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Developing an In Vitro Model of CKD-MBD Induced αKlotho Suppression

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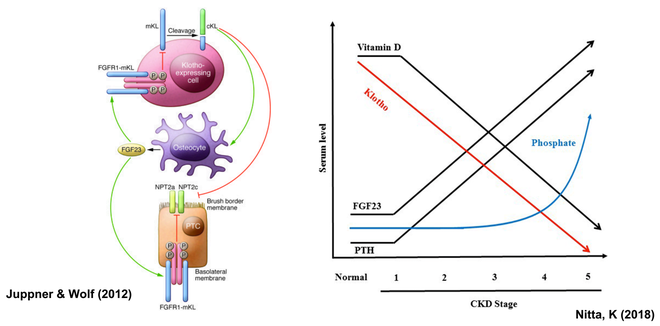
ABSTRACT

Chronic Kidney Disease (CKD) affects approximately 1 in 10 Americans. Diabetic nephropathy is also associated with the development of chronic kidney disease-mineral bone disorder (CKD-MBD). CKD-MBD disrupts the normal bone-kidney endocrine axis responsible for regulating mineral metabolism, and hyperphosphatemia develops in late stage disease. Important clinical hallmarks of the CKD-MBD progression include elevated bioactive Fibroblast growth factor-23 (FGF23) and suppression of FGF23's co-receptor, αKlotho (αKL). In healthy individuals the hormone FGF23, primarily produced by bone, and aKL aid in maintaining normal phosphate and vitamin D homeostasis. It is currently unknown what drives the suppression of αKL expression, however increasing αKL expression in CKD-MBD models is being investigated as a novel therapeutic. Our study sought to develop a novel in vitro model of one of the clinical hallmarks of the progression of CKD-MBD, αKL suppression, to investigate both possible stimuli of its repression and downstream signaling events. The Human Embryonic Kidney (HEK) cell line was used to determine if changes in fluid shear stress, similar to those that occur in diabetic nephropathy, could lead to reduced αKL expression. HEK cells were plated and exposed to oscillatory fluid shear stress (OFSS) for intervals between 0-60 min to examine protein expression or 0-2 hours to assess gene expression. HEK cells were sensitive to mechanical stimulation as pathways including increased ERK phosphorylation occurred in response to OFSS. In response to longer bouts of OFSS αKL expression was significantly ($p<0.05$) reduced. Dramatic changes in fluid shear stress may serve as a stimulus for reduced αKL expression in CKD-MBD. Further studies are underway to investigate downstream signaling events related to αKL suppression. Understanding both the stimuli of αKL suppression and related downstream signaling events could provide novel therapeutic targets for the treatment of CKD-MBD.

BACKGROUND



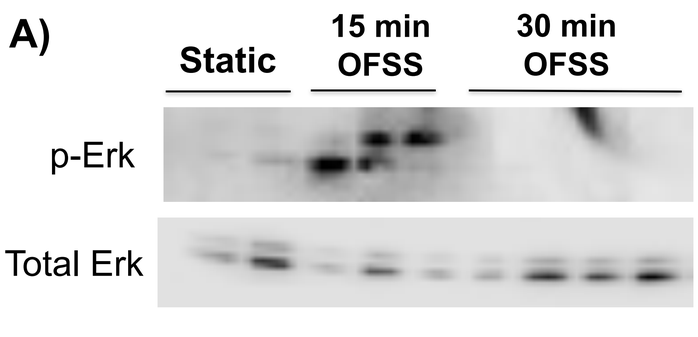
- Approximately 1:10 Americans have CKD-MBD
- CKD-MBD disrupts the normal bone-kidney endocrine axis and hyperphosphatemia develops in late stage disease
- Important clinical hallmarks of the CKD-MBD progression include elevated bioactive Fibroblast growth factor-23 (FGF23) and suppression of FGF23's co-receptor, αKlotho (αKL)
- FGF23 and aKL aid in maintaining normal phosphate and vitamin D homeostasis.
- It is currently unknown what drives the suppression of αKL expression, however increasing αKL expression in CKD-MBD models is being investigated as a novel therapeutic.

HYPOTHESIS

Oscillatory fluid sheer stress serves as a stimuli for reduced αKlotho expression in kidney cells.

RESULTS

HEK cells are responsive to OFSS stimulation



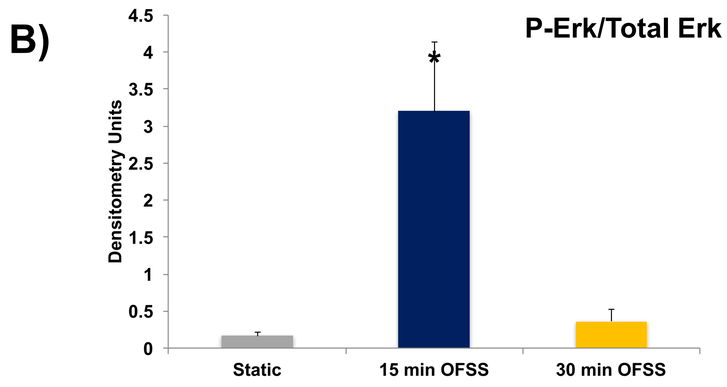


Figure 1. A) HEK cells were exposed to either static or OFFS conditions (15 min or 30 min) and harvested immediately for protein analysis. Western blot analysis was run to examine p-Erk and total Erk. B) The software program ImageJ was used to quantify p-Erk and total Erk (*$p<0.05$).

OFSS stimulation suppresses αKlotho expression

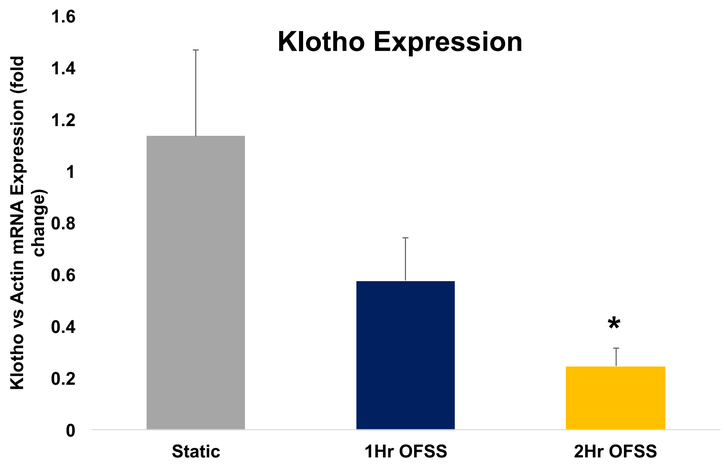


Figure 2. HEK cells were exposed to either static or OFFS conditions (1 or 2 hrs) and harvested the next day. qPCR analysis was performed examining Klotho expression and Actin (*$p<0.05$).

SUMMARY & CONCLUSIONS

- Western blot showed increased ERK phosphorylation occurred in response to fluid shear stress
- qPCR demonstrated a significant decrease in αKL gene expression after 2 hours of fluid stress and a pattern of decreased gene expression after 1 hour
- Shear stress showed significant gene expression and changes in αKL matching those seen in CKD-MBD
- These preliminary findings show trends consistent with a novel in vitro model of CKD-MBD with decreases in αKL expression

FUTURE DIRECTIONS

- Repeat Western blot and qPCR experiments
- Repeat experiment in HK-2 (proximal tubule) cell line
- Examine expression levels of more signaling molecules to confirm other changes seen in CKD-MBD

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