NFκBiz protein downregulation in acute kidney injury: Modulation of inflammation and survival in tubular cells.

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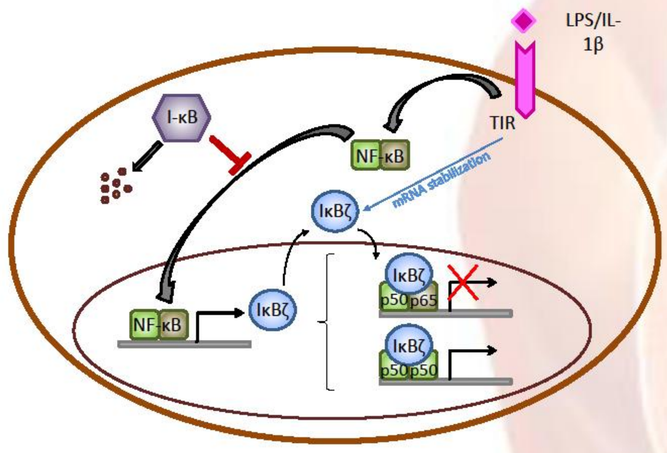
INTRODUCTION

Acute kidney injury (AKI) and chronic kidney disease (CKD) are the most severe forms of kidney disease. AKI is characterized by a sudden loss of renal function. AKI patients present increased short- and long-term mortality and risk of CKD progression. Both AKI and CKD are associated with interstitial inflammation and tubular injury. A wide range of stimuli relevant to kidney injury activate NF-κB, resulting in the transcriptional regulation of hundreds of genes related to inflammation, cell proliferation, apoptosis, etc. IκBζ is encoded by the NF-κBiz gene and belongs to the IκB family of NF-κB regulatory proteins. Unlike the classical IκB family members, IκBζ does not inhibit NF-κB translocation to the nucleus but regulates NF-κB DNA binding and transactivation. IκBζ inhibits the transcriptional activity of the NF-κB p50/p65 heterodimer but forms stable ternary complexes with the NF-κB p50 homodimer and κB DNA, promoting gene transcription by p50 homodimers.



OBJECTIVE

The aim of this work is to characterize the expression and function of IκBζ, an NFκB regulator, in kidney disease.

CONCISE METHODS AND MATERIALS

Cells and reagents

MCTs mouse tubular epithelial cells were grown on RPMI 1640 medium with 10% heat inactivated fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, in 5% carbon dioxide at 37°C. For experiments cells were rested in serum-free media 24 hours prior to the addition of stimuli. Cells were stimulated with IL-1β (1ng/ml), LPS Escherichia coli 0127:B8 (100ng/ml), TWEAK (100ng/ml), interferon-γ (INFγ) (30 UI/ml) and TNFα (30ng/ml).

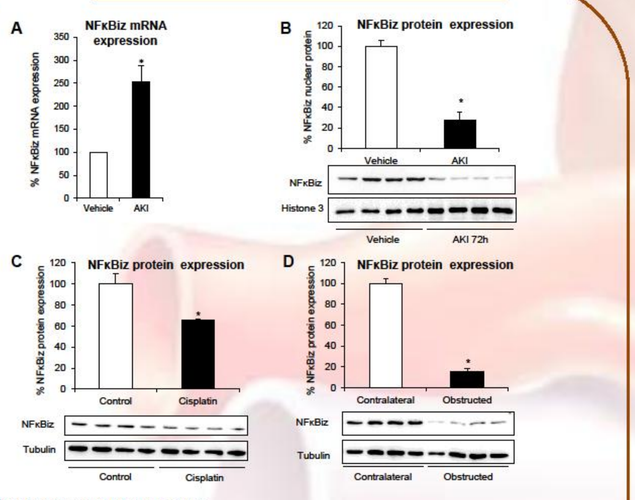
Experimental acute kidney injury

C57/BL6 mice (12- to 14-week-old) received a single i.p. injection of folic acid 250 mg/kg in sodium bicarbonate 0.3 mol/L (AKI) or vehicle alone (controls) and were sacrificed 1, 2, 3, 7 and 14 days later. Kidneys were cold saline perfused in situ before removal. One kidney from each mouse was fixed in buffered formalin, embedded in paraffin and stained with hematoxylin-eosin or used for immunohistochemistry. The other kidney was snap-frozen in liquid nitrogen for protein and RNA studies. The study was approved by the IIS-FJD animal ethics committee and followed Directive 2010/63/EU on the protection of animals used for scientific purposes.

