Connective tissue growth factor in renal development and injury

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General Introduction
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1. Prevalence of chronic kidney disease (CKD) and chronic renal failure

CKD is a worldwide public health issue. In 2008, an estimated 1.77 million patients worldwide received dialysis (1). Global dialysis populations in countries including the United States, European countries and Japan have been estimated to have increased 2-fold from about 1 million to 2 million in the past 10 years (2), and the condition is associated with poor outcomes and high cost, particularly in the elderly (2, 3). The healthcare cost for the maintenance dialysis population over the 10 years from 2001 to 2010 has been estimated at about 1.1 trillion dollars (2). The financial and human resources that will be needed to care for patients with end-stage renal disease (ESRD) in 2015 will be considerably greater than those in 2005 (4). Incidence and prevalence rates per million population for ESRD differ substantially between countries (5-10) (Figure 1). Nephropathy associated with type 2 diabetes is the most frequent cause of ESRD all over the world (5). In both United States (USA) and Japan, more than 44% of patients starting dialysis therapy in 2009 had diabetic nephropathy (5, 8, 11, 12). In contrast, the prevalence of diabetic nephropathy in ESRD was lower in the Netherlands and the United Kingdom (UK) (5-7, 10)(Figure 2). Nephrosclerosis is also a common cause of ESRD and the prevalence in ESRD is much higher for the USA than for the UK, the Netherlands or Japan (5-10) (Figure 3). The prevalence of CKD in the USA, the Netherlands and Japan has been estimated as 11.6% of the adult population (23 million), 10.6 % (1.3 million), and 10.6% (11 million), respectively. These are surprisingly high numbers of individuals of whom some will go on to develop end stage renal failure, but most as a consequence of their CKD will develop severe cardiovascular complications leading to premature death (13-15). To prevent the increasing incidence and prevalence of renal failure, many societies around the world, such as the International Society of Nephrology (ISN), Kidney Disease Improving Global Outcomes (KDIGO) and National Kidney Foundation (NKF), have called for attention and have developed programs to prevent kidney diseases and improve the care and outcomes for kidney disease patients worldwide (16). Recently, angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) have become the standard for therapies against proteinuric renal disease (17). Mineralocorticoid receptor blockade (18-20) and renin inhibitors (21, 22) have been introduced to reduce renal injury, but these agents do not completely inhibit the progression of chronic renal disease. From this perspective, investigation into the mechanisms underlying the progression of renal fibrosis and therapeutic approaches against new specific molecules are important and may have additive beneficial effects. In terms of the modalities of renal replacement therapy, considerable differences exist in the prevalence of peritoneal dialysis (PD) and transplantation worldwide (5-10) (Figure 4). The prevalence of PD is >70% in Mexico and Hong Kong, but is much lower in Japan (5).
Figure 1
Incidence (A) and prevalence (B) of endstage renal disease (ESRD) from 2001 to 2008 (ref 1-5).
* Data for the UK include only England.
Figure 2
Incidence (A) and prevalence (B) of endstage renal disease in 2008 for all patients (red bars) and for patients with diabetic nephropathy (blue bars) (ref 1-5).
* Data for the UK include only England.
Figure 3
Incidence (A) and prevalence (B) of endstage renal disease in 2008 for all patients (red bars) and for patients with hypertension (blue bars) (ref 1-5).
* Data for the UK include only England.
** Data for Japan represent nephrosclerosis likely due to hypertension (blue bars).
Figure 4
Differences in modalities of renal replacement therapy in the US, UK, the Netherlands and Japan (ref 5-10).
HD, hemodialysis; PD, peritoneal dialysis; Tx, transplantation.
2. Mechanisms of kidney disease progression

