KLOTHO ATTENUATES PARATHYROID HORMONE SIGNALLING

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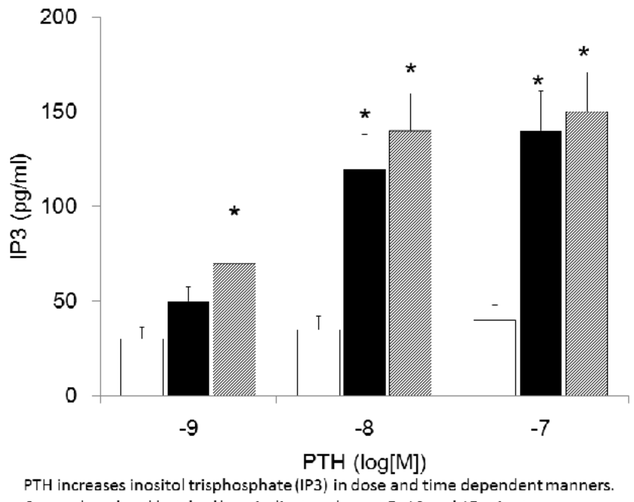
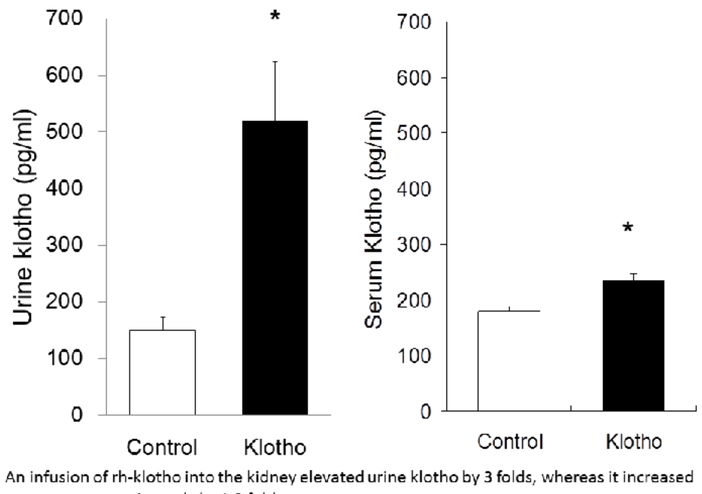
Background

- Fibroblast growth factor (FGF) 23 decreases serum phosphate by enhancing phosphate excretion and suppressing 1,25-dihydroxy-vitamin D (1,25VD) .
- Our recent data indicate that FGF23 elevates renal abundance of membrane klotho as well as serum free klotho.
- However, the mechanisms how FGF23 reduces 1,25VD remained unclear.
- Klotho binds to various membrane proteins, such as receptors and channels.
- The present study was carried out to examine the hypothesis that klotho interacts with PTH receptors to regulate 1,25VD.

