Early transcriptional changes in a novel mouse model of adenine-induced tubulointerstitial nephropathy

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Conclusions

• Several early transcriptional changes were detected in the kidney after 14 days with adenine chow, including a marked change in matrix metalloproteinase/tissue inhibitor of metalloproteinases (Mmp/Timp) ratio and an upregulation of matrix proteins, resulting in an imbalance in extracellular matrix organization homeostasis. These findings suggest that tissue remodeling and fibrosis development is an early pathophysiological event in renal failure.

• Genes involved in the complement and coagulation cascade were enriched, implying that kidney damage at an early stage predispose to transcriptional changes associated with cardiovascular disease.

• Serum FGF23 increased early, within 12 hours of adenine exposure, yet its transcript levels in bone (femur) remained unchanged after 2 weeks. This indicates that early increments in circulating FGF23, as observed in CKD patients, may be due to altered transport mechanisms or cellular processing of FGF23.

• Kidney damage resulted in reduced transcriptional levels of Klotho in the kidney as well as induction of genes involved in inflammation and the Nfκb pathway. This corroborates clinical findings in CKD showing a reduction in Klotho level and activation of several inflammatory pathways.

Objective

Mice given dietary administration of adenine mixed with casein for 8 weeks develop renal injury, mainly tubulointerstitial damage, with many phenotypic characteristics observed in patients with chronic kidney disease (CKD) (increased serum levels of FGF23, inorganic phosphorous and PTH, decrease in active vitamin D levels and bone abnormalities). The aim of this study was to examine early transcriptional changes in kidney and bone attributed to this model.

Methods

BL6 mice were fed either a casein diet (control group) or an adenine rich diet (0.3%) for two weeks. Serum levels of FGF23 were measured with ELISA, and transcriptional changes in kidney and femur were analyzed with microarray technology and subsequent pathway analyses.

Results

FGF23 levels

Serum FGF23 was increased at an early stage within 12 hours of adenine exposure (Fig 1). However, no changes were detected in femur transcript levels (data not shown).

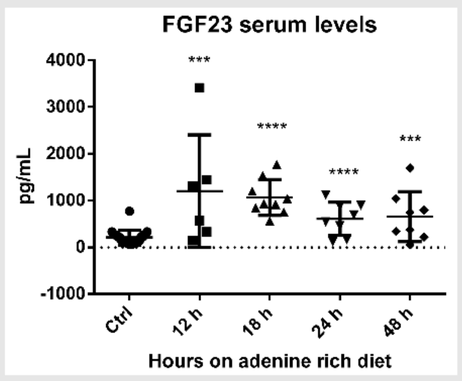

Figure 1. Early changes in FGF23 serum levels. A significant increase of FGF23 was detected within 12 hours on the adenine rich diet. Ctrl: serum samples taken at baseline and after two weeks with adenine chow. *** < 0.001, **** < 0.0001.

Imbalance in extracellular matrix (ECM) organization

Renal fibrosis can be found in almost all types of CKD and is the result of excessive formation of extracellular matrix. Several components of the ECM, as well as regulators of these components, were upregulated, Table 1.

Table 1. Imbalance in ECM organization. Components of the ECM that are upregulated in the kidneys of mice with early renal failure. FC = fold change relative to control mice.

Component type	Gene	FC	p-value
Collagen	Col1a1	5.4	2.40E-9
	Col1a2	3.8	1.59E-8
	Col3a1	4.3	1.99E-8
	Col4a1	2.6	9.86E-9
	Col4a2	2.5	1.45E-7
	Col6a1	3.1	2.45E-6
	Col6a2	2.4	1.63E-6
	Col6a3	2.2	5.68E-6
	Col8a1	2.3	2.01E-5
	Col12a1	4.2	4.06E-7
	Col15a1	3.3	7.05E-8
	Col18a1	2.4	2.52E-7
Tissue inhibitor of metalloproteinases	Timp1	21.3	1.60E-9
	Timp2	2.0	2.89E-6

Component type	Gene	FC	p-value
Fibronectin	Fn1	5.2	9.86E-9
Laminin	Lama2	1.5	6.71E-4
	Lama5	2.0	4.99E-6
	Lamb3	1.6	4.51E-3
	Lamc1	1.8	1.70E-5
	Lamc2	3.6	2.01E-8
Matrix metalloproteinase	Mmp2	3.7	2.28E-6
	Mmp3	1.6	3.35E-3
	Mmp7	9.5	2.67E-7
	Mmp9	2.3	2.50E-4
	Mmp12	4.4	1.45E-5
	Mmp14	4.1	4.38E-8
	Mmp19	1.8	5.24E-4
	Mmp23	1.7	2.74E-5

Inflammation

Adenine induced kidney failure resulted in reduced renal Klotho transcripts as well as induction of genes involved in inflammation and the Nfκb pathway (Fig 2). Klotho suppresses inflammation in diabetic mice through negative regulation of NFκB[1].

