Genetic and molecular markers of proteinuria and glomerulosclerosis

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The use of extracellular matrix (ECM) probes and ECM-related probes for assessing diagnosis and prognosis in renal diseases

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Abstract

Purpose of review

Scarring in the kidney results from excessive local synthesis and exogenous accumulation of extracellular matrix components. Once chronic damage is present in the biopsy, therapeutic intervention for the renal patient encounters severe limitations. It is therefore essential to determine clinical outcome preferably at a time point before the development of overt scarring. Clinical parameters and morphologic alterations in the biopsy are currently used as tools for the diagnosis of the renal disease entity and for assessment of the patient's prognosis. Expression levels of extracellular matrix and matrix-related components may serve as additive and even superior prognostic indicators to conventional parameters. We will elaborate on studies supporting this concept.

Recent findings

Several investigators have shown in experimental models for renal disease that extracellular matrix probes and related probes reflect disease progression and predict outcome. In this review, we will provide an update on the most recent studies of human renal biopsies showing that expression of extracellular matrix components, regulators of matrix degradation, and cytokines effecting matrix deposition may be employed for discrimination of diagnostic groups and predicting prognosis.

Summary

Molecular techniques are expected to be used more and more for diagnostic and prognostic purposes in nephrological practice to supplement the histopathological analysis of the renal biopsy. Assessment of expression of matrix molecules, matrix-regulating cytokines, and metalloproteinases in renal kidney biopsies is helpful to distinguish patients who are at risk to develop progressive renal failure from patients who are likely to recover from renal tissue injury by natural remodeling mechanisms.

Introduction

Several studies have shown that renal function at time of biopsy highly correlates with the severity of tubulointerstitial alterations, which include interstitial fibrosis and tubular atrophy.[1-3] In a large cohort of patients with IgA nephropathy the extent of interstitial fibrosis was the most important parameter for assessment of prognosis.[4] Tubulointerstitial fibrosis and glomerulosclerosis result from accumulation of extracellular matrix (ECM) molecules in response to renal injury.[5] Both interstitial ECM deposition and the deposition of periodic acid-Schiff-positive ECM in the glomeruli correlate well with renal function.[6;7] The network of ECM includes fibronectin, and collagens I, III, and IV. Collagen I and collagen III are the main components of renal fibrotic lesions [8], and fibronectin is one of the main ECM components in glomerulosclerotic lesions.[9]

Excessive ECM protein deposition in the kidney may result from increased messenger RNA synthesis of ECM molecules, from decreased degradation of ECM proteins by reduced activity of matrix metalloproteinases (MMPs) or by reduced ability of MMPs to degrade ECM proteins that have undergone posttranslational modifications, or from a combination of these factors. The MMPs belong to a large family of zinc-dependent ECM-degrading enzymes, which include the interstitial collagenases (MMP-1, MMP-2, MMP-8, and MMP-13), stromelysins, gelatinases (MMP-2 and MMP-9), and elastases.[10] Changes in MMP expression or activity will directly translate into altered ECM turnover, which may lead to scarring and a decline in renal function.[11] Cytokines, such as transforming growth factor (TGF)-β,[12] contribute to ECM accumulation by altering the balance between ECM synthesis and degradation.[13;14]

In this review we will discuss experimental studies, which have shown that ECM probes and ECM-related probes reflect renal disease progression and, more importantly, can be used to predict outcome. We will give an overview of studies in which expression of ECM molecules, MMPs, and ECM-regulating cytokines has been used in human renal biopsies for discrimination of diagnostic groups and for assessment of prognosis. Finally, we will elaborate on future integration of gene expression measurement in nephrological practice for diagnostic and prognostic purposes.

