REVIEW ARTICLE

Renal fibrosis

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Abstract

Tubulointerstitial renal fibrosis, characterized as a progressive detrimental connective tissue deposition on the kidney parenchyma, appears to be a harmful process leading inevitably to renal function deterioration, independently of the primary renal disease which causes the original kidney injury. Epithelial to Mesenchymal Transition (EMT) of tubular epithelial cells which are transformed to mesenchymal fibroblasts migrating to adjacent interstitial parenchyma constitutes the principal mechanism of renal fibrosis along with local and circulating cells. Proteinuria as well as hypoxia is included among the main mechanisms of EMT stimulation. TGFβ-1 through the SMAD pathway is considered as the main modulator regulating the EMT molecular mechanism, probably in cooperation with hypoxia inducible factors. Hepatocyte Growth Factor (HGF) and Bone Morphogenetic Factor-7 (BMF-7) are inhibitory to EMT molecules which could prevent in experimental and clinical level the catastrophic process of interstitial fibrosis. Interesting data emerge indicating that HGF and BMF-7 administration prevents the peritoneal fibrosis of mesothelial cells. Hippokratia 2009; 13 (4): 224-228

Keywords: cytokines, growth factors, fibrosis, hypoxia, angiotensin II, epithelial mesenchymal transition, TGF-β, proteinuria

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Non reversible damage of parenchymal tissues as well as the replacement of highly differentiated cells by scarring connective tissue constitutes the common pathogenetic mechanism in chronic failure of parenchymal organs such as liver, kidney and the lungs[1-3]. It seems that in spite of the adequate reserve of cells and mechanisms that these organs deserve for repairing the damaged areas, in the most of the cases, they use the easy solution of fibroblasts and the functionally insufficient connective tissue. For example, in case of the renal parenchyma, besides the glomerular epithelial cells (podocytes) the rest of the cells (endothelial, messangial, tubular) are easily and quickly multiplied offering adequate structural elements for curing properly the damage caused by several disorders. However, due to unknown deficiencies, the tissue restoration mechanisms use in the vast majority of cases the connective tissue and its major component, the fibroblast. Therefore, they usually do not achieve the most desirable effect, maybe because of cell programme discrepancies.

For obvious reasons, it seems necessary for the medical researchers to elucidate precisely the mechanisms and the mediators (growth factors, cytokines) which are involved in the development of tissue damage from the very beginning of histological lesion to the final scarring. It seems essential for medical practice to establish pharmacological interventions that could prevent the progression of tissue damage induced by the catastrophic development of connective tissue over the original lesions.

In the vast majority of chronic renal disease patients the principal tissue lesion that causes the development of organ insufficiency is the non reversible scarring, independently of the original histological findings. This process within glomeruli is manifested as secondary glomerulosclerosis whereas within tubulointerstitial space exhibits the characteristics of fibrosis. Common feature between the previously referred conditions is the synthesis of extracellular matrix proteins[4]. We herein review the main mechanisms and causative factors (mediators, growth factors, and cytokines) that are involved in renal interstitial fibrosis and participate in this non reversible connective tissue development that leads inevitably in chronic progressive renal disease, the common final pathway in the majority of primary renal diseases.

Tubulointerstitial fibrosis and epithelial to mesenchymal transition (EMT)

The pathogenesis of chronic renal disease is characterized by progressive decline of renal function and continuous accumulation of extracellular matrix, which leads to a diffuse fibrosis. Tubulointerstitial fibrosis is especially interesting because it has bean proved that its extent is better related to the decline of renal function in animal models and humans[4]. Independently of the pri-