Development of renal fibrosis
Fibrosis is the final common pathway for almost all forms of renal disease that progress to end-stage renal failure, including immunologically mediated glomerulonephritis and tubulo-interstitial nephritis, hemodynamic disorders, metabolic diseases, and hereditary diseases. After the initial injury, the affected kidney tissues undergo a series of events in an attempt to recover from the damage. Mechanisms in these processes include activation of kidney resident cells, which leads to the production and secretion of cytokines and growth factors. Chemotactic cytokines recruit inflammatory cells in the affected glomeruli and tubulo-interstitial area. These cells produce and secrete reactive oxygen species, in addition to chemokines, and inflammatory and fibrogenic cytokines, which activate mesangial cells and fibroblasts leading to glomerulosclerosis and tubulo-interstitial fibrosis. Severe damage or continued injurious stimuli lead to accumulation of extracellular matrix (ECM) in the glomeruli and tubulo-interstitial area which is usually associated with disease progression (23). Although numerous specific causes initiate renal injury, many such disorders appear to share common pathogenic pathways in disease progression. Understanding these issues is not only important to elucidate the pathogenic mechanisms underlying CKD, but may also provide novel insights into developing new therapeutic strategies.

Glomerular injury
Many different glomerular disorders exist, including immunologically mediated glomerulonephritis, metabolic renal diseases and hypertensive nephrosclerosis. Glomerular injury by these disorders can represent the initial event in the progression of renal fibrosis. Glomerular injury can be classified according to the localization of injury by immune complexes or other stimuli. Endothelial cell injury, which is typically demonstrated in post-infectious glomerulonephritis, sever hypertension, hemolytic-uremic syndrome and lupus nephritis (WHO III/IV), may induce rapid decline in renal function. In contrast, podocyte injury, such as membranous nephropathy and minimal-change nephritic syndrome, is often accompanied by heavy proteinuria. MCNS does not progress to renal failure, although a number of patients with this diagnosis are found to have FSGS on subsequent biopsies (24). Immunoglobulin (Ig)A nephropathy is a renal disease of mesangial injury, and represents mesangial proliferative glomerulonephritis characterized by diffuse mesangial deposition of IgA. IgA nephropathy is common among both Caucasians in Europe and Japanese. One quarter of patients will have ESRD by 20 years, while the evidence shows clinical remission in one-third of patients with mild disease (25).

Tubulo-interstitial injury
Clinical parameters are generally accepted to correlate better with interstitial changes than with glomerular changes (26, 27). Tubulo-interstitial injury is thus an important
parameter in the assessment of renal damage. Furthermore, understanding the mechanisms of tubulo-interstitial fibrosis is essential in establishing novel therapeutic strategies for preventing progressive kidney diseases. Fibrosis usually develops due to imbalances between the synthesis, deposition and degradation of ECM. The mechanisms have been extensively studied using many animal models. A number of cytokines (tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6), Platelet-activating factor (PAF), vasoactive compounds (angiotensinogen, angiotensin II, endothelin, thromboxane A2), chemoattractant factors (monocyte chemoattractant protein-1(MCP-1), osteopontin), growth factors (platelet derived growth factor (PDGF), transforming growth factor (TGF)-β, connective tissue growth factor (CTGF), basic fibroblast growth factor (bFGF), transcription factors nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and renin-angiotensin-aldosterone system (RAAS) are involved in the progression of tubulo-interstitial fibrosis (28, 29). Accumulation of ECM depends on the balance of production and degradation. ECM turnover is regulated by matrix metalloproteinases (MMPs) and their endogenous inhibitors, tissue inhibitors of metalloproteinase (TIMPs), along with the plasmin-plasminogen activator-plasminogen activator inhibitor (PAI) cascade (28, 29). Among the cells that accumulate in the renal interstitium, fibroblasts are a major contributor to matrix production and deposition in tubulo-interstitial fibrosis. The presence of myofibroblasts within the renal interstitium correlates with the extent of tubulo-interstitial scarring and fundamental outcomes in both clinical (30) and experimental glomerulonephritis (31, 32). In the last decade, it has been hypothesized that tubular epithelial cells, via epithelial-mesenchymal transformation (EMT), play an important role in the development of interstitial fibrosis by producing collagen and other components of the ECM (33, 34).

