Involvement of bone-marrow-derived cells in kidney fibrosis

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Takashi Wada[¶#], Norihiko Sakai[¶]*, [#]Yoshio Sakai, Kouji Matsushima[#], Shuichi Kaneko*, Kengo Furuichi[¶]*

[¶]Division of Nephrology, [#]Department of Laboratory Medicine, *Department of Disease Control and Homeostasis, Institute of Medical, Pharmaceutical and Health Sciences, Faculty of Medicine, Kanazawa University, Kanazawa, [#]Department of Molecular Preventive Medicine, University of Tokyo, Tokyo, Japan.

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Corresponding author: Takashi Wada

Division of Nephrology, Department of Laboratory Medicine, Institute of Medical, Pharmaceutical and Health Sciences, Faculty of Medicine, Kanazawa University, Kanazawa, 13-1 Takara-machi, Kanazawa 920-8641, Japan

tel: +81-76-265-2498

fax: +81-76-234-4273

e-mail: twada@m-kanazawa.jp

Abstract

Cellular mechanisms have been proposed in the pathogenesis of fibrotic processes in kidney. In this setting, cell sources underlying the generation of matrix-producing cells in diseased kidneys have been categorized as activated resident stromal cells (e.g., fibroblasts, pericytes), infiltrating bone marrow-derived cells (e.g., fibrocytes, T cells, macrophages), cells derived from epiethelial-mesenchymal transition/endothelial-mesenchymal transition. Among these cell sources, accumulating evidence has shed light on the involvement of bone marrow-derived cells including monocytes/macrophages and a circulating mesenchymal progenitor cell, fibrocyte, in the progression of fibrosis in kidney. Bone marrow-derived cells positive for CD45 or CD34, and type I (pro)collagen dependent on chemokine system and renin-angiotensin system migrate into diseased kidneys and enhance the synthesis matrix protein, cytokines/chemokines and pro-fibrotic growth factors, which may promote and escalate chronic inflammatory processes and possible interaction with resident stromal cells, thereby perpetuating kidney fibrosis.

Introduction

Fibrosis is a characteristic hallmark that determines the prognosis of any kind of progressive kidney diseases, leading to kidney failure. The histological characteristics of interstitial fibrosis in kidney are evidenced by the presence of tubular atrophy and dilation, interstitial leukocyte infiltration, accumulation of fibroblasts, and increased interstitial matrix deposition (1). In this aspect, there is accumulating evidence that cellular mechanisms driving fibrosis are involved (2). Cell sources underlying the generation of matrix-producing cells in diseased kidneys have been categorized as follows; 1) Activated resident stromal cells (e.g., fibroblasts, pericytes), 2) infiltrating bone marrow-derived cells (e.g. fibrocytes, T cells, macrophages), 3) epithelial-mesenchymal transition, EMT/endothelial-mesenchymal transition, EndMT (3-5) (Figure 1).

Among cells responsible for kidney fibrosis, fibrocytes, originally identified as a circulating bone marrow-derived, $CD34^+$ cell population of fibroblast-like cells in 1994, was reported to infiltrate from inflammatory exudates into subcutaneously implanted wound chambers (6). Accumulating evidence suggests that fibrocytes, uniquely comprising a minor fraction of the circulating pool of leukocytes (less than 1%), are a candidate for participating in organ fibrosis associated with conditions in lungs, skin and visceral fibrosis, heart, liver and kidneys, as well as in the physiological roles, such as wound repair, cochlear physiology and auditory function (7-8). In addition, a recent study reveals that the delicate balance of peroxisome proliferators-activated receptor gamma and transforming growth factor (TGF)-beta 1 activation drives the selection of an adipocyte or myofibroblast differentiation pathway through SAPK/JNK signaling (9).

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Originally, fibrocytes were identified by CD34 and collagen-1 co-expression (6, 7). A recent study revealed that markers (CD45RO, 25F9, S100A8/A9) that distinguish monocyte-derived fibrocytes from monocytes, macrophages, and fibroblasts (10). However, identification of markers specific to fibrocytes remains to be investigated. Fibrocytes may be identified by dual positivity of CD34 or CD45 and collagen-1 or pro-collagen-1 (6, 7).

Herein, in this manuscript, we focus on the involvement of bone marrow-derived cells, contributing to the pathogenesis of kidney fibrosis.

