Mechanism of Apoptosis in Development of Glomerular Damage in Nephrotic Children

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Abstract

Nephrotic syndrome is a disorder of the glomerular filtration barrier. Podocytes are key link to the filtration mechanism of the glomerular filtration barrier.

Here we report that nephrotic patients reveal a high level of podocytes loss. Moreover, this index is dependent to the level of glomerulosclerosis. Podocytes loss is associated with pro-apoptotic markers Bax and caspase-3 activation.

We conclude that primary albumin-induced glomerular damage in nephrotic children is podocytes apoptosis activated view mitochondrial pathway and caspase-3 activation. This leads to glomerulosclerosis and kidney function impairment progression.

Keywords: Nephrotic Syndrome; Apoptosis; Albumin; Toxicity; Caspase-3; Bax; Immunostaining; Podocytes

Introduction

Albuminuria is a pathological condition wherein the protein albumin is abnormally present in the urine. It is a type of proteinuria. Albumin is a major plasma protein (normally circulating in the blood); in healthy people, only trace amounts of it are present in urine, whereas larger amounts occur in the urine of patients with kidney disease [1,2].

The possibility that proteinuria may accelerate kidney disease progression to end-stage renal failure has received support from the results of increasing numbers of experimental and clinical studies. Evidence indicating that this process occurs through multiple pathways, including induction of tubular chemokine expression and complement activation that lead to inflammatory cell infiltration in the interstitium and sustained fibrogenesis, apoptosis is reviewed [2-4].

The past 20 years of research in nephrology have reported substantial information on the mechanisms by which persisting dysfunction of an individual cell cycle regulation (i.e. apoptosis) in the glomerulus and tubule is one of the main disorders leading to irreversible kidney damage [3].

Podocytes and proximal tubular cells at the glomerular-tubular junction are considered the main targets for albumin toxicity. Glomerular sclerosis is the progressive lesion beginning at the glomerular capillary wall, the site of abnormal filtration of plasma proteins. Injury is transmitted to the interstitium favoring the self-destruction of nephrons and eventually of the kidney. These processes associated with tubulointerstitial injury that are activated by ultrafiltered protein load of tubular epithelial cells [1,5].

Here we study the primary processes underlying irreversible kidney damage in children with nephrotic syndrome with main focus of apoptosis development and it's outcomes.

Materials and Methods

Patients

An examination of renal biopsies of 53 patients (aged 10 to 15 years) with nephrotic syndrome hospitalized in Pediatric Nephrology unit of the Children Clinical Hospital №7 (Kyiv, Ukraine) was done. Complex examination other than conventional methods (inspection, monitoring blood pressure, general and biochemical blood tests, determination of daily proteinuria, urinary sediment study and concentration ability of the kidneys, ultrasound of the abdomen etc.), immunohistochemical assessment of apoptosis-dependent glomerular and tubule-interstitial damage were done.

The level of kidney function impairment (stage of Chronic Kidney Disease, CKD) was assessed by the value of glomerular filtration rate (GFR). GFR was calculated by Schwartz formula.

Immunoblotting for detection of Bax, caspase-8

Proteins solubilized in Laemmli sample buffer were resolved in polyacrylamide gels by SDS-PAGE and transferred to a polyvinylidene difluoride membrane. Membranes were then blocked in 5% non-fat milk in TBS-T (136 mM NaCl, 10 mM Tris, 0.05% Tween 20) and immunoblotted using the Bax and caspase-8 Ab (Cell Signaling Technology, Danvers, MA USA) and actin mouse mAb (BD, Lexington KY, USA) for 1 h at room temperature. The actin mouse mAb were used as a loading control. After three washes with TBS-T, the membranes were incubated with secondary anti-rabbit or anti-mouse antibodies labeled with horseradish peroxidase for 1 h at room temperature. Membranes were washed three times with TBS-T. The protein bands were visualized by chemiluminescent substrate ECL. Quantitation of the protein content was done by densitometric analysis.

