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Cellular and molecular events leading to renal tubulointerstitial fibrosis

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Abstract Connective tissue remodeling of the extracellular matrix (ECM) is an essential and dynamic process associated with both physiological responses, such as wound healing, and pathological conditions, such as renal tubulointerstitial fibrosis (TIF). Data from the published literature indicate that collagens and several non-collagenous ECM glycoproteins actively contribute to TIF. The early phase of TIF is usually associated with an inflammatory process mediated by soluble factors released by activated resident cells and by infiltrating cells. Fibrogenic cytokines and growth factors secreted by inflammatory cells and fibroblasts are actively involved in connective tissue remodeling, possibly by regulating the rate of synthesis and degradation of the ECM. An uncontrolled balance of this process usually results in TIF. We review the physiology of wound healing and the pathology of fibrosis, emphasizing TIF.

Key words Tubules · Interstitium · Fibrosis · Kidney · Fibrogenic factors

Introduction

In clinical practice, fibrotic diseases present a major challenge, because effective antifibrotic agents are not yet widely available. Fibrosis is characterized by excessive deposits of the extracellular matrix (ECM), which are partly due to uncontrolled synthesis and incomplete degradation. The pathogenesis of fibrotic disorders is similar regardless of the tissue involved. Extensive research has been carried out to clarify the molecular mechanisms of fibrosis, and one molecule that has received most attention is collagen. This molecule belongs to an ECM protein family, present in almost all normal tissues in humans and multicellular animals. Collagens form the architectural framework of the vertebrate body and are an essential component for maintenance of the structural integrity of cells and tissues.

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Wound healing

Wound healing is a complex process that comprises an ordered sequence of events. Neutrophils infiltrate the wound site and basal epithelial cells proliferate; with time, neutrophils are replaced by macrophages, granulation tissue appears, neovascularization occurs, fibroblasts proliferate, and collagens continue to accumulate. In granulation tissue, the appearance of myofibroblasts contributes to wound contraction, resulting in marked reduction of the original wound size.[1] Myofibroblasts have features intermediate between those of fibroblasts and smooth muscle cells. Their cytoplasm contains actin and myosin bundles resembling those of smooth muscle cells. These myofibroblasts, along with proliferating fibroblasts, produce collagens and other ECM constituents during the repair process.[2,3]

Several growth factors, such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β, fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF), regulate various stages of the healing process. EGF is mitogenic for epithelial cells and fibroblasts,[4] whereas PDGF helps in the proliferation and migration of fibroblasts and myofibroblasts during wound healing.[5] FGF and VEGF help in neovascularization,[6,7] and TGF-β induces increased synthesis and decreased degradation of matrix proteins[8] and thus assists fibrogenesis. A monocyte-derived cytokine, tumor necrosis factor-alpha (TNF-α), might have a detrimental role, possibly by exerting chemotactic effects on fibroblasts.[9] Cytokines such as interleukin 8 (IL-8)[10] are also instrumental in the repair process.

The presence of infection, diabetes mellitus, impairment of blood supply, steroid therapy, and poor nutritional status (low protein and vitamin C intake), alone or in combination, can compromise the quality of wound healing.

Fetal wound healing

Fetal wounds heal without any histological evidence of scarring in early gestation, and may hold the key to scarless repair. Adult dermal wounds, in contrast to fetal wounds, heal with scar formation. The fetal immune response during wound healing differs from the adult response, with a primarily mononuclear cell infiltrate and decreased activity of polymorphonuclear leukocytes. In addition, the cytokine profile of the fetal wound differs from that of the adult wound. In early gestation, fetal skin wound healing occurs rapidly, in a regenerative fashion, without scar formation; in mid to late gestation there is a transition to fibrotic repair.[11]

The accelerated rate of healing, relative lack of an acute inflammatory response, and absence of neovascularization distinguishes fetal wound healing from adult wound healing. Two factors involved in scar formation are IL-8 and TGF-β. A low level of IL-8 is thought to be responsible for the lack of cellular recruitment and inflammation seen in fetal wound healing and may eventually contribute to scarless wound repair,[10] whereas TGF-β promotes wound contraction. Fetal skin fibroblasts cause a collagen gel to contract, activate, and secrete TGF-β to a lesser extent than do adult skin fibroblasts.

The exact mechanism that mediates rapid re-epithelialization in a scarless repair process is unknown. Integrins are a family of cell surface receptors which bind fibronectin, tenascin, collagen, and other ECM proteins, and these proteins are deposited rapidly in fetal wounds. Cass and coworkers[12] have proposed that epidermal integrin receptors specific for fibronectin and other wound matrix proteins are upregulated rapidly during human fetal repair. Early upregulation of integrins in fetal wounds may permit rapid keratinocyte migration and re-epithelialization, and may be important in limiting the induction of inflammatory mediators and resulting scars.[12] Once the biology of fetal wound healing is fully understood, attempts to manipulate the adult wound will progress rapidly, and scarless repair may become a clinical reality in children and adults.[13]

Tubulointerstitial fibrosis

Tubulointerstitial fibrosis (TIF) is characterized by recurrent episodes of inflammation and progressive peritubular fibrosis, as a result of excessive interstitial deposition of matrix proteins. Progression of TIF results in widening of the interstitial matrix and eventual compression and destruction of tubules, resulting in impaired renal function.

In such pathological states as TIF, the proliferation of matrix-producing cells with subsequent overproduction of matrices usually results in fibrosis. In most cases, fibrosis is progressive, and gradual expansion of the fibrotic mass leads to destruction of normal tissues and organs. Although the exact molecular mechanism of fibrosis is not yet clear, numerous studies have convincingly shown that the uncontrolled synthesis and excessive deposits of collagens are mainly responsible for fibrotic changes in human and experimental lung, liver, pancreas, kidney, ocular, and skin fibrotic diseases.[14–25] Here, based on available information, we will present the cellular and molecular events of wound healing and fibrosis, emphasizing the molecular mechanisms of TIF.

