THE ROLE OF GATA3 IN RENIN CELLS IN KIDNEY DEVELOPMENT AND DURING ADAPTATION TO PHYSIOLOGICAL STRESS

Irina V. Grigorieva, Elena F. Grigorieva, Brigitte Langer, Renate Kain, Andrew Rees

Medical University of Vienna, Clinical Institute of Pathology, Währinger Gürtel 18-20, 1090 Vienna, Austria

Introduction and Objective

- GATA3 is a dual-zinc finger transcription factor that regulates developmental gene expression in a variety of tissues[1].
- In the kidney, GATA3 is essential for ureteric bud branching and its deficiency in mice causes renal agenesis[2,3].
- In man, autosomal dominant *GATA3* mutations can cause renal dysplasia as part of the hypoparathyroidism-deafness-renal dysplasia (HDR) syndrome[4].
- We have recently shown that GATA3 is expressed in all descendant cells of the renal forkhead box d1 lineage stromal cells including renin-producing cells.
- **Objective:** To analyse the role of GATA3 in renin-producing cells and its response to fluid and electrolyte stress.

Methods

- *Immunohistology:* Expression pattern of GATA3 was assessed in embryonic and adult mouse and human kidneys by immunohistochemical, immunofluorescence and immunoelectron microscopy using specific antibodies to GATA3, renin, alpha-smooth muscle actin (α-SMA), and platelet-derived growth factor receptor beta (PDGFR-β).
- *Renin-angiotensin system (RAS) stimulation:* 8-12 week-old C57BL/6 mice were fed a diet low in sodium (0.001%) plus enalapril (ACE inhibitor) in drinking water or a normal salt (0.6%) no enalapril diet. After 10 days kidneys were excised for immunohistology or RNA isolation.
- *In vitro studies*: mouse As4.1 clonal cell line, with endogenous renin production, was treated with or without TNF-α (10 ng/ml) for 24 hrs before harvesting cells for RNA or cell extract preparation for qRT-PCR and Western blot analysis, respectively.
- *Chromatin immunoprecipitation (ChIP):* chromatin from As4.1 cells was inculabed with 3 different GATA3 antibodies or IgG and quantitative PCR was perfomed on immunopecipitated DNA using primers for GATA3 motif-containing sequences in the *Ren1c* 5' upstream region.

Results

- GATA3 is co-expressed with the aspartyl-protease renin in juxtaglomerular (JG) cells in adult human and mouse kidneys and in the developing arterioles in the embryonic kidneys (Fig. 1).