**Tubular EMT**

EMT is an essential process in embryogenesis (35), and is beneficial in normal wound healing (36), but pathogenic in malignancy (37, 38) and fibrosis (38). The pathological significance of tubular EMT in renal fibrosis has increasingly been recognized (39, 40). EMT is a cellular program consisting of a loss of cell-cell and cell-matrix interactions and cell polarity, cytoskeletal rearrangement, and basement membrane degradation with subsequent migration or invasion (39). Markers for EMT have recently been categorized and include loss of the epithelial adhesion protein E-cadherin and upregulation of mesenchymal markers such as fibroblast-specific protein 1 (FSP-1) (41). E-cadherin expression is regulated at multiple levels, including gene expression and both extra- and intracellular protein cleavage. E-cadherin gene expression is suppressed by a family of regulatory proteins, including Snail (42), Slug (43), Twist (44), ZEB1, and ZEB2 (45). These proteins are, in turn, regulated by growth factors such as PDGF, TGF-β, and Wnt proteins (37, 45). Mechanical stress, cytokines and various other factors induce fibroblasts to acquire a myofibroblast phenotype (46). A key feature of myofibroblasts is their contractile function, which is aided by the expression of α-smooth muscle actin (α-SMA), vimentin and desmin, which are intermediate filaments (46, 47).
contrast, myofibroblasts lose a quiescent fibroblast marker, membrane-bound ecto-5’-nucleotidase (47, 48).

During development of renal fibrosis in a unilateral ureteral ligation model, about 36% of new fibroblasts were shown to be derived from EMT, and 14-15% from bone marrow, and remaining from local proliferation (33, 39). However, findings from lineage tracing and in vivo reporter studies have suggested that resident interstitial mesenchymal cells, including residential fibroblasts, pericytes and perivascular cells, are an important source of myofibroblasts (18-20). Resident peritubular fibroblasts transform to myofibroblasts and provide an important contribution to the development of interstitial fibrosis (48). Resident renal mesenchymal stem cells might represent another source of myofibroblasts. Such cells reside in perivascular niches in humans (49), potentially explaining why fibrosis is often accentuated in perivascular areas. In these respects, the origin of fibroblasts remains controversial. The role of myofibroblasts in the kidney is less clear. Following the induction of unilateral ureteral obstruction (UUO) in transgenic reporter mice expressing enhanced green fluorescent protein (GFP) under the regulation of α1-type I collagen promoter, about 75% of GFP-positive cells expressed α-SMA (50). This suggests that although α-SMA-positive myofibroblasts might be a major source of ECM, other cell types such as more mature fibroblasts, tubular epithelial cells, macrophages or fibrocytes can also contribute to ECM deposition. In addition, the roles and regulation of α-SMA may be more complicated (51). In vitro, lack of α-SMA in renal fibroblasts results in more prominent motility, proliferation and type I collagen expression (51). In vivo, mice lacking α-SMA display more fibrosis and interstitial cell proliferation following UUO (51). In addition, α-SMA gene transfer into UUO kidneys significantly ameliorates interstitial fibrosis. These phenomena are considered to show that α-SMA deficiency is modulated by compensatory up-regulation of other smooth muscle-related protein (51).

**TGF-β in renal fibrosis**

TGF-β is the central mediator of fibrosis, mostly by inducing ECM production and proliferation of myofibroblasts and fibroblasts, but also through immunoregulatory functions. Thus, while direct inhibition of TGF-β might have undesired adverse effects, elucidation of the complex regulation of TGF-β might provide specific antifibrotic targets (29). Although the profibrotic actions of active TGF-β are well-described, recent reports have documented the interesting complexity of TGF-β regulation. TGF-β1 is produced in a latent form consisting of a TGF-β1 dimer bound to a latency-associated peptide (LAP), which inhibits binding to the TGF-β receptor. This complex is bound to latent TGF-β-binding protein (LTBP), which inhibits binding to the ECM. Transgenic mice that overexpress latent TGF-β1 show reduced expressions of IL-1β, TNF-α, and MCP-1, substantially reduced fibrosis in UUO and anti-GBM nephritis via Smad-7-mediated inhibition of NF-kB-dependent renal inflammation and TGF-β/Smad2/3-dependent fibrosis (52, 53). Regulation of TGF-β in renal fibrosis has not yet been fully elucidated.
Nephron number
After glomerular filtration rate falls below about half of normal, further loss of renal function continues, even if the original disease has become inactive. Increased glomerular intracapillary pressure is considered to be a key mediator of progressive renal sclerosis in this self-perpetuating vicious cycle. The increase in glomerular pressure may induce proteinuria, mediating the accompanying tubular and interstitial cell injury (54). In models of nephron loss, sclerosis indices for individual glomeruli were found to correlate with either maximum single-nephron glomerular filtration rate (GFR), glomerular pressure, or average values for these parameters as assessed by repeated micropuncture in the same glomeruli (55). Glomerular capillary hypertension is often maintained by angiotensin-dependent mechanisms via increased systemic pressure and vasoconstriction of efferent arterioles. ACEIs and ARBs are effective in protecting against glomerular sclerosis even if systemic pressure is not elevated. Although angiotensin II functions as a central mediator of glomerular hemodynamic changes, subsequent non-hemodynamic changes, such as mechanical and oxidant stress, NF-kB activation, chemokines, and TGF-β expression, will cause eventual glomerular sclerosis (54).