The process and progression of TIF can be tentatively divided into three phases or events which sometimes overlap: initial triggering events, inflammatory events, and fibrotic events.

Initial triggering events

Fibrotic diseases are mostly progressive, and they usually lead to irreversible endstage organ failure, a major cause of morbidity and mortality. Understanding the molecular mechanisms and treating these fibrotic diseases is a challenge in modern medicine. The ideal approach would be to identify the primary cause of a particular fibrosis and control this at the early stages. However, the primary causes of the fibrosis are very diverse: e.g., alcohol and viral infections are major causes of liver fibrosis;[26,27] renal scarring results from glomerulonephritis, diabetic mellitus, or hypertension.[28–30] Hypertension can cause diffuse cardiac fibrosis,[31] which is usually associated with progressive heart failure. Toxic vapors and inorganic dusts induce pulmonary fibrosis. The etiology of idiopathic pulmonary fibrosis is still not known. Commonly used drugs, such as cisplatin, gentamicin, and bleomycin, can induce fibrosis in the kidney and lung.[32–36] Physical or chemical injuries and immunological disorders can lead to cutaneous fibrosis, including keloids, hypertrophic scars, and scleroderma.

Tubulointerstitial nephritis (TIN) is characterized by morphologic changes, affecting predominantly the tubules and the interstitium, after recurrent episodes of inflammation. Chronic TIN is usually associated with TIF. The etiology of TIN-triggering events is diverse, ranging from bacterial infection to drugs and heavy metals. In Table 1, we list common agents and factors associated with chronic TIN. A number of these causative agents for TIN may well also be triggering factors for cellular activation or injury which could initiate inflammatory responses that might induce mitogenic and fibrogenic factors. These factors, in turn, could act on tubular epithelial cells and interstitial fibroblasts to proliferate, differentiate, and produce excessive amounts of matrix proteins, resulting in TIF.

Inflammatory events

Infiltrating inflammatory cells play a major role in the pathogenesis of TIF by secreting mitogenic and fibrogenic

Table 1. List of common agents, factors, and disorders associated with chronic tubulointerstitial nephritis (TIN)

1. Infections	• Pyelonephritis
2. Drugs/toxins	• Aspirin
	• Acetaminophen
	• Cyclosporine
	• Tacrolimus
	• Cisplatin
	• Gentamicin
	• Nitrosoureas
	• Lithium
	• Streptazotocin
3. Metabolic diseases	• Uric acid nephropathy
	• Nephrocalcinosis
	• Hypokalemic nephropathy
	• Oxalosis
4. Immunologic diseases	• Lupus nephritis
	• Transplant rejection
	• Sjogren's syndrome
5. Physical factors	• Chronic urinary tract obstruction
	• Radiation nephritis
6. Hematologic disorders	• Sickle-cell hemoglobinopathies
	• Multiple myeloma
	• Post-transplant lymphoproliferative disorder
7. Granulomatous diseases	• Sarcoidosis
	• Tuberculosis
	• Wegener's granulomatosis
	• Xanthogranulomatous pyelonephritis
	• Renal candidiasis
	• Heroin nephropathy
	• Phenytoin hypersensitivity
8. Hereditary disorders	• Polycystic kidney disease
	• Medullary cystic disease
	• Medullary sponge kidney
	• Familial juvenile nephronophthisis
	• Hereditary nephritis
9. Heavy metals	• Lead
	• Cadmium
	• Arsenic
	• Barium
	• Bismuth
	• Copper
	• Mercury
	• Silicon

factors, which then act on matrix-producing cells to produce and increase levels of ECM. It has been shown that inflammatory mononuclear cells recovered by Ficoll-Hypaque centrifugation on day 13 of TIN, induced by bovine tubular basement membrane (TBM) antigens, contained 60% T-helper cells, 10% B cells, 9% T-suppressor cells, and 9% monocytes and macrophages.[37] The ratio of T-helper cells to T-suppressor cells was higher in the nephritic kidney than in the spleen or peripheral blood. Monocytes and macrophages became prominent only at the latest stages of the disease.[37] A similar pattern of inflammatory infiltration of lymphocytes and macrophages, with the induction of adhesion molecules, such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) in the tubular epithelial cells, has been detected in renal tissues obtained from patients with TIN.[38] Both VCAM-1 and ICAM-1 are ligands for integrins expressed on memory-activated T cells and monocytes, and thus could create a microenvironment for the interaction of inflammatory cells and tubular epithelial cells.

All these inflammatory infiltrates usually play a role in subsequent TIF by triggering the increased production of certain fibrogenic cytokines, responsible for inducing the fibrotic cascade. For example, T-cell clones generated from human skin affected by scleroderma were CD4+ and produced high levels of IL-4, but not interferon (INF)-γ; IL-4 has been reported to increase the proliferation and chemotaxis of fibroblasts, and also to increase type I collagen, tenascin, and decorin synthesis.[39–41] A fibrogenic role for IL-4 has been shown in human and experimental renal scarring.[42]

