INCREASED OR REDUCED PODOCYTE ZHX2 EXPRESSION EXACERBATES FOCAL SEGMENTAL GLOMERULOSCLEROSIS

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INTRODUCTION

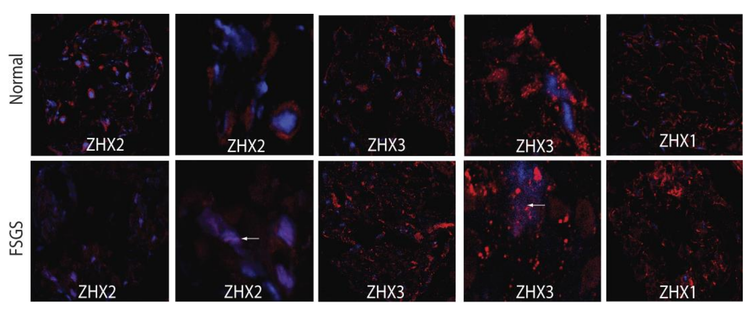
Zinc fingers and homeoboxes (ZHX) transcriptional factor family members, major regulators of podocyte gene expression, are tethered to the cell membrane as heterodimers and homodimers. ZHX2-ZHX1 heterodimers are mostly present in the podocyte body, and ZHX2-ZHX3 at the slit diaphragm. Changes in overall ZHX2 expression leads to loss of heterodimerization, that promotes nuclear entry and changes in gene expression.

METHODS

Human kidney biopsies from focal glomerular sclerosis (FSGS) were stained for ZHX proteins to assess cellular localization and changes in protein expression during disease.
To study loss of heterodimerization due to low ZHX2 expression, Balb/cJ mice (low ZHX2 content) and Balb/c mice (normal ZHX2 content) were treated with Adriamycin (ADR) as a model of FSGS.
To study loss of heterodimerization due to ZHX2 overexpression, podocyte-specific ZHX2 transgenic rats were generated using the human NPHS2 promoter. Following baseline characterization, we induced Adriamycin nephrosis (ADR)
Also, ZHX2 transgenic rats were backcrossed 10 generations into the Buffalo/Mna background, another model of FSGS.
mRNA ZHX2 expression was assessed by Real-time PCR. For urine collection, rats and mice were housed in metabolic cages in fasting during 18h. Total urine protein was measured using Bradford-based methods and albuminuria was assessed by ELISA.

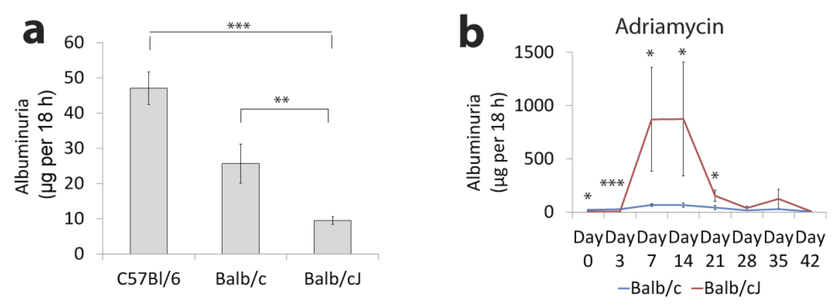
RESULTS

1. ZHX2 expression and cellular localization in human kidney biopsies is different in FSGS compared with control patients



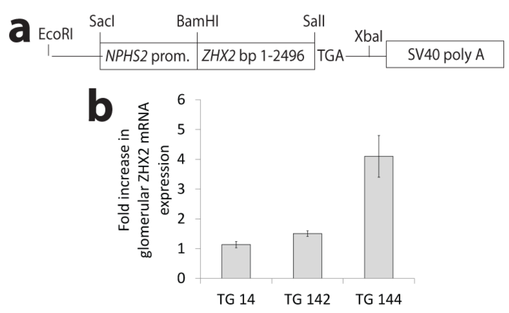
Glomerular ZHX family proteins expression in human kidney biopsies from FSGS patients. Overall ZHX2 protein expression in FSGS was either increased or decreased, often with major redistribution of ZHX2 and/or ZHX3 to the podocyte nucleus.

2. ZHX2 deficient mice developed higher albuminuria after ADR treatment



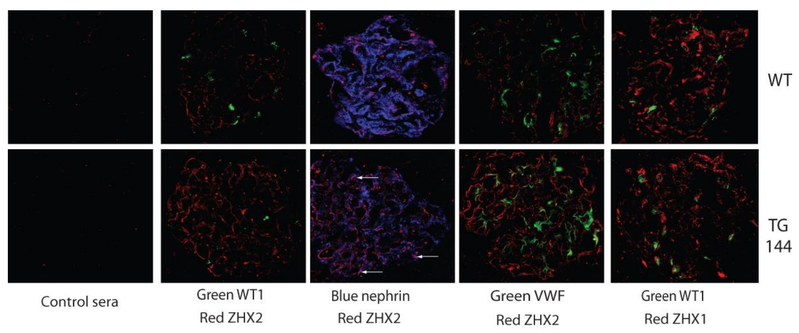
Albuminuria in Balb/c and Balb/cJ mice before and after ADR treatment. a. Balb/cJ mice (with lower ZHX2 expression) has lower albuminuria than Balb/c (normal ZHX2 expression) at baseline. b. When treated with ADR, Balb/cJ mice developed higher albuminuria levels at day 14 after treatment (873.53 ± 413.46 µg per 18 h) compared with Balb/c (65.64 ± 18.91 µg per 18 h) ($p<0.05$)