Chronic ischemia
In human diseases such as hypertensive nephrosclerosis, patients can develop tubulo-interstitial fibrosis leading to end-stage renal failure without heavy proteinuria. In such situations, chronic hypoxic injury might play the critical role in the progression of renal failure. Chronic ischemia in the tubulo-interstitium can be induced by impairment of the glomerular capillary bed and loss of peritubular capillaries. The mechanisms underlying this reduction in the capillary bed is considered to be related to vascular endothelial growth factor (VEGF)-A. Models of chronic renal injury have shown an early increase in VEGF, but this was reduced in chronic advanced disease (56, 57). Tubulo-interstitial fibrosis is characterized by loss of renal tubular epithelial cell integrity, loss of peritubular capillaries, and an increase in the myofibroblasts that are the main contributors to ECM deposition. Hypoperfusion of peritubular capillaries induces chronic hypoxia, which can induce and accelerate transdifferentiation of tubular epithelial cells (58, 59). Hypoxia-inducible transcriptional factors (HIFs) are elevated as transcriptional responses to hypoxia, inducing the regulation of erythropoietin, metabolic adaptation, and neoangiogenesis (60). Acute renal ischemic injury and acute nephrotoxic tubulo-interstitial injury also decrease peritubular capillary density in association with tissue hypoxia, leading to tubulo-interstitial fibrosis, and this might represent one of the mechanisms lowering renal function after recovery from acute ischemic kidney injury (61, 62).

3. Hypertension in renal disease
Nephrosclerosis is a disorder usually associated with chronic hypertension. Hypertension represents a major public problem in the United States, European countries and
Japan (63). Furthermore, 90% of patients with CKD experience hypertension (blood pressure>130/80 mmHg) during the course of the disease, which will accelerate kidney damage (64). In this respect, hypertension is a major cause and accelerator of renal morbidity and mortality. Hypertensive nephrosclerosis is characterized histologically by vascular, glomerular, and tubulo-interstitial involvement. Recent data suggest that two different processes can lead to glomerulosclerosis (65, 66).

Initial glomerular injury may relate to glomerular ischemia rather than to hyperfiltration and hypertension. In this early stage of hypertensive renal damage, renal pathology shows wrinkling and thickening of the glomerular basement membrane, with consequent hypoxia in the parenchyma leading to tubular atrophy and interstitial fibrosis (67, 68). Glomerular changes in the later stage of nephrosclerosis consist of an increase in the mesangial matrix, which results in either segmental or global sclerosis of the glomerular tuft, sometimes accompanied by hyalinosis lesion (65, 69). These changes are considered to be related to increased glomerular pressure by loss of renal autoregulation associated with afferent arteriolar dilatation and increase in glomerular capillary size and glomerular hypertrophy, and represent a secondary form of focal segmental glomerulosclerosis (FSGS) (70). Vascular and glomerular diseases are often associated with severe interstitial fibrosis. Glomerular ischemia leads to hypoxia in the postglomerular vasculature at an early stage with macrophage infiltration, matrix deposition and tubular atrophy (68).

