INTEGRIN LINKED KINASE REGULATES THE TRANSCRIPTION OF AQP2 BY NFATC3

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INTRODUCTION AND AIMS

We recently published (Cano-Peñalver et al. FASEB J 2014 and Mamuya et al. AJP 2016) that depletion of the intracellular scaffold protein integrin-linked kinase (ILK) diminishes the water channel Aquaporin 2 (AQP2) abundance and its membrane translocation, resulting in polyuria and urine osmolality decrease, both alterations related to Nephrogenic Diabetes Insipidus. We previously showed (Albertoni-Borghese et al. Nephron Extra 2011) that AQP2 expression is regulated by the nuclear factor of activated T cells (NFATc). The NFATc nuclear shuttle is a balance between the nuclear import by calcineurin and the nuclear export by glycogen synthase kinase 3β (GSK3β), the last able to be phosphorylated by ILK, thus inactivated. Therefore, we tested the hypothesis that ILK is required for NFATc-regulated AQP2 expression.

METHODS

Adult conditional ILK knock-down (cKD-ILK) and NFATc3 isoform KO (NFATc3-KO) mice were housed in metabolic cages. After 24h, urine volumes and osmolality were compared with their corresponding littermates under physiological conditions. Their excised inner medullas were processed to determine the proteins levels of AQP2, NFATc3 and P-GSK3β (ser9)/GSK3β.

Mouse inner medullary collecting duct cell line (mIMCD3), subjected to ILK depletion with siRNAs, were transfected with a luciferase-based reporter plasmid for NFAT transcriptional activity and 24h later treated with the GSK3β inhibitor LiCl (0,1M) for 24h more. The luciferase activity was determined (in relative luciferase units, R.L.U.) and normalized to total protein content.

Values are represented as the means ± SEMs . Data were analyzed by Student's t test or one-way ANOVA followed by Bonferroni's multiple-comparisons test. P values less than 0.05 were considered statistically significant (*= P<0.05 vs WT or CT; #= P<0.05 vs CT-treated cells)

RESULTS

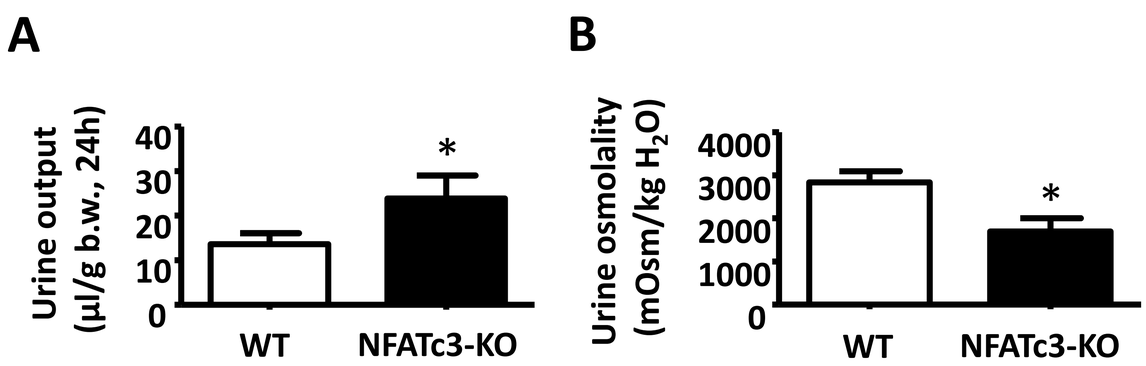

Fig 1: Increased basal polyuria (A) and decreased urine osmolality (B) in NFATc3-KO 24h collected urine compared to WT. Similar results were obtained in cKD-ILK model (Cano-Peñalver et al. FASEB J 2014).

