Klotho Suppresses Renal Angiotensin by Inhibiting Wnt Signaling in Adriamycin Nephropathy

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Introduction

- Membrane klotho protein is cleaved by ADMA, releasing its extracellular domain as free klotho that is detectable in the blood.
- Free klotho binds to WNT and the receptors for IGF and TGF-β, inhibiting their signals.
- Both renal klotho expression and serum klotho concentration decrease as the kidney function worsens.
- Klotho gene transfer shows renoprotection in various kidney disease models.
- However, the effects of klotho protein supplement on kidney diseases have not been fully examined.

Methods-1

Experiments were performed to assess how klotho protein supplement protects the kidney from its injury. Adriamycin (5 mg/Kg) was injected into Wister rats to induce nephropathy.

Human recombinant klotho (A+K, 30 μg/kg/day), klotho and 4-benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione (TDZD, GSK-3β blocker, 0.2mg/kg/day, A+K+T) or vehicle (A) was administered into animals with adriamycin nephropathy.

Rats which were untreated with Adriamycin were used as control (C). Animals were killed 4 weeks later by excess amount of anesthesia to assess early changes in the kidney and aorta.

Methods-2

Body weight, systolic blood pressure (tail cuff), albuminuria, 8-epi-PGF2α excretion, creatinine clearance, angiotensin II and klotho levels were measured.

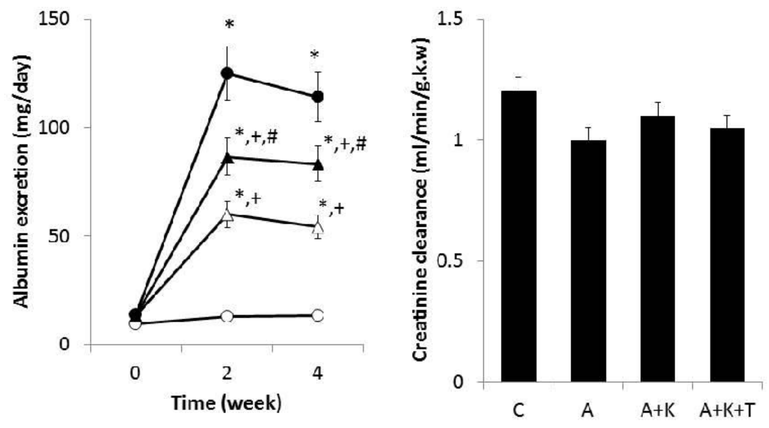
Aortic SOD and whole kidney expressions of Wnt1, TGF-β1 and fibronectin were assessed with RT-PCR against GAPDH.

Kidney abundances of β-catenin and Twist were estimated using Western blot against beta-action.

The expressions of nephrin and TRPC6 were examined in laser-assisted microdissected glomeruli using PCR.

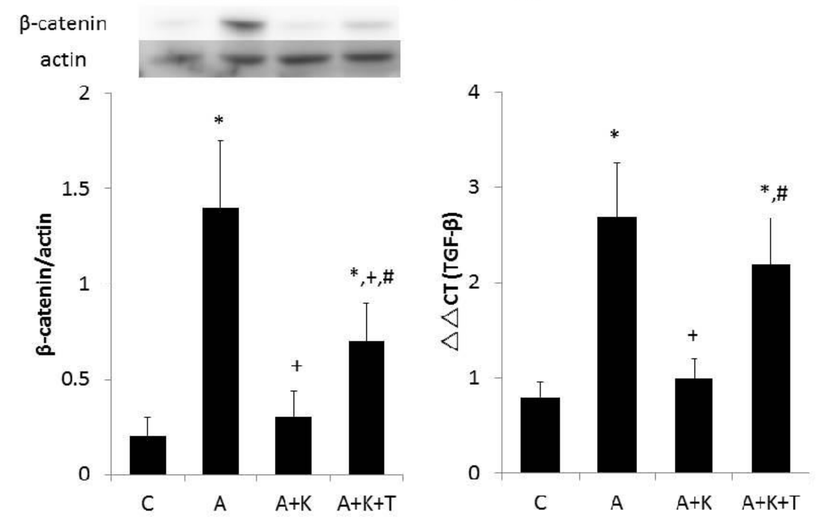
Immunohistochemistry was also utilized to investigate the localization of Wnt1 expression.