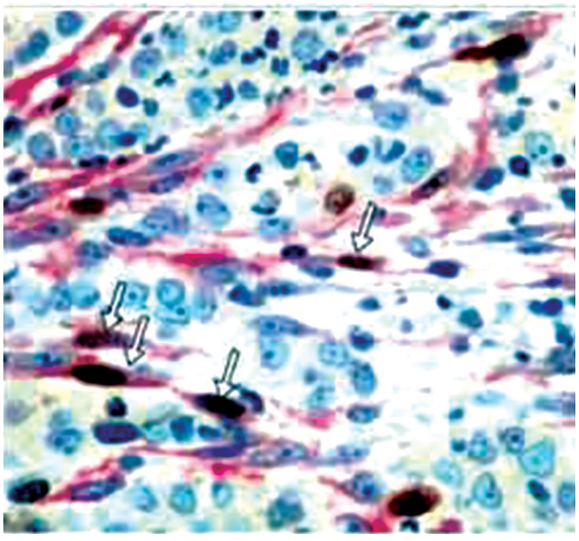

Figure 1: Renal fibrosis characterized by connective tissue expansion through tubulointerstitial parenchyma as well as by fibroblasts infiltration (arrows).

mary cause leading to renal damage, interstitial fibrosis is the common process, in most of the cases, which is characterized by stimulation of myofibroblasts, which are positive in actin reaction of smooth muscle cells (Figure 1). These cells are the main source of interstitial matrix accumulation in various pathological states. Therefore, the possible intervention in the above mentioned stimulation could be an effective inhibitory strategy of renal disease progression[4].

While the role of myofibroblasts in renal fibrosis is generally accepted, there is a lot of ambiguity regarding the origin of these cells. Some researchers assume that myofibroblasts originate from the stimulation of native fibroblasts of interstitial space or even from the invasion of circulating cells. However, innovative studies by Strutz et al, 15 years ago, revealed that tubular epithelial cells could also exhibit fibroblast-like characteristics in various renal diseases, supporting the theory of the epithelial to mesenchymal transition (EMT)[5]. Therefore, by definition, tubular EMT is a process in which the tubular renal cells lose their phenotypical epithelial characteristics and acquire new ones, which are characteristics of the mesenchymal cells. So these cells exhibit a transformation capacity, after which they can produce extracellular matrix.

It is interesting that the renal tubules in the adult kidney, besides the collecting tubules, originate embryologically from the metanephrical mesenchyma and consequently they are transformed into epithelial ones. Therefore, EMT could be considered as a reversed embryogenesis. Although EMT in renal fibrosis was initially suggested as a scientific theory, according to studies within the last few years, it was proved to be the main mechanism of interstitial fibrosis in various renal diseases. It must be emphasized that in most cases the experiments concerning EMT were conducted using embryonic cells or cells of tumor metastases[4].

Current findings in renal biopsies support that there are tubular cells with EMT characteristics in patients with chronic tubular disease, which means that these cells are presented with evidence of smooth muscle cells a-actin expression. Rastaldi et al working on 133 human renal biopsies in various renal diseases, independently of the histological lesions, found that EMT of epithelial tubular cells was present in many samples and, even more important, that EMT extention was correlated with the degree of the interstitial damage[6]. Iwano et al, 5 years ago, using sophisticated labelling techniques, proved that tubular epithelial cells, following proper experimental handling, exhibited EMT characteristics and presented immigration infiltrating the tubulointerstitial space, where the cells expressed also fibroblast-like characteristics[7]. It seems that in the initial phase of fibrosis, the main role belongs to the native fibroblasts, whereas in the advanced stages, as long as the primary renal cause insists, the tubular epithelial cells are principally involved in the process of renal fibrosis within the interstitial space, after EMT transformation.

The fibroblasts of interstitial space and epithelial tubular renal cells generally express radical differences in morphology and their phenotype. The genes, which are modified during the EMT, should be many hundreds or thousands and this fact has been actually confirmed. However, the concise process and the important clues of EMT are not precisely known. So far, the model of a process which is completed in four stages is suggested: 1) loss of epithelial cells adhesive properties, 2) expression of fibroblast-like characteristics, such as the smooth muscle a-actin (Figure 2), 3) rupture of the tubular basal mem-