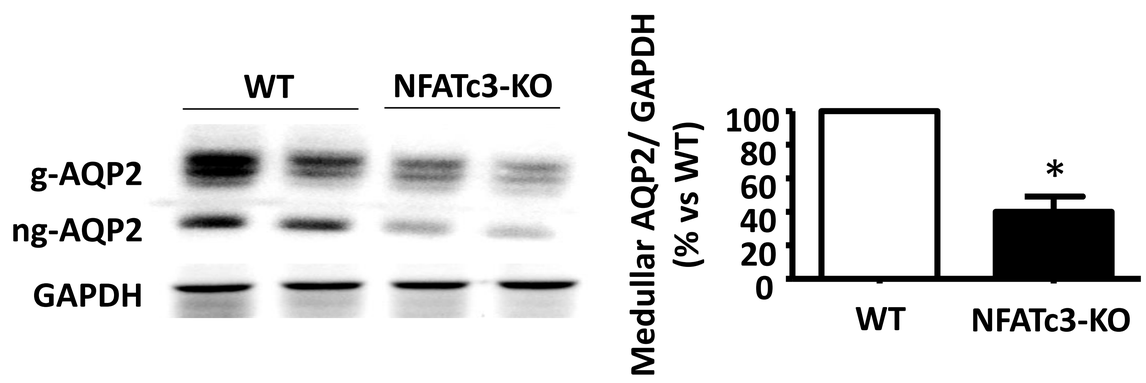

Fig 2: Expression of AQP2 is significantly reduced in renal medullas isolated from NFATc3-KO compared to WT mice. Representative immunoblots and densitometric analysis of both non-glycosylated and glycosylated AQP2 normalized to GAPDH . An analogous decrease of AQP2 protein levels was observed in cKD-ILK (Cano-Peñalver et al. FASEB J 2014).

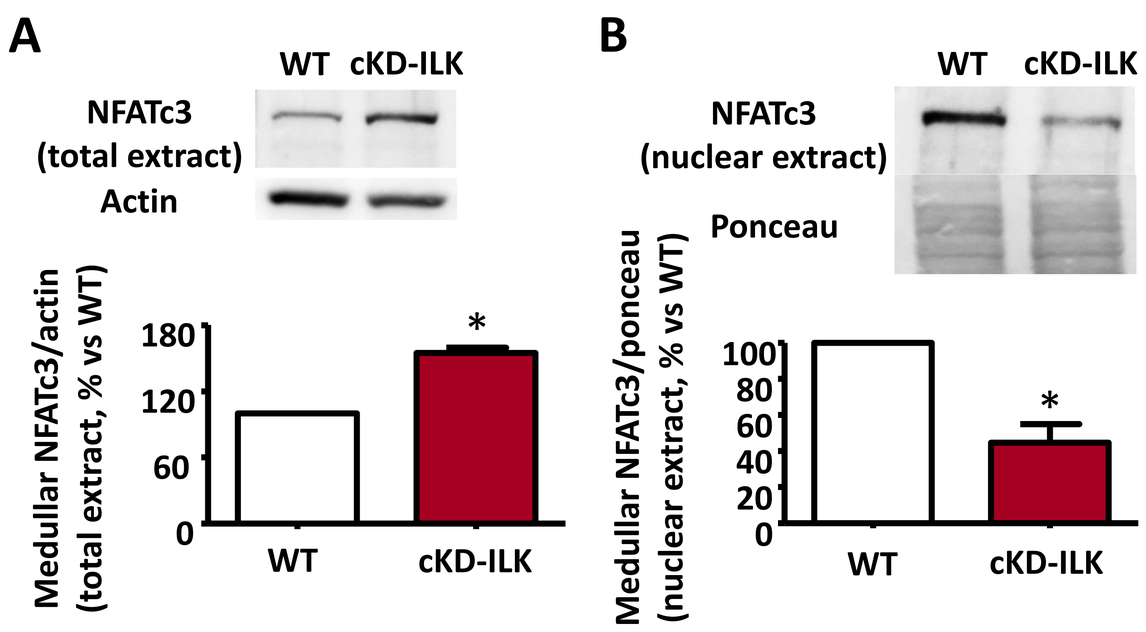

Fig 3: Increase in NFATc3 protein levels in the total protein extract, but decreased in nuclear extracts from medullas of cKD-ILK compared to WT. Representative immunoblots and densitometric analysis of (A) NFATc3 total protein extract normalized to Actin or (B) NFATc3 in nuclear enriched protein extract normalized to Ponceau.