Detection of cells dual positive for CD34 or CD45 and type I collagen in kidney fibrosis

The detection and role of fibrocytes in the progressive fibrosis in kidney remains investigated thus far. We have uncovered CD45- and type I collagen-dual positive cells (CD45$^+$/ColI$^+$) infiltrated the interstitium, especially the corticomedullary regions in progressive kidney fibrosis induced by ureteral ligation in mice by immunostainings and flow cytometry analyses (11) (Figure 2a). The number of infiltrating fibrocytes increased with the progression of fibrosis after a ureteral ligation, reaching a peak on day 7 (Figure 2b). To further verify the existence of fibrocytes, dual immunostainings of CD34 and type I collagen were also performed. The infiltration of CD34- and type I collagen-dual positive cells was also observed in the interstitium and correlated with disease progression as determined by CD45- and type I collagen-dual immunostainings (11).

These findings in mice prompted us to explore the presence of CD45- and type I procollagen-dual positive cells (CD45$^+$/proColI$^+$) infiltrating into human kidneys, including diabetic nephropathy. The number of infiltrating CD45$^+$/proColI$^+$ in the interstitium correlated well with the severity of tubulointerstitial lesions, such as interstitial fibrosis, the number of CD68-positive macrophages as well as urinary monocyte chemoattractant protein-1 (MCP-1)/CCL2 levels in patients with chronic kidney disease. In particular, there was an inverse correlation between the number of interstitial CD45$^+$/proColI$^+$ and kidney function at the time of biopsy (12). The numbers of interstitial CD45$^+$/proColI$^+$ and macrophages as well as urinary MCP-1/CCL2 levels were significantly decreased during convalescence induced by glucocorticoid therapy. Collectively, these results suggest that cells dual positive for CD34 or CD45 and type I (pro)collagen may be involved in the pathogenesis of chronic kidney disease, leading to kidney fibrosis through the interaction with macrophages as well as MCP-1/CCL2.

Chemokine system involved in infiltration of bone marrow-derived cells dual positive for CD34 or CD45 and type I collagen into kidney

It is of note that fibrocytes, isolated from human and mice, express chemokine receptors such as CCR2, CCR3, CCR5, CCR7, and CXCR4, thereby regulating the recruitment to sites of fibrosis (7, 11, 13-15). Supporting this notion, intradermal instillation of SLC/CCL21 was firstly described to induce the recruitment of fibrocytes at the injected site (16). In addition to skin lesions, CCR7-expressing infiltrating cells, also positive for type 1 collagen (CCR7$^+$/ColI$^+$), were detected in diseased kidneys 7 days after ureteral ligation in wild-type mice (11). 37.8% of infiltrating cells expressed CCR7 (number of CCR7$^+$/ColI$^+$ divided by the number of CCR7$^+$ or CXCR4$^+$ or

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CCR2$^+$/ColI$^+$). In wild-type mice, the ratio of CCR7$^+$/ColI$^+$ in obstructed kidneys was increased to 7.9% of the total isolated renal cells compared with that in normal kidneys (0.25%) and contralateral kidneys (0.21%).   Of these CCR7-expressing cells in obstructed kidneys, 66.5% of cells were CXCR4$^+$/CCR2$^+$, 16.8% of cells were CXCR4$^+$/CCR2$^-$, 4.3% of cells were CXCR4$^-$/CCR2$^+$, and 12.4% of cells were CXCR4$^-$/CCR2.   The impact of CCL21/CCR7 signaling on progressive kidney interstitial fibrosis was further examined.   Mean interstitial fibrosis as well as the amount of hydroxyproline were reduced by almost 50 % in mice treated with anti-CCL21 antibodies compared with that in wild-type mice 7 days after an ureteral ligation, which was confirmed by the similar reduction in CCR7-null mice (11). Accordingly, based on the finding that treatment with anti-CCL21 antibodies or CCR7 deficiency resulted in over 50% reduction in the number of CD45$^+$/ColI$^+$, thereby CCL21/CCR7 signaling is thought to be the major pathway attracting CD45$^+$/ColI$^+$ into the kidney in this particular model (11). Interestingly, blockade of CCL21/CCR7 signaling reduced the number CCR2-expressing infiltrates in immunohistochemical studies along with renal transcripts of MCP-1/CCL2. *In vitro* studies also revealed that stimulation of cultured CD45$^+$/ColI$^+$ with angiotensin II enhanced the expression of mRNA of type 1 collagen and TGF-beta (17). These findings further suggest that CD45$^+$/ColI$^+$ may contribute to kidney fibrosis by producing MCP-1/CCL2 and TGF-beta, which may be responsible for chronic persistent inflammatory processes and activation of resident stromal cells (e.g., fibroblasts, pericytes) and the process to EMT/EndMT, in addition to collagen synthesis of CD45$^+$/ColI$^+$.   All these events may orchestrates fibrotic down stream events, eventually resulting in kidney fibrosis (Figure 1).

