ALBUMIN DOWNREGULATES KLOTHO IN TUBULAR CELLS

Beatriz Fernandez-Fernandez[1], M. Concepción Izquierdo[2], Dimitra Nastou[3], Lara Valiño-Rivas[2], Laura Gonzalez Lafuente[2], Ana B. Sanz[2], Alberto Ortiz[1], M.Dolores Sanchez-Niño[2],

[1]IIS-Fundación Jiménez Díaz-Universidad Autónoma de Madrid, Nephrology, Madrid, SPAIN, [2]IIS-Fundación Jiménez Díaz-Universidad Autónoma de Madrid, Renal and Vascular Research

INTRODUCTION AND AIMS

✓Kidney tubular cells are the main sources of Klotho, a protein with phosphaturic actions.

✓ Genetic Klotho deficiency causes premature cardiovascular aging in mice.

✓ Human chronic kidney disease (CKD) is characterized by acquired Klotho deficiency.

✓Despite the lack of uremic toxin accumulation, stage 1 CKD (normal GFR) is already associated with decreased Klotho and premature cardiovascular aging.

✓We have explored whether albuminuria, a criterion to diagnose CKD when GFR is normal, may directly decrease Klotho expression in human CKD, preclinical models and cultured tubular cells.

METHODS

✓ Murine proximal tubular epithelial (MCT) cell cultured, experimental murine protein-overload nephropathy in mice, nephrosis induced by injection of puromycin in rats, immunohistochemistry in paraffin-embedded tissue for CD68 and F4/80 positive macrophages staining, quantitative reverse transcription-polymerase chain reaction and Western blot were performed in the laboratory.

✓ Urinary Klotho protein measurement was assessed in human urine from four groups of CKD patients according to KDIGO categories.

RESULTS

✓ In a CKD cohort, albuminuria correlated with serum phosphate after adjustment for GFR, age and sex.

✓ In this regard, urinary Klotho was decreased in patients with pathological albuminuria but preserved glomerular filtration rate. (Figure 1)

✓Proteinuria induced in rats by puromycin aminonucleoside (PAN) and in mice by albumin overload was associated with interstitial inflammation and reduced total kidney Klotho mRNA expression. (Figure 2)

✓Western blot disclosed reduced kidney Klotho protein in albumin-overloaded mice and immunohistochemistry localized the reduced kidney Klotho expression to tubular cells in proteinuric animals. (not shown)

✓ In cultured murine tubular cells, albumin directly and dose-dependently decreased Klotho mRNA and protein expression. (Not shown)

✓This was inhibited by trichostatin A, an inhibitor of histone deacetylases (HDAC), but unlike cytokine-induced Klotho downregulation, not by NF-κB inhibitors. (Not shown)

