Changes in the Tubular Proteome of 2K1C Hypertensive Rats

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Aim of study

Tubular atrophy and interstitial fibrosis are the final common stage in most forms of progressive kidney disease. Whereas much is known about the pathophysiological role of the interstitial cells, less is known regarding changes in the tubular proteome. In this study, we investigated changes in the tubular proteome of normal or minimally damaged tubular tissue.

Methods

Normal or minimally damaged tubulointerstitial FFPE tissue from the non-clipped kidney of 2K1C hypertensive rats (n=4) 24-weeks after surgery, and age-matched controls (n=6) was laser capture microdissected. Proteins were extracted and analyzed by LC-MS/MS in a label-free quantitative manner using fold change in protein abundance between 2K1C and controls.

Results

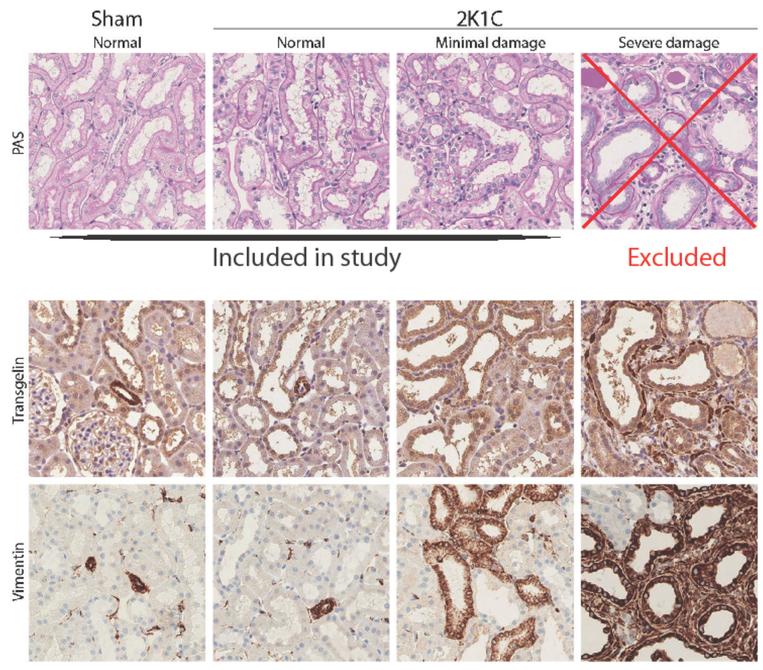

Figure 1. Morphologic changes (row 1, PAS) and immunohistochemical patterns (row 2 transgelin, row 3 vimentin) in sham (column 1) and 2K1C non-clipped (column 2 - 4) kidneys. Column 1 and 2 show normal histology, arterioles are markedly stained for both transgelin and vimentin. Column 3 shows discrete changes in some tubules that have enlargement of tubular epithelial cell nuclei and reduction of the brush border. Tissue from column 1 - 3 would be eligible for microdissection. Immunohistochemistry shows focal positivity in tubular epithelial cells both for transgelin and vimentin. Column 4 shows advanced changes with enlargement and increased cellularity of the interstitium, flattening of tubular epithelial cells and thickening of the tubular basement membrane; this tissue was not eligible for microdissection

Table 1. Top 10 proteins more or less abundant in 2K1C compared with sham, data shown for FFPE tubuli tissue from the present study and for fresh-frozen cortical tissue from the same experiment published previously

Gene	Protein	2K1C vs sham			
		FFPE tubuli		Frozen cortex	
		P-value	Fold Change	P-value	Fold Change
More abundant in 2K1C					
Tagln	Transgelin	2E-02	3.32	6E-05	3.55
Krt18	Keratin, type I cytoskeletal 18	7E-03	3.23	4E-05	3.05
Vim	Vimentin	5E-02	3.19	0.10	1.68
Ftl1	Ferritin light chain 1	1E-04	3.10	0.04	1.93
Hspa1a;Hspa1b	Heat shock 70 kDa protein 1A/1B	5E-03	2.92	2E-06	3.00
Ckb	Creatine kinase B-type	1E-03	2.80	2E-05	3.65
Anxa2	Annexin A2	2E-02	2.09		n.d
Gls	Glutaminase kidney isoform, mitochondrial	2E-04	2.02	0.06	-1.16
Fth1	Ferritin heavy chain	1E-03	2.02		n.d
Bdh1	D-beta-hydroxybutyrate dehydrogenase, mitochondrial	7E-03	1.98		n.d
Less abundant in 2K1C					
Mgam	Protein Mgam	1E-02	-2.18	0.06	-1.33
Nit1	Nitrilase homolog 1	1E-02	-2.34	2E-03	-1.79
Bhmt	Betaine--homocysteine S-methyltransferase 1	2E-02	-2.46	9E-03	-1.89
Ccbl2	Kynurenine--oxoglutarate transaminase 3	1E-02	-2.95	4E-04	-2.27
-	Major urinary protein	4E-03	-3.15	7E-04	-2.78
Mep1a	Meprin A subunit alpha	4E-03	-3.36	3E-03	-2.50
Bhmt2	S-methylmethionine--homocysteine S-methyltransferase BHMT2	4E-02	-3.69	2E-03	-2.27
Slc5a8	Protein Slc5a8	5E-03	-4.13		n.d
Hmgcs2	Hydroxymethylglutaryl-CoA synthase, mitochondrial	3E-03	-5.41	2E-03	-1.92
Hp	Haptoglobin	1E-02	-19.7		n.d