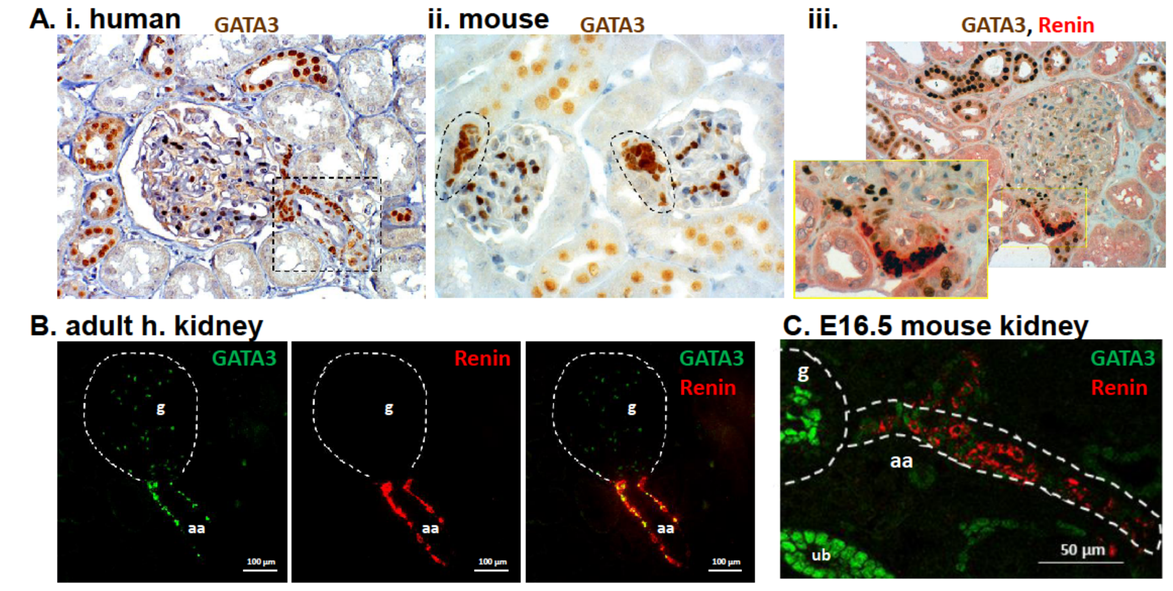

Figure 1. Expression of GATA3 in renin-producing cells in mouse and human kidneys. A. Immunohistochemical detection of GATA3 (brown) in adult (i) human kidneys and (ii) mouse kidneys showing expression of GATA3 in JG cells (*outlined*). **iii.** Co-immunohistochemical detection of renin expression (red) and GATA3 (brown) in sections of adult human kidneys. Boxed region shows magnification of the JG region. **B.** Co-immunofluorescence with anti-GATA3 (*green*), anti-renin (*red*) antibodies on adult human kidney sections showing co-localisation (*yellow*) of GATA3 with renin in JG cells of the afferent arteriole (aa). g – glomerulus. **C.** Co-immunofluorescence with anti-GATA3 (*green*), anti-Renin (*red*) antibodies on embryonic (E) 16.5 mouse kidneys.

- In the developing kidneys GATA3 is expressed in vascular smooth muscle cells (VSMCs) that co-express α-SMA and PDGFR-β, before renin expression is detected, and continues to do so in the renal vasculature of mature kidneys, suggesting a role in renal vessel formation (Fig. 2).

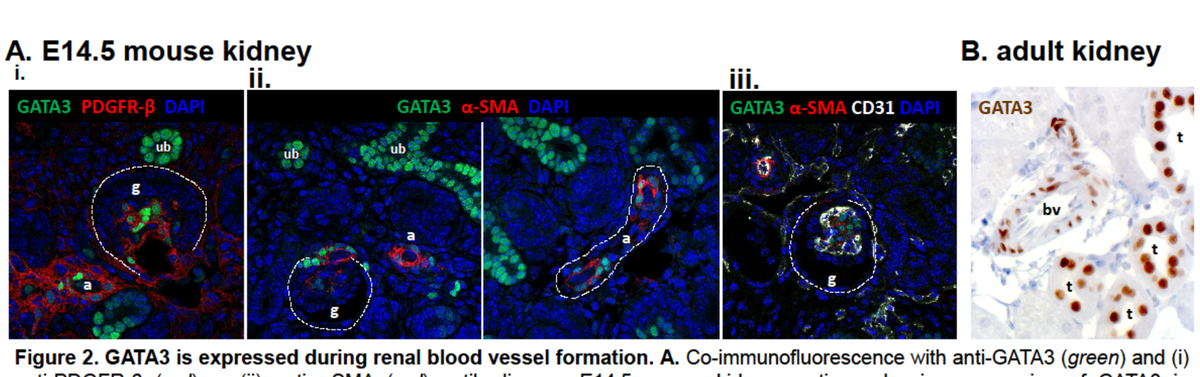

Figure 2. GATA3 is expressed during renal blood vessel formation. A. Co-immunofluorescence with anti-GATA3 (*green*) and (i) anti-PDGFR-β (*red*) or (ii) anti-α-SMA (*red*) antibodies on E14.5 mouse kidney sections showing expression of GATA3 in developing arterioles (a); (iii) triple co-localisation with endothelial marker CD31 revealed that GATA3 is expressed in VSMCs and not endothelial cells. g – glomerulus, ub – ureteric bud. **B.** Immunohistochemical detection of GATA3 (brown) in sections of adult mouse kidneys showing that GATA3 expression is maintained in mature blood vessels (bv). GATA3 is also expressed by epithelial cells of the distal and connecting tubules (t), as well as collecting ducts.

- RAS stimulation by salt deprivation and enalapril treatment in 8-week old mice induced strong nuclear expression of GATA3 in afferent arteriolar VSMCs that acquired renin expression (Fig. 3).
- Striking localisation of GATA3 outside of the nuclei in the cytoplasm of JG cells and afferent arteriolar VSMCs was observed in mice fed a normal salt diet (Fig. 3A)
- Immunoelectron microscopy confirmed the extra-nuclear localisation of GATA3 in JG cells in human kidneys and identified it in renin-containing secretory vesicles (Fig. 3B).