Detection of apoptotic podocytes

Renal tissue (3-µm sections) was deparaffinized and rehydrated prior to processing. Antigen retrieval was performed by boiling in citrate buffer for 20 minutes. After three PBS washes, the sections were incubated with blocking buffer for 1 hour. The mouse monoclonal anti-WT1 primary antibody (Santa Cruz, USA) was applied overnight at 4°C. The sections were then incubated with a secondary Alexa Fluor 488 goat anti-mouse IgG (*Invitrogen*, Grand Island, USA) for 1h at room temperature. The nuclei were counterstained with DAPI (Santa Cruz Biotechnology, Inc., California, USA. All of the samples were stained for the same length of time under identical conditions. The staining was assessed on the same day using identical gain settings. The immune-labeled cells were observed using a Zeiss LSM 510 laser scanning confocal microscope and a 40X/1.2NA water-immersion objective.

Statistics

Statistical analysis was done using the method of variation statistics (STATISTICA 6.0) and nonparametric statistical approaches (Mann-Whitney test). Results are presented as Mean ± SEM. $P < 0.05$ was considered as statistically significant.

Results

### Expression of proapoptotic factor Bax in kidney tissue of patients with nephrotic syndrome

We have analyzed the levels of expression and localization of proapoptotic factor Bax in patients with morphological variant of nephrotic syndrome - focal segmental glomerulosclerosis. Stages of FSGS were determined by level of glomerular sclerotic area. Level of sclerosis corresponding to ≤ 25% of the glomerular area was assumed as I stage of FSGS, II stage of FSGS - 25 - 50%, III stage - 50 - 75% and IV stage - 75 - 100%. Analysis of Bax expression in kidney biopsies from children with focal segmental glomerulosclerosis show the presence of high level of Bax expression in both glomerular and tubule-interstitial segments. Higher level of immune signal was recorded in glomeruli as compared to tubule-interstitial segment in FSGS I-II stages (43,57 ± 0,88 a.u. vs 24,9 ± 0,41 a.u., $P < 0.01$). When complete glomerular sclerosis presents a high level of Bax was documented in the surrounding tubule-interstitial segment (13,7 ± 0,42 a.u. vs 22,5 ± 0,65 a.u., $P < 0.01$) (Figure 1).
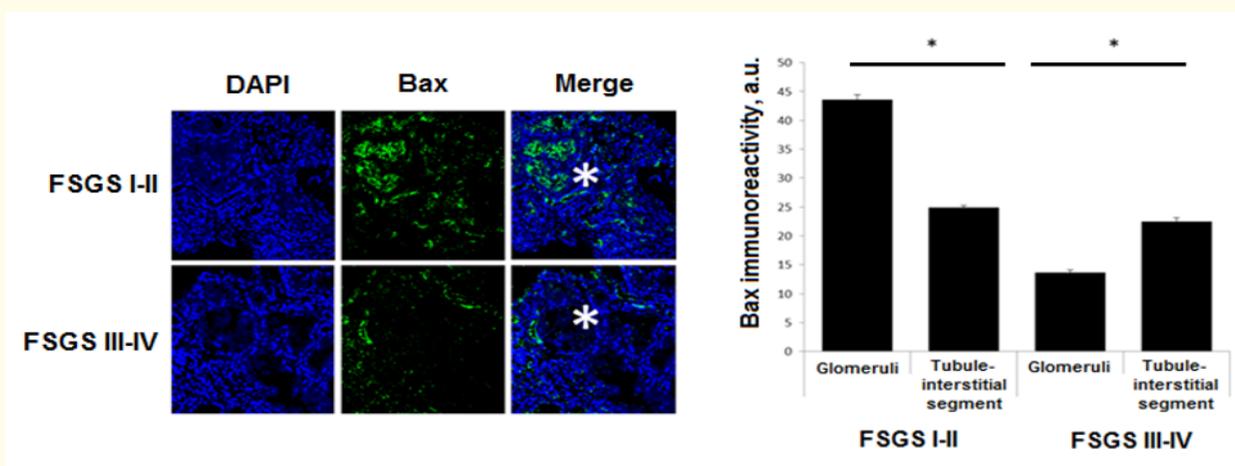
Figure 1: *Topical characteristic of the Bax expression in different stages of FSGS. DAPI – $_{visualization}$ of nuclei; Bax - Bax immune signal in kidney tissue. * - glomeruli, *p < 0,05.*