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Renin-angiotensin system and bone marrow-derived cells dual positive for CD45 and type I collagen

Renin-angiotensin system is one of the major pathway in the pathogenesis of fibrotic conditions, possibly dependent on two major distinct receptors, designated as angiotensin II type 1 receptor (ATR1) and angiotensin II type 2 receptor (ATR2). We hypothesized that $CD45^+/ColI^+$ might contribute to kidney fibrosis dependent on the renin-angiotensin system. In a murine model of kidney fibrosis, the extent of kidney fibrosis in AT2R deficient mice was more evident, concomitantly with the larger number of infiltrating $CD45^+/ColI^+$ in fibrotic kidneys (17). Interestingly, $CD45^+/ColI^+$ numbers in bone marrow were also increased in mice treated with ureteral ligation, especially in AT2R deficient mice (Figure 2c). In the points of therapeutic views, pharmacologic inhibition of AT1R reduced the degree of kidney fibrosis as well as the number of $CD45^+/ColI^+$ in both the kidney and the bone marrow. Supportingly, AT1R inhibition decreased the angiotensin II-stimulated expression of type I collagen synthesis in isolated human $CD45^+/ColI^+$, while an AT2R inhibitor augmented the expression of mRNA of type I collagen. These results suggest that AT1R/AT2R signaling may contribute to the pathogenesis of kidney fibrosis by at least two mechanisms: (1) by regulating the number of $CD45^+/ColI^+$ in bone marrow, and (2) by activation of these cells (17).

Fibrocytes: possible clinical biomarker for fibrotic disease

Clinical biomarkers for reflecting fibrogenic activity and indicating disease progression are required in various fields. Moeller et al. reported that fibrocytes defined as cells positive for CD45 and collagen-1 were significantly elevated in patients with stable

idiopathic pulmonary fibrosis (IPF), with a further increase during acute disease exacerbation. Fibrocyte numbers were an independent predictor of early mortality. The mean survival of patients with fibrocytes higher than 5% of total blood leukocytes was 7.5 months compared with 27 months for patients with less than 5%. Thus, they conclude that circulating fibrocytes in patients with IPF are an indicator for disease activity of IPF and might be useful as a clinical marker for disease progression (18). Supporting this notion, the number of interstitial $CD45^+/proColI^+$ was significantly decreased during convalescence induced by glucocorticoid therapy in patients with chronic kidney diseases (12). Further studies would be required for providing the evidence that counting fibrocyte numbers might be a biomarker for activity and progression of fibrotic conditions.

Involvement of monocytes/macrophages in kidney fibrosis

Recent studies reveal that macrophage diversity in response to their microenvironment uncovers their roles in kidney injury, raising therapeutic possibilities to attenuate kidney fibrosis (19-20). With our viewpoint, human peripheral CD14-positive monocytes/macrophages directly contribute to producing type I collagen, resulting in fibrogenesis, which are dependent on an amplification loop of MCP-1/CCL2-CCR2 (21). In addition, the presence of MCP-1/CCL2 expression is suggestive of a chronic stage of disease, especially in tubulointerstitial lesions and urinary levels of protein excretion, despite of renal etiologies through the recruitment and activation of macrophages. As is well-known, urinary protein excretion promotes and escalates tubulointerstitial lesions, resulting in kidney fibrosis. Moreover, the measurement of urinary MCP-1/CCL2 levels is a useful clinical tool for monitoring the disease activity