The pathogenic role of chemokines in recruiting interstitial inflammatory cells during TIF has been suggested in numerous studies. For instance, the pathogenic roles of tubular-epithelial-cell-derived monocyte chemoattractant protein-1 (MCP-1), osteopontin, regulated on activation normal T-cell expression and secreted (RANTES), and endothelin have been demonstrated in various human and experimental tubulinterstitial diseases. A significant correlation between the increased interstitial expression of MCP-1 and the interstitial accumulation of monocytes and macrophages has been demonstrated in human diabetic nephropathy with tubulointerstitial damage.[43] Upregulation of the expression of MCP-1, osteopontin, and RANTES in the tubular epithelial cells of patients with chronic progressive membranous glomerulonephritis was associated with the increased interstitial infiltration of inflammatory cells.[44] Similar to MCP-1, the increased interstitial expression of osteopontin was closely associated with the interstitial accumulation of macrophages in both human and experimental models of TIF.[45–46] For instance, in human lupus nephritis and IgA nephropathy, increased osteopontin expression by tubular epithelial cells correlated with the degree of interstitial monocyte and macrophage infiltration.[47] Urinary levels of MCP-1 have been shown to be significantly correlated with the degree of interstitial fibrosis in patients with IgA nephropathy.[48] In a rat model of anti-glomerular basement membrane (anti-GBM) nephritis, increased expression of MCP-1 and osteopontin by tubular epithelial cells was associated with interstitial inflammation; MCP-1 and osteopontin expression was blocked after the rats were injected with antisense oligodeoxynucleotides to reduce interstitial inflammation, providing evidence of a pathologic role for both MCP-1 and osteopontin in tubulointerstitial inflammation.[49] Similar roles for other chemokines, such as RANTES, have been reported during early interstitial injuries.[50]

Macrophage colony-stimulating factor (m-CSF) is a hematopoietic growth factor best known as a regulator of monocyte and macrophage survival, proliferation, and chemotaxis.[51] The increased expression of m-CSF by tubular epithelial cells has been shown to be closely associated with the interstitial accumulation and proliferation of macrophages in experimental anti-GBM nephritis and unilateral ureteral obstruction models.[52] Similar to m-CSF, the expression of macrophage migration inhibitory factor (MIF) is upregulated in both human and experimental models of tubulointerstitial injury, which is associated with interstitial macrophage and T-cell accumulation.[53,54] From the

above information, it is clear that numerous tubular epithelial cell-derived chemokines and growth factors serve to recruit inflammatory cells, which can contribute to subsequent TIF by triggering the increased production of fibrogenic factors responsible for inducing fibrotic events.

Fibrotic events

The synthesis and systematic accumulation of collagen and noncollagenous matrix proteins are essential for normal tissue development, homeostasis, and wound repair. Excessive matrix accumulation can lead to pathologic fibrosis, as seen in systemic sclerosis, keloids, cirrhosis of the liver, and renal fibrosis.[15,17,18,20,21,23–25] The persistent activation of the genes encoding for ECM proteins distinguishes controlled wound repair from the uncontrolled connective tissue deposition leading to pathologic fibrosis. The fibrotic process results in the disruption of the normal architecture of the tissues and ultimately leads to their failure to perform their physiological functions.

In the fibrotic phase, the activated tubular epithelial cells, interstitial fibroblasts, and myofibroblasts start to produce excessive matrix proteins, which are deposited in the interstitial space, and irreversible TIF gradually develops. The increased expression and deposition of type I, type III, and type IV collagens are found in such experimental fibrotic rat kidney diseases as cisplatin-induced TIF,[36] gentamicin-induced TIF,[33] age-associated TIF in Fischer 344 (F-344) rats,[55] hypertension in Dhal rats,[56] and streptozotocin-induced chronic diabetes.[57] In addition, such noncollagenous proteins as fibronectin, laminin, secreted protein acidic and rich in cysteine (SPARC), and thrombospondin contribute to the interstitial fibrotic process.[58] Studies using human renal biopsies have shown that the increased deposition of type I, type III, type IV, and type VI collagens in such renal diseases as hypertensive nephrosclerosis, IgA nephropathy, and diabetic nephropathy results in a widening of the normally narrow interstitium.[59–61] In situ hybridization studies indicate that both tubular epithelial cells and interstitial cells are the main source of these collagens in the fibrotic interstitium both in human and experimental animals.[62,63] The matrix remodeling in various fibrotic diseases is not only due to the excessive synthesis and deposition of matrix proteins but is also related to the activity of matrix-degrading enzymes. Below, we present the general role of these enzymes in fibrotic diseases.

Matrix remodeling during fibrosis

Fibrosis involves the excessive production and deposition of newly synthesized ECM components, as well as the inadequate removal, degradation, and clearance of ECM components; an abnormal balance of these processes results in the alteration of the structure and function of the involved tissues and organs.[64] Generation of the ECM is predominantly achieved through collagen production,[65] whereas resorption of the ECM is mediated predominantly by the matrix metalloproteinases (MMPs). In addition, tissue inhibitors of metalloproteinases (TIMPs) also play an active role in matrix remodeling by blocking MMP activities. These two classes of molecules play a crucial role in the fine regulation of ECM turnover, which is altered in most pathological states associated with abnormal ECM accumulation (fibrotic diseases) or tissue destruction (rheumatoid arthritis).