Kidney function and temporal changes in albuminuria



Open circles, closed circles, open triangles and closed triangles depict the data from C, A, A+K and A+K+T, respectively.
*, + and # indicate significant difference from C, A, and A+K.

Abundance of β-catenin and expression of transforming growth factor-β (TGF-β) in whole kidney



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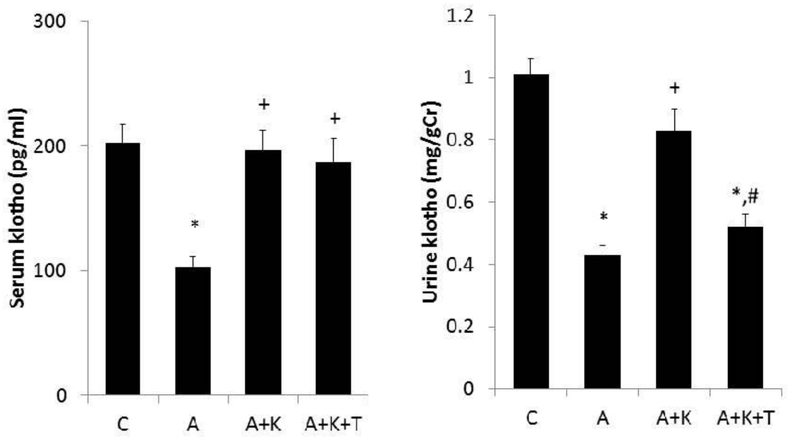
Serum klotho levels and urinary excretion of klotho



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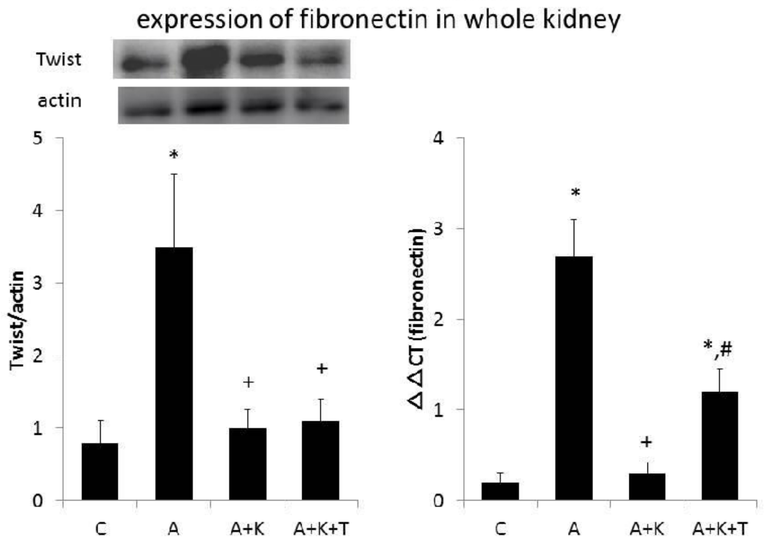
Renal expression klotho shows similar trends with urine klotho

Abundance of Twist and expression of fibronectin in whole kidney



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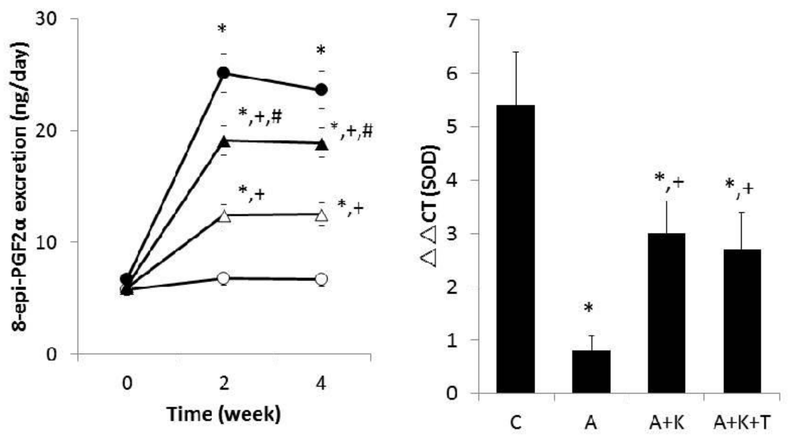
Aortic superoxide dismutase (SOD) expression and temporal changes in oxidative stress



