DIFFERENTIAL PROTEOMICS OF EXPERIMENTAL HYPER-TENSIVE RENAL DISEASE REVEALS DISTINCT PROTEIN SIGNATURES AND PERIOSTIN AS A DAMAGE MARKER

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OBJECTIVES

Hypertension-induced nephrosclerosis adds to the increasing prevalence of end-stage renal disease (ESRD).

The aims of this study were to investigate the proteome of damaged kidneys due to hypertension and to define a protein classifier for hyper-tension induced kidney disease.

METHODS

The renal cortex proteome from juxtamedullary cortex (JMC) and outer cortex (OC) of hypertensive Two-Kidney, One-Clip (2K1C) male Wistar-Hannover rats (n=4) was compared to normotensive, sham-operated controls (n=6) using mass spectrometry based quantitative proteomics.

We combined a high abundant plasma protein depletion strategy with an extended liquid chromatographic gradient to improve peptide and protein identification. Immunohistology was used for independent confirmation of protein abundance. The 2K1C rat model was described by us previously (1-3).

RESULTS

We identified 1,724 proteins, of which 1,434 were quantified with ≥2 unique peptides. Comparative proteomics revealed 608 proteins, including the PDGFR-β pathway, with different abundance between the non-clipped kidney of hypertensive 2K1C rats and of controls ($p<0.05$, absolute fold change ≥1.5). Additional results are depicted in Figure 1 A-C and Table 1.

Among most significantly altered proteins in whole cortex were periostin, transgelin and creatine kinase B-type (Figure 2A and Tables 1 and 2). Relative abundance of periostin alone indicated clear classification of 2K1C and controls (Figure 2A and B). Enrichment of periostin in 2K1C was verified by immunohistology with positivity around fibrotic vessels (Figure 2C).

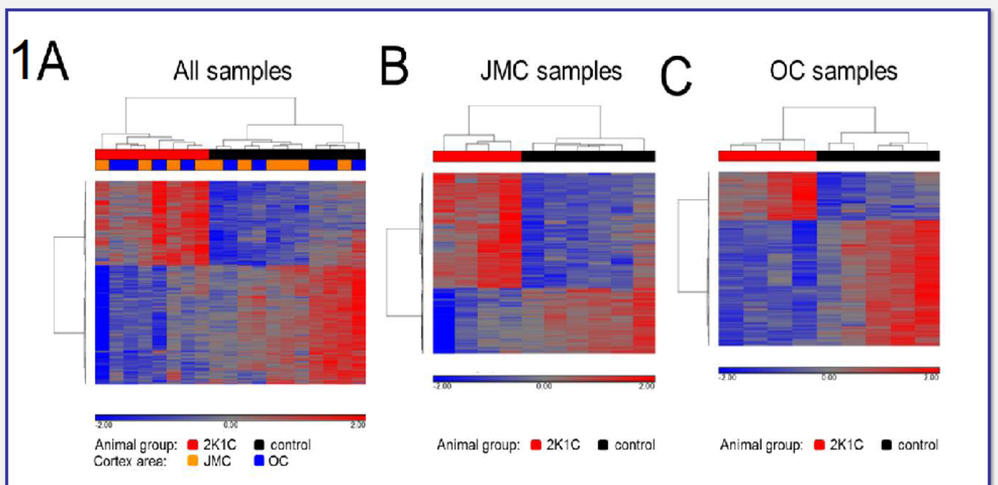

Figure 1. Unsupervised hierarchical cluster analysis of 2K1C- and control animals.

(A) 608 quantified proteins separate samples by animal group. (B) 475 proteins with differential abundance in JMC-tissue. (C) 445 proteins in OC-tissue. *Upregulated proteins are depicted in red, down-regulated in blue.*

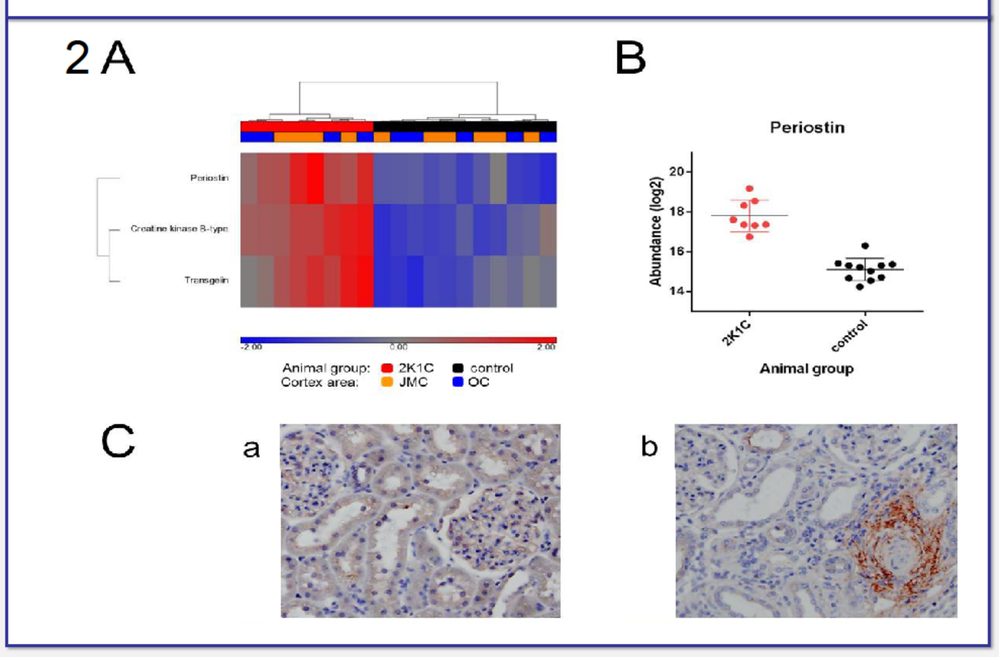

Figure 2. Classifier of hypertensive nephrosclerosis.