4. Diabetic nephropathy
Diabetic nephropathy is a major cause of end-stage renal failure all over the world and is a worldwide public health issue. Pathological abnormalities are noted in patients with long-standing diabetes mellitus. The major histological changes in glomeruli in diabetic nephropathy are mesangial expansion, glomerular and tubular basement membrane thickening and glomerular sclerosis (71). Glomerular arteriolar hyalinosis is also prominent (72). Diabetic nephropathy seems to occur as a result of interactions between metabolic and hemodynamic factors (73).

Chronic hyperglycemia
Chronic hyperglycemia is a major initiator of diabetic microvascular complications. Numerous studies have indicated that hyperglycemia induces an elevation of TGF-β in the glomeruli and in cultured mesangial cells, and through the same cytokine, stimulates the production of matrix proteins (72, 74-80). In addition, overexpression of bone morphogenic protein 7, which is decreased in the diabetic kidney, prevents glomerulosclerosis and tubulo-interstitial collagen accumulation by acting counter to TGF-β (81). High glucose levels also evoke an intrinsic pathway of pro-apoptotic signaling in mesangial cells, which may contribute to renal injury (82). Hyperglycemia also impairs endothelial thrombomodulin-dependent activated protein C formation. Loss of this pathway has been reported to interrupt cross-talk between the vascular compartment and podocytes, causing glomerular cell apoptosis and proteinuria.
Conversely, maintaining high activated protein C levels during long-term diabetes protects against diabetic nephropathy (83).

**Advanced glycation end-products (AGEs)**

Advanced nonenzymatic glycation has been implicated in the pathogenesis of long-term complications of diabetes mellitus, including diabetic glomerular disease. AGEs have been shown to accumulate in the glomeruli and tubulo-interstitium by crosslinking with collagen, in association with the severity of the renal disease (84-86). AGEs are able to mediate diabetic complications by stimulating a number of mediators, including oxygen free radicals, cytokines, chemokines, adhesion molecules, TGF-β1, and CTGF (87). Induction of TGF-β1 appears to be the key intermediate step for many AGE-receptor for AGE (RAGE)-mediated effects on cell growth and matrix homeostasis (88). Inhibition of AGEs reduce overproduction of TGF-β1 in diabetic animals independent of the glycemic status (89). In addition, neutralizing antibodies to TGF-β1 are able to block AGE-induced EMT (90).

**Hemodynamic factors**

Hemodynamic factors that contribute to the development of diabetic nephropathy include increased systemic and intraglomerular pressure, as well as activation of vasoactive hormone pathways such as the renin-angiotensin-aldosterone system (91) and endothelin (92).

The early stage of diabetic nephropathy is characterized by glomerular hemodynamic abnormalities that result in glomerular hyperfiltration, leading to microalbuminuria even if the renal function is preserved. Hyperglycemia-induced hyperfiltration is due to the dilatation of afferent arteriole to a greater extent than the dilatation of efferent arterioles (93). Nitric oxide (93, 94), atrial natriuretic peptide (ANP) (95), insulin-like growth factor (IGF)-1 (96) and vasodilatory prostaglandin (97) have been shown to contribute to glomerular hyperfiltration and hypertension.

Other proposed mechanisms by which hyperglycemia might promote the development of diabetic nephropathy include activation of protein kinase C, and upregulation of heparanase expression, which accelerates degradation of heparan sulfate. The latter may lead to altered availability of various growth factors, which require the presence of heparan sulfate for proper signaling via their high affinity receptors (98-100). Sodium-glucose cotransporter 2 (SGLT2) is involved in transporting glucose across the apical membrane of the cell membrane. SGLT2 inhibitor may prevent the progression of diabetic nephropathy by lowering the toxicity of glucose in renal cells (101).