ECM degradation is also an integral part of wound healing. A delicate balance has evolved between matrix-degrading enzymes (MMPs), and their inhibitors (TIMPs) to ensure adequate removal of damaged matrix components during the healing process.[66] An imbalance in their production and utilization rates could lead to an excessive accumulation of matrix proteins. As mentioned earlier, increased deposition of collagens significantly contributes to TIF, but possible roles for MMPs and TIMPs in interstitial matrix remodeling are not yet clear. Considering that both MMPs and TIMPs have a generalized role in other human fibrotic diseases, it is likely that these molecules may also contribute to matrix remodeling in TIF. However, studies of the role(s) of MMPs and TIMPs in TIF were not always conclusive. In the rat unilateral ureteral obstruction model of interstitial fibrosis, it has been shown that levels of MMP-1 and MMP-9 expressed by tubular epithelial cells remained unchanged, while TIMP-1 expression was upregulated.[67] A similar expression pattern is also demonstrated in other models of progressive renal diseases.[68,69] In chronic cyclosporine A-induced nephropathy in rats, the increased expression of TIMP-1, with no significant changes in the expression of stromelysin and interstitial collagenase, was associated with TIF.[70] On the other hand, TIMP-1 deficiency does not attenuate TIF in mice with obstructive nephropathy.[71]

The ADAMs (a disintegrin and metalloproteinase domain) are type-I membrane proteins, containing a disintegrin and a metalloproteinase domain, about which little is now known.[72] ADAMs are thought to be involved in important cellular events, such as cellular adhesion and membrane protein shedding.[73] Approximately half of the ADAMs identified are in the metzincin superfamily of metalloproteinases, which also includes the MMPs. Recently, a subgroup of eight ADAMs have been identified, and designated ADAM-TS (a disintegrin and metalloproteinase domain, with thrombospondin type-1 modules). The ADAM-TS group, unlike typical membrane-anchored ADAMs, lacks a transmembrane domain and a cytoplasmic domain at the C terminus. Instead, these metalloproteinases contain a varying number of thrombospondin type-1 domains.[74] ADAMs and ADAM-TSs may assist in the proteolysis of matrix proteins. To understand the matrix remodeling in TIF, it is important to identify the numbers of MMPs, ADAMs, ADAM-TSs, and TIMPs, and their roles in the interstial fibrotic process. The significance of studying these molecules in TIF would be to determine if specific or limited numbers of these molecules, independently or in combination, are involved. If numbers are limited, biochemical manipulations can be developed to restore the balance of these proteins to prevent either the excessive

deposition or removal of the ECM. If this balance is restored, the pathological process may be arrested and further tubulointerstitial damage could be prevented.

Factors regulating fibrosis

Complex networks of cellular and molecular interactions regulate the fibrotic process. As noted above, mediators such as cytokines, chemokines, and growth factors released by resident cells or infiltrating inflammatory cells usually play a major role in ECM remodeling. Changes in the microenvironments during fibrosis may alter the cellular functions, and could further contribute to or enhance the fibrotic process. For instance, a type-I collagen-rich microenvironment has activated matrix-producing hepatic stellate cells,[75] and these activated cells express increased levels of collagen receptors of α1β1 and α2β1 integrins.[76] Some of these collagen receptors of integrins may also be involved in the signal transduction of molecules that regulate the proliferation, migration, and adhesion of matrix-producing cells, and thus influence and contribute to the fibrotic process. Profibrotic factors and cytokines such as TGF-β1, connective tissue growth factor (CTGF), endothelin, and heat shock protein 47 (HSP47) have the potential to mediate both human and experimental fibrotic diseases.[77–79] Of these molecules, TGF-β1 is the most extensively studied.

Transforming growth factor-β

The TGF-β family of multifunctional regulatory peptides controls cell growth and differentiation, morphogenesis, and the remodeling of connective tissues. Although five isoforms of TGF-β have been discovered, only three have as yet been identified in mammals. All three isoforms are structurally made up of a 25-kDa homodimer composed of two 12.5-kDa biologically active subunits linked by disulfide bonds.[80] All three TGF-β isoforms show a high level of sequence conservation. For instance, TGF-β1 and TGF-β2 share 74% amino-acid homology, TGF-β2 and TGF-β3 have 82% homology, and TGF-β1 and TGF-β3 have 78% homology.[81]

TGF-β1 is expressed at high levels during tissue remodeling, and affects the formation of connective tissue by stimulating the transcription of ECM genes. Both in-vitro and in-vivo studies have convincingly shown that blocking TGF-β1 suppresses collagen production and subsequently modulates the fibrotic process.[82,83] A fibrogenic role for TGF-β1 is reported in the kidneys of patients with such renal diseases associated with TIF as diabetic nephropathy, membranous nephropathy, HIV-associated nephropathy, and obstructive nephropathy.[84] An increased level of TGF-β has been found in the cortical tissue of renal allografts undergoing chronic rejection, and is thought to play an important role in the development of TIF.[85] Renal biopsies of patients with hypertensive nephrosclerosis have shown increased interstitial expression of TGF-β1, associated with increased deposits of type III, type IV, and type VI collagens in TIF.[60,61,86] Blocking the biologic activities of TGF-β by using antisense oligodeoxynucleotides significantly decreases interstitial collagen accumulation in experimental models of ureteral obstruction,[87] and experimental nephritis.[88] Existing information suggests a fibrogenic role for TGF-β in TIF. However, the roles of TGF-β isoforms in the synthesis of matrix proteins and their nuclear signaling events in TIF are not yet completely understood. Although further studies of TGF-β1 in appropriate in-vitro and in-vivo settings are highly desired for understanding its pathogenic roles in TIF, at this stage, blocking TGF-β1 is an effective approach to prevent the progression of TIF.

Connective tissue growth factor

Connective tissue growth factor (CTGF) is a heparin-binding 38-kDa cysteine-rich peptide that promotes the proliferation of fibroblasts, collagen synthesis, and chemotaxis by mesenchymal cells.[89] CTGF protein is induced by TGF-β in connective tissue cells, but not in other cell types. The regulation of CTGF appears to be controlled primarily at the level of transcription, and a brief exposure of fibroblasts to TGF-β is sufficient to induce a prolonged high level of CTGF expression.[90] In human dermal and mouse NIH 3T3 fibroblasts, CTGF protein or mRNA was observed only in the presence of TGF-β1.[91,92] This upregulation depends on sequences present in the 5′ upstream region of the CTGF promoter.[93] CTGF is overexpressed in numerous human renal fibrotic disorders, such as diabetic nephropathy and proliferative glomerulonephritis with interstitial fibrosis, but not membranous glomerulonephritis or post-infectious glomerulonephritis.[94,95] Future studies of the detailed mechanism by which CTGF influences the terminal cascade of events leading to TIF and of CTGF as a target for future antifibrotic therapy would be important.

