Podocyte-specific NF-κB inhibition ameliorates proteinuria in adriamycin-induced nephropathy in mice

Tadashi Yoshida, Maho Yamashita, Matsuhiko Hayashi
Apheresis and Dialysis Center, School of Medicine, Keio University, Tokyo Japan

Background

Podocytes play a central role in the formation of the glomerular filtration barrier in the kidney, and their dysfunction has been shown to result in proteinuria. In the present study, we sought to determine the cell-autonomous role of NF-κB, a proinflammatory signaling, within podocytes in proteinuric kidney disease.

Methods

The IκBΔN is a truncated form of IκB and lacks two phosphorylation sites required for its degradation. Therefore, it blocks the activation of NF-κB continuously. We previously generated IκBΔN mice, which contain the IκBΔN transgene separated from a universal CAG promoter by a floxed STOP sequence (Yoshida et al., J Am Heart Assoc., 2013). By breeding Nephrin-Cre mice and IκBΔN mice, podocyte-specific IκBΔN mice were generated. Male podocyte-specific IκBΔN mice and control mice at 12 - 13 weeks of age received intravenous injection of adriamycin (ADR), and their phenotype was analyzed 14 days after the treatment.

Primary culture of murine podocytes was performed as previously described (Takemoto et al., Am J Pathol., 2002). Podocytes were extracted using Dynabeads M450, and they were treated with 0.25 μg/mL ADR or saline for 24 hours.

Results

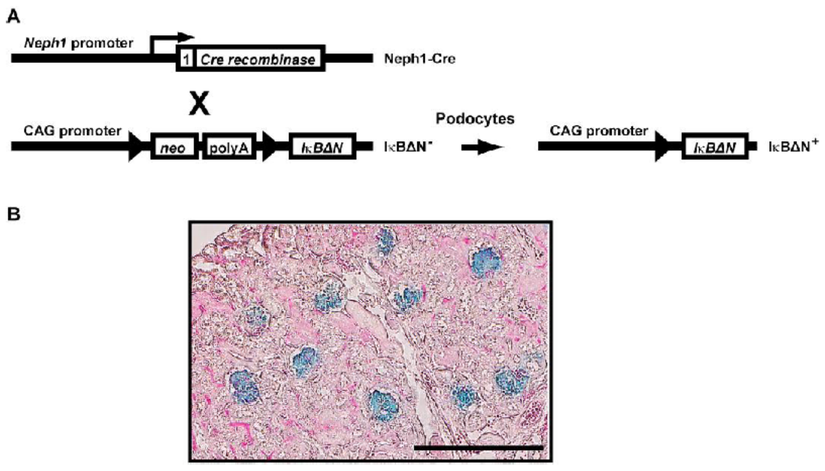

Fig 1. Podocyte-specific IκBΔN mice were generated by breeding Nephrin-Cre mice and IκBΔN mice.

(A) A schematic representation of podocyte-specific recombination of the IκBΔN transgene gene is shown. (B) Podocyte-specific LacZ expression is shown in the kidneys from mice bred by Nephrin-Cre mice and ROSA-floxed LacZ mice. Bar 200 μm.

Results

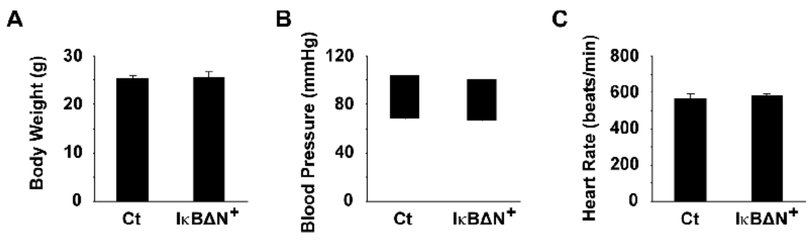

Fig 2. Podocyte-specific IκBΔN mice were phenotypically normal before adriamycin treatment.

Body weight (A), blood pressure (B), and heart rate (C) were not different between podocyte-specific IκBΔN mice and control mice.

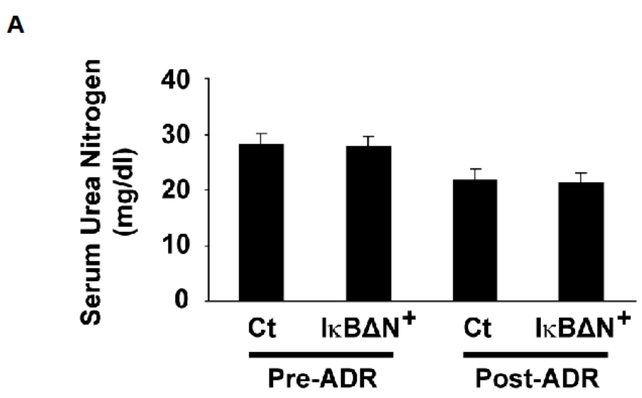

Fig 3. Podocyte-specific inhibition of NF-κB decreased albuminuria in adriamycin-induced nephropathy.