3. Glomerular ZHX2 mRNA expression is higher in ZHX2 podocyte-specific transgenic rats



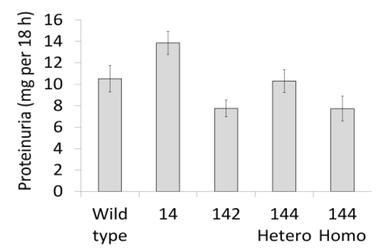
a. To generate ZHX2 podocyte-specific transgenic rats, NPHS2 promoter was cloned into pTREtight-MP between SacI and BamHI sites and rat Zhx2, excluding the sequence for the last 4 amino acids, and including a new stop codon, was cloned between BamHI and Sal. b. Three founder lines of ZHX2 podocyte-specific transgenic rats were characterized (TG 14, TG 142, TG 144). Glomerular RNA expression of ZHX2 in heterozygous rats showed a fold-increase of 1.13 ± 0.10 in TG 14, 1.50 ± 0.09 in TG 142 and 4.09 ± 0.69 in TG 144.

4. TG 144 rats revealed increase expression of ZHX2 in podocyte cell membrane localization



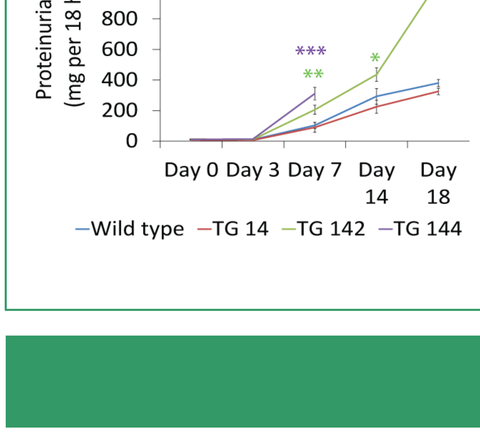
ZHX2 is elevated in 144 TG rats compared with Wild type. ZHX2 is expressed in podocyte membrane and slit diaphragm (co-localization with nephrin indicated with white arrows). There is no ZHX2 expression in endothelium. Expression of ZHX1 is unchanged in TG 144 rats compare with Wild type.

5. None of the ZHX2 transgenic rat lines had proteinuria at baseline



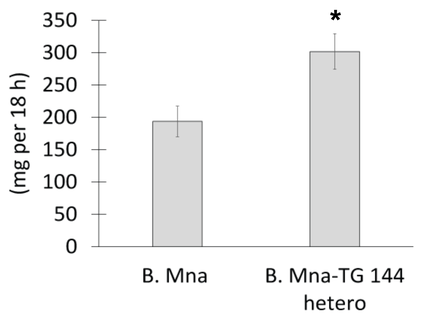
Proteinuria in ZHX2 transgenic rats at baseline. 18 hours urine proteinuria from Wild type and podocyte-specific transgenic rats 14, 142 and 144 3-months male rats at baseline. None of the ZHX2 transgenic rat lines had proteinuria at baseline.

6. When compared with Sprague Dawley rat, ZHX2 TG rats had more proteinuria and more severe glomerular disease than controls after Adriamycin treatment.



Proteinuria from Wild type and podocyte-specific transgenic rats 14, 142 and 144 4 months old male rats after Adriamycin treatment. One-single injection of 7.5 mg/kg of Doxorubicin hydrochloride (Adriamycin) were administrated intravenously. 18 hours urine samples were collected at day 3, 7, 14 and 18 after treatment. When compared with WT Sprague Dawley rat, proteinuria in Adriamycin nephrosis was most severe at day 7 after treatment in TG 144 with 310.4 ± 41.5 mg/ml in 18 h ($p<0.01$) and was associated with more mortality, more severe in TG 142 with 204.8 ± 29.95 mg/ml in 18 h ($p<0.01$) and indistinguishable from control (102.6 ± 19.5 mg in 18 h) in TG 14 (89.1 ±31.95 mg in 18 h).

7. Backcross of the ZHX2 transgene into the Buff/Mna rat background was associated with more proteinuria



Proteinuria in TG 144 backcrossed with Buff/Mna rats. TG 144 were backcrossed into the Buff/Mna rat background, a model of FGSG, for 10 generations. 18 hours urine were collected from 8 month-old Wild type Buff/Mna and TG 144-Buff/Mna male rats. TG 144-Buff/Mna rats had more proteinuria (301.9 ± 27.4 mg in 18 h) than non-transgenic Buff/Mna (193.8 ±23.7 mg in 18 h) ($p<0.05$).

CONCLUSIONS

Loss of heterodimerization caused by overexpression or deficiency of ZHX2 in podocytes worsens the development of FSGS. These findings suggest the importance of optimal ZHX2 expression levels in avoiding relapse or worsening of FSGS.

REFERENCES

1. Liu G, Clement LC, Kanwar YS, Avila-Casado C, Chugh SS. ZHX proteins regulate podocyte gene expression during the development of nephrotic syndrome. J Biol Chem. 2006 Dec 22;281(51):39681-92.
2. Clement LC, Liu G, Perez-Torres I, Kanwar YS, Avila-Casado C, Chugh SS. Early changes in gene expression that influence the course of primary glomerular disease. Kidney Int. 2007 Aug;72(3):337-47.

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