

PROCESSES OF FIBROGENESIS IN GLOMERULAR NEPHROPATHIES

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REZUMAT

Progresia afecțiunilor renale se asociază cu dezvoltarea de leziuni fibroase la nivel glomerular – glomeruloscleroză, și la nivel interstițial – fibroza interstițială. De mecanismele implicate în aceste procese sunt răspunzătoare atât celulele inflamatorii, cât și celulele rezidente: celula mezangială glomerulară, fibroblastul interstițial, celula tubulară epitelială și celulele musculare netede vasculare. Celule rezidente se modifică fenotipic, aceste modificări ducând la proliferarea lor și la producția de matrice extracelulară. Un rol important în fibrogeneza renală îl au și factorii de creștere: TGF β , CTGF, PDGF, FGF, BMP7. Pe de altă parte sistemul renină – angiotensină – aldosteron stimulează fibrogeneza, și prin intermediul unor acțiuni diferite de cele bine cunoscute implicate în hemodinamică și echilibrul hidro – electrolitic. Strategiile terapeutice îndreptate împotriva acestor mecanisme, pot fi benefice în reducerea fibrozei renale care acompaniază afecțiunea renală progresivă.

Cuvinte cheie: glomeruloscleroză, fibroză tubulo-intestinală, miofibroblast, factori de creștere, angiotensină II

ABSTRACT

Progressive renal disease is associated with the development of fibrosing lesions in the glomerulus- glomerulosclerosis, and in the interstitium- interstitial fibrosis. The mechanisms involved in this processes are shared by the inflammatory cells and the resident cells: glomerular mesangial cell, interstitial fibroblast, tubular epithelial cell and vascular smooth muscle cell. These resident cells undergo phenotypic changes, which lead to proliferation and increased production of the extracellular matrix. An important role in fibrogenesis is played by various growth factors, such as TGF β , CTGF, PDGF, FGF, BMP7. On the other hand, the renin-angiotensin system stimulates renal fibrogenesis, with actions that are beyond the well-known hemodynamic and salt/water homeostasis. Treatment strategies focused on any one of these mechanisms, could be beneficial in reducing the ongoing renal scarring that accompanies progressive renal diseases.

Key Words: glomerulosclerosis, tubulointerstitial fibrosis, myofibroblast, growth factors, angiotensin II

Renal fibrosis is the final common pathway for almost all forms of kidney diseases that progress to end-stage renal failure. As a result of inflammation and injury, humoral factors are released by infiltrating and resident renal cells that stimulate kidney tissue to produce extracellular matrix (ECM) molecules, overproduction of which generates fibrosis, leading to the permanent loss of normal integrity and function of the kidney.¹

As a good model for the development of tissue fibrosis could serve the normal wound healing process, which has been shown to involve three major phases: induction of tissue injury, inflammation with deposition of ECM, and resolution of the inflammatory process. During the induction phase an increased secretion of chemokines and growth factors by resi-

dent cells produces an influx of mononuclear cells, as well as an increase in the number of interstitial fibroblasts. The second phase is characterized by the synthesis of ECM. This process may be reversible due to increased matrix degradation in the resolution phase. However, often matrix deposition continues for too long, eventually resulting in destruction of normal organ architecture. In that case, resolution is not initiated in time to conserve organ function.²

Fibrotic lesions occur not only in the glomerulus, but also in the interstitial and vascular compartments of the kidney. In the current paper we will try to discuss these processes, with emphasis on the types of cells involved and the role played by different mediators of fibrogenesis. Understanding these mechanisms could provide insight for developing therapeutic means aimed at slowing the progression of the renal disease.

GLOMERULOSCLEROSIS

The term glomerulosclerosis is generally used to designate glomerular scarring. The structure of the normal glomerulus can be injured by many mechanisms, which can be divided into two groups, that is,

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immunologically mediated and non-immunologically mediated. In both cases, the final effect will be increased production and secretion of ECM.³

It is currently assumed that progressive kidney scarring is due to the interactions between resident renal cells and infiltrating, mainly inflammatory, cells. Within the glomeruli, injury is followed by potential damage to resident cell lines with consecutive release by endothelial cells of pro-inflammatory cytokines, chemokines and growth factors, attracting inflammatory cells to the glomerular capillaries and initiating a microinflammatory process.⁴ The infiltration of the glomerular capillaries by monocytes/macrophages leads to their interaction with all glomerular cell lines (endothelial, mesangial and epithelial) to stimulate the proliferation of some (endothelial and mesangial) and the synthesis of extracellular collagenous matrix by most of them (endothelial, mesangial and epithelial). Such increased synthesis in the face of a presumed decreased breakdown would lead to irreversible glomerular sclerosis.⁵

Changes in the phenotypic characteristic of cells play a key role in the glomerular scarring process. El Nahas tried to explain the origin in the glomerulus of α -smooth muscle actin (SMA) - positive cells, responsible for ECM synthesis. He found out that there could be an *intrinsic* and an *extrinsic* pathway of producing glomerulosclerosis.