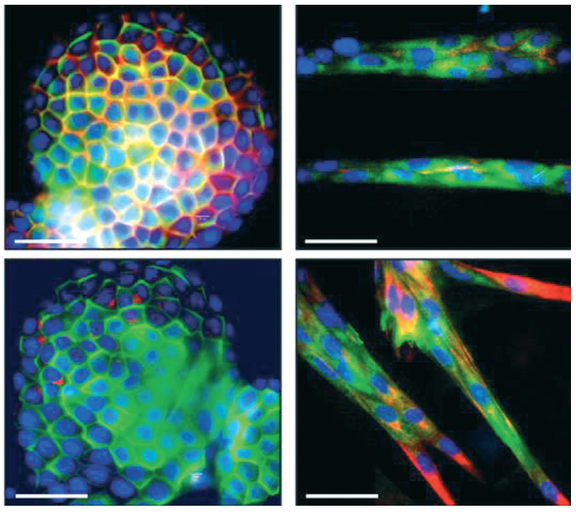

Figure 2: Epithelial to Mesenchymal Transition (EMT). Epithelial cells in 3D culture (left) acquire new (mesenchymal) properties such as shape, cytoskeleton and migration capacity (right). Additionaly they are positive to smooth muscle cells a-actin.

brane and 4) transmigration of epithelial cells into the renal interstitial space[4]. Iwano et al, using an experimental model, showed that myofibroblasts, which originate from tubular epithelium, were at least 36% of the total population of myofibroblasts[7]. Nevertheless the experimental interruption of EMT process resulted in effective fibrosis inhibition. Furthermore, it was shown that stimulation of native and circulating cells, which were transformed into fibroblasts, are necessary for fibrosis, but the cornerstone of the process is the EMT mechanism.

Factors regulating EMT

EMT is regulated by many growth factors, cytokines, hormones and other molecules in various ways. Among many factors, which have been implicated, the major one appears to be TGF-β1. The TGF-β family includes molecules that are proteins of 25 kilodaltons which are involved in development and differentiation of several tissues[8]. The molecule was isolated originally from thrombocytes, consequently, however, was found in cell cultures of monocytes-macrophages as well as messangial renal cells. In mammalians there are three isoforms of TGF-β: TGF-β1, TGF-β2 and TGF-β3. In humans TGF-β1 is the most significant isoform and is secreted as inactivated complex of high molecular weight.

The final activated molecule of TGF-β1 emerges from the complex form and constitutes the ligand for specific receptors which are found on the cytoplasmatic membrane of several cells. This significant growth factor is secreted by an autocrine and paracrine way whereas its secretion is regulated and controlled by a feedback mechanism under normal conditions. TGFβ-1 is the main regulator of tissue healing following the injury induced by various disease stimuli. However its overproduction, in case of malfunction of its switch-off mechanism, promotes matrix extracellular protein synthesis resulting in undesirable development of connective tissue which provokes the damage of the adjacent parenchyma.

Indeed, in a large number of experimental models the scarring process has been in accordance with TGF-β1 levels. On the other hand TGF-β1 overproduction by the kidneys which results in renal collagen synthesis and sclerosis could be down regulated by specific anti-TGF-β1 antibodies resulting in deterioration of renal damage. TGF-β1 action on renal tubular epithelial cells is critical: it provokes cellular apoptosis, down regulates the matrix extracellular proteins dissolution, stimulates the interstitial fibroblasts and triggers the reversal differentiation of epithelial cells to mesenchymal myofibroblasts (epithelial to mesenchymal transition). TGF-β1 plasma levels estimation is not considered adequately precise because part of the molecule concentration in the estimated blood sample comes from thrombocyte cytolysis. Nevertheless there is no difference in TGF-β1 plasma levels between patients with renal disease and normal controls. On the contrary significant elevations of TGF-β1 levels have been found in urine samples of patients with diabetic nephropathy, rejection of kidney transplant and acute glomelural inflammation[9]. It is suggested that in these cases the elevated levels are due to TGF-β1 augmented production by the kidney itself. On the other hand in cases of patients with IgA nephropathy under immunosuppressive therapy, in nephrotic patients after remission as well as in patients with glomerular disorder under treatment with ACE inhibitors the urine TGF-β1 levels have been found significantly diminished.