Cell death assays

For assessment of apoptosis cells were rested in serum-free media for 24 hours and then stimulated for 24 hours. A lethal cytokine cocktail (TWEAK/TNFα/INFγ) was used as a positive control. For morphological characterization of apoptosis cells were fixed with formalin and nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) to observe the typical morphological changes. For assessment of hypodiploid apoptotic cells, adherent cells were pooled with spontaneously detached cells and incubated in 100 µg/mL propidium iodide (PI), 0.05% NP-40, 10 µg/mL RNAse A in PBS at 4°C for >3 hours. This assay permeabilizes the cells, allowing PI to stain both alive and dead cells. The percentage of apoptotic cells with decreased DNA staining (hypodiploid cells) was counted by flow cytometry using BD CellQuest Software.

IκBζ expression in experimental AKI

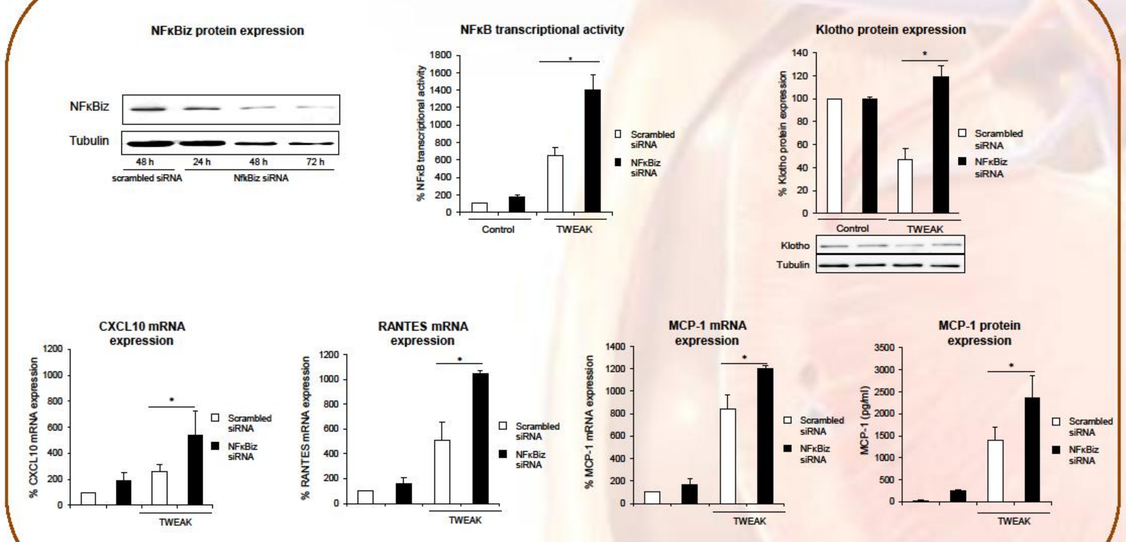


IκBζ is expressed by kidney tubules.
A) Transcriptomics analysis of NFκBiz mRNA expression in kidney samples from experimental AKI induced by a folic acid overdose or vehicle control kidneys. Mean±SEM of 3 mice per group at day 1.
B) Representative Western blot of NFκBiz in nuclear renal tissue lysates. Mean±SEM of 5 mice per group.
C) Cisplatin-induced AKI. Representative Western blot and quantification of NFκBiz in renal tissue lysates. Mean±SEM of 5 mice per group.
D) UUO. Representative Western blot and quantification of NFκBiz in renal tissue lysates at day 5. Mean±SEM of 8 mice per group.