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of inflammatory renal disorders, including diabetic nephropathy (22-25). This was supported by the fact that blockade of MCP-1/CCL2 prevented leukocyte migration to the kidney, urinary protein excretion and TGF-beta expression, thereby preventing glomerulosclerosis and interstitial fibrosis (23, 26-28). In addition to MCP-1/CCL2-CCR2, blockade of fractalkine-CX3CR1 also reduced kidney fibrosis, which was concomitant with reduction in macrophage infiltration (29). CCR2 receptor is expressed in glomerular podocytes, suggesting MCP-1/CCL2 activation of CCR2 on podocytes may underlie induction of MMP-12, leading to glomerular basement membrane damage and urinary protein excretion (30). Finally, it is of note that there were significant correlations between the numbers of CD45^{+}/proColI^{+} and macrophages in human kidneys as well as urinary levels of MCP-1/CCL2, suggesting the close relationship of CD45^{+}/proColI^{+} with macrophages. Based on these results, we propose MCP-1/CCL2-CCR2 axis on the recruitment and activation of bone marrow-derived cells, especially macrophages, play a role in the pathogenesis of tubulointerstitial lesions, resulting in kidney fibrosis despite of kidney etiologies (Figure 3).

Involvement of T cells in kidney fibrosis

The degree of fibrosis is related to leukocyte infiltration. Tapmeier et al. examined the role of different T cell populations on kidney fibrosis in the mouse model of unilateral ureteral obstruction (31). They found a critical role for CD4^{+} T cells in kidney fibrosis. In addition, Nikolic-Paterson describes modulation of T cells in the process of kidney fibrosis; 1) T cells may act directly fibroblasts and pericytes to promote their migration, proliferation, and differentiation, resulting in the accumulation of alpha-SMA[+] myofibroblasts, which synthesize and deposit interstitial matrix, 2) T cells may induce a

profibrotic phenotype in the infiltrating macrophage population, which, in turn, secrete pro-fibrotic and pro-proliferative cytokines and growth factors that induce fibroblast migration, proliferation, and differentiation, 3) T cells may act directly on tubular epithelial cells to induce secretion of cytokines and growth factors that, in turn, act on fibroblasts (32).

Stromal cell activation and myofibroblast generation in kidney fibrosis

Activation of local stromal cells (e.g., fibroblasts and pericytes) and generation of myofibroblasts from epithelial cells (via EMT), pericytes, endothelial cells (via EndMT) and bone marrow-derived cells are key processes in tubulointerstitial fibrosis (33). For 15 years, EMT has been viewed as a principle source of fibroblasts in tissue fibrosis (34). In addition to tubular epithelial cells, glomerular podocytes (35) and endothelial cells (36-37) undergo transition after injury, which have been reported to be involved in kidney damage, resulting in fibrosis. More recently, in the process of perpetuation of fibrogenensis, hypermethylation of *RASAL1*, encoding an inhibitor of the Ras oncoprotein, is associated with the perpetuation of fibroblast activation and fibrogenesis in the kidney (38). In contrast, Duffiels et al. describe that pericytes and perivascular fibroblasts are primary source of collagen-producing cells in kidney fibrosis (39-40). Therefore, further studies would be required for determining the degree to which processes including those resulted from bone marrow-derived cells, contributes to kidney fibrosis.

Concluding remarks and future directions

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A deep insight of bone marrow-derived cells dependent on chemokine system and renin-angiotensin system provides a key for the novel pathogenesis of progressive organ fibrosis including kidney fibrosis. In addition to those systems, our recent unpublished data suggest the possible involvement of $CD45^+/ColI^+$ in diabetic nephropathy, which is supported by the evidence that $CD45^+/proColI^+$ are detected in human diabetic nephropathy (12). Further studies for biology of bone marrow-derived cells and the interaction of resident stromal cells would be required for a better understanding and the therapeutic benefit for kidney fibrosis.

Acknowledgements

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Figure legends

Figure 1. Possible cellular mechanims involved in fibrosis in kidney

Figure 2. Cells dual positive for CD34 or CD45 and type I collagen, detected in kidneys

and bone marrow, may play a role in kidney fibrosis.

(a) In wild-type mice, $CD45^+/ColI^+$ infiltrated the

Interstitium, especially the corticomedullary regions after ureteral ligation. (b) The

number of infiltrating $CD45^+/ColI^+$ as well as mean interstitial fibrosis in kidney were

reduced in mice treated with anti-CCL21 antibodies and in CCR7-null mice compared

with that in wild-type mice 7 days after ureteral ligation. Values are the mean ±SEM.

(c) By flow cytometry analyses, fibrocytes increased in number at bone marrow cells,

which was enhanced in AT2R-null mice with ureteral ligation.

Figure 3.   Macrophages dependent on MCP-1/CCL2-CCR2 axis contribute to

progressive kidney diseases, resulting in fibrosis.