It seems that angiotensin II, acting as a local growth factor, mainly on renal tubular epithelial cells, stimulates cell hypertrophy and type IV collagen production and as far as the mechanisms involved in this action, among others, TGF-β1 path activation is certainly included. Indeed this mechanism could explain the TGF-β1 level decrease in urine samples after corticosteroids or ACE inhibitors administration. Nevertheless combination therapy with ACE inhibitors and angiotensin II receptor blockers intensifies significantly this phenomenon. The main two factors that trigger TGF-β1 secretion are: angiotensin II and proteinuria. In fact patients with diabetic nephropathy and proteinuria exhibit high TGF-β1 levels in urine collection whereas this phenomenon is not observed in patients with diabetes of identical duration as well as regulation, but without proteinuria.

Strong enough evidence reveals that at least five mechanisms are involved in tubulointerstitial damage in proteinuric glomerulopathies. 1) Direct damage of tubular cells which consume large quantities of proteins by phagocytosis as far as they deserve available lysosoms to deposit and catabolize the protein molecules. Beyond this threshold the overwhelming proteins destroy the tubular cells. 2) Tubular ischemia constitutes a usual phenomenon secondary to glomerural injury in proteinuric glomerulopathies. 3) A large constellation of chemokines and several growth factors arrive downstream to the tubular cells. 4) Neoantigens and adhesion molecules with proinflammatory characteristics are expressed on tubular cells after protein attachment. 5) Finally, epithelial to mesenchymal transition of tubular cells, triggered by urine proteins and simultaneous stimulation of complement which also activates the tubular cells to be transformed through the EMT mechanism[10-15].

Molecular studies using gene detection techniques have shown that the largest density of TGF-β receptors is expressed by the epithelial tubular cells, proving that these cells emerge as the main target of TGF-β1[4]. This factor alone is capable to stimulate and accomplish the entire fibrosis process, which as it is already refered is completed in four stages. This remarkable ability of TGF-β1 underlines that EMT progress occurs through the main path of TGF-β1, which leads to the interstitial fibrosis, under pathological conditions. The Epidermal Growth Factor (EGF) and the Fibroblasts Growth Factor-2 (FGF-2) can also trigger EMT, however in a lesser degree. They can both increase, collectively or synergically, TGF-β1 action. Interleukin-1 and gelatinase are two factors, which can lead to EMT, however this action is inhibited by anti-TGF-β1 antibodies. In conclu-

sion, it appears that TGF-β1 functions as a common path for the actions of many other mediators, which lead to EMT. Angiotensin-II alone cannot induce EMT even under high concentrations. However, it is a very potent catalyst, which increases significantly TGF-β1 action on inducing EMT[4].

Apart from the soluble factors, there are others, such as collagen type I or the tubular basal membrane rupture which promote EMT. On the contrary, there are endogenous factors, with inhibitory effects on EMT process. So, the Hepatocytes Growth Factor (HGF) and the Bones Morphogenetic Factor-7 (BMF-7) suppress tubular EMT both in vitro and in vivo. It must be stated that very high concentrations of HGF and BMF-7 were used over cell cultures in the aforementioned studies.

Molecular mechanisms

Our knowledge regarding the molecular mechanisms, which regulate EMT is not sufficient. Many intracellular paths have been related to this regulation. The main route, which is directly related to EMT regulation, is the TGF-β1 pathway. This factor stimulates two transmembrane receptors (serine-threonine kinases type I and II), which consequently activate two intracellular mediators, Smad II and Smad III[16]. The activation of these mediators causes their phosphorylation, which results to their incorporation to each other and to another third mediator, Smad IV. This triple complex is transported to the cell nucleus, where it regulates the stimulation of genes, which are responsible for the TGF response that is the synthesis of proteins necessary for the EMT modification.