Open circles, closed circles, open triangles and closed triangles depict the data from C, A, A+K and A+K+T, respectively.
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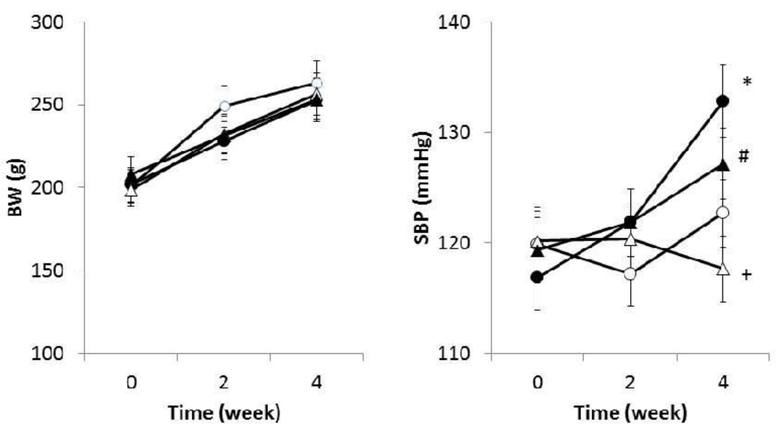
Summary

1. Klotho supplement reduced blood pressure and albuminuria in adriamycin nephropathy.
2. Supplemental klotho reversed decrements in renal klotho expression and urine klotho.
3. Nephrin expression was improved by klotho but diminished by GSK-3β inhibition.
4. Supplemental klotho ameliorates oxidative stress and glomerular expression of TRPC6, possibly through IGF receptor repression.

Temporal changes in body weight (BW) and systolic blood pressure (SBP)



Open circles, closed circles, open triangles and closed triangles depict the data from C, A, A+K and A+K+T, respectively.
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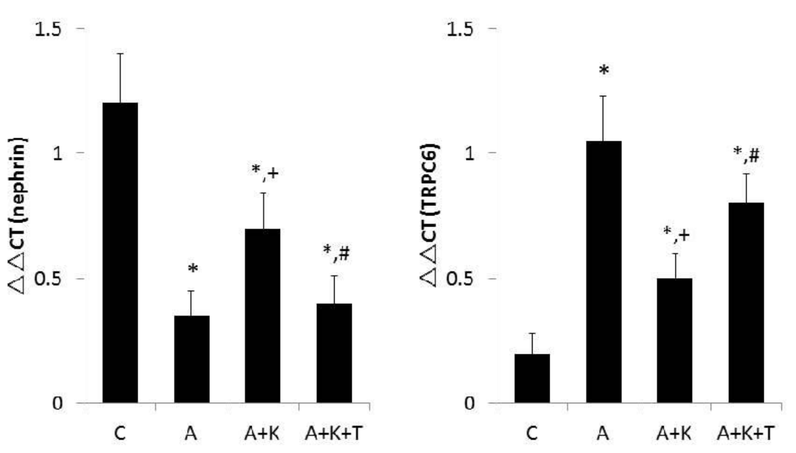
Glomerular expression of nephrin and transient receptor potential channel 6 (TRPC6)