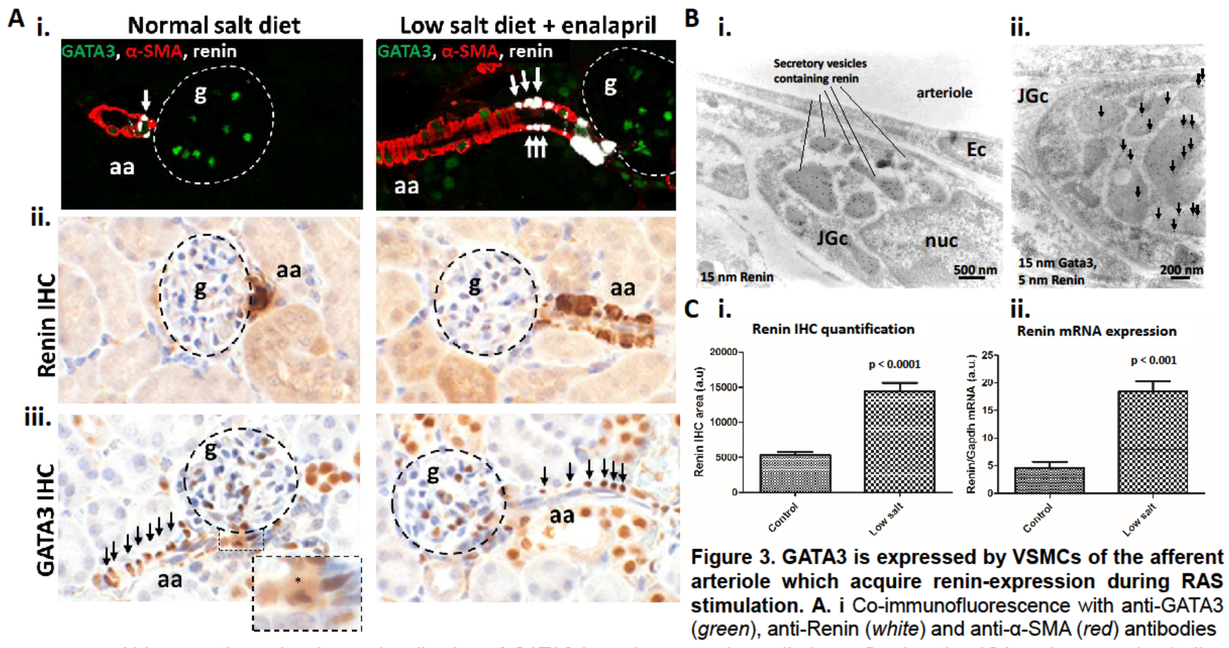

Figure 3. GATA3 is expressed by VSMCs of the afferent arteriole which acquire renin-expression during RAS stimulation. A. i Co-immunofluorescence with anti-GATA3 (*green*), anti-Renin (*white*) and anti-α-SMA (*red*) antibodies on mouse kidney sections showing co-localisation of GATA3 in renin-expressing cells is confined to the JGA under normal salt diet, and in VSMCs of the afferent arteriole (aa, *arrows*) that have the capability to transform to renin-secreting cells under RAS stimulation. **ii.** IHC detection of renin expression (brown) showing extension of the renin immunoreactivity along the aa during RAS stimulation. **iii.** IHC detection of GATA3 (brown) showing expression in JG cells and VSMCs of the aa that transform to renin-secreting cells during salt deprivation. Boxed region is magnification of the JG cells region showing cytoplasmic localisation of GATA3. **B. i.** Immunoelectron microscopy on 70 nm sections of JG cells in human kidneys with anti-Renin antibody and gold particle (15 nm) labelling showing localisation of renin in electron dense secretory vesicles. nuc – nucleus. Ec – endothelial cell. **ii.** Co-labelling with anti-GATA3 and anti-Renin antibodies and secondary antibodies coupled to 15 nm and 5 nm gold particles to detect expression of GATA3 (*arrows*) and renin, respectively. **C. i.** Quantification of renin IHC signal in kidney sections showing that the diet regime results in >50% increase in renin immunoreactivity, n=~20 aa regions/mouse in 4mice/group. **ii.** Quantification of renin mRNA expression in kidneys showing the diet regime results ~75% increase in renin expression levels. n=6 mice/group.

- Expression of GATA3 is retained *in vitro* in the renin-secreting As4.1 cell line as shown by RT-PCR and Western blot analysis (Fig. 4). GATA3 protein in these cells localised predominantly to the nuclei.
- To determine whether GATA3 has a role in regulating renin (*Ren1c*) gene expression, As4.1 cells were treated with TNF-α, a known inhibitor of renin expression and this induced a 5-fold reduction in GATA3 expression to near undetectable levels. Concomitantly, *Ren1c* expression was down-regulated suggesting that GATA3 might regulate *Ren1c* expression.