The intrinsic pathway

The cells involved are resident glomerular cells. Mesangial cells trans-differentiate in response to injury from a mature, adult, pericyte phenotype to an embryonic myofibroblastic one (the mesangioblast), characterized by proliferation and contraction, as well as the expression of cytoskeletal cell markers, such as α SMA. This mesangioblastic phenotype, observed in experimental and clinical nephropathies, is associated with proliferative and sclerotic changes. In particular, the mesangioblast is capable of releasing interstitial collagens type I and III, normally not detected within healthy glomeruli. The deposition of collagens I and III is irreversible as glomeruli are devoid of collagenases - metalloproteinases capable of breaking down such collagens. In other words, the trans-differentiation of adult mesangial cells to embryonic mesangioblasts is characteristic of mesangial injury and is the forerunner of mesangial and glomerular sclerosis. Mesangial cells trans-differentiate in response to injury.⁵

The activation of mesangial cells is associated with the presence of cytoskeletal proteins, such as desmin, vimentin, moesin, or radixin. These are known to play an important role in cell movement, migration, and

cell-matrix interactions and have been associated with mesangial cells activation in experimental glomerulonephritis and in culture. The expression by mesangial cells of the non-muscular type myosin heavy chain embryonic isoform (Smemb) has been described in immune- and non-immune models of glomerulosclerosis.⁶

Similar phenotypic changes have been described in glomerular epithelial cells shown to express desmin and Smemb.⁷

The factors involved in the phenotypic changes of these cells leading to the expression of α SMA are growth factors, such as the platelet derived growth factor (PDGF) and transforming growth factor β (TGF β), as well as ECM components, such as fibronectin.⁶

The resulting cells have a modified expression of the cellular receptors (integrins).⁶ Enhanced expression of beta-1 integrins by activated mesangial cells may contribute to the pathological mesangial remodelling characterized by mesangial cell proliferation and matrix deposition in human glomerulonephritis (GN).⁸

Glomerular modifications are potentially reversible. Progression of glomerulosclerosis may depend on the balance between the proliferation and death, through apoptosis, of the activated mesangial cells. It may also depend on the balance between the synthesis and breakdown of glomerular ECM.⁶

The extrinsic pathway

Alternatively, the presence of α SMA immunostain within the glomeruli may reflect their invasion by periglomerular and interstitial myofibroblasts.⁹

Severe tubulointerstitial changes with the abundant expression of interstitial fibroblasts can often be seen, in humans and rats, to precede significant glomerulosclerosis. El Nahas postulated that interstitial myofibroblasts contribute to glomerulosclerosis through the infiltration of the glomeruli. A similar hypothesis has been put forward suggesting that periglomerular inflammatory cells, including lymphocytes and monocytes, find their way into the glomeruli through holes in the Bowman's capsule. It is probable that such holes, caused by mediators released from the periglomerular inflammatory cells, also allow the passage of interstitial myofibroblasts into the glomeruli. This extrinsic pathway to glomerulosclerosis would also explain the appearance of interstitial (type I and III) collagen within scarred human glomeruli in the presence of capsular adhesions. Qualitative and quantitative changes in glomerular fibronectin, may contribute to the migration of myofibroblasts into the

glomeruli during renal scarring. Very close correlations have been noted between glomerular fibronectin and α SMA containing cells within the glomeruli of rats with experimental glomerulonephritis.⁷

The presence of myofibroblasts in the crescents of patients with crescentic glomerulonephritis has been reported by Alpers.¹⁰ The detection of myofibroblasts in glomerular crescents appeared to be linked, in a study performed by Goumenos et al., to the presence of disrupted Bowman's capsules. Positive correlations were found between the percentage of fibrotic crescents, the percentage of glomeruli with a disrupted Bowman capsule and the expression of α SMA-positive cells in the interstitium, suggesting a causal link. Accordingly, interstitial myofibroblasts may play a role in the pathogenesis of fibrous crescents, through their migration into the Bowman space of the glomeruli with disrupted capsules.⁹ This mechanism is similar to that shown to lead to glomerulosclerosis.

It is likely that interstitial myofibroblasts are attracted to glomeruli by growth factors such as PDGF and TGF β , as well as by changes in ECM, including those affecting fibronectin. This is supported by the observations made by Wiggins and his colleagues in rabbits with glomerulonephritis, where glomerular inflammation was associated with the proliferation of perivascular cells (probably myofibroblasts) synthesizing collagen I and migrating into the interstitium and ultimately into the glomeruli thus contributing to their sclerosis.⁶ Such vascular-interstitial-glomerular pathway to glomerulosclerosis would explain the prognostic importance of vascular and interstitial renal lesions in the progression of chronic renal insufficiency.⁶

Recent studies indicate the possibility of migration into injured glomeruli of haematopoietic progenitor cells suggestive of stem cells. These appear to be involved in the normal turnover of mesangial cells and in the mesangium response to injury. Following experimental mesangiolysis, it appears that a significant percentage of cells repopulating the mesangium are derived from a pool of progenitor cells located at the vascular pole and apparently haematopoietically derived.⁷ The migration of these cells into the injured glomerulus seems to require PDGF, as well as basic fibroblast growth factors (bFGF). Whilst PDGF plays an important role in the migration of the progenitor cells into the glomerulus, bFGF has been linked to the proliferation of repopulating mesangial cells.⁵