Caspase-3 levels in nephrotic children

We detected the increased level of apoptotic effector caspase-3 in all nephrotic children in its dependence on kidney function deterioration. The level of caspase-3 in children with CKD I exceeded control value by 60,3±7,5% and by 90,1 ± 9,8% in group with CKD II-III (p < 0,01 та p < 0,001, respectively) .
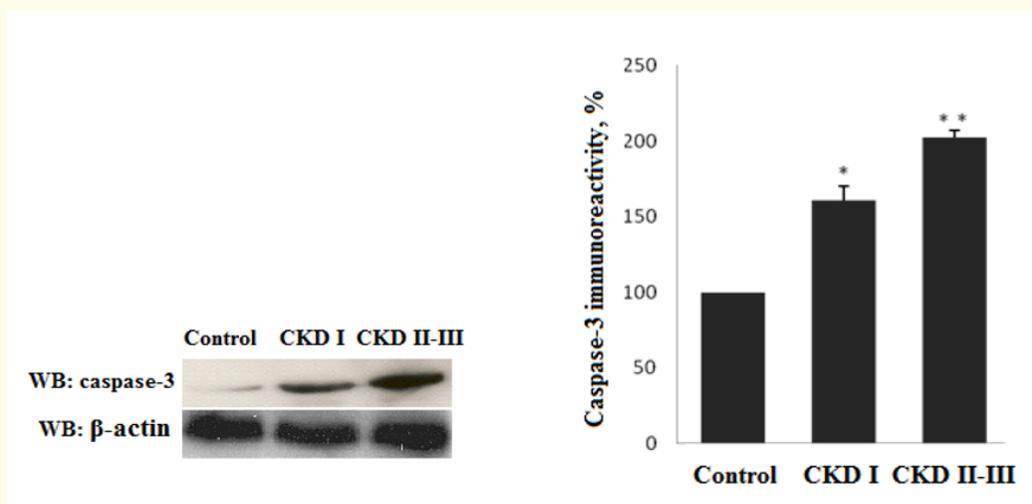
*Figure 2: Caspase-3 levels in children with nephrotic syndrome. *p <0,01, **p < 0,001.*

Levels of podocytes damage in children with nephrotic syndrome

The level of podocytes damage analyzed by counting the number of immuno-labeled podocytes/glomerulus. As a result of quantitative evaluation we found that the average number of podocytes/glomerulus is 9,4 ± 0,8 podocytes/glomerulus in nephrotic children with at the FSGS I-II stage and 3,8 ± 0,4 podocytes/glomerulus (p < 0,01) (Figure 3).
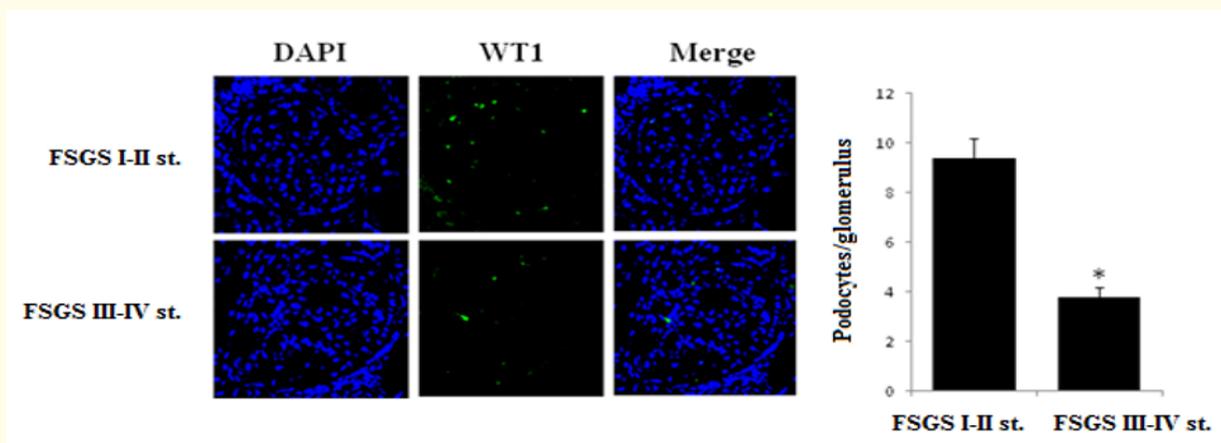
*Figure 3: Immunostaining of podocytes in nephrotic children. *p < 0,01.*

