Compartmental Differences within the COL3A1 Network in Proteinuric Kidney Disease; Informing Drug Activity Using the Jaccard-Tanimoto Index

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Background

Glomerular accumulation of collagen type III is the predominant finding in collagenofibrotic glomerulopathy, also known as collagen type III glomerulopathy, and nail-patella syndrome [1, 2]. Both these rare diseases are characterized by overt proteinuria and need for renal replacement, typically within 10 years of diagnosis. Curiously, in proteinuric kidney disease, glomerulosclerosis is eventually accompanied by tubulointerstitial matrix deposition. The presence of scar in the tubulointerstitium accelerates the progression of kidney fibrosis, and is associated with poorer prognosis [3, 4]. Understanding the qualitative and quantitative changes in collagen type III accumulation in such diseases might spur development of targeted and effective therapeutics. The present study evaluates the relation between proteinuria and glomerular collagen type III accumulation, and compartmental differences in collagen type III signaling in the rat model (5) of puromycin aminonucleoside (PAN) nephropathy.

Methods

Animal Model: Adult male Wistar rats (~6 weeks old) were administered PAN (100 mg/kg in in 0.5 mL saline, IP, *n* = 8) or saline (0.5 mL, IP, n=4) and sacrificed after 21 days and the left kidney harvested.

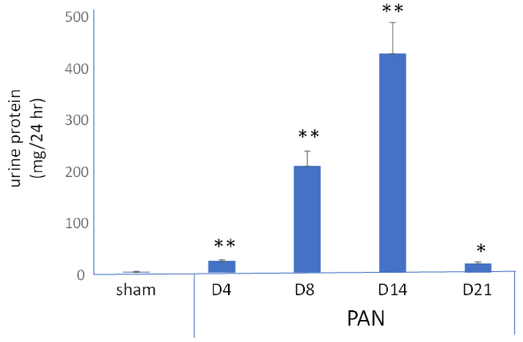
Renal Dysfunction: Twenty-four urine samples were obtained on Days 4, 8, 14 and 21 after PAN administration. Urine protein was determined using the Bradford assay. Urine protein obtained from the sham cohort on Days 1 and 21 and levels were averaged.

Collagen Analysis: At sacrifice, the left kidney was retrieved for transcriptomic and histopathologic analyses. Quantitaive polymerase chain reaction (qPCR) was performed on sample cDNA in triplicate with the Applied Biosystems (ThermoFisher Scientific, Waltham, MA, USA) TaqMan™ Fast Advanced Master Mix following manufacturer's protocol. Analysis was performed for collagen type 1 alpha chain 1 (*COL1A1*) and collagen type 3 alpha 1 chain (*COL3A1*) and were normalized by the housekeeping gene peptidylprolyl isomerase A (PPIA). Commercially available and validated TaqMan™ probes were used for *COL1A1* (Thermofisher, Waltham, MA, USA; Mm00801666_g1), *COL3A1* (Thermofisher, Waltham, MA, USA, Mm00802300_m1), and PPIA (Thermofisher, Waltham, MA, USA, Mm02342430_g1). All qPCR was run on an Applied Biosystems QuantStudio 3 Real-Time PCR system. Peroxidase 3, 3'-diaminobenzidine (DAB) immunohistochemistry was performed with a rabbit anti collagen type 3 primary antibody from MyBioSource (San Diego, CA, USA) and SignalStain® Boost IHC Detection Reagent (HRP, Rabbit) #8114 by Cell Signaling (Danvers, MA, USA) acting as a secondary antibody. All Images (25×) were taken with a Leitz (Leica-Microsystems; Wetzlar, Germany) Laborlux D light microscope and AmScope (Irvine, CA, USA) FMA050 camera.

Gene Ontology Network Analysis: HumanBase was used to build glomerular (G) and tubular (T) *COL3A1* transcriptomic networks. Network analysis was restricted to 51 elements each, inclusive of *COL3A1*, with a minimum interaction confidence of 0.01. The Jaccard–Tanimoto similarity index was used to calculate common elements within the two compartments

Results

Figure 1. PAN-induced Proteinuria. Compared to the sham cohort, the PAN cohort exhibited increased urine protein during measurements made on Days 4, 8, 14, and 21 following PAN administration. Only significant differences vs. sham are highlighted. **, $p <$ 0.01 vs. sham; *, $p <$ 0.05 vs. sham.