TUBULOINTERSTITIAL SCARRING

As with glomerular sclerosis, it is assumed that the interactions between resident cells (tubulo-epithelial and fibroblastic) and infiltrating inflammatory cells (lympho-monocytic) lead to the initiation and progres-

sion of tubulo-interstitial scarring.⁵

Tubulointerstitial fibrosis, comprising tubular atrophy, infiltration by inflammatory cells, accumulation of ECM, and proliferation of mesenchymal cells in the interstitium, is a major characteristic of most progressive chronic renal diseases leading to end-stage renal failure, regardless of cause.¹ It has been shown that in all forms of progressive renal failure tubulointerstitial fibrosis not only occurs but also better parallels the decline in glomerular filtration rate than does the degree of glomerulosclerosis.¹⁴

Interstitial fibrosis has some common pathogenic mechanisms with glomerulosclerosis, although these two processes are not identical. Both are characterized by an accumulation of ECM. The complex interstitial scar contains normal matrix proteins (collagen I, III, V, VII; fibronectin; tenascin), but also proteins located normally in the tubular basement membrane (collagen IV, laminin). In fibrogenesis an important role is played by qualitative changes of the ECM.

Fibronectin has been shown to have important biologic effects on a variety of target cells. The differentiation of some cells is affected by it: chondrocytes become fibroblastic, smooth muscle cells lose their contractile phenotype, and malignant and virus-transformed cells revert to a normal-appearing phenotype in the presence of fibronectin. Both plasma and cellular fibronectin are potent chemoattractants for fibroblasts *in vitro*. It also promotes the anchorage and growth of fibroblasts and other normal cells in culture, the establishment of basement membranes polarity, and the migration of a variety of cells. Depending on the alternative spliced regions of the fibronectin molecule it could have different properties, such as: increased glycosylation, different proteases and binding characteristics.¹⁵ In the production of these proteins a very important role is played by myofibroblasts.

Fibroblasts

Renal fibroblasts play a major role in maintaining organ integrity as well as in the progression of inflammatory and non-inflammatory renal diseases towards end-stage renal failure. Whereas fibroblasts in healthy kidneys are in a quiescent state and seem to be responsible mainly for the maintenance of ECM homeostasis, they become activated in fibrogenesis.¹⁶

Fibroblasts in inflammation and fibrosis may undergo characteristic changes that distinguish them from regular fibroblasts. There are two possible explanations for this process in fibrogenesis: either a subset of fibroblasts may be favoured from a preexisting heterogeneous population, or fibroblasts may differentiate. In human granulation tissue, fibroblasts may acquire morphologic and functional features of smooth muscle cells, hence the term myofibroblasts.¹⁷

Myofibroblasts are terminally differentiated cells with morphologic features intermediate between those of fibroblasts and smooth muscle cells. The cell retains the biologic properties of fibroblast, synthesizing interstitial collagens I and III, and at the same time express SMA.¹⁸

As already mentioned, interstitial fibrosis is characterized by the increased deposition of ECM, which consists largely of fibrous collagens. Collagen production by myofibroblasts increases by four to five times and also changes qualitatively. Müller et al. noted a shift in relative amounts from type I to type III (and moderately type V as well).¹⁷

Human fibroblasts may express α SMA constitutively *in vivo*, as shown by Alpers.¹⁰ Therefore, α SMA could serve as a marker for a subset of human fibroblasts that eventually proliferate and participate in the deposition of ECM.¹⁷

The interstitial expression of α SMA appears initially periglomerular and peritubular, whereas in vessels, where it is normally expressed, it disappears in parallel with the progression of vascular sclerosis and myonecrosis.

Ando et al. have identified a new marker for myofibroblasts: caldesmon. This is a major calmodulin- and actin-binding protein found in smooth muscle and non-muscle cells, which plays a vital role in the Ca^{2+} -dependent regulation of the contraction. Co-localization of caldesmon and α SMA expression in the interstitium is important to further characterize the phenotype of interstitial myofibroblasts.¹⁹

The evolution of myofibroblasts is either back to a non-muscular phenotype or to apoptosis. The latter seems to be responsible for the disappearance of myofibroblasts in advanced stages of wound healing and the evolution of the granulation tissue towards a scar. When contraction stops and the wound is fully epithelialized, myofibroblasts containing α SMA disappear, probably as a result of apoptosis, and the scar classically becomes less cellular and composed of typical fibroblasts with well-developed rough endoplasmic reticulum but with no more microfilaments.²⁰ On the other hand, myofibroblasts persist in hypertrophic scars and fibrotic lesions, so the persistence of cells, that express vimentin and α SMA could be associated with fibrosis. The regulation of apoptotic phenomena during wound healing may be important in scar establishment and development of pathological scarring.²¹

In chronic inflammation and fibrosis the heterogeneity of fibroblasts is accentuated with the selection of highly proliferative subsets, as well as fibroblast differentiation. Further research in this area may enable us to selectively inhibit renal fibrosis without inhibiting regular tissue repair.¹⁷