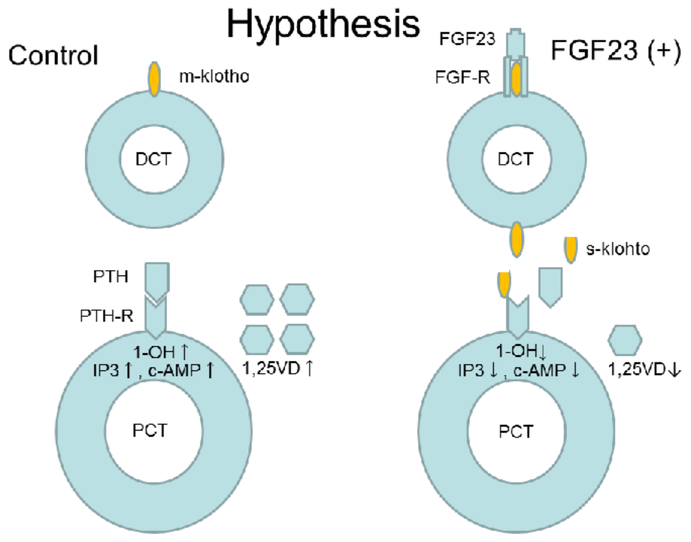


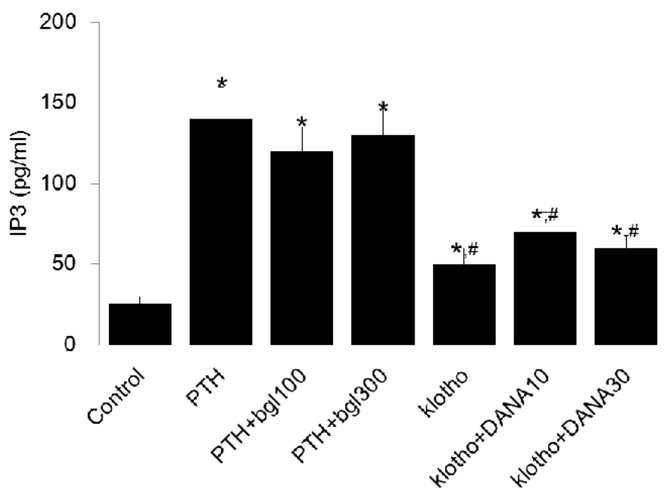
PTH increases inositol trisphosphate (IP3) in dose and time dependent manners. Open, closed and hatched bars indicate values at 5, 10 and 15 minutes



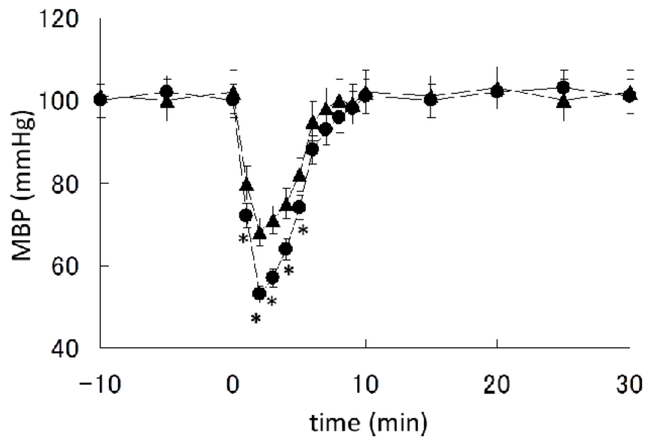
An infusion of rh-klotho into the kidney elevated urine klotho by 3 folds, whereas it increased serum concentration only by 1.3 folds

Hypothesis





PTH-induced increments of IP3 were suppressed by 1 nM klotho, but not altered by β-glucosidase (bgl) or 2,3-didehydro-2-deoxy-N-acetyl-neuraminic acid (NADA)



High doses of rh-klotho ameliorated PTH-induced maximal reduction of blood pressure

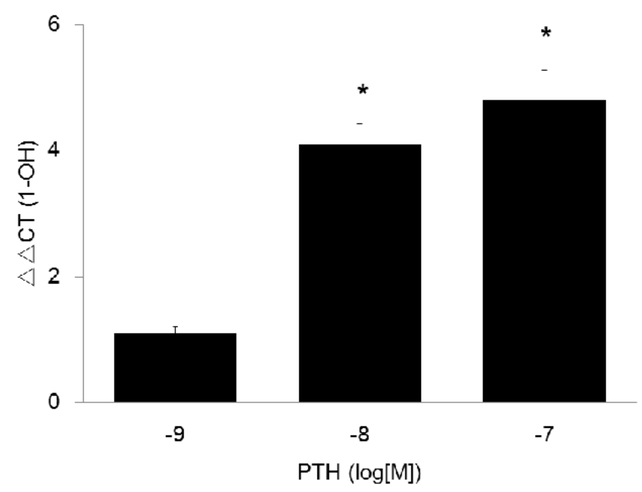
Method 1

- In vitro cell-free study for klotho binding

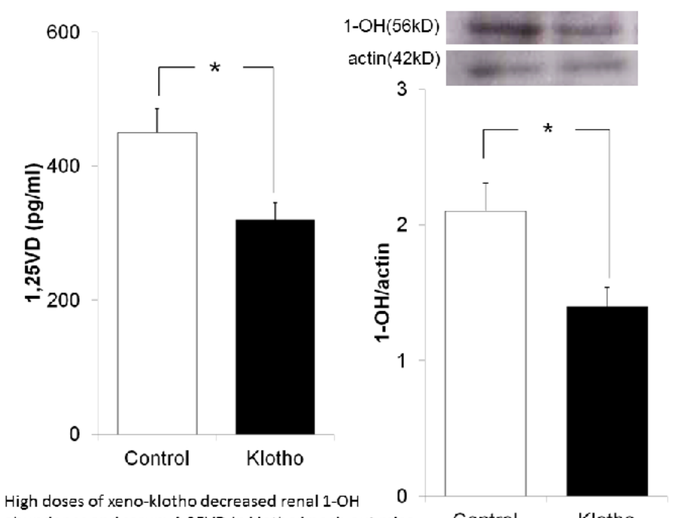
Protein G sepharose beads were coated with antibody against PTH receptor. Then, human PTH receptor and recombinant human klotho were added to the beads. Finally, proteins resided on the beads were detected.