Endothelin 1

Endothelin (ET)-1, a 21-amino-acid peptide, is the predominant isoform of the endothelin peptide family. ET-1 is ubiquitously expressed and stimulates vasoconstriction and cell proliferation. A number of studies have demonstrated an increase in the level of ET-1 in fibrotic diseases such as idiopathic pulmonary fibrosis, atherosclerosis, and scleroderma.[96–98] ET-1 stimulates the proliferation of normal airway epithelial cells, which can be blocked by phosphoramidon.[99] Inflammatory cytokines such as TNF-α and IL-1 have been shown to increase in human pulmonary fibrosis and in experimental models of pulmonary fibrosis;[100,101] these cytokines induce the expression and release of ET-1 in the lung.[102] Patients with idiopathic pulmonary fibrosis revealed an increase in the production of ET-1, which is thought to contribute to the fibrotic process by promoting the growth of epithelial cells and fibroblasts and increasing collagen production.[103,104] An important role of ET-I has also been reported in TIF. ET-1 appears to contrib-

ute actively to the TIF by inducing TGF-β expression and by stimulating matrix synthesis. Modulating the biological activities of ET-1 by blocking its receptors resulted in reduced tubulointerstitial damage in a rat model of chronic transplant nephropathy.[105] Besides, transgenic mice overexpressing human ET-1 have been reported to develop TIF.[106]

Heat shock protein 47

Heat shock protein 47 (HSP47) is a collagen-specific molecular chaperone, and is involved in the biosynthesis and secretion of procollagens.[107] Substantial in-vivo evidence now points to HSP47 as an important molecule in the fibrotic process in lung, liver, and kidney.[108–110] For instance, the expression of HSP47 augmented CCl_4-induced liver cirrhosis, bleomycin-induced pulmonary fibrosis, and antithymocyte serum-induced glomerulosclerosis in rats.[108–112] The upregulation of HSP47, coordinated with the increased interstitial accumulation of collagens, is also seen in human and experimental renal fibrotic diseases. For instance, upregulation of the expression of HSP47 and the increased accumulation of interstitial collagens (types I and III) has been reported in the kidneys of cisplatin-treated rats, gentamicin-treated rats, aged F-344 rats, and hypertensive rats.[55–59] Moreover, blocking and modulating the expression of HSP47 have been shown to reduce the renal fibrotic process.[113,114] Although further studies of the fibrogenic role of HSP47 are warranted, at this stage, HSP47 appears to be a reasonable future therapeutic target for developing an antifibrotic agent.