The origin of interstitial myofibroblasts

The origin of interstitial myofibroblasts remains unknown. Interstitial resident fibroblasts, tubular cells and pericytes are all candidates.⁷ (Fig. 1)

Alpers and his colleagues have suggested that myofibroblasts are derived from *interstitial fibroblasts* in view of their peritubular distribution and structural similarities.²²

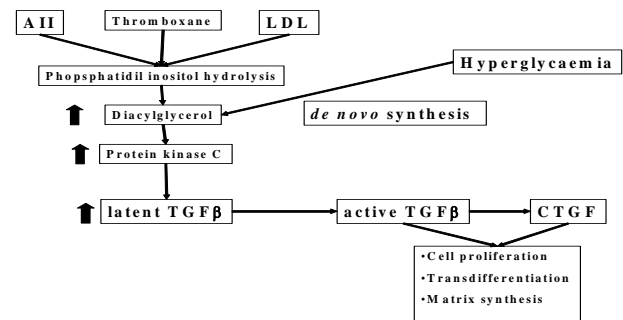


Figure 1. The origin of α SMA positive cells in the glomerulus and interstitium

Concerning *vascular cells*, the activation or changes in the phenotype of these cells has been noticed. Cells expressing α SMA and vimentin migrate from the vascular walls to the renal interstitium and contribute to tubulointerstitial fibrosis. This has been shown in rabbits with experimental glomerulonephritis, where vascular adventitial cells synthesizing collagen were seen to infiltrate the renal interstitium. These cells were found to release TGF β .²³

Arteriolar obliteration could play an important role in the progression of renal diseases. Ischaemic injury may contribute to the progression of tubulointerstitial fibrosis through either the migration of myofibroblasts into the renal interstitium or the modulation of a resident fibroblast population. This fibrosis may in turn contribute to further obliteration of the interstitial vasculature, as well as to the inhibition of compensatory dilatation of the remaining arterioles.²⁴

Transdifferentiation of tubular epithelial cells into fibroblastic cells has been postulated by Neilson and his colleagues. This hypothesis is supported by the *in vitro* observations of transition of tubular cells with fibroblastic characteristics and by the *in vivo* detection of vimentin in tubular cells after their exposure to nephrotoxins.⁶

It has been assumed for a long time that a differentiated cell may not change its phenotype. However, many cellular biology experiments have shown that the differentiated state of a cell, although it is stable, is not irreversible.³ Transdifferentiation is defined as the loss of one phenotype and the gain of another.²⁶

Nadasdy et al. have described mesenchymally appearing cells within the interstitium of human kidneys with marked fibrosis. Ng and coworkers showed a *de novo* expression of myofibroblast markers in some

tubular epithelial cells, these cells lost apical-basal polarity and tight junctions and acquired a fusiform cell shape characteristic of fibroblasts. Moreover, these cells separated from neighbouring cells, lost contact with the tubular basement membrane and seemed to migrate to the interstitium. The process of trans-differentiation was restricted to areas where the tubular basement membrane was disrupted, underlining the importance of an intact anchoring of tubular epithelial cells. The process of epithelio-mesenchymal transdifferentiation could be induced by cytokines like FGF-2, TGF β , and EGF. Studies by Strutz determined that tubular epithelial cells did synthesize fibronectin and collagen type I after stimulation with EGF, FGF-2 or TGF β .²⁷

Preliminary data offered by El-Nahas have also implied a contribution of *bone marrow-derived mesenchymal cells* (MSC) to the renal interstitial fibroblastic pool.

Thus, stem cells along with quiescent renal interstitial fibroblasts, adventitia pericytes and proximal tubular epithelial cells, may all contribute to the pool of interstitial myofibroblasts, characteristic of interstitial renal fibrosis.

THE MEDIATORS OF FIBROSIS

Transforming growth factor β (TGF β)

A cytokine that received intense interest for its role in matrix metabolism is TGF β . It stimulates the *in vitro* mesangial cell synthesis of proteoglycans, fibronectin, and collagens, as well as glomerular epithelial cell synthesis of fibronectin, type I, III and IV collagen, laminin, and biglycan. Monocytes that infiltrate glomeruli during inflammatory and degenerative glomerular processes are major producers of TGF β . An association of increased TGF β production with matrix expansion has been described in several different experimental models of glomerular disease. Treatment of rats with anti TGF β antibody or a naturally occurring TGF β antagonist acutely reduced the ECM accumulation in a mesangioproliferative nephritis model. Transfection of glomeruli of normal rats with a TGF β cDNA, as well as chronic exposure to elevated circulating concentrations of TGF β in mice, that are transgenic for this growth factor, both resulted in prominent expansion of the ECM. TGF β may not only contribute to ECM expansion by inducing synthesis, but also by decreasing the production of matrix-degrading enzymes, such as plasmin, and by upregulating protease-inhibitor synthesis, such as plasminogen inhibitor I (PAI-1) and increased expression of the tissue inhibitor of metalloproteinase-1.^{28,29}

TGF β correlates with the deposition of fibronectin and PAI-I. This correlation demonstrates

that in renal fibrosis advanced overproduction of TGF β leads to a continuous synthesis of ECM and inhibition of the degradation of ECM. TGF β also induces glomerular expression of integrins that enhances cell-matrix interaction and stimulates ECM assembly.³⁰

