Organ-Specific Collagen Expression: Implications for Renal Disease

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Abstract

Chronic kidney disease is characterized by progressive accumulation of extracellular matrix and scarring, leading to the loss of kidney function. Excess deposition of the collagen family of proteins is the hallmark of kidney fibrosis. In this review, we survey the collagens that are associated with renal disease and we highlight the use of a transgenic approach to identify *cis*-acting sequences in the collagen type I promoter which are capable of directing collagen type I expression specifically in the kidney. Ultimately it may be possible to use this approach to halt the accumulation of collagen selectively in this organ.

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Introduction

A striking feature of chronic kidney disease is the accumulation of extracellular matrix (ECM) resulting in interstitial fibrosis [1]. Even when the primary damage is to the glomeruli, the degree of interstitial fibrosis correlates well with renal function and prognosis. This has led to the

notion that interstitial scarring and fibrosis constitute a final common pathway for many different renal disorders [2], and that accumulation of matrix components, mainly collagen type I, plays a key role in the progression of kidney disease [3].

The renal interstitium consists of cells embedded in a complex ECM. In the normal interstitium there are capillary endothelial cells, dendritic cells, and structural cells which we refer to here as fibroblasts. Collagen molecules play a major role in the ECM, mainly contributing to structural integrity but also modulating cell differentiation, cell growth and adhesion. In normal kidney the production and assembly of ECM is balanced by degradation, thus maintaining connective tissue homeostasis. In normal adult kidney, the most abundant protein component of basement membranes is type IV collagen. Rather little type I and type III collagen is synthesized normally, but along with excess type IV collagen expression is often markedly increased in pathological conditions. Other collagens are also present in the kidney, as summarized in table 1.

In this review, we will focus on collagens associated with renal disorders. We highlight recent transgenic studies, which show that collagen production is very tightly specified by lineage-specific, *cis*- and *trans*-acting elements. Understanding how this is achieved could allow approaches aimed to reduce the deposition of collagen molecules in a kidney-specific manner.

Table 1. Expression of collagens in normal adult kidney and in renal fibrosis

Renal compartment	Collagen types					
	I	III	IV	VI	XV	XVIII
Adult normal kidney						
Glomerular BM			$\alpha 1, \alpha 2$ [5] ^a $\alpha 3\text{--}\alpha 5$ [5] ^a	traces [8]	+ [9]	+ [5]
Glomerular mesangium	– [6, 7]	– [6]	$\alpha 1, \alpha 2$ [5]			
Bowman's capsule		traces [6]	$\alpha 1, \alpha 2$ [5] $\alpha 5, \alpha 6$ [5] $\alpha 3, \alpha 4$ [5] ^{a,c}		traces [9]	
Tubular BM			+ [6]	traces [8]	+ [9]	+ [5]
Proximal tubule			$\alpha 1, \alpha 2$ [5] $\alpha 3\text{--}\alpha 5$ [5] ^a			
Distal tubule			$\alpha 1\text{--}\alpha 6$ [5]			
Interstitial or tubulointerstitium	+ [6, 10]	+ [6, 7]				
Vessel structure	+ [6]		+ [6]		+ [9] ^d	
Fibrotic kidney						
Glomerular BM			+ [7]	+ [11]		
Glomerular mesangium	+ [6, 7, 10]	+ [6, 7]	+ [6]	+ [11]		
Bowman's capsule	+ [6]			– [11]		
Tubular BM			+ [7]	– [11]		
Proximal tubule						
Distal tubule						
Interstitial or tubulointerstitium	+ [6, 7, 10]	+ [6, 7]	+ [12]	+ [11]	+ [13]	
Vessel structure				+ [11] ^b		

Some of the many reports of collagens present in normal and fibrotic kidneys are summarized (+ indicates presence, – indicates absence followed by references in brackets). Italicized references are studies of human kidney. Type V collagen is absent from normal mouse kidney but present in trace amounts in the mesangium of glomerulosclerotic mice [6] and type VIII collagen has been described in the intact glomerulus [14]. Type XVIII α 1 mRNA is upregulated in kidney following ureteric obstruction [15].

