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

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**Recommended Citation**

Tobin, Kirby OMS-2; Waite, Timothy OMS-2; Oberhelman, Forrest OMS-3; and Hum, Julia Ph.D, "Developing an In Vitro Model of CKD-MBD Induced αKlotho Suppression" (2018). MU-COM Research Day. 105.

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Developing an in vitro model of CKD-MBD induced aKlotho 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 αKL 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.

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

RESULTS
HEK cells are responsive to OFSS stimulation

A) Static vs OFSS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>p-Erk</th>
<th>Total Erk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Static</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OFSS 15 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OFSS 30 min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B) OFSS stimulation suppresses aKlotho expression

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Klotho Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Static</td>
<td></td>
</tr>
<tr>
<td>1Hr OFSS</td>
<td>*</td>
</tr>
<tr>
<td>2Hr OFSS</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. A) HEK cells were exposed to either static or OFSS 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).

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

ACKNOWLEDGEMENTS
Current and Former Lab Members:
Madison Kelly, Mehdi Shadmand, Curren Sharma, Tyler Lucas, Collin Young, Cecelia Bender, Vincent Marshall, Bryan Wacker

Funding Sources:
Faculty Research Development Grants - Marian University College of Osteopathic Medicine