Proteomic analysis of kidneys from spontaneously hypertensive and normal rats reveals CLIC4, as a putative biomarker in hypertensive nephropathy

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Hypertensive nephropathy is a major cause of declining kidney function. We aimed to identify novel markers associated with pathogenesis and development of hypertensive nephrosclerosis in experimental animals. In order to elucidate the biological processes involved in kidney damage Spontaneous Hypertensive Rats (SHR) were used as a model of human hypertension. Comparative proteomic analysis of kidney tissue at three time points (6, 13, and 20 weeks) of SHR and normotensive controls (Wistar Kyoto rats, WKY) yielded a significant number of differentially expressed proteins. The most prominent finding was CLIC4 (Chloride intracellular channel 4) which is significantly overexpressed at all time points in SHR kidneys. CLIC4 differential expression was confirmed by immunological assays (WB, IHC and IF).

Objective

The purpose of this study is to identify novel markers associated with pathogenesis and development of hypertensive nephrosclerosis in SHR.

Physiological parameters

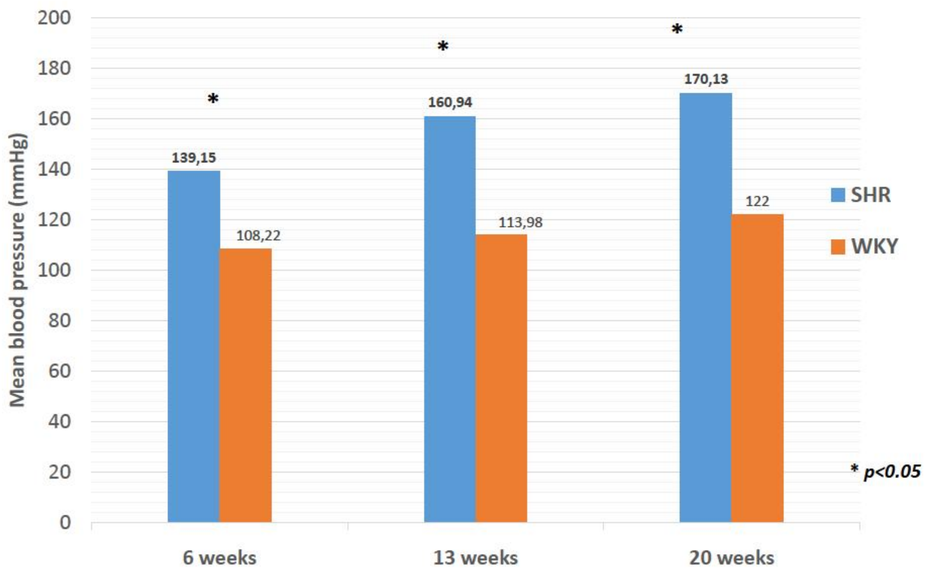
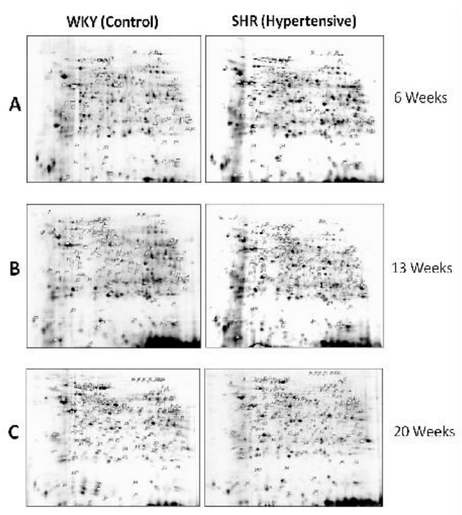


Figure 1: *From 6 weeks of age the SHR animals already had significantly higher mean BP than WKY animals marking the onset of hypertension. The SHR animals went on to reach mean BPs of approximately 170 mmHg by 13 and 20 weeks of age while the WKY animals remained normotensive (n=8 for each group)*

Proteomic analysis - Results



Differentially expressed proteins			
	Up-regulated	Down-regulated	Total
6 weeks	119	38	157
13 weeks	241	5	246
20 weeks	57	268	325