Altered insulin signaling also plays an important role in podocyte regulation and microalbuminuria (38-40). In recent reports, podocyte insulin sensitivity has been shown to play a critical role in the development of diabetic nephropathy. Mice with podocyte-specific deletion of the insulin receptor, even in the presence of normoglycemia, develop albuminuria with glomerular mesangial expansion, accumulation of matrix, podocyte apoptosis, and glomerulosclerosis (102). Recent evidence indicates that epigenetic
mechanisms, such as post-transcriptional histone lysine acetylation and deacetylation, histone methylation and demethylation, ubiquitination, histone sumoylation, DNA methylation, and activation of microRNAs, which constitute metabolic memory responses to episodes of transient hyperglycemia, will become major targets for treatment and prevention of diabetic complications, including diabetic nephropathy (41-43).

5. Peritoneal dialysis as a modality of renal replacement therapy
The characteristic feature of chronic peritoneal damage in peritoneal dialysis (PD) treatment is decreased ultrafiltration capacity associated with submesothelial fibrosis, accumulation of ECM and neoangiogenesis (103). The decrease in ultrafiltration capacity seen after prolonged PD often results in its discontinuation (104). According to a multi-center survey conducted in Japan, 34% of PD discontinuations are due to overhydration resulting from ultrafiltration failure or poor compliance with salt and fluid restriction (104, 105).

**Impact of uremia in the peritoneum (Figure 5-1)**
The pathophysiological mechanisms underlying changes to the peritoneal membrane are not fully understood (106), but both human biopsy studies and animal studies have suggested that uremia alone induces fibrotic changes in the peritoneum (Figures 6A, 6B) (103, 107). In addition, pre-dialysis uremic peritoneum showed predominant infiltration of CD68-positive macrophages, which is an important factor in determining baseline peritoneal permeability (108). The degree of associated peritoneal inflammation determines changes in peritoneal function before and after initiation of PD (108-111).

**Effects of peritonitis and acute inflammation in the peritoneum (Figure 5-2)**
Histologically, acute peritoneal inflammation can cause morphological damage to the peritoneum (Figures 6C, 6D) (112, 113). In addition, episodes of peritonitis are correlated with chronic peritoneal fibrotic changes (114). Both retrospective and prospective studies have found that peritonitis damages the peritoneal membrane and impairs its ultrafiltration capacity (115) and is an important risk factor for ultrafiltration failure (109, 115).

**Effects of peritoneal fluid on the peritoneum (Figure 5-3)**
In addition, the high glucose concentration, low pH, and presence of glucose degradation products in peritoneal dialysis solutions have all been implicated in modulation of the peritoneal membrane.

Morphological changes to the peritoneal membrane that occur over time in patients on PD include fibrosis and neoangiogenesis in association with changes in mesothelial cells.
Functional alteration of mesothelial cells in PD treatment (Figure 5-A)
Mesothelial cells are the main component of peritoneum and play an important role in peritoneal homeostasis, including antigen presentation, clearance of fibrin, and synthesis of cytokines, growth factors, and matrix proteins (116). Data is increasingly being accumulated on the role of mesothelial cells in determining functional alterations to the peritoneum during PD. Aging has also been shown to be accompanied by the production of inflammatory cytokines by mesothelial cells from patients without renal failure (117). Studies have demonstrated that peritoneal mesothelial cells undergo EMT after exposure to injury (118, 119) or associated growth factors to form fibroblasts (119). Furthermore, EMT of peritoneal mesothelial cells is associated with angiogenic stimuli (120) and altered solute transport (121). Evidence increasingly suggests that treatment to prevent EMT may also ameliorate peritoneal fibrosis and EMT is one of the treatment targets in tubulo-interstitial fibrosis (122).