(A) Unsupervised hierarchical cluster analysis of transgelin, creatine kinase B-type and periostin separating 2K1C from controls. (B) Scatter plot for periostin in 2K1C and controls. (C) Periostin immunoreactivity was negative in controls (a), and positive around fibrotic blood vessels in 2K1C (b).

Table 1. Over-represented in 2K1C

		2K1C/control		JMC		OC	
Accession	Protein	p-value	Fold Chan	p-value	Fold Chan	p-value	Fold Change
P36201	Cysteine-rich protein 2	2E-07	12.8	2E-05	18.4	2E-03	8.7
D3ZGK7	Carboxylesterase 1C	1E-08	10.6	1E-05	11.4	7E-04	9.8
Q6P7C7	Transmembrane glycoprotein NMB	4E-08	8.8	7E-06	12.1	2E-03	6.2
P63255	Cysteine-rich protein 1	6E-06	7.7	6E-04	5.3	3E-03	11.5
Q5RJR9	Serpin H1	2E-09	6.8	3E-05	8.2	3E-05	5.6
D3ZAF5	Periostin	8E-08	6.6	3E-04	7.1	3E-04	6.1
Q5XI38	Lymphocyte cytosolic protein 1	8E-08	5.5	5E-06	6.2	3E-03	4.7
Q63621	Interleukin 1 receptor accessory protein b	3E-06	5.3	7E-04	4.9	3E-03	5.7
P52925	High mobility group protein B2	3E-08	5.2	4E-06	5.7	2E-03	4.8
Q499V1	Uridine phosphorylase 1	9E-07	5.2	1E-04	6.0	4E-03	4.4
Q9R1T3	Cathepsin Z	4E-07	5.1	4E-05	5.2	3E-03	5.0
M0R9D5	Protein Ahnak	2E-06	5.0	9E-04	5.3	1E-03	4.7
B2GVB9	Fermt3 protein	2E-06	5.0	7E-05	6.6	1E-02	3.8
P07335	Creatine kinase B-type	2E-07	4.7	2E-05	5.4	3E-03	4.0
D3ZPS3	Protein Pglyrp2	2E-06	4.7	1E-03	4.7	2E-03	4.8
Q5M860	Rho, GDP dissociation inhibitor (GDI) beta	4E-06	4.5	8E-05	5.1	1E-02	4.0
P31232	Transgelin	7E-06	4.3	3E-04	4.7	1E-02	3.9
Q99MI5	Spermidine synthase	4E-06	4.1	1E-04	4.9	1E-02	3.4
Q4TU93	C-type mannose receptor 2	1E-06	4.0	4E-05	4.4	7E-03	3.6
G3V904	Phospholipase D4	4E-06	3.8	9E-05	4.3	1E-02	3.3

Table 2. Classification Method

	Normalized Correct Rate	Normalized Correct Rate	Normalized Correct Rate	Normalized Correct Rate
	(Periostin, Transgelin, Creatine kinase B-type)	(Creatine kinase B-type)	(Transgelin)	(Periostin)
K-Nearest Neighbor with Absolute Value/City Block distance measure and 3 neighbors	100.00	95.45	87.50	100.00
K-Nearest Neighbor with Absolute Value/City Block distance measure and 5 neighbors	100.00	95.45	87.50	100.00
K-Nearest Neighborwith Euclidean distance measure and 3 neighbors	100.00	95.45	87.50	100.00
K-Nearest Neighbor with Euclidean distance measure and 5 neighbors	100.00	95.45	87.50	100.00
Linear Discriminant Analysis with Equal Prior Probabiity	100.00	95.45	93.75	10000
Nearest Centroid with Equal Prior Probability	100.00	95.45	93.75	100.00
Partial Least Squares Linear Discriminant Analysis with Equal Prior Probability	100.00	95.45	93.75	100.00
K-Nearest Neighbor with Absolute Value/City Block distance measure and 1 neighbor	100.00	95.45	89.20	89.20
K-Nearest Neighbor with Euclidean distance measure and 1 neighbor	100.00	95.45	89.20	89.20

CONCLUSIONS

There exists distinct protein signatures in experimental hypertension induced kidney damage.

We propose periostin, especially in combination with transgelin and creatine kinase B-type, as possible proteomic classifier to distinguish hypertensive nephrosclerosis from normal tissue. This classifier needs to be further validated with respect to early fibrosis diagnosis and to prognosis as well as for its potential as a novel therapeutic target.

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