Extracellular matrix probes and extracellular matrix-relating probes reflect disease progression and predict outcome in experimental renal disease

Several studies in animal models for renal disease have shown the usefulness of ECM probes and ECM-regulating probes as markers for disease progression and predictors of renal outcome. Teppo *et al* [15•] investigated an amino-terminal propeptide, PIIINP, in the urine. During the synthesis and deposition of collagen III, PIIINP is degraded from the collagen and secreted into the urine. The authors showed that urinary PIIINP-to-creatinine ratio reflects the ongoing fibrotic processes in the kidney. They concluded that measurement of urinary excretion of PIIINP is useful as an early non-invasive indicator of renal fibrosis after kidney transplantation. ECM molecules and ECM-regulating cytokines may serve as prognostic tools in a way that changes in their messenger RNA levels, detected early in the course of the disease, could herald alterations at the protein level and morphologic changes, which occur at a later time point. This concept was presented by Striker [16] in an excellent overview, forming one of the forefronts on the topic at the time. An increase of TGF-β1 messenger RNA levels preceded morphologic alterations in a model of chronic renal fibrosis.[17] In studies by the groups of Striker and Killen the level of collagen messenger RNA in an early phase of models for progressive glomerulosclerosis predicted the severity of the disease at a later stage.[18;19] Collagen I messenger RNA proved to be an even stronger predictor for outcome in anti-glomerular basement membrane (α-GBM) disease than clinical and morphologic parameters.[19] Bergijk *et al* [20] showed that timely application of medication in mice with lupus nephritis-induced glomerulosclerosis, based on early upregulation of ECM messenger RNA levels, can be beneficial for prevention of renal disease progression.[20] The observation that the elevated ECM messenger RNA levels at early phases of the disease do not immediately lead to increased ECM deposition may be explained by a concomitant heightened activity of MMPs, which counteracts deposition of ECM proteins.

The appearance and distribution of fibronectin messenger RNA isoforms have been investigated in the unilateral ureter obstruction model in rats [21], which is a model for interstitial fibrosis, and in human renal biopsies.[22] Different fibronectin messenger RNA isoforms result from alternative splicing at the ED-A region, the ED-B region, and the V regions of fibronectin messenger RNA. TGF-β effects decreased exclusion of the ED-A region from the primary fibronectin messenger RNA molecule.[23] In human renal diseases, oncofetal fibronectin and ED-A- and ED-B-positive isoforms of fibronectin (ED-A+ fibronectin and ED-B+ fibronectin, respectively) accumulate at locations of chronic lesions, independent of the cause of the disease. In the rat unilateral ureter obstruction model, progression of fibrosis

Setting	Probe	Site	Findings	Study
Diagnostic				
Native kidney diseases	Decorin[a]	Cortex	Increased in MCD versus FSGS	41
	TGF-β_1	Cortex	Increased in FSGS versus MCD. Associated with steroid-resistance	40
Kidney transplantation	α-SMA[b]	Cortex	Increased in CR versus other renal diseases	45••
	Collagen I[c]	TI	Differentiates CR from CsAT toxicity	43•
		TI	Differentiates CR from CsAT toxicity	42
	Collagen IIII[c]	TI	Differentiates CR from CsAT toxicity	43•
		TI	Differentiates CR from CsAT toxicity	42
	Collagen α3(IV)	TBM	*De novo* in CR, not in CsA toxicity	42
	Collagenase type IV[b]	Cortex	Increased in CR versus other renal diseases	45••
	Laminin-β2	Cortex	Increased in CsA toxicity versus CR	44
	Laminin-β2[c]	TBM	*De novo* in CR, not in CsA toxicity	42
	LTBP1[c]	TI	AR versus stable grafts	46
	MMP-2	Glomeruli	AR versus grafts with no AR	47
	TGF-β_1	Cortex	Increased in CsA toxicity versus CR	44
	TIMP-1	TI	Separation of AR from CAN	48
Prognostic				
Native kidney diseases	Collagen α2(IV)	Glomeruli	Disease progression in DN	60
	CTGF	Glomeruli	Disease progression in DN	60
	Decorin[c]	TI	Predicts fibrosis and renal failure	6
	Fibronectin	Glomeruli	Marker for favorable prognosis	58••
	Integrin β4[b]	TI	Progression marker	59••
	MMP-7[4]	TI	Progression marker	59••
	MMP-9[b]	TI	Progression marker	59••
	TGF-β_1	TI	Marker for favorable prognosis	58••
Kidney transplantation	Collagen III[c]	TI	Predicts long-term graft function	53
	Fibronectin	Cortex	Reflects disease progression	54
	PAI-1	Glomeruli	Correlates with delta creatinine	55
	TGF-β_1	Cortex	Marker for favorable prognosis	56
	TGF receptor 1[b]	Cortex	Marker during AR for bad prognosis	57••
	Thrombospondin-1	Cortex	Reflects disease progression	54