Fibrosis and neoangiogenesis in ultrafiltration failure in PD (Figure 5-B)
Fibrosis and neoangiogenesis are known to constitute a major common pathway inducing ultrafiltration failure in long-term PD patients (Figures 6E, 6F) (103, 123). Many factors are involved in these processes. Studies have noted that increased dialysate concentrations of TGF-β, one of the most important growth factors for fibrosis, correlate with peritoneal membrane solute transport in PD patients (124, 125). In mesothelial cells, TGF-β is induced by glucose (126, 127), which is present in the PD fluid. The uremic milieu, along with non-physiological PD solutions, leads to the appearance of AGEs, which in turn induce TGF-β in peritoneal tissues. These AGEs bind to a cognate receptor (RAGE), and this interaction induces fibrosis, which is blocked by anti-RAGE antibodies (128). Another important pathological change in the peritoneal membrane is neoangiogenesis, which has been demonstrated in human biopsy specimens (103). In addition, daily infusion of 4.25% dextrose PD fluid induced peritoneal neoangiogenesis with abnormal peritoneal transport in rats (129, 130). VEGF concentrations in dialysate fluid reportedly correlate with solute transport in PD patients (131). Glucose degradation products have been demonstrated to be toxic to mesothelial cells, but also increase the production of VEGF by the same cells (132, 133). The interaction between fibrosis and angiogenesis seems to be linked through common initiating growth factors, and inflammatory cytokines, such as TGF-β (120, 134) and IL-1β (135), which up-regulate VEGF production and angiogenesis. Interventions that reduce angiogenesis also reduce fibrosis (136, 137). Endostatin peptide, an inhibitor of angiogenesis, has been shown to prevent the progression of peritoneal sclerosis in a mouse experimental model (137). Angiogenesis and fibrosis also seem to be linked with the process of EMT (138). Understanding the mechanisms of fibrosis and the interaction with angiogenesis is therefore important to developing therapeutic strategies in order to preserve the peritoneum as a dialysis membrane.
Figure 5
Mechanisms underlying deterioration of peritoneal membrane function in PD patients.
Figure 6
Peritoneum histology of peritoneal dialysis (PD) patients.
A, B: Peritoneal membrane of a patient with predialysis renal failure obtained at insertion of PD catheter.
C, D: Peritoneal membrane of bacterial peritonitis.
E, F: Peritoneal membrane of ultrafiltration failure after 8 years of PD treatment.
A, C, E: Hematoxylin-Eosin stain; B, D, F: Masson's trichrome stain; x100
6. Aim of the study

Connective tissue growth factor (CTGF/CCN-2) is a 349-amino acid cysteine-rich polypeptide belonging to the CTGF/Cyr61/Nov (CCN) family (139). CTGF was first identified by Bradham et al. in conditioned media of endothelial cells as a 36- to 38-kDa polypeptide containing chemotactic activity toward fibroblasts (140). The CCN family consists of six regulatory proteins, which participate in diverse biological processes such as angiogenesis and wound healing, and are involved in control of migration, cell proliferation and differentiation (139, 141, 142).

The aim of the studies described in this thesis was to investigate the possible involvement of CTGF in renal fibrosis and peritoneal fibrosis in PD patients.

- Tissue repair seems to recapitulate developmental programs at play in organogenesis. We studied expression of CTGF and relationships between CTGF and TGF-β isoforms in glomerulogenesis, normal adult glomerulus and various human glomerulopathies, using human and rat kidney specimens (Chapter II).
- We investigated the expression of CTGF mRNA in human renal biopsy specimens of various renal diseases using in situ hybridization (Chapter III).
- To investigate the expression and precise localization of CTGF and interactions with TGF-β isoforms in proliferative glomerulonephritis, we analyzed the well-established model of proliferative glomerulonephritis induced by anti-Thy-1.1 antibody in rats. Regulation of CTGF expression by TGF-β isoforms was studied in cultured cells (Chapters IV and V).
- We focused on diabetic nephropathy and hypertensive nephrosclerosis, as the main causes of renal failure in the United States, Japan and the European countries. We studied CTGF expression in human type 2 diabetic nephropathy and hypertensive nephrosclerosis using renal tissues, urine, plasma and cultured cells. For hypertensive nephrosclerosis, we also analyzed CTGF in uninephrectomized spontaneous hypertensive rats, similar to the late stage of the human hypertensive nephrosclerosis (Chapters VI and VII).
- Finally, we studied the possible roles of CTGF in the peritoneal fibrosis and peritoneal transport dysfunction in peritoneal dialysis patients (Chapter VIII).
- Understanding the expression of CTGF in human materials and animal models of renal fibrosis and peritoneal fibrosis in PD patients may provide us with new potential therapeutic targets for preventing the progression of renal disease and peritoneal injury.
REFERENCES

General Introduction


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