BM = Basement membrane. ^a Not observed in all species. ^b Not in the medial layer. ^c Low levels. ^d Intertubular blood vessels.

Collagens

Collagens are abundant proteins and typically represent 25% of the total protein content of mammals. Currently, there are 28 collagen molecules which are grouped in subfamilies depending on their structure and function [4]. In the context of kidney, the most abundant collagens are type I and III collagen which belong to the fibrillar collagen family, while type IV collagen is a network-forming collagen and is a major component of basement membranes [5].

Collagen and Related Diseases

The physiological importance of correct expression of collagen genes in kidney is clearly illustrated by inherited renal disorders caused by defects in collagen genes. The best characterized are mutations in the *COL4A3*, *COL4A4* and *COL4A5* collagen genes (for review, see [16]). The *COL4A5* gene is located on the X chromosome, and defects in this account for X-linked Alport's; affected males usually develop end stage renal failure and carrier females are not phenotypically entirely normal. Autosomally inherited Alport's, and benign familial hematuria are due to mutations in the *COL4A3/COL4A4* genes. Thus ab-

normal collagen IV chains lead to a spectrum of phenotypes [17].

Collagen type I gene defects underlie osteogenesis imperfecta and have not generally been associated with renal manifestations. Intriguingly, it was recently found that the *oim/oim* mouse (a model of osteogenesis imperfecta) develops a characteristic renal abnormality [18]. The *oim/oim* mice do not synthesize functional pro α 2(I) collagen chains resulting in homotrimeric type I collagen α 1(I)₃. In addition to the musculoskeletal deformity, collagen type I accumulates strikingly in the glomeruli of these mice. The mechanisms involved in this excessive collagen accumulation and why it should manifest itself in kidney are not yet fully understood. It is interesting to note that studies of collagen molecules synthesized by cultured mesangial cells showed that ~95% were of type I. Strikingly, 50% of this was homotrimeric α 1(I)₃ [19]. This is reminiscent of wound healing; possibly the high rate of synthesis of alpha chains leads to malformation of secreted collagen type I. It remains to be seen whether *Colla1* homotrimers occur in renal fibrosis in humans.

Another fibrillar collagen, type III collagen, has been associated with a human glomerulopathy, which in some cases progresses to renal failure [20].

Renal Fibrosis

Renal fibrosis is a complex phenomenon that involves a large number of cells that normally do not synthesize collagen proteins. Growth factors and cytokines are important in driving collagen production, in particular, transforming growth factor beta and connective tissue growth factor [21, 22]. In the glomerulus, the epithelial, endothelial and mesangial cells do not synthesize collagen type I under normal circumstances. However, as disease progresses and glomerulosclerosis occurs, collagen type I deposition is characteristic and in end-stage renal failure is found in the glomeruli and interstitium [10].

A fascinating set of observations suggest that tubular epithelial cells can undergo transition in renal disease to acquire mesenchymal characteristics and contribute to interstitial fibrosis. In this process, cells lose epithelial characteristics and express alpha smooth muscle actin and vimentin. Strutz et al. [23] have made extensive contributions in this area, based largely on studies of a protein they have termed FSP-1 (fibroblast-specific protein 1). This protein is identical to the cytoskeleton-associated calcium binding protein S100A4, and is not expressed in normal renal tubular cells. The name FSP-1 is potential-

Fig. 1. Histological section of a transgenic embryo (E15.5) harboring part of the human *Col1a2* enhancer fused to the proximal promoter (P. Prom) and driving expression of beta-galactosidase transgene. Specific blue staining is seen almost exclusively in the developing kidney (arrow). The right panel shows an enlargement of this region.

ly misleading, since expression of S100A4 is not restricted to fibroblasts. Despite this, studies of FSP-1/S100A4 have yielded strong support for the idea that epithelial to mesenchymal transition (EMT) does occur [23, 24]. In experimental models, as many as 36% of interstitial matrix-elaborating cells may arise from local EMT, with a further 14–15% originating from the bone marrow, and the rest from local proliferation of resident interstitial fibroblasts [25].