Table 2: *Number of proteins up-regulated and down-regulated with hypertension*

Figure 2: *Proteomic profiles of hypertensive (SHR) and normotensive (WKY) rats. Representative 2D gel electrophoresis from proteomic analysis of WKY and SHR rats at 6 weeks **(A)**, 13 weeks **(B)** and 20 weeks **(C)**.*

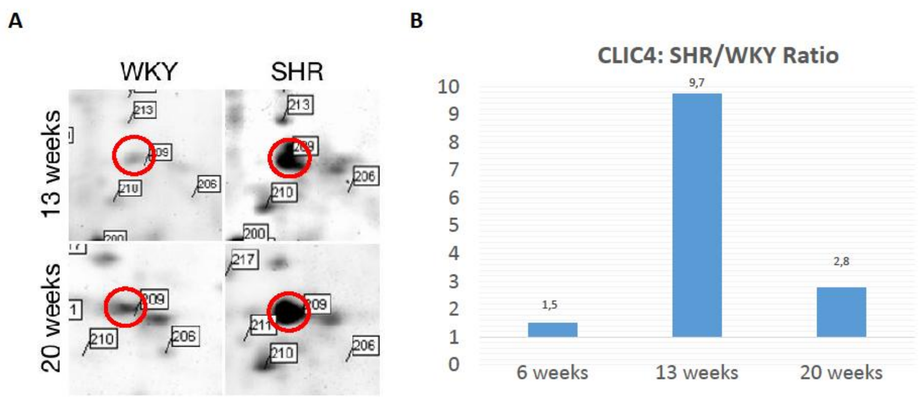


Figure 3: (A) *The most prominent finding of proteomic analysis was the the overexpression of Chloride intracellular channel protein 4 (CLIC4) in SHR animals (spot in red cycle).* ***(B)*** *Quantification of proteomic analysis*

CLIC4:

- Can insert into membranes and form poorly selective ion channels that may also transport chloride ions
- Is an early marker of hypoxia induced pulmonary hypertension
- Is a component of apoptotic response to oxidative stress

Validation of proteomic analysis

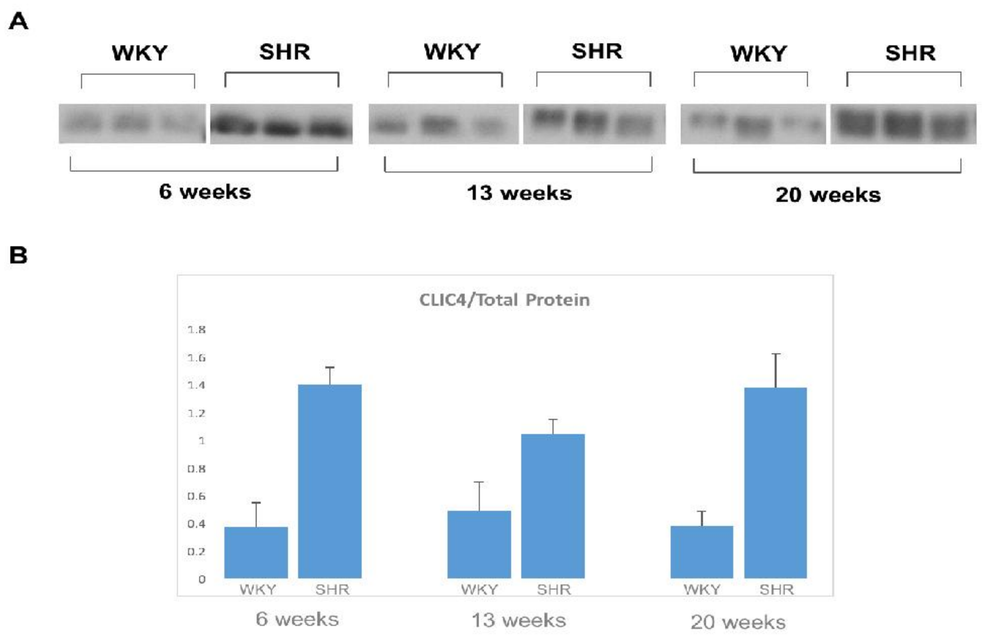


Figure 4: (A) *Western Blot analysis of CLIC4 expression in SHR and WKY rats shows that CLIC4 is up-regulated with hypertension at all time points of age.* ***(B)*** *Quantification data of the protein bands following normalization according to total protein content (p<0.05).*