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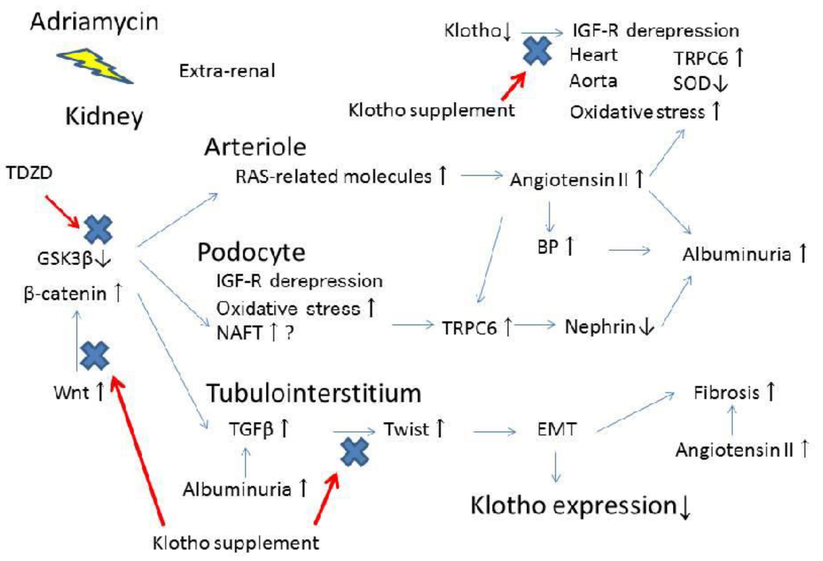
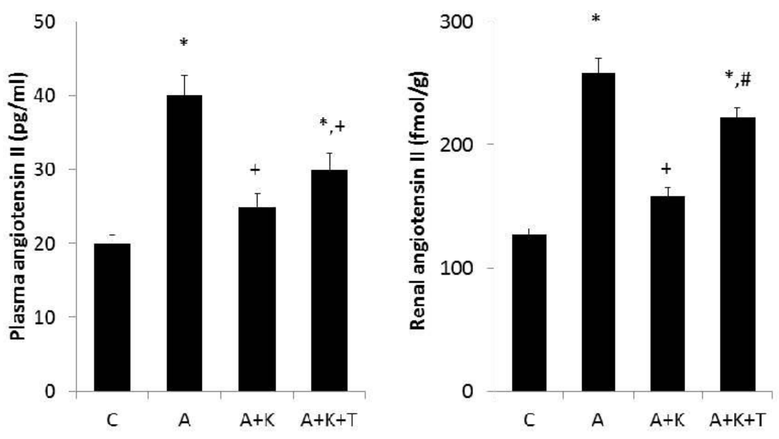
Working hypothesis



Plasma and renal angiotensin II concentrations



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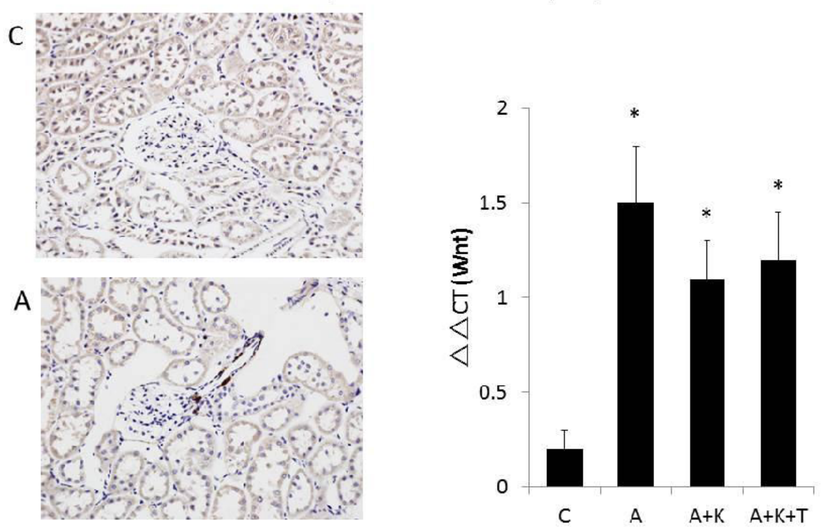
Immunohistochemistry and whole kidney expression of Wnt



* indicated significant difference from C.
Wnt was considerably expressed in the afferent arteriole.

Conclusions

The present results suggest that

1. Wnt/catenin signal is transduced at least partly via GSK-3β in adriamycin nephropathy.
2. Klotho supplementation suppresses renal angiotensin II by inhibiting Wnt.
3. Supplemental klotho inhibits both Wnt and TGF-β signaling, thereby suppressing epithelial-mesenchymal transition.
4. Angiotensin II induces renal fibrosis, at least partly in the manner independent of TGF-β.

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