Markers were investigated at the messenger RNA level unless otherwise noted. MCD, minimal change disease; FSGS, focal and segmental glomerulosclerosis; TGF, transforming growth factor; CR, chronic rejection; TI, tubulointerstitium; CsA, cyclosporine A; TBM, tubular basement membrane; AR, acute rejection; CAN, chronic allograft nephropathy; DN, diabetic nephropathy.[a] Investigated at the messenger RNA level and at the protein level. [b] Gene was identified by microarray analysis and validated by RT-PCR. More genes are reported in the publication. [c] Investigated at the protein level.

Table 1. ECM-related probes used as molecular diagnostic and prognostic markers in the clinical setting.

was accompanied by increasing deposition of ED-A and ED-B positive fibronectin protein.[21] ED-A and ED-B positive fibronectin messenger RNA levels were increased early after disease induction, preceding the development of fibrotic lesions.[21]

In chronic glomerulonephritis in rats MMPs are involved in expansion of the glomerular mesangial matrix. Tomita *et al.*[24•] studied acute and prolonged mesangial proliferative glomerulonephritis, induced by a single injection or two consecutive injections of anti-Thy-1.1 monoclonal antibody, respectively. Impaired expression of MMP-9 was observed at an early stage of the prolonged model. A similar impairment of MMP-9 expression was seen in anti-glomerular basement membrane-induced glomerulosclerosis.[25] Tomita and colleagues[24•] further found that the level of

collagen I-degrading activity in the urine was negatively correlated with the amount of mesangial matrix expansion. These findings suggest that impaired MMP-9 expression contributes to glomerular ECM deposition and that analysis of collagen I-degrading activity in urine is a suitable method for determining the severity of mesangial matrix expansion. In conclusion, the studies discussed above show that expression of ECM probes and ECM-related probes can be used to predict outcome in experimental renal disease.

Based on the findings in animal models it was proposed that messenger RNA levels of ECM components and regulators of ECM may serve as prognostic tools in patients with chronic renal diseases. Several comprehensive review articles have recently appeared that discuss the prospect of integrating molecular techniques in nephrological practice for diagnostic and prognostic purposes.[26-38] An overview of studies showing the use of ECM-related probes as molecular diagnostic and prognostic indicators in the clinical setting is presented in Table 1. In the following paragraphs we will give an update on clinical studies that have shown the applicability of ECM probes and ECM-regulating probes as molecular markers in nephrology.

Expression of extracellular matrix probes and extracellular matrix-regulating probes may improve diagnostic practice

From a practical point of view, the use of molecular techniques in nephrology is particularly appealing for those cases in which the techniques can provide a substantial addition to currently used clinical variables and histopathological information from the biopsy with respect to determining diagnosis. Important examples in daily practice are the difficulty in some cases of discriminating idiopathic focal and segmental glomerulosclerosis (FSGS) and minimal change disease (MCD) in native kidney disease and that of differentiating between chronic rejection and chronic cyclosporine A (CsA) toxicity in renal allografts. Improvement of diagnostic efficacy in these cases by analysis of ECM expression may help in offering individualized therapeutic regimes. Studies performed in the context of these two examples will be discussed in the next paragraphs.

Differentiation between minimal change disease and idiopathic focal and segmental glomerulosclerosis

MCD and FSGS are non-hereditary proteinuric diseases. In MCD the biopsy does not show visible alterations by light microscopy. In idiopathic FSGS light microscopy reveals focal and segmental glomerular lesions.[39] It is not yet clear whether idiopathic FSGS represents a separate disease entity or is part of a continuous spectrum of abnormalities in which MCD and FSGS represent the extremes. Distinction between

MCD and FSGS is difficult in cases that show only non-sclerosed glomeruli in the biopsy and where clinical presentation does not provide the solution.