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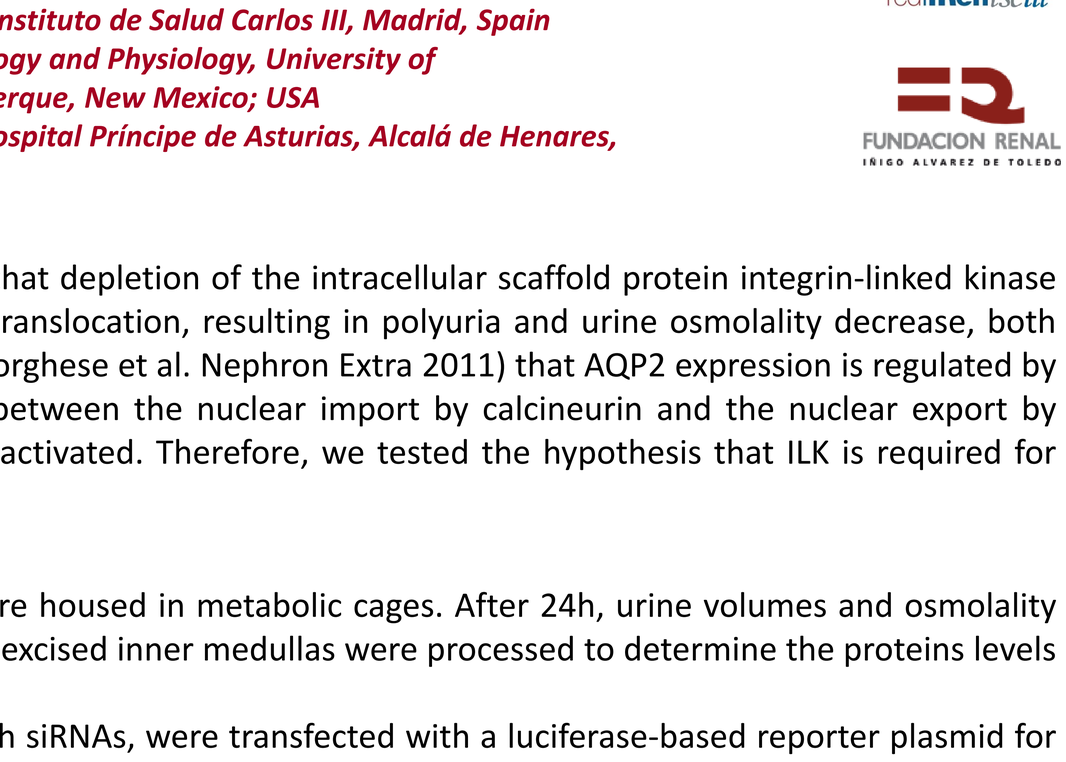


Fig 4: Basal decrease of GSK-3β phosphorylation at serine 9 (P-GSK3β) in (A) medullas from cKD-ILK and (B) ILK-depleted mIMCD3 cells. Representative immunoblots and densitometric analysis of P-GSK3β normalized to GSK3β are shown.

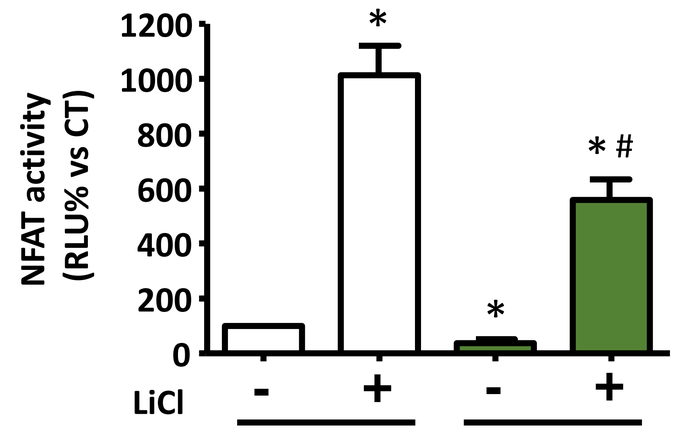
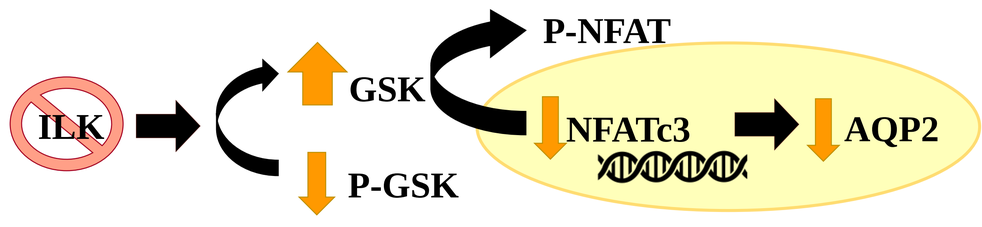


Fig 5: ILK-depleted mIMCD3 cells with siRNA-ILK (si-ILK) or controls (CT) were transfected with the luciferase-based NFAT activity reporter and treated or not with the GSK-3β inhibitor LiCl for 24h. Luciferase (R.L.U.) was determined and normalized to total protein content. Si-ILK have significantly reduced NFAT activity compared to CT. The NFAT activity is increased when cells are treated with LiCl although LiCl-treated si-ILK did not completely reach the LiCl–treated CT values.

CONCLUSION

ILK inactivation may decrease GSK3β-mediated nuclear NFATc3 presence, and hence its transcriptional activity on AQP2.



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