Localization of CLIC4

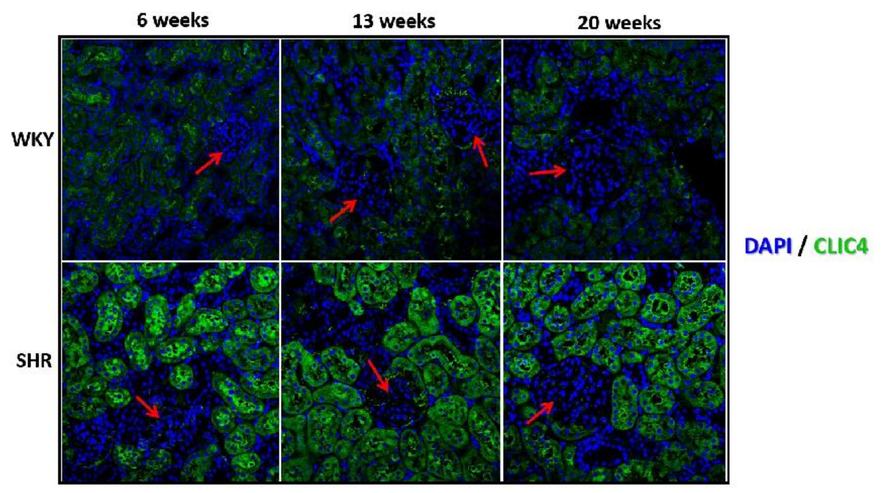


Figure 5: *Representative IF images of CLIC4 staining on WKY and SHR animals show significant up-regulation of CLIC4 in hypertensive rats. CLIC4 is mainly expressed in the cortex and up-regulated in the proximal tubules of SHR animals. Glomeruli (red arrows) show no significant expression of CLIC4 in both SHR and WKY animals*

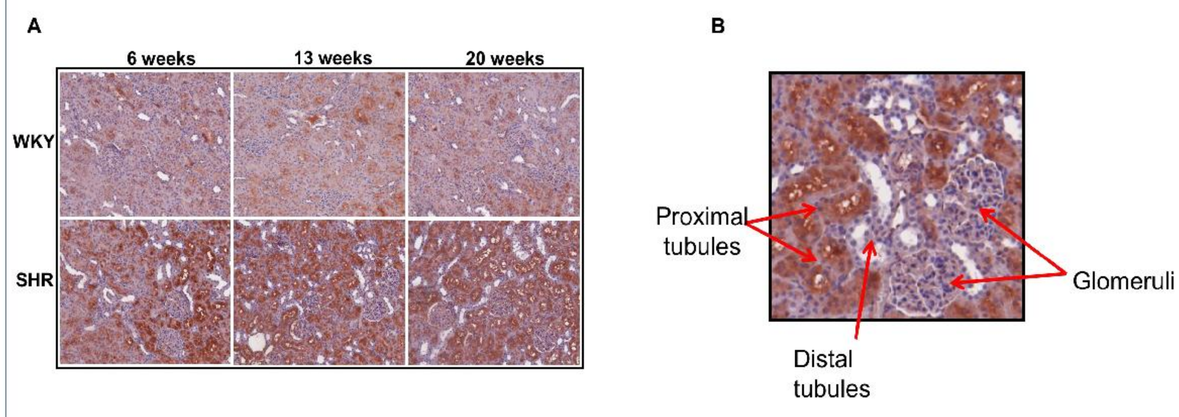


Figure 6: (A) *Representative expression patterns of CLIC4 in WKY and SHR animals.* ***(B)*** *In hypertensive animals CLIC4 is mainly expressed in the proximal tubules of renal parenchyma. The distal tubules and glomeruli show no significant staining.*

Conclusions

- Proteomic analysis of SHR and WKY kidney tissue yielded a significant number of differentially expressed proteins
- CLIC4 was found to be overexpressed in the proximal tubules of SHR animals from the early stage of hypertension and maybe involved in the pathogenesis of hypertension and the development of Hypertensive Nephrosclerosis.

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