- Cell culture

Confluent cultured human proximal tubular cells were challenged by increasing doses of human PTH in the presence or absence of klotho. Then, cells were pretreated with either β-glucuronidase (100 and 300 μg/ml), 1 nM klotho with or without 2,3-didehydro-2-deoxy-N-acetyl- neuraminic acid (DANA: 10 and 30 pM) for 6 hrs.



PTH induces 1-αhydroxylase (1-OH) expression in a dose dependent manner



High doses of xeno-klotho decreased renal 1-OH abundance and serum 1,25VD in klotho knock-out mice

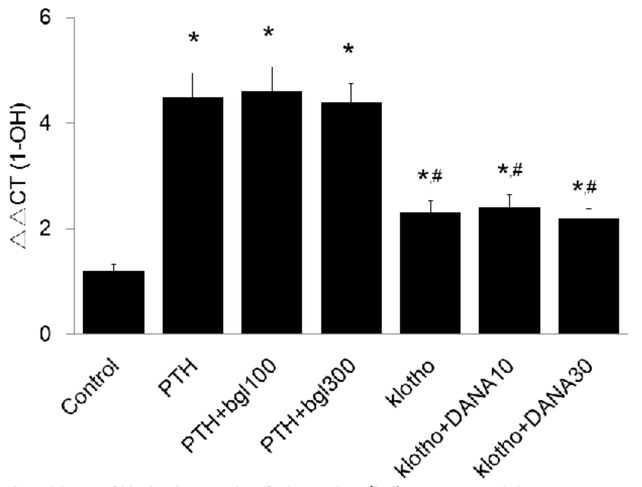
Method 2

- In vivo study

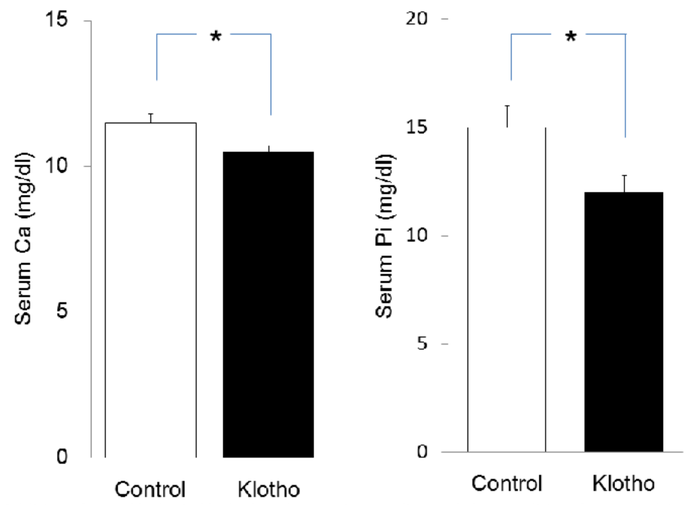
Renal interstitial catheters were implanted on 3 days before acute experiments. On the day of acute experiments, the infusion of supplemental dose of klotho (10 μg/kg/day) or vehicle into rat renal interstitium was started.

In the first series of experiments, PTH (100 μg/kg) was injected as a bolus via jugular vein under anesthesia. Urine was collected 4 times every 10 min interval (before PTH, 0-10, 10-20, 20-30 min).

In second series of studies. After 1 hr of equilibration periods, human PTH (100 μg/kg) was injected subcutaneously. Urine was collected for 24 hr. Blood samples were taken 23 hr later from PTH administration.



The addition of klotho, but neither β-glucosidase (bgl) nor NADA, inhibits increments of 1-OH expression by PTH



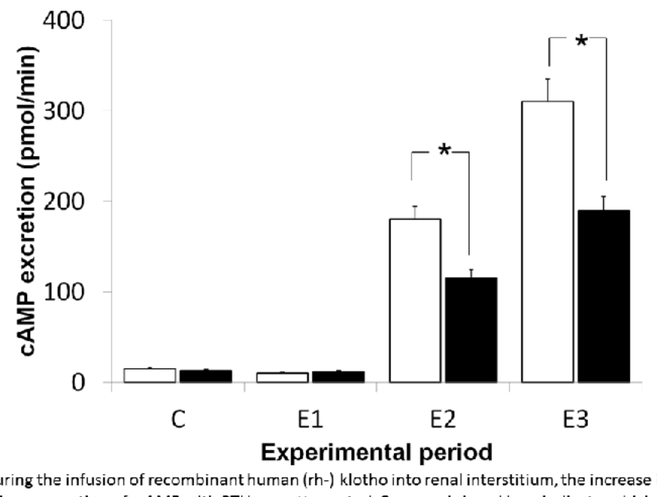
Addition of xeno-klotho in klotho knock-out mice ameliorated abnormalities of serum calcium and phosphate