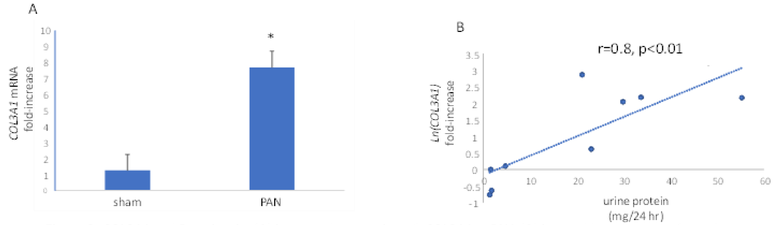
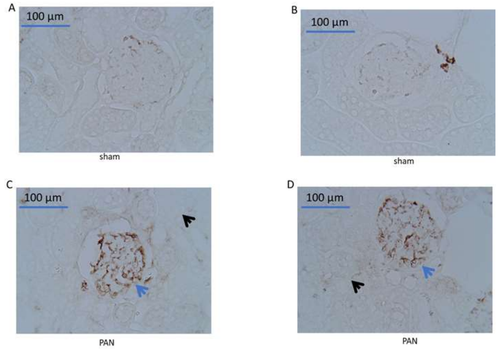


Figure 2. *COL3A1* and Proteinuria. (**A**) increased expression of *COL3A1* mRNA (**A**) in renal homogenates from the PAN cohort *, $p <$ 0.05 vs. sham. (**B**) There was a significant and direct correlation between renal *COL3A1* mRNA and proteinuria.

Figure 3. Collagen Type III Immunohistochemistry. (**A**,**B**) representative kidney sections from sham animals showing little staining for collagen type III; (**C**,**D**) representative kidney sections from PAN-treated animals showing robust staining for collagen type III (blue arrows) predominantly localized to the glomerulus. The tubular compartment (black arrow) has little or no staining for collagen type III.



Results (2)

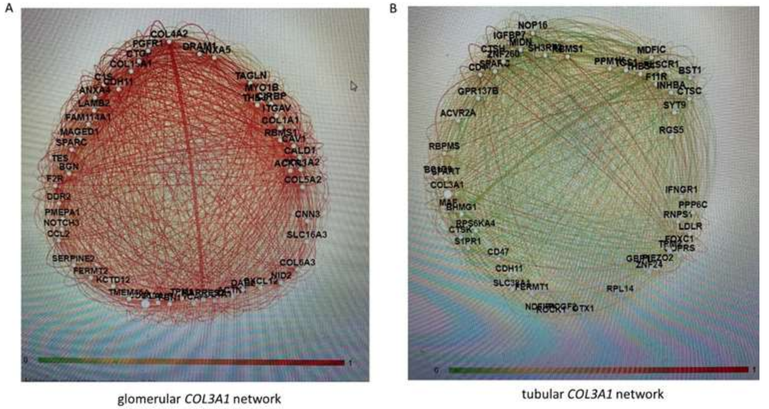


Figure 4. *COL3A1* Transcriptome. HumanBase was utilized to generate glomerular and tubular *COL3A1* transcriptomes i.e., the network of genes/proteins modulated by a change in the *COL3A1* expression level from baseline/healthy kidneys. The glomerular (**A**) *COL3A1* transcriptome (average strength of 0.8 ± 0.08, degree of redness) is relatively robust i.e., is more strongly coupled with associated members within its transcriptome compared to its tubulointerstitial (**B**) counterpart (average strength of 0.56 ± 0.01).

Conclusion

- The PAN model of proteinuric kidney disease is associated with a robust increase in in urine protein.
- Increase in renal *COL3A1* mRNA is directly associated with the increase in urine protein.
- Collagen type III deposition is restricted to the glomerulus which may be explained at least in part by a robust *COL3A1* transcriptomic network in that compartment.
- Urine protein may serve as a noninvasive biomarker of glomerular *COL3A1* upregulation in this model.
- Results from this study may inform the therapeutic potential of drugs targeting the glomerular *COL3A1* network

References

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