Cytokines and LPS increase IκBζ expression in cultured tubular cells

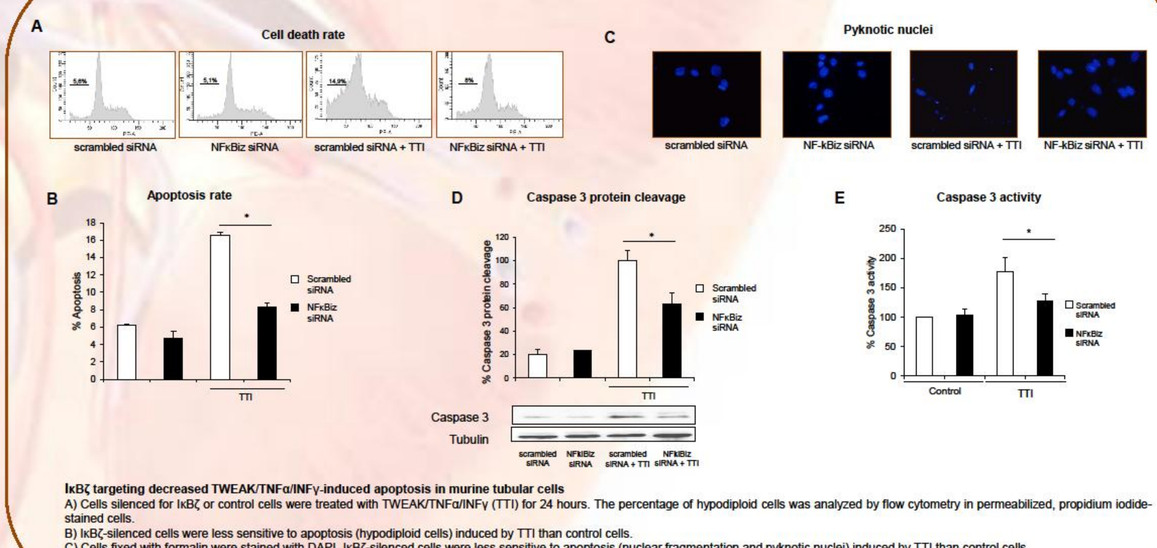
Cytokines and LPS upregulate IκBζ expression in murine tubular cells
Murine tubular cells were serum-depleted for 24 hours and then treated with TWEAK, LPS, IL1β or a combination of TWEAK/TNFα/IFNγ. IκBζ expression was studied both, at transcriptional (A-D) and translational level (E-I). Mean ±SEM of 3 independent experiments. Representative western blots are shown.

IκBζ silencing results in increased NFκB transcriptional activity, Klotho expression and inflammatory gene expression in response to TWEAK



IκBζ targeting increased NFκB transcriptional activity, klotho expression and the pro-inflammatory action of TWEAK in murine tubular cells.
Cells were silenced for NFκBiz and 72 hours later treated with 100 ng/ml TWEAK for 3 hours. NFκB transcriptional activity was assessed by use of a reporter gene assay. Klotho protein expression was measured by Western blot. CXCL10, RANTES and MCP-1 mRNA levels were measured.

IκBζ silencing decreases apoptosis in cultured tubular cells



IκBζ targeting decreased TWEAK/TNFα/INFγ-induced apoptosis in murine tubular cells
A) Cells silenced for IκBζ or control cells were treated with TWEAK/TNFα/INFγ (TTI) for 24 hours. The percentage of hypodiploid cells was analyzed by flow cytometry in permeabilized, propidium iodide-stained cells.
B) IκBζ-silenced cells were less sensitive to apoptosis (hypodiploid cells) induced by TTI than control cells.
C) Cells fixed with formalin were stained with DAPI. IκBζ-silenced cells were less sensitive to apoptosis (nuclear fragmentation and pyknotic nuclei) induced by TTI than control cells.
D) Caspase-3 cleavage was assessed by Western blot. There was less caspase-3 cleavage in IκBζ-silenced cells stimulated with TTI than in control cells.
E) Caspase-3 activity assay confirmed a decrease in caspase-3 activity in NFκBiz silenced cells stimulated with TTI when compared to control cells

CONCLUSSIONS

- ✓ **IκBζ is constitutively expressed in some cell types in the kidney cortex, specifically in tubular epithelial cells, and its expression involves complexe post-transcriptional and post-translational mechanisms**
- ✓ **IκBζ suppresses chemokine production in tubular cells in a pro-inflammatory environment**
- ✓ **IκBζ promotes cytokine-induced cell death**
- ✓ **Constitutively expressed or low level IκBζ suppresses klotho expression in tubular cells**
- ✓ **The observation that IκBζ inhibites the pro-inflammatory action of NF-κB-activating cytokines while promoting cytokine-induced cell death postulates IκBζ as a therapeutic target to limit inflammation in kidney disease**

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