1 • LA QUINASA LIGADA A INTEGRINAS (ILK) REGULA LA TRANSCRIPCIÓN DE AQP2 A TRAVÉS DE LA EXPORTACIÓN NUCLEAR DE NFATC3 MEDIADA POR GSK3BETA

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Introducción: La diabetes insípida nefrogénica (DIN) es una alteración presente en diversas enfermedades renales, caracterizada por la micción de grandes cantidades de orina con una osmolalidad baja.

Recientemente, hemos publicado que la deleción de la quinasa ligada a integrinas (ILK) disminuye los niveles totales de Acuaporina-2 (AQP2) y su translocación a membrana, originando un cuadro compatible con la DIN (Cano-Peñalver et al. FASEB J 2014; Mamuya et al. AJP 2016). Previamente hemos descrito (Albertoni Borghese et al. Nephron Extra 2011) la regulación transcripcional de AQP2 por la subfamilia de factores nucleares dependientes de calcineurina NFATc, cuya fosforilación y salida del núcleo es mediada por la quinasa glicógeno sintasa 3-beta (GSK3beta), un substrato directo de ILK. Aquí estudiamos el papel de ILK en la regulación de la actividad transcripcional de la isoforma NFATc3 sobre el promotor de AQP2.

Material y Métodos: Ratones adultos con deleción condicional de ILK (cKD-ILK) o ausentes de NFATc3 (NFATc3-KO). De estos animales y sus respectivos controles se determinaron los volúmenes y osmolalidades de las orinas de 24h y se analizaron los niveles de distintas proteínas en sus extractos medulares. Células de túbulo colector en cultivo (mIMCD3) con la ILK delecionada mediante la transfección de RNAs interferentes, transfectadas posteriormente con plásmidos reporteros y tratadas con cloruro de litio (LiCl) fueron procesadas para determinar la actividad transcripcional de NFAT.

Resultados: Similar a cKD-ILK, NFATc3-KO resultaron un modelo de DIN, mostrando basalmente poliuria, y baja expresión medular de AQP2. Las médulas renales de cKD-ILK mostraron mayores niveles de NFATc3, sin embargo su presencia en el núcleo estaba disminuida y la actividad de GSK3beta aumentada (expresada como una disminución de la isoforma fosforilada en serina 9) In vitro, la deleción de ILK en mIMCD3 disminuye la expresión de AQP2 (Cano-Peñalver et al. FASEB.J 2014), la fosforilación de GSK3beta y la actividad del reportero luciferasa de NFAT. El inhibidor de GSK3beta LiCl revirtió la actividad transcripcional.

Conclusión: La inactivación de ILK podría aumentar la exportación nuclear de NFAT mediada por GSK, disminuyendo su presencia nuclear y por lo tanto su actividad transcripcional sobre AQP2.

2 • EFFECTIVENESS OF MTT IN LIVER PHENOTYPE IN A MODEL OF AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE (ARPKD)

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Introduction: Polycystic liver disease (PLD) are genetic disorders characterized by progressive bile duct dilatation and cyst development in hepatic parenchyma. PLD are inherited in a dominant or recessive form and can develop alone or in association with polycystic kidney disease (PKD). A number of different mechanisms have been related to the pathogenesis of Polycystic Disease, which we focused on alteration in the extracellular matrix (ECM).

MTT is an inhibitor of the metalloproteinases of ECM, previously, we have shown the effectiveness of MTT in models of Autosomal Dominant Polycystic Kidney Disease (ADPKD), both in renal and hepatic phenotype. In this work, we have focused in the PLD associated to Autosomal Recessive Polycystic Disease (ARPKD) and its treatment.

Matherial and methods: In this study, we use a model of Autosomal Recessive Polycystic Kidney Disease (ARPKD), Pkhd1 del3-4/del3-4 (Pkhd1-KO), to test the effectiveness of MTT in hepatic cystogenesis.

Results: First, we have studied the liver phenotype in our model of ARPKD and we have found difference between males and females, depending on the timing of disease. This fact is crucial for the correct design of futures experiments and for knowledge of the disease.

Then we have tested MTT, alone and in combination with Tolvaptan. We probe the therapy MTT in different points of life of the Pkhd1-KO model inhibiting the hepatic cystogenesis.

Conclusión: In SEN 2016, our group showed the effect of MTT in Autosomal Dominant Polycystic Kidney Disease (ADPKD) inhibiting the hepatic cystogeneis and collecting duct cyst (DBA+ cyst). With this work, we have demonstrated the effectiveness of MTT in the inhibition of hepatic phenotype of Autosomal Recessive Polycystic Kidney Disease.

Key words: cystogenesis, therapy, PLD, ARPKD, MTT

3 • CYTOKINE TWEAK PROMOTES CYSTOGENESIS IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD) IN A TIME DEPENDENT MANNER

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Introduction: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common monogenic disorder in which kidneys develop fluid-filled cyst derived from the tubule epithelial cells. The three causative genes for ADPKD, PKD1, PKD2 and GANAβ, encode protein products polycystin-1 (PC1), polycystin-2 (PC2) and glucosidase IIα subunit (GIIα). Several mechanism are associated with cyst initiation and cyst progression, recent findings aim inflammation as one the most important molecular mechanism in the progression of the ADPKD.

TWEAK (tumor necrosis factor TNF-like weak inducer of apoptosis) is a TNF-like cytokine and member of the TNF superfamily. TWEAK promotes inflammation, proliferation, cell death and angiogenesis. However, the role of TWEAK in ADPKD is unknown.

Matherial and methods: We have studied the effect of proinflammatory cytokine TWEAK in our ADPKD animal model, Pkd1cko/cko Tam-Cre. This model presents a cystogenesis developmental switch, because the inactivation of Pkd1 gene in different points of life determines the cystic phenotype. Based on this, we show that TWEAK promotes the progression of ADPKD.

Results: We tested TWEAK in different developmental windows of our animal model (Pkd1cko/cko Tam-Cre) we show that proinflammatory cytokines can be accelerates the progression of ADPKD. The mice that received TWEAK presented higher levels of BUN and worst survival rate than the group of control mice.

In addition, TWEAK promotes the progression of hepatic cystic phenotype (associated to ADPKD). These results suggest that TWEAK, a proinflammatory cytokine, not online acts at the kidney level.

Conclusión: Doing use of Pkd1cko/cko Tam-Cre animal model, we demonstrated that TWEAK promotes the progression of ADPKD in a time dependent manner. We results shown how inflammation may be modulation the severity of ADPKD and the possibility of a treatment with anti-inflammatory therapy.

Key words: inflammation, TWEAK, cytokine, cystogenesis, ADPKD.