*Ksp-KL*$^{-/-}$) using cre-lox recombination technology. These mice were hyperphosphatemic with elevated serum FGF23 levels, indicating that distal tubular Klotho affects phosphate reabsorption in the proximal tubules. In contrast to *Ksp-KL*$^{-/-}$ mice, β *-KL*$^{-/-}$ mice (systemic Klotho knockout mice) exhibited the phenotype of existing Klotho null mice confirming that the Klotho gene functions as anti-aging gene. The mechanism of this proposed distal-to-proximal tubular signaling remains to be explored (**Study I**).

To further elucidate the role of renal Klotho in the development of aging phenotype, we generated mice with Klotho deleted throughout the nephron (*Six2-KL*$^{-/-}$). *Six2-KL*$^{-/-}$ mice were infertile, kyphotic, growth retarded and had a decreased life span, closely resembling the phenotype seen in systemic *Klotho* knockout mice. Further, the serum and urine biochemistries, low serum Klotho levels, as well as profound histological abnormalities, were indistinguishable from systemic *Klotho* knockout mice, unravelling the kidney as the principal contributor to circulating Klotho and as the mediator of Klotho anti-ageing traits (**Study II**).

In order to prevail the significant controversies regarding the presence or absence of Klotho in the vascular system and whether the vascular tissue is directly responsive to FGF23 endocrine action, we analyzed the expression of the FGF23 co-receptor Klotho in mouse arteries and generated a novel mouse model, harboring a vascular smooth muscle cell-specific deletion of Klotho (*Sm22-KL*$^{-/-}$). Arterial Klotho transcripts were detected at very low levels, whereas the corresponding protein levels were undetectable. FGF23-Klotho signaling was collectively absent in the mouse arteries, and the vascular phenotype was unaffected by FGF23 treatment. Thus, our data do not support direct, Klotho-dependent FGF23 vasculotoxicity although confirmative studies in humans are warranted (**Study III**).

Lastly, to better define the role FGF23-Klotho axis in the regulation of inflammation, we utilized chemotherapy-based conditioning, (Bu)-cyclophosphamide (Cy) murine model for acute graft versus host disease (aGVHD). FGF23-Klotho axis was dysregulated in the aGVHD-induced kidney injury (AKI). Renal Klotho was ablated in aGVHD mice. Further, serum biochemistry was also indistinguishable in the LPS-induced AKI model. Thus, our findings demonstrate that FGF23-Klotho signalling is substantially disturbed in aGVHD which might contribute to the development AKI (**Study IV**).

Taken together, the studies included in the present thesis substantially contribute to the understanding of the role FGF23-Klotho axis and its mechanism of action in several disease conditions. Urgent studies to identify current and novel therapeutic interventions that restitute the deranged FGF23-Klotho axis in CKD and the inflammatory disease, aGVHD-associated AKI and identify individuals who will benefit the most from such treatment are warranted.

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