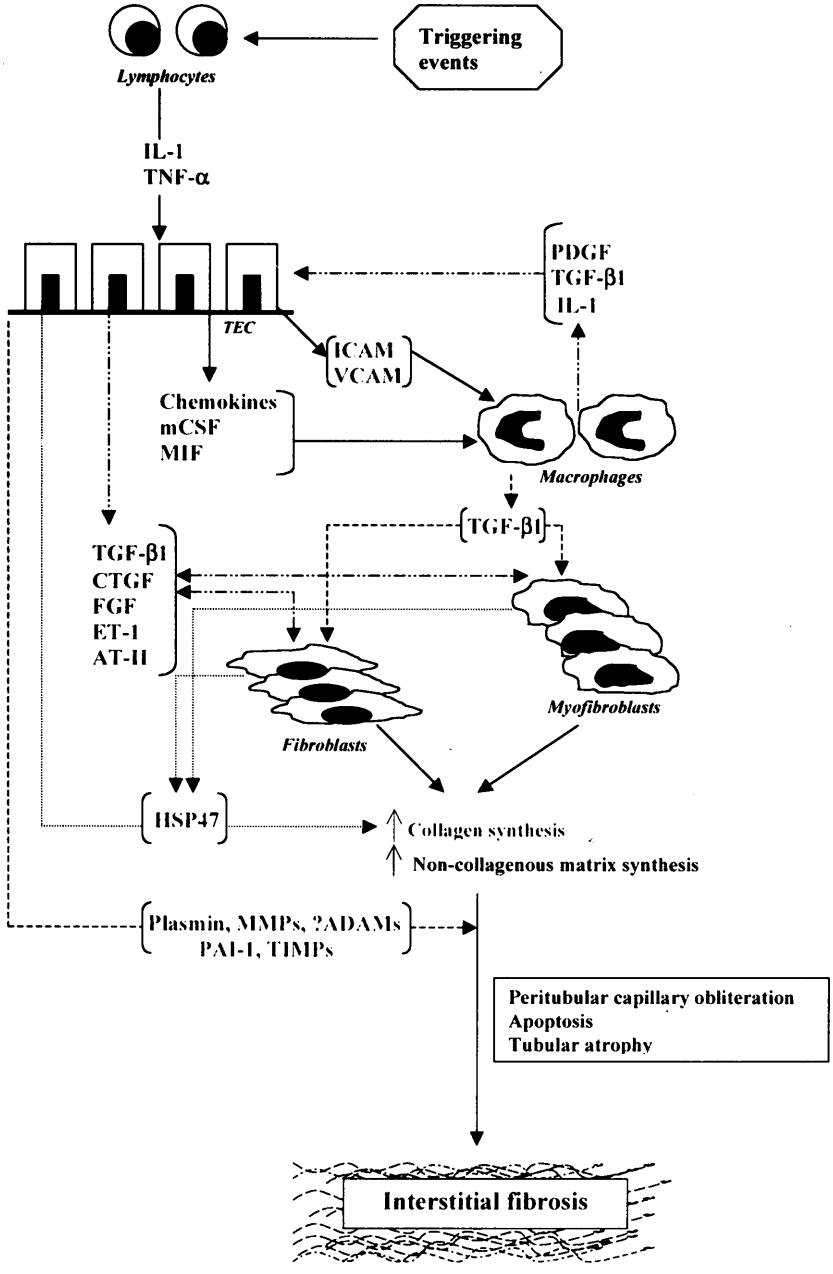

Fig. 1. Schematic diagram of possible molecular interactions during fibrogenesis. There are additional factors, which may have significant roles in the interstitial fibrotic process, but to make the diagram simple, we did not include those. *IL-1*, Interleukin-1; *TNF-α*, tumor necrosis factor alpha; *mCSF*, macrophage colony-stimulating factor; *MIF*, macrophage migration inhibitory factor; *ICAM*, intercellular adhesion molecules; *VCAM*, vascular cell adhesion molecules; *TGF-β1*, transforming growth factor beta 1; *PDGF*, platelet-derived growth factor; *CTGF*, connective tissue growth factor; *FGF*, fibroblast growth factor; *ET-1*, endothelin-1; *AT-II*, angiotensin II; *HSP47*, heat shock protein 47; *MMP*, matrix metalloproteinase; *TIMP*, tissue inhibitor of metalloproteinase; *ADAM*, a disintegrin and a metalloproteinase; *PAI-1*, plasminogen activator inhibitor-1; *TEC*, tubular epithelial cell

Other factors

Besides the above-mentioned factors, platelet-derived growth factor (PDGF), interleukin 1 (IL-1), IL-4, IL-8, IL-10, interferon-γ (INF-γ), tumor necrosis factor (TNF), insulin-like growth factor (IGF), angiotensin II (AT-II), tissue transglutaminase (TTG), and FGF also play roles in the inflammatory and fibrotic phases of TIF.[115,116] Of these, AT-II seems to contribute actively to renal interstitial fibrosis by inducing TGF-β expression and by stimulating matrix synthesis.[116] Blocking the bioactivities of AT-II by means of either AT-II receptor antagonists or angiotensin-converting enzyme (ACE) inhibitors could attenuate TIF, possibly by suppressing TGF-β production.[117] Genetic manipulations of AT-II have suggested its roles in renal diseases. For instance, AT-II-deficient mice produce less TIF than wild mice in experimental models of unilateral ureteral obstruction[118] and anti-GBM nephritis.[119] In Fig. 1, we schematically summarize some important molecules that interact with each other during fibrogenesis.

Modulation of fibrosis

When the initial cause of a particular fibrosis is recognized, the first step would be to eliminate or reduce it. However, so many causes of fibrosis exist that the early detection of the pathogenic factor initiating the process is not always possible. So, the next reasonable target for therapy would be to reverse and block the early fibrosis. To block the cascade of events during the fibrotic process, one option would be to develop new therapeutic agents that are able to interfere with the interaction of fibrogenic factors with their specific cell surface receptors. The administration of an IL-1 receptor antagonist can suppress liver fibrosis in rats, possibly by modifying the biological effects of IL-1.[120] In a similar study, IL-1 receptor antagonist treatment markedly reduced experimental nephritis and subsequent renal fibrosis.[121] Soluble TGF βII receptor therapy has been shown to be an effective inhibitor of hepatic fibrogenesis.[122] Thus, modulating the biological effects of various fibrogenic factors by blocking their receptors may provide a novel therapeutic approach to the treatment of progressive fibrotic diseases.

Another potential target might be to block the transmission of external signals in the cell through the signal transduction cascade. Studies have shown that AG1295, a PDGF receptor kinase blocker, can reduce interstitial fibrosis in rat kidneys.[123] Other commonly used options are to block the synthesis and secretion of fibrogenic factors. A beneficial effect of blocking PDGF, IL-1, TGF-β, and HSP47 has been shown in various fibrotic diseases, including TIF. For instance, a fibrogenic role for the TGF-β family has been convincingly demonstrated in a unilateral ureteral ob-

