**Exploring the effect of tacrolimus on the renal kinome: identification of novel phosphoproteins**

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Tacrolimus is a calcineurin inhibitor (CNI), and the main immunosuppressant used in solid organ transplantation. However, it causes complications such as hypertension, acidosis, hyperkalaemia, diabetes mellitus and hypercalciuria. These complications (which mirror the metabolic syndrome) may be mediated by altered renal tubular transport mechanisms.

As calcineurin is a protein phosphatase, and tacrolimus has been shown to alter the activity of serine/threonine kinases, we decided to use a multiplexed quantitative approach using tandem mass tags and mass spectrometary to identify novel phosphoproteins involved in pathways that lead to the adverse effects caused by tacrolimus.

From the renal cortices isolated from C57BL/6J mice following vehicle or tacrolimus treatment, LC-MS/MS detected 417 unique phosphopeptides with 44% present in two or three replicates. 22% of the identified phosphopeptides showed a significant increase and 27% showed a decrease in phosphorylation post-tacrolimus treatment.

Significant increases in phosphorylated proteins involved in regulating sodium transport and blood pressure (NHERF1: 16-39%, ACE: 27.6%), bone mineral regulation (Osteopontin: 33%) and glucose metabolism (Fructose-biphosphate aldolase: 17.8%) were identified.

Phosphoproteins significantly depleted in tacrolimus animals included those involved in proximal tubular bicarbonate reabsorption (Electrogenic sodium bicarbonate cotransporter 1/SLC4a4: 16-49%), glucose regulation (AMPK subunit beta1: 25.3%, Glycogen synthase: 35.7%), potassium transport (KCNJ16: 33.2%) and proximal tubular phosphate reabsorption (NaPi-IIa: 45-71%).

These data suggest that the effects of calcineurin inhibition on solute transport and cellular metabolism are diverse, profound and may relate to the adverse effects of CNIs. Further investigation of the role of these novel candidates in the effects of CNIs is warranted.