Similar to its effects on glomerular cells, TGF β can increase ECM synthesis of tubulointerstitial cells. Cell culture data show that TGF β increases the production of interstitial collagens (type I and III), by a renal epithelial cell line and also by renal fibroblasts. The latter seem to be the most important cells contributing to interstitial fibrosis. The *in vitro* data is supported by *in vivo* observations showing that there is increased expression of TGF- β mRNA and protein in parallel with the development of periglomerular and cortical fibrosis in chronic glomerular disease or in primary interstitial disease models.²³

Moreover, it seems that apical tubular membranes express specific growth factor receptors. Immunohistological studies have demonstrated that apical membranes in proximal and distal tubules and in collecting ducts express specific receptors for TGF β , but also for IGF-1 and HGF. The expression of specific signaling receptors in apical membranes sets the stage for activation of tubular cells in conditions with glomerular proteinuria.³¹

Honkanen et al. have proposed urinary TGF β measurement, which could be useful in assessing the rate of progression of the disease, as well as the effects of treatment. TGF β excretion is increased in membranous glomerulonephritis. Highly increased excretion suggested persistently active and/or progressive clinical course, whereas less elevated values suggested normal GFR and remission. Furthermore, immunosuppressive treatment significantly decreased urinary TGF β . Presuming that urinary TGF β reflects ongoing sclerosing and fibrosing processes in the kidney, its determination could be used as a non-invasive tool to assess the progression of renal disease, to select patients for immunosuppressive therapy, and to follow-up the effects of treatment.³² Similar results have been obtained by Haramaki et al. in IgA nephropathy.³³

According to Bertelli et al., TGF β upregulates the expression of two major collagen components which accumulate in tubulointerstitial fibrosis (type III and V):

- TGF β upregulates transcription of type V collagen in tubular epithelial cells, and of type III collagen in tubulointerstitial fibroblasts;
- Posttranscriptional mechanisms, such as increased mRNA stability, for both types of collagen;
- TGF β inhibits the collagenolytic pathway.³⁴

Using immunohistology and *in situ* hybridization Yamamoto et al. analysed the expression of the TGF β

isoforms 1,2 and 3 in human renal biopsy specimens from patients with chronic glomerulonephritis and diabetic nephropathy. Increased glomerular and tubulointerstitial TGF β protein and RNA expression was found in patients with fibrotic renal disease from IgA nephropathy, focal and diffuse lupus nephritis, and diabetic nephropathy, while in normal kidneys and in kidneys of patients with nonfibrotic kidney diseases there is a negative or scant staining. According to Peters et al. isoform 1 was predominant, while the role of TGF β 2 and TGF β 3 is not well established.³⁵ A study performed by Yu et al. demonstrates a clear profibrotic effect of all three TGF β isoforms. TGF β 2 and TGF β 3 effects may be partially mediated by TGF b1. This data suggests that a blockade of all 3 isoforms may have the best therapeutic effect in reducing renal fibrosis.³⁶

An important factor in the cascade of events leading to augmented ECM production may also be represented by the changes in TGF β receptors. Riser et al. showed on rat mesangial cell cultures the effects of exposure to high-glucose medium and/or mechanical strain on TGF β receptor expression and binding.³⁷

A central component of TGF β stimulated mesangial cell fibrogenesis is the Smad signal transduction family. Smad protein complexes activated by the receptor, accumulate in the nucleus, where they facilitate the transcriptional activation of target genes.³⁸

There are multiple sites for the regulation of TGF β action that include synthesis, secretion, and activation of the latent molecule, binding, and signal transduction. Active TGF β has an autocrine action on mesangial cells, thus stimulating matrix synthesis, inhibiting matrix degradation and contributing to the pathogenesis of glomerulosclerosis and tubulointerstitial fibrosis. (Fig. 2)

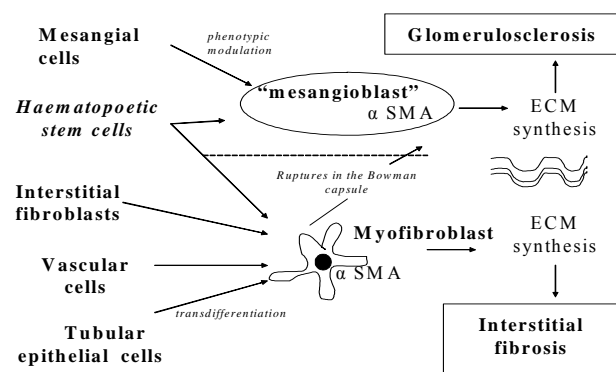


Figure 2. Factors implicated in the activation of TGF α

Connective tissue growth factor (CTGF)

Connective tissue growth factor (CTGF) is a member of a new family of growth regulators, that induces kidney fibroblast proliferation and ECM synthesis. Effects of TGF β on fibroblasts were found to be par-

tially mediated by CTGF. Three different cell types including interstitial fibroblasts, mesangial cells and epithelial cells have been shown to express CTGF mRNA. The majority of CTGF positive cells in the tubulointerstitial area were myofibroblasts, as defined by coexpression of α SMA. This suggests both autocrine and paracrine inductions of this factor by TGF β .³⁹