n.d protein not detected in previous study
[a]Skogstrand et.al 2013, Journal of Hypertension

Table 2. Most significantly changed pathways

Ingenuity Canonical Pathways	p-value	z-score*
Tryptophan Degradation III (Eukaryotic)	5E-11	n/a**
Fatty Acid β-oxidation I	2E-09	n/a
EIF2 Signaling	1E-08	3.0
Remodeling of Epithelial Adherens Junctions	4E-07	2.6
Regulation of Actin-based Motility by Rho	7E-07	3.3
RhoA Signaling	2E-06	2.5
Protein Ubiquitination Pathway	4E-06	n/a
Signaling by Rho Family GTPases	2E-05	2.1

From the top 15 pathways only those with at least 10 significantly changed proteins that were changed in a consistent direction were included. The complete list of canonical pathways with p-values less than 0.001 are shown in Supplemental table S2.

* z-score indicates accordance with the Ingenuity Knowledge Base, in respect to the relative abundance of the observed proteins. Absolute z-score above 2.0 indicate significant activation. Negative z-score indicate deactivation.

** n/a, no experimental data exist to predict activation or deactivation of the current pathway

Energy metabolism
Protein turn-over
Rho-signalling

Regulation of actin-based motility by Rho

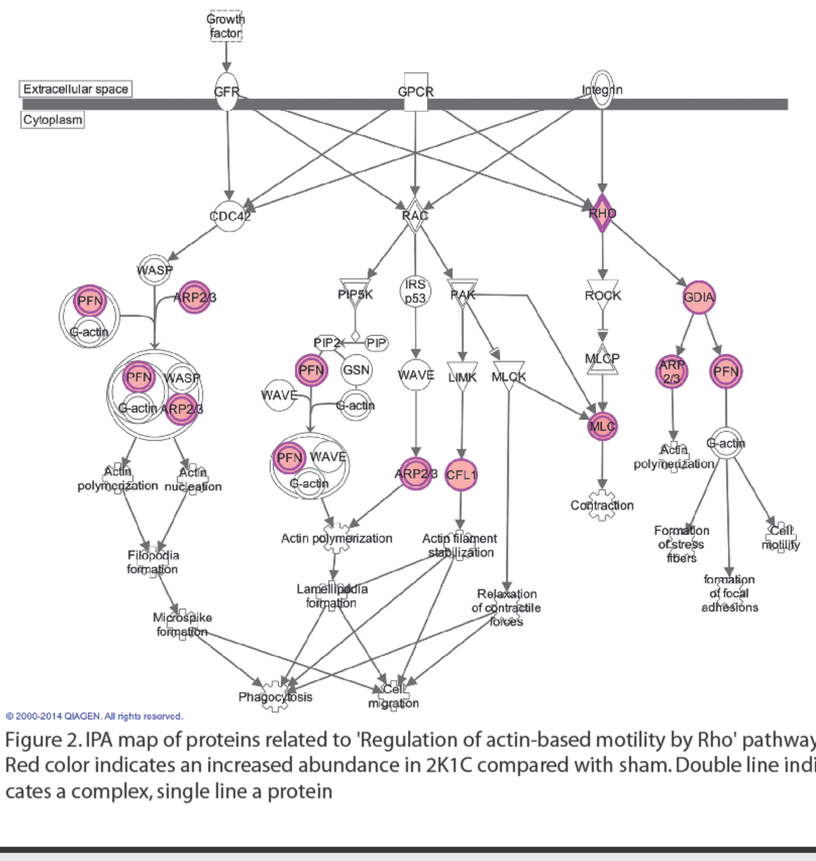

Figure 2. IPA map of proteins related to 'Regulation of actin-based motility by Rho' pathway. Red color indicates an increased abundance in 2K1C compared with sham. Double line indicates a complex, single line a protein

Conclusions

The present study made use of microdissected FFPE tubulointerstitial tissue without discernable interstitial expansion or pronounced tubular alterations to investigate proteomic changes in the normal or minimally changed tubules of in chronic hypertensive kidney disease. The study demonstrated increased abundance of several damage markers, including transgelin and vimentin. Canonical pathway analyses indicated possible changes to energy metabolism, increased protein turnover and activation of Rho signaling.

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