Besides the main path, which was previously mentioned, there are other additional ones, which support the main path. Quite recently, the integrin-linked kinase (ILK) path was described, which acts complementary to the previous route regarding the EMT process completion. ILK reacts with integrins and other cell skeleton substances controlling the regulation of a number of processes, which are dependent on the integrins. Cell adhesion, changes of the cell shape, expression of genes and the accumulation of extracellular matrix are included in these processes. The ILK stimulation promotes specific key-processes, which include the loss of the epithelial E-Catherin, the expression of fibronectin, its accumulation extracellularly, the expression of metalloproteinase 2, its excretion and the increase of the cell migration, which means that this action includes the main events, which occur during EMT following cell stimulation by TGF-β1.

In conclusion, the combination of the TGF and ILK paths supplements each other in a complexed path, where the Smad mediators hold an important role and the tubular EMT is the final outcome. ILK, according to the above mentioned mechanisms, can control the activities of the main path leading to the stimulation of transcription, regulating the expression of all the genes, which are responsible for EMT. Furthermore, ILK can phosporylize directly the light chains of myosin under a mechanism, which is dependent on the calcium concentration and consequently regulates directly the motility, contractibility and migration of the cells, which are modified during EMT[4].

Which is the outcome for the cells undergone EMT

It seems that there are three possible options. The first one leads to reverse transformation, the process by which myofibroblasts are transformed to tubular epithelial cells, when the primary damage diminishes. The second leads to multiplication and increase of fibroblast population, whereas the third leads to the suicide of cells by the mechanism of apoptosis. Concerning the first option, it appears that EMT is reversible at least at the initial stages. Indeed, several studies support that EMT is directly dependent on the duration and intensity of exposure of the tubular epithelial cells to the renal damage factor. For instance, fibrosis caused by chronic cyclosporin administration until a specific threshold, could be reversible following the interruption of the medication intake. However, that critical point is not precisely known. If the renal damage factor persists, it appears that the reverse change of fibroblasts to epithelial cells is impossible. In that case, the second or third option occurs, which are the multiplication and the increase of myofibroblasts population, or their death.

Hypoxia and Fibrosis

Recent evidence revealed that hypoxia is a significant factor involved in interstitial fibrosis in chronic renal disease progression[17]. In spite of the high blood perfusion of renal parenchyma, due to particular structure of this organ, there are extended regions, where the O_2 delivery is under low tension (10 mmHg), whereas at the same time the renal cells of these areas have high oxygen demands. This fact predisposes the renal tissue to be highly sensitive in acute and chronic ischemic damage. Hypoxia has been shown to contribute to several chronic renal diseases and in many experimental models. This fact is attributed to the vasoconstriction, the capillary net deterioration, the vessel lumen stenosis due to atherosclerotic damage and the decline of O_2 transportation due to anemia[17].

The cellular response to hypoxia includes specific mediators, namely the factors that are induced by hypoxia (Hypoxia-Inducible Factors, HIF). Among these factors, the most extendedly studied are the HIF-1 and HIF-2. As regulators of the O_2 homeostasis, HIFs promote the separation of the O_2 from the blood and its delivery to tissues; regulate angiogenesis, erythropoiesis, anaerobia glucolysis, cellular multiplication and apoptosis. In spite that acute HIF stimulation is proved to contribute to the cell survival, as in the ischemia-reperfusion model of various organs, its functional role in chronic diseases appears to be harmful.

The impressive presence of HIF-1 was confirmed immunohistochemically in an experimental model of renal fibrosis and in renal biopsies of patients with diabetic and IgA nephropathy. In this study, the population of cells, which expressed HIF-1, was related to the degree of the

tissue fibrosis. Gene study of the same biopsies revealed the presence of approximately 45 genes related to HIF. Conclusively, it could be suggested that another significant factor of renal fibrosis is the HIF stimulation[18].