Method 3

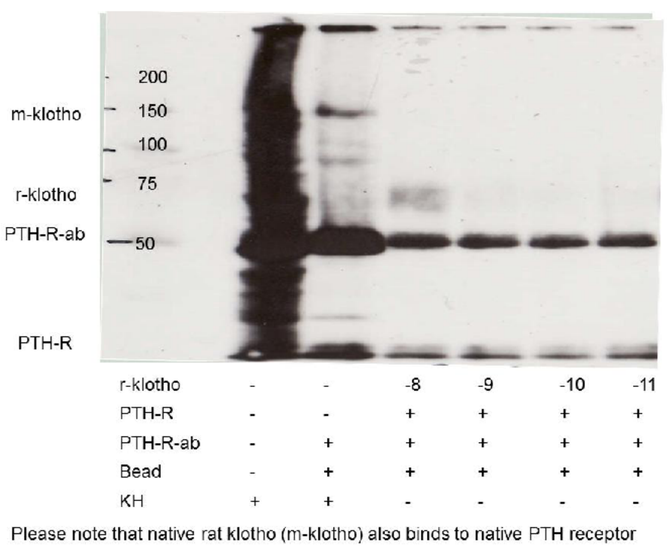
- In vivo study

In the third series of investigations, rh-klotho (100 μg/kg) or vehicle was administered as bolus injection directly into aorta through femoral artery. After 30 min of equilibration periods, PTH (100 μg/kg) was injected as a bolus via jugular vein. Urine was then collected for 1 hr to measure klotho.

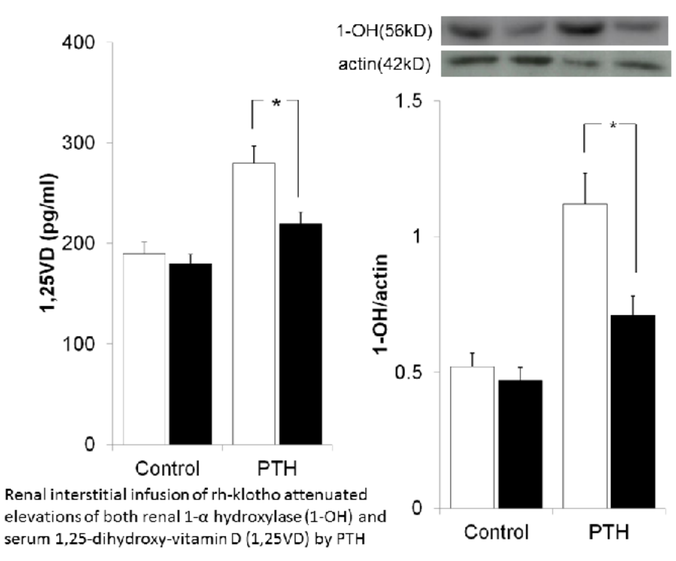
In the fourth study, klotho knock-out (B6.129-Kltm1Yin/Jcl) mice were subcutaneously treated with pharmacological dose of rh-klotho (100 μg/kg/day) or vehicle. A week later, animals were killed with anesthesia overdose to obtain blood and kidney samples.



During the infusion of recombinant human (rh-) klotho into renal interstitium, the increase in urinary excretion of c-AMP with PTH was attenuated. Open and closed bars indicate vehicle and klotho-infused groups



Please note that native rat klotho (m-klotho) also binds to native PTH receptor



Renal interstitial infusion of rh-klotho attenuated elevations of both renal 1-α hydroxylase (1-OH) and serum 1,25-dihydroxy-vitamin D (1,25VD) by PTH

Summary

- Human klotho protein binds to human PTH receptors.
- In human proximal tubular cells, human klotho suppresses PTH-stimulated generation of inositol trisphosphate in vitro.
- In addition, klotho inhibits the expression of 1-OH by PTH in vitro and in vivo.
- Moreover, human klotho diminished the magnitude of PTH-induced increments of cyclic AMP excretion in rats.
- Klotho also lowered PTH-induced elevations of 1,25-dihydroxy-vitamin D (1,25VD) levels in rats.
- Finally, the administration of free klotho protein ameliorated abnormally high levels of 1,25VD in klotho-deficient mice.

Conclusion

- The present results suggest that free klotho plays a mediatory role in FGF23-induced inhibition of 1,25VD synthesis.

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J5) Chronic Kidney Disease. Bone disease.

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