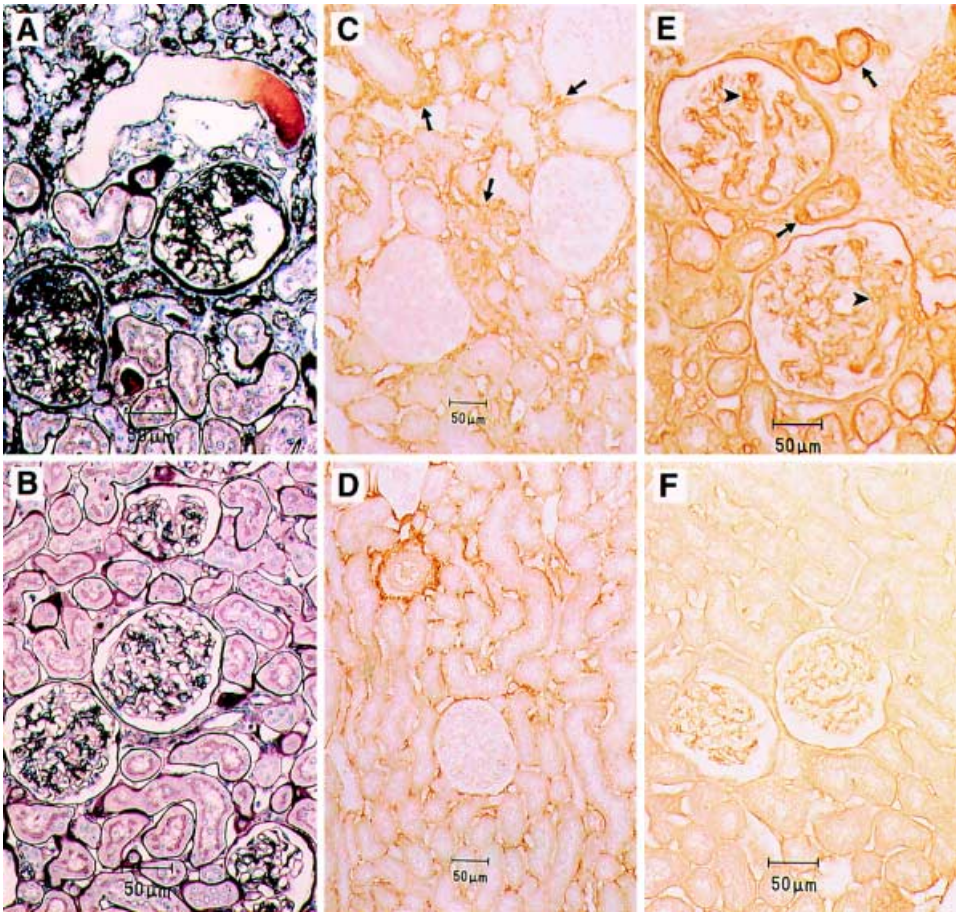

Fig. 2A–F. Diet restriction modulates age-associated renal fibrosis. Histological features of kidneys obtained from 24-month-old F-344 rats fed a standard diet (**A**) and 24-month-old 30% diet-restricted F-344 rats (**B**). Note severe inflammatory cell infiltration, glomerulosclerosis, tubular basement membrane thickening, and interstitial fibrosis in the kidney of the rat fed a standard diet (**A**), but in the diet-restricted rat, renal structural damage is minimum (**B**). Increased accumulation of type I, collagen (**C**) and type IV collagen (**E**) is noted in glomerulosclerosis (*arrowheads* in **E**), tubular basement membrane thickening (*arrows*, in **E**), and interstitial fibrosis (*arrows* in **C**), in the kidney of a rat fed a standard diet. In contrast, relatively less accumulation of type I collagen (**D**) and type IV collagen (**F**) is seen in the kidney of a diet-restricted rat. **A**, **B** Periodic acid-Schiff methenamine silver (PAM) staining; **C**–**F**, Immunoperoxidase staining

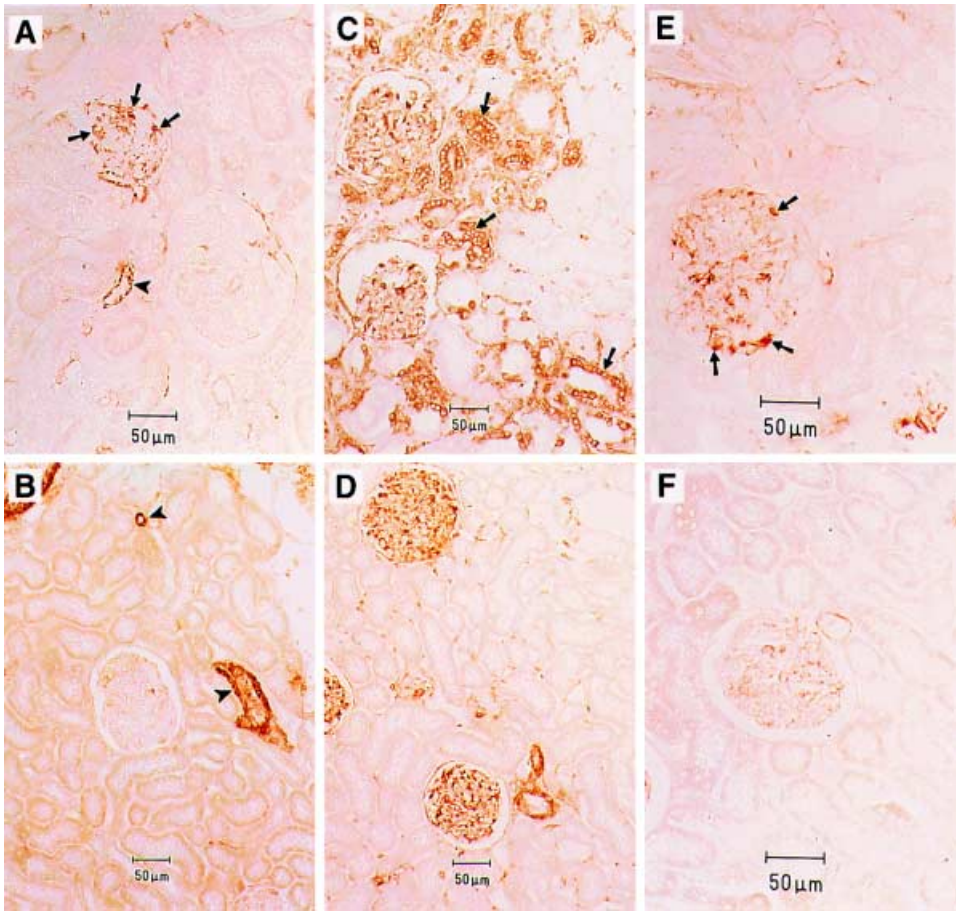

Fig. 3A–F. Diet restriction modulates age-associated phenotypic alteration of resident renal cells. Immunohistochemistry evaluations show α-smooth muscle actin (**A, B**), vimentin (**C, D**) and desmin (**E, F**) in kidneys obtained from 24-month-old F-344 rats fed a standard diet (**A, C, E**) and 24-month-old 30% diet-restricted F-344 rats (**B, D, F**). Positive staining of α-smooth muscle actin is present in mesangial cells (*arrows*) and interstitial cells in the kidney of a rat fed a standard diet (**A**). In the kidney of a diet-restricted rat (**B**), the expression of α-smooth muscle actin is mainly present in blood vessel walls (*arrowheads*), and is mostly negative in mesangial cells and interstitial cells. Positive staining for vimentin is noted in the tubular epithelial cells (*arrows*) in a kidney from a rat fed a standard diet (**C**); in comparison, most of the tubular epithelial cells are negative in a kidney from a diet-restricted rat (**D**). In contrast to the strong immunostaining for desmin in glomerular epithelial cells (*arrows*) noted in a kidney from a rat fed a standard diet (**E**), staining was weak or absent in the glomerular epithelial cells of a kidney from a diet-restricted rat (**F**). **A–F**, Immunoperoxidase staining

