Background

Collagen accumulation of collagen type III is the predominant finding in collagenolysis/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 therapies. 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 purpuric aminocollodians (PAN) nephropathy.

Methods

Animal Model: Adult male Wistar rats (6–7 weeks old) were administered PAN 100 mg/kg 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-hour urine samples were obtained on Days 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 transcriptional and histopathologic analyses. Quantitative 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 alpha 1 chain 1 (COL1A1) and collagen type alpha 1 chain (COL1A1) 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; MM00810666_g1), COL1A1(ThermoFisher, Waltham, MA, USA; MM008102300_m1), and PPIA(ThermoFisher, Waltham, MA, USA; MM02342430_g1). All qPCR was run on an Applied Biosystems QuantStudio 3 Real-Time PCR System (ThermoFisher Scientific). PPA (SignalStain - Diaminobenzidine – 050) immunohistochemistry was performed with a rabbit anti collagen type III 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 (25x) were taken with a Leica (Leica Microsystems; Wetzlar, Germany) Laborlux D light microscope and AmScope (Irvine, CA, USA) FMA5050 camera.

Results

Col3a1 Network in Proteinuric Kidney Disease; Informing Drug Activity Using the Jaccard-Tanimoto Index

Figure 1. PAN-induced Proteinuria.
Compared to the sham cohort, the PAN cohort exhibited increased urine protein during measurements made on Days 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 (B) in renal tissue samples from the PAN cohort vs. sham. (B) There was a significant and direct correlation between renal COL3A1 mRNA and protein.

Figure 3. COL3A1 Immunohistochemistry.
Representative sections from sham (A) and PAN (B) are shown. Staining for COL3A1 was increased in PAN samples. Representative sections from sham (C) and PAN (D) are shown. Staining for COL3A1 was increased in PAN samples. COL3A1 expression was localized to the glomerulus. The tubular compartment (black arrow) has little or no staining for collagen type III.

Conclusion

• The PAN model of proteinuric kidney disease is associated with a robust increase 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 collagenolysis 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

1. Narayan, P1, Pellicano AJ1 and Goldberg ID1
1 Angion Biomedica Corp., Uniondale, NY, USA

Figure 4. COL3A1 Transcripts. 
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 baseline/healthy kidneys. The glomerular (A) COL3A1 transcriptome (average strength of 0.8 ± 0.86, 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).

Glomerular COL3A1 Network

Tubular COL3A1 Network

Glotter Biomedica Corp., Uniondale, NY, USA

Table 1. Differences in the COL3A1 Network in Proteinuric Kidney Disease; Informing Drug Activity Using the Jaccard-Tanimoto Index

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