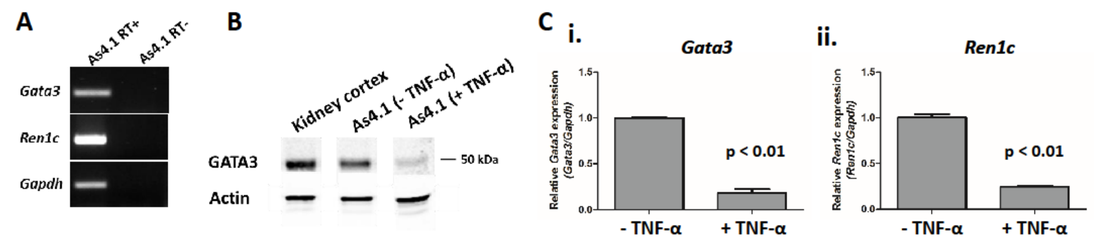

Figure 4. Expression of GATA3 is retained *in vitro*. A. Reverse transcriptase (RT)-PCR analysis showing expression of *Gata3* in the renin-expressing As4.1 cell line. **B.** Western blot analysis using cell extracts from mouse kidney cortex (control) and As4.1 cells treated with or without TNF-α. **C.** Quantitative (q) RT-PCR analysis on As4.1 cells treated with or without TNF-α to detect expression of (i) *Gata3* and (ii) *Ren1c*.

- Putative GATA3 binding motifs are present in the promoter and enhancer elements of the *Ren1c* gene (Fig. 5A). ChIP with anti-GATA3 antibodies and chromatin prepared from As4.1 cells revealed enrichment of the *Ren1c* enhancer DNA sequences containing GATA3 binding motifs (Fig. 5B).
- Two GATA motifs occupied by GATA3 were found to flank a CRE-response element[5] in the distal enhancer regulatory region of *Ren1c.*

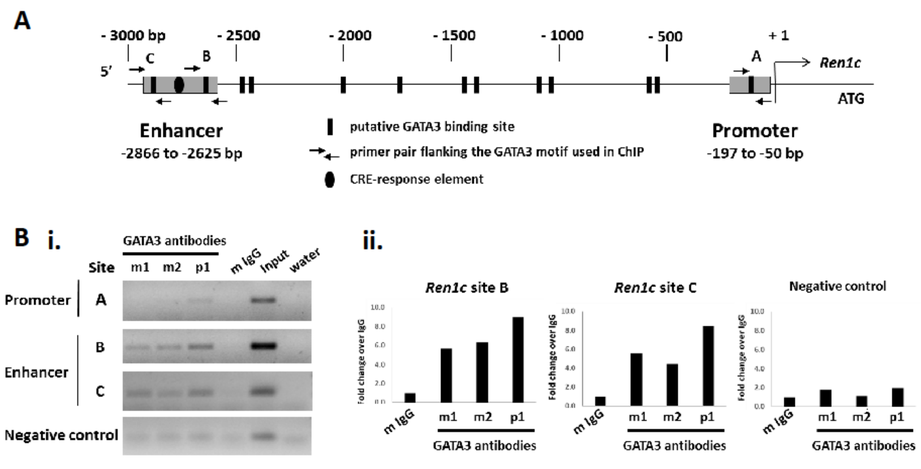

Figure 5. A. Analysis of *Ren1c* 5' upstream sequence reveals putative GATA3 motifs located within the promoter, enhancer and spacer regions. **B. i.** PCR analysis of DNA sequences immunoprecipitated from As4.1 chromatin using 3 different GATA3 antibodies (m1/m2-mouse monoclonal, p2 - goat polyclonal) revealed that GATA motifs B and C, located within the *Ren1c* enhancer, are enriched by all 3 GATA3 antibodies, while GATA motif A shows weak enrichment and no specific enrichment was detected with negative control loci. **ii.** qPCR analysis showing 8-fold enrichment of DNA sequences containing GATA motifs B and C with GATA3 antibodies compared to IgG.

Conclusions

Our studies reveal a previously undescribed role for GATA3 in JG cells which produce renin in embryonic and adult kidneys and by cells of the afferent arterioles which acquire renin expression during stimulation of RAS. *In vitro* studies reveal that GATA3 may directly regulate expression of the renin gene by binding to the *Ren1c* enhancer.

References

1. Pandolfi, P.P., et al., *Targeted disruption of the GATA3 gene causes severe abnormalities in the nervous system and in fetal liver haematopoiesis.* Nat Genet, 1995. 11(1): p. 40-4.
2. Grote, D., et al., *Pax 2/8-regulated Gata 3 expression is necessary for morphogenesis and guidance of the nephric duct in the developing kidney.* Development, 2006. 133(1): p. 53-61.
3. Grote, D., et al., *Gata3 acts downstream of beta-catenin signaling to prevent ectopic metanephric kidney induction.* PLoS Genet, 2008. 4(12): p. e1000316.
4. Van Esch, H., et al., *GATA3 haplo-insufficiency causes human HDR syndrome.* Nature, 2000. 406(6794): p. 419-22.
5. Castrop H et. al., *Physiology of Kidney Renin.* Physiol Rev. 2010. 90(2): p.607-73.

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Irina Grigorieva
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