Gore-Hyer et al. studied the effects of TGF β and CTGF on ECM expression in cultures of primary human mesangial and human proximal tubule epithelial cells. Thus, both growth factors have similar profibrogenic effects on ECM production in mesangial cells, while promoting divergent effects in tubular epithelial cells. CTGF induction of TN-C, a marker of epithelial-mesenchymal transdifferentiation, with no significant induction of collagenous protein synthesis in tubular cells, may suggest a more predominant role for CTGF in the process of transdifferentiation rather than induction of excessive collagen deposition by tubular cells during renal fibrosis.⁴⁰

The urinary level of CTGF (like that of TGF β) could be useful in the follow-up of renal diseases. Gilbert showed that the magnitude of urinary CTGF-N excretion was related to the severity of diabetic nephropathy. In the context of its known profibrotic actions, these findings suggest that CTGF may contribute to the chronic tubulointerstitial fibrosis that accompanies proteinuric renal disease.⁴¹

Hepatocyte growth factor (HGF) can block, at least partially, renal fibrogenesis promoted by TGF β 1 in the remnant kidney, via attenuation of CTGF induction.⁴²

It seems that CTGF plays an important role in the profibrotic action of TGF and could become a possible therapeutic target against tubulointerstitial fibrosis.

Bone morphogenetic protein (BMP-7)

BMP-7 (bone morphogenetic protein) belongs to the TGF β cytokines superfamily, and has osteoinductive properties. Exogenous administration of recombinant human bone morphogenetic protein (BMP)-7 was recently shown to ameliorate renal glomerular and interstitial fibrosis in rodents with experimental renal diseases. This action takes place by antagonizing the profibrogenic effects of TGF β . Wang et al. showed that in cultured mesangial cells with TGF β the coincubation of BMP-7 decreases ECM proteins and CTGF.⁴³

Basic fibroblast growth factors (β FGF)

Fibroblast growth factors constitute a large family of signaling molecules that participate in the regulation of basic cellular processes such as proliferation, survival, migration and differentiation. They are also

implicated in modulating morphogenetic processes involving cellular rearrangements and tissue remodeling.

Fibroblasts express FGF-2 and one of its high affinity receptors. FGF-2 cause a robust induction in proliferation in these cells and induces α SMA expression as marker for myofibroblast formation, but has no major effect on synthesis of collagen type I and fibronectin. Proliferative activity in tubular epithelial cells is increased in diseased human kidneys and correlates with staining for FGF-2. Basal proliferation of fibroblasts can be reduced by the addition of a neutralizing antibody to FGF-2, pointing to a possible autocrine loop in human renal fibrogenesis. Autocrine stimulation of cell proliferation has been described in a number of cells, including endothelial and vascular smooth muscle cells.⁴⁴

Strutz et al. have demonstrated that bFGF is a potent mitogen for renal fibroblasts *in vitro* and may mediate autocrine fibroblast proliferation. A robust increase in bFGF protein could be detected in the interstitium by immunohistochemistry of kidney biopsies with interstitial scarring. *In situ* hybridization studies revealed that interstitial fibroblasts themselves upregulate bFGF expression. Moreover, the degree of β FGF expression *in vivo* correlated with the proliferation index and with interstitial volume. Primary fibroblast cultures that grew out of biopsies with interstitial fibrosis contained more β FGF than fibroblasts from control kidneys. The same authors found an induction of β FGF by TGF β .¹⁶

Kriz et al. studied the link between FGF-2 and podocyte injury. In contrast to mesangial cells and glomerular endothelial cells, podocytes have little proliferative capacity *in vivo*. Long-term treatment (8 and 13 weeks) of rats with FGF-2 led to albuminuria and to increase in serum creatinine indicating the development of chronic renal failure. Histologically, the classic picture of focal segmental glomerulosclerosis was found. Since an increase of cell number of podocytes was not evident, the authors conclude that FGF-2 stimulates podocytes to re-enter the cell cycle and to undergo mitosis (nuclear division). However, podocytes -probably due to their highly differentiated cell shape in the adult- are unable to complete cell division (cytokinesis) resulting in bi- or multinucleated cells; in others, cell division may totally fail leading to podocyte degeneration. Most podocytes in FGF-2-treated rats exhibited degenerative changes including cell body attenuation, extensive pseudocyst formation, widespread foot process effacement, as well as detachments from the glomerular basement membrane.⁴⁵

FGF exerts its effects via low and four high-affinity receptors (FGFR1- FGFR4). The high-affinity

receptors consist of four transmembrane tyrosine kinases. Heparan sulphate proteoglycan is a low affinity receptor for FGF-2.