Strehlau *et al* [40] showed that in pediatric patients with nephrotic syndrome intrarenal TGF-β1 messenger RNA levels are significantly higher in patients with FSGS than those in patients with MCD. Relatively high intrarenal TGF-β1 messenger RNA levels are also associated with steroid resistance.[40] The expression of the ECM component decorin has been investigated in MCD and FSGS. Decorin is a proteoglycan that binds TGF-β and thereby can counteract its actions. It was found that expression of decorin in the renal cortex is significantly higher in patients with MCD than that in patients with FSGS, whereas TGF-β1 levels are similar between the diseases.[41] This finding may provide an explanation for the absence of tubulointerstitial fibrosis in MCD. Altogether, expression of TGF-β1 and decorin, and the expression ratio between the two may represent molecular markers for improvement of discrimination between idiopathic FSGS and MCD. The discriminating power of these markers will have to be further validated in a prognostic fashion in an independent group of patients.

Differentiation between chronic rejection and chronic cyclosporine A toxicity

Expression of ECM molecules and regulatory cytokines may help in improving diagnostic assessment in renal transplant biopsies with acute or chronic damage. An overview of studies in transplantation on this topic is given in Table 1.[42-48] Late allograft loss remains a major problem in renal transplantation. Failure of the kidney graft is usually preceded by a slow deterioration of renal function over time. The morphologic changes seen in biopsies from deteriorating renal grafts are generally less specific. Chronic allograft nephropathy is a histologic diagnosis, which encompasses interstitial fibrosis, tubular atrophy, and glomerular abnormalities.[49] Several factors, including an ongoing chronic rejection and the use of immunosuppressive medications such as CsA, may contribute to these pathologic changes, as reviewed elsewhere.[50] The pathologic differentiation between chronic CsA toxicity and chronic rejection is difficult to make. Although in practice the biopsy often shows morphologic signs of both entities, differences in ECM protein composition or ECM messenger RNA levels may provide a useful addition to the evaluation of transplant biopsies and facilitate therapeutic decision-making.

Abrass and colleagues [42] showed that analysis of deposition of collagens I, III, and (α3)IV, and of laminin-β2 is helpful in distinguishing chronic rejection from CsA toxicity. In more recent publications, Koop and Bakker performed studies in which stronger criteria were set for defining patients suffering from chronic rejection or from CsA toxicity. The extent of protein deposition of collagens I and III in the interstitial fibrotic lesions are different between patients with morphologic signs of

chronic rejection and those with morphologic signs of CsA toxicity [43•]. Messenger RNA levels of TGF-β1 and laminin-β2 discriminate CsA toxicity from chronic rejection with high sensitivity and specificity.[44] In conclusion, the extent of protein deposition of collagens I and III, and the levels of messenger RNA for TGF-β1 and laminin-β2 may be used to improve diagnosis in late allograft failure. Due to the strict selection criteria of the patient groups in the studies mentioned, the messenger RNA and protein expression will have to be measured in a randomized population of renal biopsies with chronic allograft failure to further validate their discriminating ability.

Extracellular matrix probes and extracellular matrix-related probes reflect and predict disease progression in the clinical setting

Gene expression levels may function optimally as additive parameters in those cases where they supplement clinical parameters and morphologic information from the biopsy for prediction of prognosis of the patient.

Scherer and colleagues [51••] compared two groups of transplanted patients by microarray analysis, which showed normal histology at 6 months after transplantation. They identified 8 genes, some of which are involved in differentiation and proliferation of vascular smooth muscle cells, in the 6-month biopsies suitable to predict outcome at 12 months.[51••] Other studies [52;53] in protocol biopsy specimens from transplanted kidneys showed that interstitial fibrosis, and collagens I and III as its main constituents, can be regarded as reliable predictors of long-term graft function. Baboolal and colleagues [54] studied protocol renal allograft biopsies taken between 3 and 12 months. Messenger RNA levels for TGF-β, thrombospondin, and fibronectin were analyzed, and interstitial fibrosis was quantified by morphometric analysis. The messenger RNA levels of the transcripts and the extent of fibrosis increased progressively over the first 12 months. The serial changes in TGF-β messenger RNA expression and structural injury were not associated with a progressive change in serum creatinine, indicating that renal function, in comparison with messenger RNA levels, does not appropriately reflect disease progression. Glomerular messenger RNA levels of plasminogen activator inhibition-1, which is frequently used as marker for TGF-β activity, predicted deterioration of graft function.[55] In acute rejection of renal transplants cortical TGF-β messenger RNA levels were found to be predictive for favorable prognosis.[56] Sarwal *et al* [57••] performed a wide gene expression study in transplant biopsies with acute rejection and identified a cluster of genes that includes TGF receptor 1, the expression of which is associated with bad prognosis. In biopsies from diseased native kidneys, we recently found that TGF-β and fibronectin mRNA levels negatively correlate with the extent of renal function deterioration after