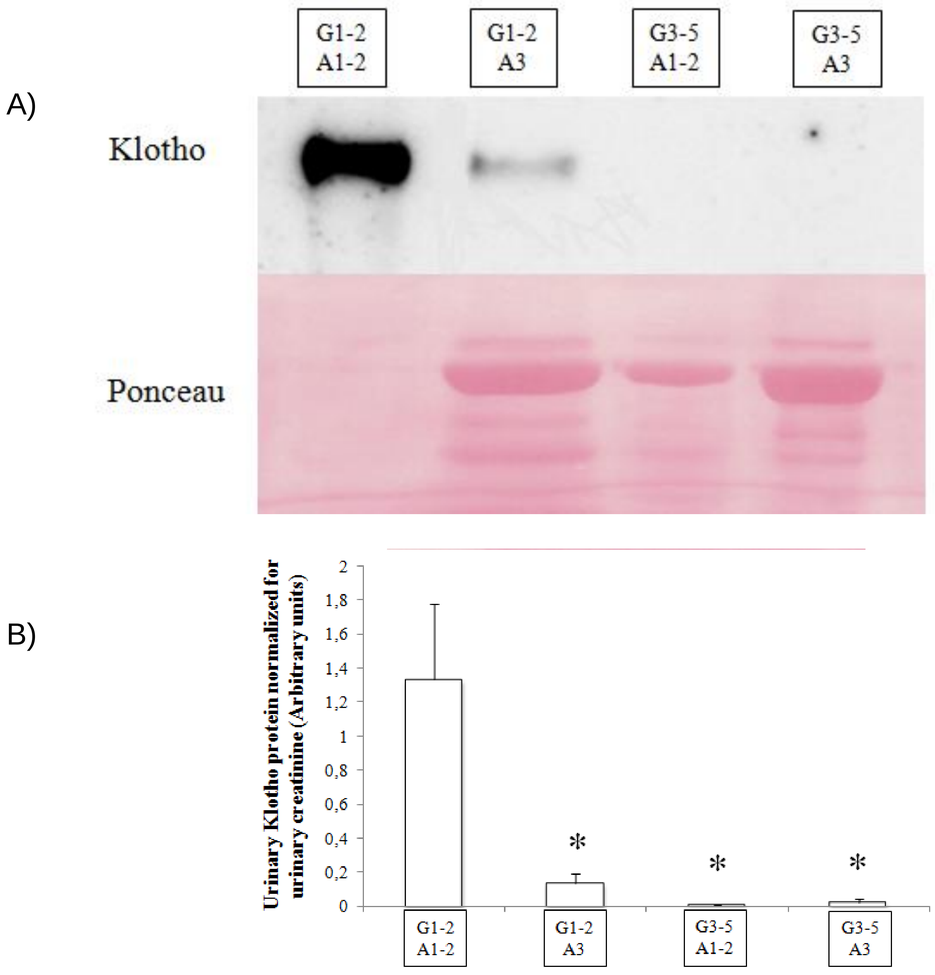

Figure 1:
A) Klotho (WB) in patients classified as 2012 KDIGO G and A categories ·
B) Quantification of Western blot results. * p<0.05 vs G1-2/A1-2.

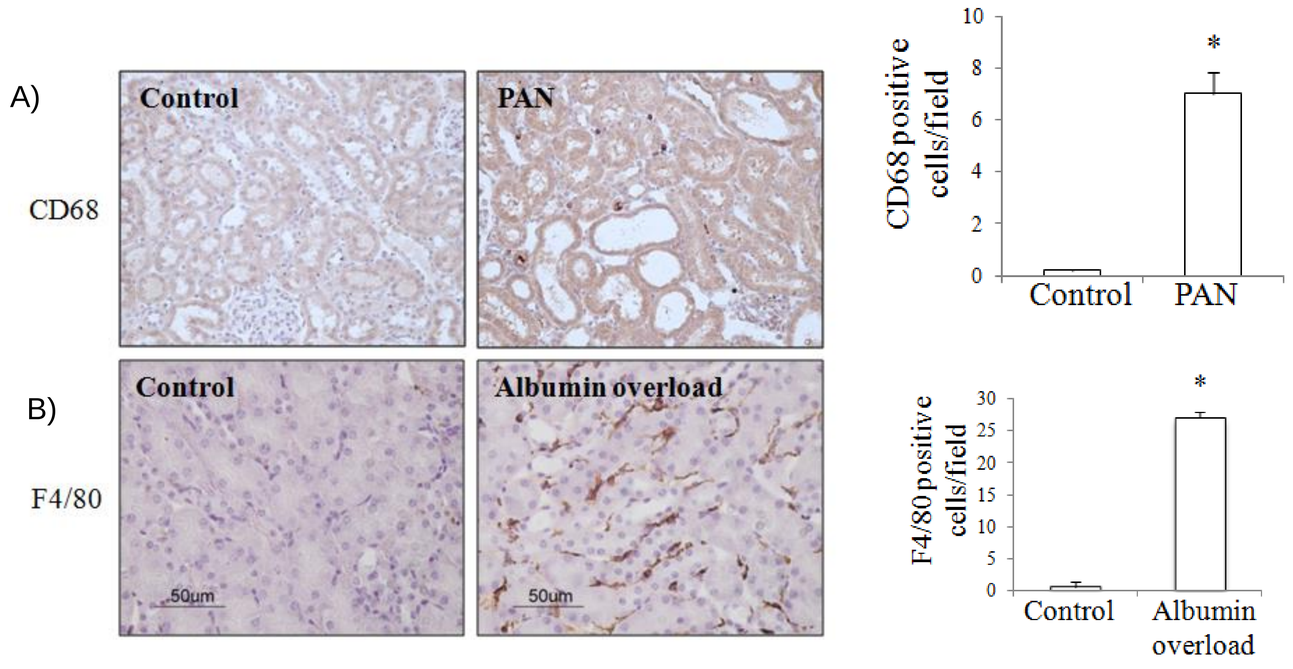

Figure 2: : A) Quantification CD68 immunohistochemistry 10 days following PAN or vehicle injection. CD68+ macrophages are increased in PAN nephrosis. *p<0.001
B) Quantification and immunohistochemistry image representative of F4/80 positive macrophages in albumin overload nephropathy at day 7.

CONCLUSIONS

Albumin directly decreases Klotho expression in cultured tubular cells, possibly through epigenetic mechanisms.

This may explain or at least contribute to decrease Klotho and to promote FGF-23 resistance in early CKD stages, as observed in preclinical and clinical proteinuric kidney disease.

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Beatriz Fernandez-Fernandez
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