PDGF

Mesangial cells produce PDGF, a growth factor which induces mesangial cell proliferation. PDGF-B chains and their receptors are overexpressed in various glomerular diseases. Infusion of PDGF-BB or glomerular transfection with a PDGF-B chain cDNA induces selective mesangial cell proliferation and matrix accumulation *in vivo*. PDGF-B chain or β -receptor knockout mice fail to develop a mesangium. Antagonism of PDGF-B chain with neutralizing antibodies can reduce mesangial cell proliferation and matrix accumulation in a rat model of mesangioproliferative nephritis.¹ The effects of PDGF-B antagonism are independent of TGF-beta.² PDGF-C a new member of the PDGF family, has been evidenced. It is expressed in the human kidney, and stimulated at the level of podocytes and interstitial cells after their injury/activation.³ PDGF-D is another isoform of PDGF, and it is expressed in mesangio-proliferative GNs, and it could act as a mitogen for the glomerular cell.⁴⁹

THE ROLE OF THE RENIN-ANGIOTENSIN SYSTEM (RAS) IN RENAL FIBROGENESIS

In the pathogenesis of adaptative glomerular hyperfiltration and hypertension a central role was attributed to angiotensin II (AII). AII-mediated glomerular efferent arteriolar vasoconstriction initiates the increase in intraglomerular capillary pressure which in turn initiates glomerulosclerosis. But the actions of the renin-angiotensin system in progressive renal disease are beyond hemodynamic and salt/water homeostasis.

AII is a known mitogen for glomerular mesangial cells in culture. The administration of AII *in vivo* leads to phenotypic changes in mesangial and epithelial cells with the expression of α SMA and desmin in these cells, respectively.

As with glomerulosclerosis, AII appears to play an important role in the development of tubulo-interstitial scarring and fibrosis. AII has been implicated in the tubular growth response as well as in tubulo-interstitial fibrogenesis. AII is capable of stimulating the hypertrophy of proximal tubular cells, also by potentiating the proliferative response of these cells to epidermal growth factor.

The tubulointerstitial fibrogenic effect of AII has been demonstrated. The incubation of proximal tubular cells with AII stimulates their synthesis of collagen IV. The long-term infusion of AII leads to increased renal interstitial deposition of collagen in rats. It also appears to activate interstitial fibroblasts into

myofibroblasts expressing α SMA in these cells. The renin-angiotensin system is now recognized to be linked to induction of PAI-1, via both type 1 and 4 receptors.⁵⁰

It has been reported that AII stimulates the expression of PAI-1 in several cell lines. PAI-1 is a major physiological inhibitor of the plasminogen activator/plasmin system, a key regulator of fibrinolysis and ECM turnover. PAI-1 induction by A II in endothelial cells seems to be mediated by AIV via a receptor that is different from A II type 1 and 2 receptors (AT1 and AT2). Therefore, it can be hypothesized that the induction of PAI-1 by A IV may be implicated in the pathogenesis of renal interstitial fibrosis in several forms of chronic glomerulonephritides. This effect is not antagonized by AT1 receptor antagonists.⁵¹

Many of the proliferative and fibrogenic effects of AII are mediated by endothelin, PDGF or TGF β . On the other hand, FGF-2 is capable of stimulating the expression of ACE in vascular smooth muscle cells.

AII regulates cell growth and fibrosis through the production of several mediators. The activation of vascular cells by AII increases the production of many agents, including growth factors, cytokines, chemokines, that participate in cell proliferation and ECM accumulation. AII also increases metalloproteinase production involved in matrix degradation. AII-induced ECM production is mainly mediated by TGF β . A new mediator of AII-induced fibrosis has been described: the above described CTGF, a profibrogenic cytokine, which acts as a downstream mediator of TGF β profibrotic activities.⁵²

Local activated RAS plays an important role in the progression of the glomerular disease. Arai et al. introduced human genes for renin and angiotensinogen into the rat kidney. Seven days after transfection, ECM was expanded in the glomeruli and a smooth muscle actin was expressed in the mesangial cells. These results suggest that locally activated RAS induces glomerular sclerosis and a phenotypic change in mesangial cells.⁵³ Weber showed that AII could be produced by myofibroblasts at sites of repair, and has important autocrine and paracrine functions.⁵⁴

There are two major classes of AII receptors, AT-1 and AT-2, both of which are present in the kidney. The blockade of the AT-1 receptor did not prevent monocyte/macrophage infiltration, but did impair fibroblast proliferation, myofibroblast differentiation and the synthesis of TGF β . Overall, AT-1 receptor antagonism diminished the increase in interstitial volume. Blockade of the AT-2 receptor did not attenuate monocyte/macrophage infiltration, TGF β synthesis or fibroblast proliferation, but blocked the

differentiation of these fibroblasts into myofibroblasts.⁵⁵

It is speculated that AT-2 plays a role in tissue remodelling or repair of vascular and cutaneous tissues under some pathophysiological conditions. Several studies indicate that AT-2 is involved in apoptosis. Ma et al. found less apoptosis in the kidneys of AT-2 null mutants, which may contribute to profound interstitial changes.⁵⁶ Data by Ruiz-Ortega et al. show that after diverse initiating insults, AT-2 over-expression is a common feature of kidney injury.⁵⁷

Peten et al. showed that in some forms of glomerulosclerosis, the lesions develop independently of AII. Under these circumstances, pharmacological inhibition of AII may aggravate the lesions through dysregulation of the levels and the balance, between matrix synthesis and degradation.⁵⁸