It is evident therefore that understanding of molecular mechanism underlying collagen genes transcription will provide an insight into the activation of such genes in kidney diseases and perhaps a way to interfere in this process.

Kidney-Specific Collagen Elements?

There is exquisite control of collagen production in individual tissues and cell types. The production of collagen is largely regulated at the level of transcription. During the past ten years, studies on the expression of collagen genes, particularly type I and IV collagen genes, have provided an informative model system to study the mo-

lecular mechanisms and cellular factors that control ECM assembly and function in normal and diseased states. These studies have been based on two experimental approaches to investigating transcriptional regulation; transfection of cultured cells and transgenic animals. Transfection studies have identified proximal promoter sequences and key elements which direct the constitutive expression of collagen and the response to specific stimuli such as cytokines and growth factors in vitro. Transgenic studies of collagen promoters have shown that separate *cis*-acting sequences direct expression to different tissues and/or cell lineages [26]. This is not only the case for type I and IV collagens but has also been shown for collagen type II, VI, and XI.

Since collagen type I is expressed in multiple cell lineages at different times during development and in response to injury in adult cells, we and others have explored the possibility of identifying the *cis*-acting sequences that control the lineage specific expression of collagen type I. Studies in our group have identified critical enhancer sequences located far upstream (~20 kb) of the start site of transcription [27]. Crucially, in transgenic mice harboring reporter genes under the control of the enhancer, expression was found in all mesenchymal cell lineages and recapitulated the endogenous gene expression during embryonic development [28]. As expected for important control elements, the chromatin structure and enhancer activity is evolutionary conserved between mouse and human. Recently, we have shown that the distant enhancer cooperates with the proximal promoter to drive *Col1a2* transcription in specific cell lineages during development [29].

Intriguingly, we have shown that a 1.4-kb sequence located in the human $\alpha 2$ chain of collagen type I (*COL1A2*) drives expression almost exclusively in the kidney of transgenic embryos. We have illustrated this using beta-galactosidase as a reporter, which generates a blue chromogen with the appropriate substrate. This 1.4-kb sequence is part of a 2.2-kb enhancer sequence that we found to express at a high level in almost all collagen producing cells [30]. Removing the 5' end of this enhancer leads to expression largely restricted to the kidney, implying that a kidney-specific *cis*-acting sequence is present within this shorter sequence. Work is underway to identify the minimal sequence that is able to drive expression exclusively in kidney and to isolate the transcription factors bind to it.

Little is known about mechanisms regulating gene expression for the alpha chains of basement membrane type IV collagen. The genes have an unusual arrangement of

head-to-head orientation in transcriptional units: *COL4A1-COL4A2*, *COL4A3-COL4A4*, and *COL4A5-COL4A6*. Each of the three pairs has a bi-functional promoter [31–33]. The mechanism of this bi-directional transcription is not fully understood. Recent studies showed that the proximal promoter (1.6 kb overlapping *COL4A5* and *COL4A6*) is sufficient for cell type specific expression in cell lines derived from different compartments of the kidney in response to stimulation with growth factors affecting expression of type IV collagen in vitro [34]. However, the same promoter did not drive expression in the kidney in transgenic mice. Unexpectedly, expression was actually observed in the upper alimentary tract, in a position independent and bi-directional manner [35]. Moreover, a further 10.6 kb of upstream sequence from *COL4A5* and 13.8 kb from *COL4A6* do not appear to contain additional *cis*-acting sequence, suggesting that control elements of kidney, skin and lower gastrointestinal tract lie elsewhere in relation to the gene, probably at a considerable distance from the start of transcription, which would be similar to *COL1A2*. The lesson from both the collagen I and collagen IV promoters is that short proximal promoters may show activity in vitro in different cell types, but still require other elements to drive temporal and tissue specific expression in vivo. As yet, none of the other type IV collagen promoters have been assessed in vivo using the transgenic approach.

Although much remains to be done, it seems plausible that expression of collagens in the kidney is controlled in a highly specific manner. Understanding how this is achieved might ultimately provide us with an elegant route to preventing collagen accumulation in the kidney, without interfering with matrix homeostasis in other organs.

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