biopsy.[58••]

Measurement and interpretation of expression profiles in whole renal tissue samples is complicated by the fact that the tissue is composed of many different cell types. Infiltrating cells that are present in variable amounts in kidney biopsies have their own expression profile, thereby complicating interpretation of expression data derived from whole biopsy specimens. Henger and colleagues [59••] compared hydronephrotic kidneys that primarily showed inflammatory activity with kidneys that showed signs of fibrosis. The authors identified genes that showed high expression during either inflammation or scarring of the renal parenchyma.[59••] These genes, which include several ECM molecules and MMPs, could additionally be used to predict clinical outcome in a separate group of renal biopsies. In a study of diabetic nephropathy, Adler *et al* [60] showed that measurement of messenger RNA levels of collagen α2(IV) and connective tissue growth factor in normoalbuminuric patients can be used to predict progression to microalbuminuria.

Regulators of ECM degradation may be used as diagnostic and prognostic tools in glomerular diseases. Levels of pro-MMP-2 and pro-MMP-3 in serum of patients with chronic transplant nephropathy reflect changes in the renal ECM.[61] In diabetic nephropathy, changes in MMP expression are correlated with the degree of ECM expansion. In the cortex of kidney biopsies from patients with diabetic nephropathy levels of MMP-3 messenger RNA are inversely correlated with the extent of matrix accumulation.[62] In addition, a marked decrease in MMP-2 messenger RNA expression was detected in glomeruli of diabetic patients.[63] A recent microarray study in glomeruli from diabetic kidneys showed that several ECM-degrading molecules are differentially regulated in diabetic nephropathy.[64] MMPs may thus be useful to identify patients at risk for developing progressive renal failure. Indeed, gene expression studies in human kidneys revealed an association of mRNA levels of MMP-7 and MMP-9 with renal outcome.[59••]

Together, the studies discussed in this paragraph demonstrate that expression of ECM probes and ECM-related probes in human renal biopsies can be used to predict renal outcome. Intrarenal messenger RNA assessment can be of significant prognostic value, even at a relatively early time point of the disease before overt sclerotic damage of the kidney has taken place.

Conclusion

Diagnostic approaches making use of molecular analyses are likely to be implemented, together with conventional strategies, in clinical practice. Assessment of expression of ECM molecules, ECM-regulating cytokines, and MMPs in renal kidney biopsies may be helpful to discriminate patients who are at risk for developing progressive renal failure from those who are likely to show a natural remodeling capacity after insults of the kidney. Gene expression levels may function as additive parameters supplementing clinical parameters and morphologic information from the biopsy for prediction of prognosis of the patient. Ideally, screening should take place at a relatively early time point of the disease before the onset of development of irreversible scarring, allowing more efficient therapeutic intervention. In kidney transplantation, implementation of protocol biopsies, for instance taken at 6 months after transplantation, will offer a useful tool to identify and validate molecular surrogate markers for outcome. In the long run, gene profiling could be envisioned to contribute in decision-making concerning the way of treatment, the tuning of medication doses, and risk assessment for the renal patient. Gene expression profiling by microarray technology could be seen as an initial step in identifying high-risk patients to application of single nucleotide polymorphism analysis of particular genes for determining renal disease susceptibility. As discussed in this review, several investigations have already provided clusters of genes, the expression of which is associated with renal outcome. Large published data sets will have to be brought back to a condensed list of genes, which need to be validated for their predictive value in follow-up studies. Before application of molecular analyses in everyday practice, the genes showing the highest predictive value from microarray data should be extensively tested in larger, randomized patient groups.

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