It seems that AII has received the greatest consideration as the mediator of injurious actions of the RAS in the kidney. However, aldosterone has been also implicated in fibrogenesis. Aldosterone does stimulate type IV collagen synthesis by mesangial cells *in vitro*. It also enhances the levels of PAI-1, thus promoting ECM accumulation. Some studies suggest that increased production of TGF β is likely to be at least partly attributable to a direct action of aldosterone on renal tissue. The specific targeting of the actions of the mineralocorticoid ameliorate heart failure, but its use for the treatment of progressive renal disease has undergone only preliminary study.⁵⁹

THERAPEUTIC IMPLICATIONS

Some indirect strategies intended to inhibit fibrosis in renal diseases have been established. These therapies are aimed to reduce blood pressure, glomerular hyperfiltration, proteinuria, hyperglycaemia and hyperlipidaemia.¹⁴

The importance of blocking the RAS is related not only to the decrease of blood pressure and proteinuria.

In vitro data indicate that AII, independent of blood pressure, increases synthesis and decreases degradation of pathological ECM components. These effects are largely, but not completely, mediated by AII induction of TGF β . In many models of renal fibrosis and in a number of human renal diseases, blockade of AII retards disease progression. Very recent studies indicate that AII blockade suppresses TGF β , whether the therapeutic agent is an angiotensin converting enzyme inhibitor or an AII type 1 receptor antagonist. This data suggest that an important antifibrotic, therapeutic effect of AII blockade is the reduction of TGF β overexpression and raise the question of whether disease progression could be

further retarded if AII blockade were optimized for maximal TGF β reduction rather than for normalization of systemic blood pressure.⁶⁰ AII receptor antagonists reduce but do not normalize aberrant TGF-beta production.¹⁴

AII blockade and low-protein diet have additive effects on disease reduction, suggesting that disease progression in humans with chronic renal failure may be slowed more effectively when AII blockade and low-protein diet are combined. Since maximal pharmacological AII inhibition was used, it is likely that dietary protein restriction further reduces pathological TGF β overexpression by mechanisms different from those of enalapril or losartan.⁶¹

Recent years have convincingly demonstrated that both glomerulosclerosis and chronic tubulointerstitial injury, once developed, can be stabilized and even reverted. In rats with spontaneous disease, angiotensin-converting enzyme inhibitors (ACEIs) given late during the animal's life, when animals were already proteinuric, decreased proteinuria and stopped the disease from progressing, as documented by a lower incidence of glomeruli affected by sclerotic lesions and less interstitial injury than in untreated controls.⁶²

A further concept of therapy is the development of agents which act directly on the target cells (mesangial cells, fibroblasts, vascular smooth muscle cells). Antagonism of growth factors – either by antibodies or by natural antagonists, such as decorin – seems to have good effects.

Border et al. studied gene therapy by injecting in the muscle of the animal cDNA, used in order to produce decorin. This protein is secreted in the circulation and has the following effects on the kidney: decrease in TGF β level, decrease in the production of ECM.⁶³

It has been demonstrated that the administration of selective inhibitors of PDGF reduces mesangial cell proliferation and matrix synthesis. Partial inhibition of PDGF may well be beneficial, while complete PDGF inhibition might effectively block normal mesangial cell proliferation and turnover.⁶⁴

Treatment with anti-TGF β antibodies or natural antagonists of TGF β reduces the accumulation of ECM. Chronic effects of abrogation of the action of TGF β might turn out to be negative, because this growth factor can potently suppress inflammation. Therefore, an alternative treatment target could be CTGF, a mediator of TGF β .¹⁴

Glomerulosclerosis is accompanied by local activation of the blood coagulation system. The blocking effect of heparin is achieved via direct interference in the interaction between plasma- fibronectin and

glomerular structures and not by its anti-coagulant effect.³

Therapy with heparins, both regular anticoagulant and non-anticoagulant derivatives, has been shown to affect renal cell proliferation, matrix synthesis, and progressive renal dysfunction, by interfering with the biological activity of cytokines such as β FGF and PDGF. However, heparins also have multiple actions that are not mediated via cytokines, and therefore it is difficult to determine whether its efficacy was mainly due to interferences with cytokines.²⁸

The manipulation of apoptosis and related changes within myofibroblasts may have therapeutic implications. In the modulation of apoptosis AT2 receptor of AII could play a role.⁶⁵

Many investigations have focused on agents introduced for quite different indications.

Statins (3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors) introduced to lower blood cholesterol have been shown to have variable effects on fibrogenesis. They reduce collagen secretion by mesangial cells and fibroblasts.

Phosphodiesterase inhibitors (theophylline) downregulate fibroblast mitogenesis and collagen synthesis.

The immunosuppressive agent mycophenolate mofetil has consistently been shown to reduce proliferation of not only T and B-lymphocytes, but also vascular smooth muscle cells and mesangial cells. The *in vivo* abrogation of fibroblast accumulation after sub-total nephrectomy suggests that it is also a direct antagonist of fibroblast proliferation.¹⁴

To sum up, understanding the mechanisms of progression of renal diseases and the factors implicated in fibrogenesis, could lead us to the finding of the means to slow progression, in some cases to remove recently formed renal scar tissue, and also reduce mortality.

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