Male podocyte-specific IκBΔN mice and control mice at 12 - 13 weeks of age received intravenous injection of adriamycin (ADR), and their phenotype was analyzed 14 days after the treatment. Serum levels of urea nitrogen (A) and albuminuria (B) were measured. *P<0.05 compared with mice without the treatment. #P<0.05 compared with control mice.

Results

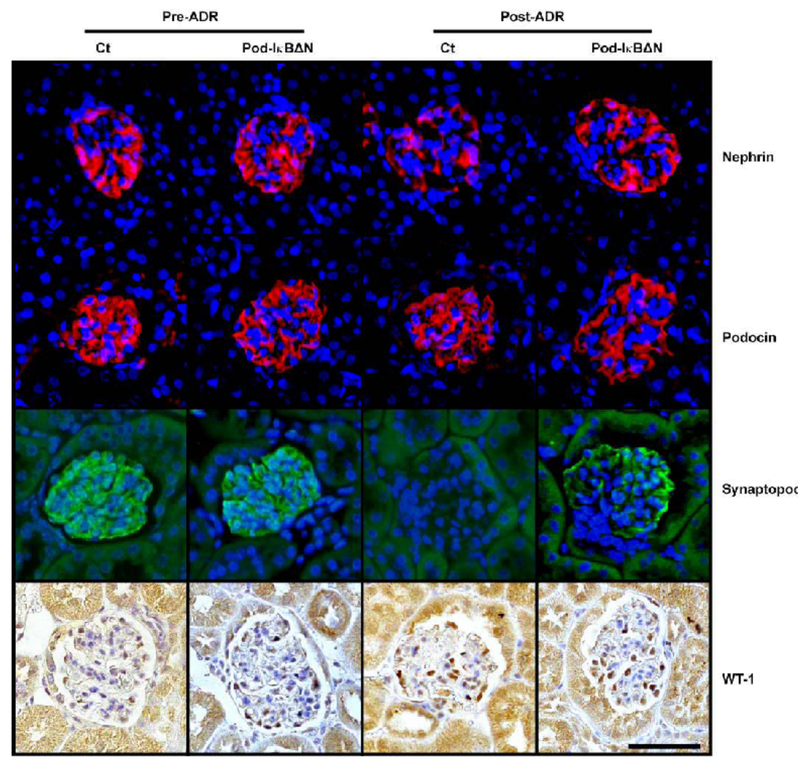

Fig 4. ADR reduced the expression of nephrin and synaptopodin in control mice, but the reduction was attenuated in podocyte-specific IκBΔN mice.

Representative pictures of immunofluorescence/immunohistochemical studies for nephrin, podocin, synaptopodin, and WT-1 are shown. Bar: 50 μm.

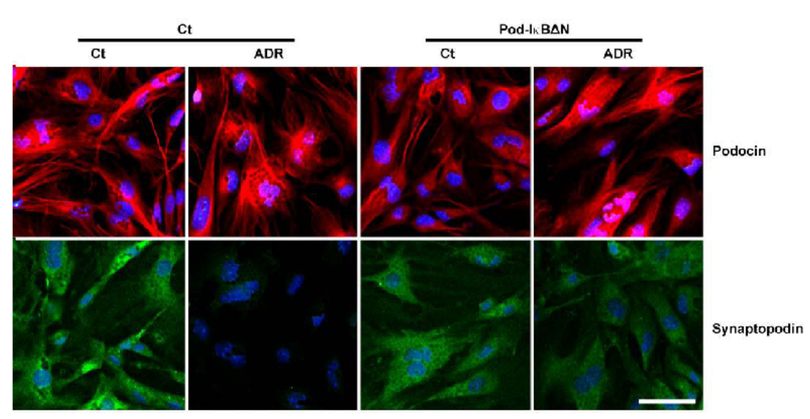

Fig 5. ADR decreased expression of synaptopodin in cultured podocytes derived from control mice, but not from podocyte-specific IκBΔN mice.

Primary cultures of podocytes were performed using podocyte-specific IκBΔN mice and control mice, respectively, and they were treated with ADR or saline for 24 hours. Expression of podocin and synaptopodin was examined by immunofluorescence studies. Bar: 50 μm.

Conclusions

Because nephrin and synaptopodin are essential for the maintenance of the slit diaphragm in podocytes, these results suggest that proteinuria in adriamycin-induced nephropathy is caused by the reduction in expression of these proteins. The results also suggest that the NF-κB signaling in podocytes cell-autonomously contributes to proteinuria through the regulation of these proteins.

Ref: Yamashita et al., Clin Exp Nephrol, 2016, in press.

53 ERA
100--MP
Renal pathology. Experimental and clinical.
Tadashi Yoshida
DOI: 10.3252/pso.eu.53era.2016
ePosters supported by F. Hoffmann- La Roche Ltd.
53rd CONGRESS ERA-EDTA VIENNA AUSTRIA MAY 21st-24th, 2016
Held jointly with Österreichische Gesellschaft für Nephrologie
PosterSessionOnline.com