Discussion

The glomerular filtration barrier (GFB) is composed of two cell types, the capillary endothelial cells and the podocytes, separated by a specialized glomerular basement membrane. In addition, mesangial cells support the structure of the glomerulus, and parietal epithelial cells line the capsule encircling the glomerular capillaries [6-8]. Podocytes are the target of many forms of injury, including antibodies to podocyte membrane antigens. In secondary forms of FSGS, such as after loss of nephron number, hypertension, and tubulointerstitial disease, podocytes are also injured [9]. There is a growing body of experimental and clinical literature showing that podocyte number is a critical determinant for the development of glomerulosclerosis [10].

In was shown previously a decrease in podocyte number in type II diabetic Pima Indians correlated closely with those patients who had microalbuminuria, the earliest manifestation of diabetic nephropathy [11]. Moreover, they showed that the decrease in podocyte number was more pronounced in patients with more advanced nephropathy. In contrast to the decrease in podocyte number, mesangial and glomerular endothelial cell number remained normal. More recently, a similar paradigm in patients with type I diabetic nephropathy was documented [12].

Here we show that podocytes number/glomerulus is lower in patients who developed high level of glomerulosclerosis (FSGS III-IV) as compared to group with FSGS I-II. This is a strong evidence that loss of podocytes in nephrotic children is directly interferes with glomerulosclerosis.

To evaluate the mechanism responsible for podocytes loss in nephrotic children we studied the level of apoptotic markers Bax and caspase-3. Bax immunostaining shows that nephrotic children have higher level of Bax expression in glomeruli while FSGS I-II st. observes. We hypothesize that apoptosis in glomerular cells including podocytes plays key role in course of glomerular damage under permanent influence of albuminuria. In contrast, higher level of Bax expression documented in children with FSGS III-IV st. meaning that tubular-interstitial damage is crucial while glomerulosclerosis appears at high level.

It is known that development of apoptosis is due to two main pathways activation – intrinsic (mitochondrial) and extrinsic [13]. In an example of the extrinsic pathway of apoptosis, the binding of Fas ligand (FasL) to its receptor Fas leads to the cleavage and activation of caspase-8 [14]. In the intrinsic pathway of apoptosis, the BCL-2 homology region 3 (BH3)-only proteins can either sequester anti-apoptotic proteins such as BCL-2, or directly activate the pro-apoptotic multi-BH3 proteins, such as BAK and BAX. The final step in this pathway is caspase-3 activation [14,15]. Thus, our data on caspase-3 levels in nephrotic children may us able to hypothesize that intrinsic apoptotic pathway plays a role in glomeruli damage.

To summarize, podocytes are located on the outer aspect of the glomerular basement membrane, one of the functions of podocytes is to provide a tensile support to the underlying glomerular capillary loop, by opposing the hydrostatic capillary pressure [16]. Thus, if podocyte number is decreased, there are insufficient podocytes to cover that specific area of basement membrane. This is a primary predisposing factor in glomerulosclerosis development [17,18].