struction model of interstitial fibrosis,[124,125] while blocking the bioactivity of TGF-βI by means of its antisense oligodeoxynucleotides reduced TIF.[87]

Progressive fibrosis of such organs and tissues as liver, lung, kidney, heart, blood vessels, and skin comprises a constellation of mechanistically related disorders. Recently, it has also been discovered that decorin is a natural regulator of TGF-β. Decorin is a leucine-rich proteoglycan that binds and neutralizes extracellular TGF-β so as to block the fibrogenic effects of this molecule.[126] Because decorin is a natural human compound, which can be produced as a recombinant molecule, it is likely not to be immunogenic, compared with antibody treatment. Therefore, decorin offers hope as a treatment for chronic fibrotic diseases associated with the overproduction of TGF-β. In fact, numerous in-vivo studies have already shown an antifibrotic role for decorin.[126,127] The transfer of decorin cDNA into the skeletal muscle of glomerulonephritic rats significantly decreased the renal accumulation of matrix proteins, suggesting a therapeutic potential of decorin as an antifibrotic agent.[127] Other recent studies demonstrating the increased expression of decorin in progressive fibrotic diseases have revealed a rather complex role for decorin in fibrogenesis.[128]

Recently, pirfenidone (5-methyl-1-phenyl-2-(1H)-pyridone) has successfully been used as an antifibrotic agent in experimental pulmonary fibrosis, renal fibrosis, and sclerosing peritonitis.[129–131] Pirfenidone markedly inhibited or prevented fibrotic changes in all the experimental models, possibly by suppressing the *TGF*-β gene at the transcriptional level.[132] Pirfenidone has also shown encouraging results in human fibrotic diseases. A beneficial effect of pirfenidone was reported in terminally ill patients with advanced idiopathic pulmonary fibrosis.[133,134] Based on the published literature, pirfenidone, at this stage, appears to be a promising new antifibrotic drug.

Recently, attempts to develop agents to block specific post-translational enzymes of collagen synthesis, to inhibit collagen accumulation in fibrotic diseases, have received enormous attention. HSP47 is a collagen-specific molecular chaperone, which binds to the triple helix region of the procollagens, and helps in the stabilization of the molecules in the endoplasmic reticulum.[107] In human and experimental fibrotic diseases, the increased expression of HSP47 is closely associated with the increased deposition of collagens.[55–59,108–112] Suppression or blocking of the expression of HSP47 in renal scarring models resulted in reduced fibrotic activity. Although further studies are warranted, agents blocking the bioactivities of HSP47 appear to have an antifibrotic therapeutic potential.[113,114] Prolyl 4-hydroxylase plays a central role in the synthesis of collagens by catalyzing the formation of 4-hydroxyproline, which is essential for the assembly of triple-helical structures. Block-

ing this enzyme activity resulted in decreased collagen accumulation, and its inhibitors are likely to be potential candidates as anti-fibrotic drugs.[135–137]

Dietary restriction is one of the few experimental manipulations known to extend life and to reduce age-associated diseases and neoplasms.[138–142] A high-protein diet aggravates uremic symptoms associated with progressive TIF (Fig. 2), and produces phenotypic alterations of resident renal cells in F-344 rats (Fig. 3); in contrast, a protein-restricted diet could slow the loss of residual kidney function in both humans and experimental animals.[113,143,144] Another anti-fibrotic drug is tetrandrine, a Chinese herbal compound, which is successfully used to treat pulmonary fibrosis and is thought to exert its antifibrotic action partly by inhibiting the proliferation of fibroblasts.[145,146] Penicillin G has also been successfully used for treating dermal fibrosis in patients suffering from systemic sclerosis, although little is known about the mechanism of its antifibrotic action.[147] In addition, a few experimental studies designed to inhibit fibrogenic mediators have provided encouraging results, which could, theoretically, form the basis for new therapeutic strategies. Preliminary information suggests the beneficial effects of blocking PDGF, IL-1, TNF, soluble fibronectin, and the IL-1 receptor in various experimental fibrotic diseases.[121,148–150]

Conclusion

TIF is characterized by the progressive accumulation of ECM proteins, mainly due to the transcriptional activation of these proteins. It has been shown that activated tubular epithelial cells, fibroblasts, and myofibroblasts are the main sources of the matrix proteins that constitute the interstitial scar tissue. Although many details of fibrogenesis remain to be elucidated, several common general features and events are noted during fibrosis. Inflammatory events initiated either locally, or at sites distant to the affected organs, facilitate the activation of fibrogenic cells in the primary affected site, and the migration of fibrogenic cells to that site; then, soluble factors released by these activated cells (by autocrine and paracrine functions) turn on the fibrotic cascade, resulting in extensive remodeling of the affected tissues and organs. Once further research has well characterized the molecules involved in TIF, this would help in developing strategies to make a focused approach to specific intervention in this complex cascade of fibrotic events.

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