It seems very interesting that hypoxia causes the process of EMT in vitro in tubular epithelial cells through HIF-1 stimulation and that genetic exclusion of the epithelial HIF-1 resulted in the decrease of protein production, which is specific for the mesenchymal type of cells and the extracellular production of matrix. Furthermore, the pharmacological exclusion of specific oxidases had the same results, which discloses that the next step in the intracellular path following HIF stimulation is the message transmission to the nucleus of the tubular cells by means of oxidases participation[19].

Besides EMT stimulation, hypoxia can lead directly to fibrosis formation, through an increase of transcriptional genes expression, which are related to the extracellular matrix. Hypoxia promotes collagen I production and decreases the matrix metalloproteinase-2 in epithelial renal cells, whereas increases plasminogen activator inhibitor-1 as well as the tissue inhibitor of metalloproteinase-1 and the growth factor of the connective tissue, through a transcriptional response which also includes HIF. The activation of transcriptional genes, which are sensitive to hypoxia, could be the result of the cooperation between the HIF and other routes, for instance the TGF-β1/Smads path[20]. In spite that the molecular basis of the functional interaction between HIF and TGF-β1 is not completely understood, the most reasonable explanation is that TGF-β1 levels increase as a response to hypoxia and consequently both routes cooperate at the transcriptional level of regulation.

Hypoxia increases Smad-III m-RNA, activating in this manner the TGF-β1 path, indicating that hypoxia and HIF could influence the TGF-β1 route in many levels. It seems that HIF and TGF-β1 route stimulation in renal cells promotes the development of renal fibrosis by at least 3 mechanisms: 1) by direct regulation of the transcriptional genes, which control the extracellular production of the matrix, 2) by functional cooperation with TGF-β-1 and 3) promoting EMT process. In conclusion, the HIF activation by chronic hypoxia promotes interstitial tissue fibrosis, in contrast to the protection which is offered by HIF during the acute ischemic damage[17].

Clinical approach

For the clinician nephrologists, the prevention or the reversibility of EMT appears to be attractive. The principal factors with known inhibitory properties against EMT, as previously have been referred, are the HGF and the BMF-7. These factors are indeed tested on experimental models and clinical practice with promising results. For instance, there is compelling evidence that EMT is among the main mechanisms of peritoneal fibrosis, the dramatic damage of mesothelial barrier in peritoneal dialysis patients. Indeed under stimulating environment (toxic solutions), mesothelial cells undergo essential transformation which includes morphological and functional changes. So mesothelial layers lose their adhesive contacts as well as their cytoskeleton components and consequently they acquire migratory identities and are transformed into connective tissue cell phenotype. Hence EMT which is an essential component of healing mechanism repairing tissue disorders after cell injury, appears in this case as a detrimental process which causes fibrotic damage in peritoneal mesothelial tissue becoming catastrophic in dialysis capacity. Hopefully, there are clinical indications that HGF and BMP-7 administration, well known antifibrotic agents, prevent fibrotic process in peritoneal dialysis mesothelial cells[21,25].

In conclusion, tubulointerstitial renal fibrosis characterized as a progressive detrimental connective tissue deposition on the kidney parenchyma appears as a harmful process leading inevitably to renal function deterioration, independently of the primary renal disease which causes the original kidney injury. Epithelial to Mesenchymal Transition (EMT) of tubular epithelial cells which are transformed to mesenchymal fibroblasts migrating to adjacent interstitial parenchyma constitutes principal mechanism of renal fibrosis along with local and circulating cells. Proteinuria as well as hypoxia is included among the main mechanisms of EMT stimulation. TGFβ-1 through the SMAD pathway is considered as the main modulator regulating the EMT molecular mechanism, probably in cooperation with hypoxia inducible factors. Hepatocyte Growth Factor (HGF) and Bone Morphogenetic Factor-7 (BMF-7) are inhibitory to EMT molecules which could prevent in experimental and clinical level the catastrophic process of interstitial fibrosis. Interesting data emerge indicating that HGF and BMF-7 administration prevents the peritoneal fibrosis of mesothelial cells.

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