Conclusion

The podocytes have different functions including glomerular filtration, biosynthesis and maintenance of the glomerular capillary architecture. The clinical manifestations of podocyte dysfunction are limited proteinuria and renal insufficiency. In summary, owing to their unique and complex cellular organization and many functions, podocytes are the most vulnerable constituent of the glomerular filtration barrier. Here we show one of the possible mechanisms of the podocytes injury in nephrotc children. Further investigation in this area have big importance as a perspective area in nephrotic syndrome pathgenesis and treatment approaches.

Bibliography

1. Fogo AB. "Mechanisms of progression of chronic kidney disease". *Pediatric Nephrology* 22.12 (2007): 2011-2022.

2. Hemmelgarn BR., *et al*. "Relation between kidney function, proteinuria, and adverse outcomes". *Journal of the American Medical Association* 303.5 (2010): 423-429.

3. Favaloro B., *et al*. "Role of apoptosis in disease". *Aging* 4.5 (2012): 330-349.

4. Christman JW., *et al*. "Redox Regulation of Nuclear Factor Kappa B: Therapeutic Potential for Attenuating Inflammatory Responses". *Brain Pathology* 10.1 (2000): 153-162.

5. Ding WY and Saleem MA. "Current concepts of the podocyte in nephrotic syndrome". *Kidney Research and Clinical Practice* 31.2 (2012): 87-93.

6. Kim JH., *et al*. "Podocyte injury induces nuclear translocation of WTIP via microtubule-dependent transport". *Journal of Biological Chemistry* 285.13 (2010): 9995-10004.

7. Neal CR., *et al*. "Glomerular filtration in the subpodocyte space is highly restricted under physiological perfusion conditions". *American Journal of Physiology–Renal Physiology* 293.6 (2007): 1787-1798.

8. Peti-Peterdi J and Sipos A. "A high-powered view of the filtration barrier". *Journal of the American Society of Nephrology* 21.11 (2010): 1835-1841.

9. Assady S., *et al*. "New Insights into Podocyte Biology in Glomerular Health and Disease". *Journal of the American Society of Nephrology* 28.6 (2017): 1707-1715.

10. Wang RM., *et al*. "Swiprosin-1 Promotes Mitochondria-Dependent Apoptosis of Glomerular Podocytes via P38 MAPK Pathway in Early-Stage Diabetic Nephropathy". *Cellular Physiology and Biochemistry* 45.3 (2018): 899-916.

11. Su J., *et al*. "Evaluation of podocyte lesion in patients with diabetic nephropathy: Wilms' tumor-1 protein used as a podocyte marker". *Diabetes Research and Clinical Practice* 87.2 (2010): 167-175.

12. Lioudaki E., *et al*. "Increased Urinary Excretion of Podocyte Markers in Normoalbuminuric Patients with Diabetes". *Nephron* 131.1 (2015): 34-42.

13. Tamiru Y., *et al*. "Review on mechanisms of regulating apoptosis in animal cells". *World Journal of Biomedicine and Pharmaceutical Sciences* 2 (2017): 33-38.

14. Ma FY., *et al*. "The role of stress-activated protein kinase signaling in renal pathophysiology". *Brazilian Journal of Medical and Biological Research* 42.1 (2009): 29-37.

15. Peña-Blanco A and García-Sáez AJ. "Bax, Bak and beyond - mitochondrial performance in apoptosis". *FEBS Journal* 285.3 (2018): 416-431.

16. Sanz AB., *et al*. "Mechanism of renal apoptosis in health and disease". *Journal of the American Society of Nephrology* 19.9 (2008): 1634-1642.

17. Trimarchi H. "Podocyturia: Potential applications and current limitations". *World Journal of Nephrology* 6.5 (2017): 221-228.

18. Al Hussain T., *et al*. "Podocyte and Parietal Epithelial Cell Interactions in Health and Disease". *Advances in Anatomic Pathology* 24.1 (2017): 24-34.