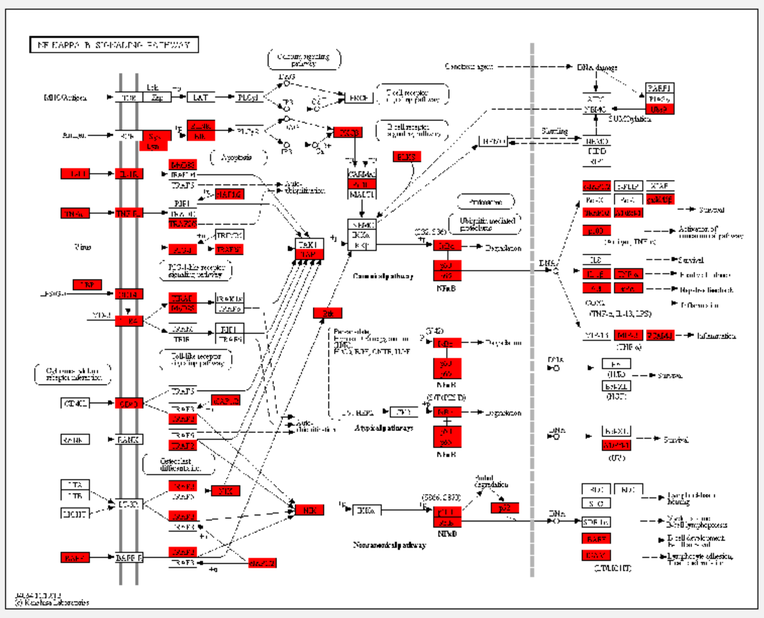

Figure 2. KEGG pathway ""NFκb signaling pathway". Several transcripts were upregulated (red) in the kidneys of mice with renal failure.

Enrichment of "Complement and coagulation cascade"

Analysis showed statistical enrichment of the KEGG pathway "Complement and coagulation cascade" (Fig 3).

The complement system regulates several steps in the inflammatory response and has been shown to play a pathogenic role in kidney disease[2].

The coagulation cascade (including PAI-1 and fibrinogen) has been associated with cardiovascular disease in patients with CKD [3-5].

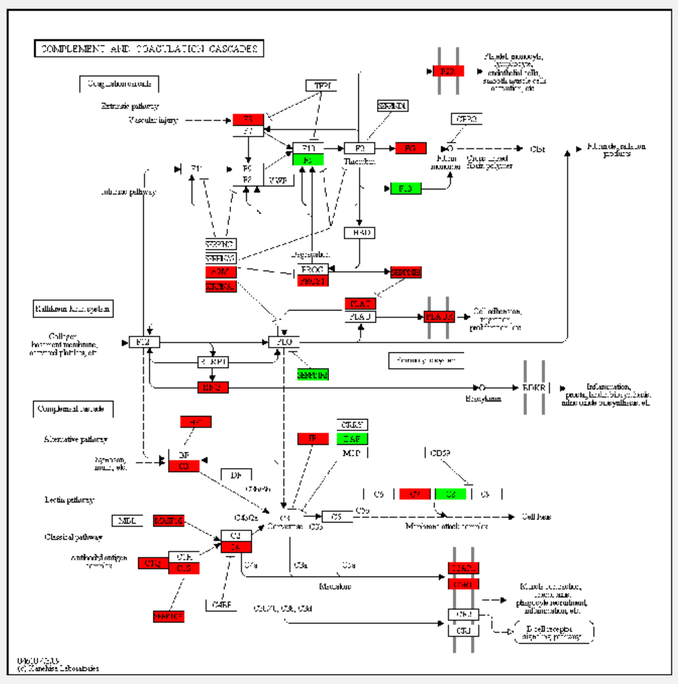

Figure 3. KEGG pathway "Complement and Coagulation cascade". Red genes were transcriptionally upregulated in the kidney following adenine induced renal injury while green were downregulated.

1) Zhao Y, Banerjee S, Dey N, LeJeune WS, Sarkar PS, et al. (2011) Klotho depletion contributes to increased inflammation in kidney of the db/db mouse model of diabetes via RelA (serine)536 phosphorylation. *Diabetes*
2) McCaughan JA, O'Rourke DM, Courtney AE (2013), The Complement Cascade in Kidney Disease: From Sideline to Center Stage, *Am J Kidney Dis.*
3) Kannel WB. (2005), Overview of hemostatic factors involved in atherosclerotic cardiovascular disease, *Lipids*
4) Mühlberger I, Mönks K, Fechete R, Mayer G, Oberbauer R, Mayer B, Perco P. (2012), Molecular pathways and crosstalk characterizing the cardiorenal syndrome, *OMICS*
5) Kirmizis D, Tsiandoulas A, Pangalou M, Koutoupa E, Rozi P, Protopappa M, Barboutis K. (2006), Validity of plasma fibrinogen, D-dimer, and the von Willebrand factor as markers of cardiovascular morbidity in patients on chronic hemodialysis, *Med Sci Monit.*

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