Mini Review

The role of fibrocytes in progressive renal fibrosis

Norihiko Sakai[1)], Takashi Wada[2,*)], Kouji Matsushima[3)], and Shuichi Kaneko[1)]

[1)]Disease Control and Homeostasis, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan
[2)]Department of Laboratory Medicine, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan
[3)]Department of Molecular Preventive Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Fibrosis is a common pathway resulting in organ failure. Renal fibrosis is a progressive and potentially lethal disease caused by diverse clinical entities. The degree of renal fibrosis well correlates with the prognosis of renal diseases independent of their etiologies. Fibrocytes are peculiar circulating cells that share markers of leukocytes as well as mesenchymal cells. A considerable number of fibrocytes dual positive for CD45 and type I collagen or CD34 and type I collagen infiltrated the interstitium along with the progression of fibrosis in an experimental murine renal fibrosis model. Most fibrocytes in the kidneys were positive for CCR7. In addition, a ligand for CCR7, secondary lymphoid tissue chemokine (SLC/CCL21) co-localized with high endothelial venule-like vessels in fibrotic kidneys. CCL21/CCR7 blockade reduced the number of infiltrating fibrocytes as well as the extent of renal fibrosis, which was confirmed by a decrease in renal transcripts of pro α1 chain of type I collagen and transforming growth factor-β_1. These findings suggest that CCL21/CCR7 signaling contributes to the progressive renal fibrosis through the regulation of fibrocytes.

Rec.1/9/2007, Acc.3/1/2007, pp494-498

*Correspondence should be addressed:
Takashi Wada, Department of Laboratory Medicine, Graduate School of Medical Science, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8641, Japan. Phone +81-76-265-2498, Fax +81-76-234-4273

Key words fibrocytes, fibrosis, CCR7, CCL21, kidney

Introduction

Fibrosis is a characteristic pathological finding of progressive organ diseases, resulting in organ failure. Renal fibrosis is a progressive and potentially lethal disease caused by diverse clinical entities[1)]. In addition, the degree of renal fibrosis correlates well with the prognosis of renal diseases independent of their etiologies[2)]. The histological picture of renal fibrosis is characterized by tubular atrophy and dilation, interstitial leukocyte infiltration, accumulation of fibroblasts, and increased interstitial matrix deposition[3)]. Currently, resident fibroblasts, epithelial-mesenchymal transition (EMT)-derived fibroblasts/myofibroblasts and monocytes/macrophages are thought to be major participants in the pathogenesis of renal fibrosis[4-5)].

A circulating bone marrow-derived population of fibroblast-like cells (termed fibrocytes), first identified a decade ago, is thought to be another candidate for participating organ fibrosis[6)]. Fibrocytes comprise a minor fraction of the circulating pool of leukocytes (less than 1%) and share the markers of leukocytes

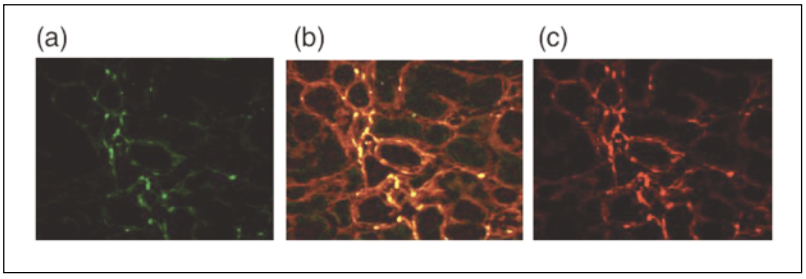

Fig.1 Fibrocytes dual-positive for CD45 and type I collagen infiltrated the kidney after ureteral ligation

CD45- and type I collagen-dual positive fibrocytes infiltrated the interstitium, especially the corticomedullary regions in mice kidney (a; CD45, b; merge, c; type I collagen).

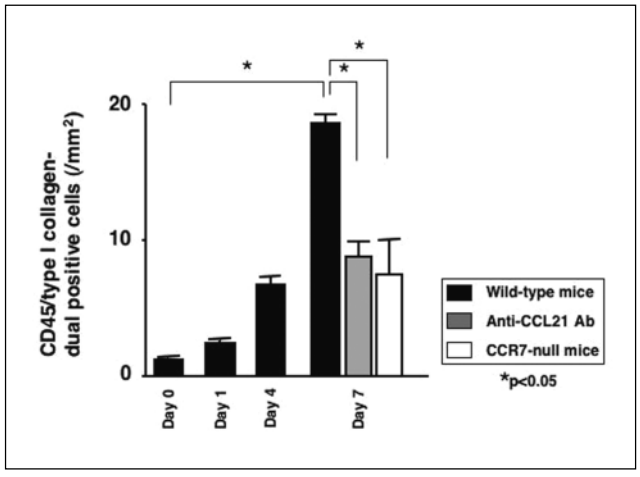

Fig.2 Fibrocytes infiltrated the kidney after ureteral ligation dependent on CCL21/CCR7 signaling

The number of infiltrating fibrocytes dual positive for CD45 and type I collagen was reduced in wild-type 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.

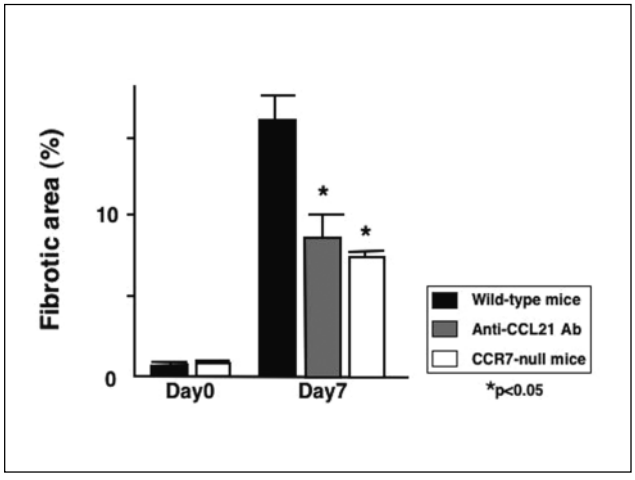

Fig.3 CCL21/CCR7 signaling contributed to renal fibrosis

The extent of renal fibrosis was reduced by 50% in wild-type mice treated with anti-CCL21 antibodies and in CCR7-null mice compared with those in wild-type mice 7 days after UUO. Values are the mean ± SEM.

(e.g., CD45, CD34) as well as mesenchymal cells (e.g., type I collagen, fibronectin)[6]. Fibrocytes are present in experimental fibrosis associated with conditions such as pulmonary fibrosis, bronchial asthma and skin wounds[7-10]. Furthermore, fibrocytes are detected in human fibrosing diseases including nephrogenic fibrosing dermopathy and burns[11-12]. Of note, fibrocytes express chemokine receptors such as CCR7, CXCR4 and CCR2[7-8]. Recent studies demonstrate that chemokine/chemokine receptor systems on fibrocytes are involved in the recruitment of circulating fibrocytes to sites of fibrosis[7-8].

Here we review the pathophysiological roles of fibrocytes in renal fibrosis and discuss their trafficking into diseased kidneys from circulation.

Fibrocytes In An Experimental Renal Fibrosis Model

1) Presence of fibrocytes in fibrotic kidneys

One of the unique characteristics of fibrocytes is the simultaneous expression of both leukocyte markers, such as CD45 and CD34, and type I collagen[6]. Renal fibrosis induced by unilateral ureteral obstruction (UUO) is a well-known renal fibrosis model in mice. Recently, we have revealed that fibrocytes dual positive for CD45 and type I collagen or CD34 and type I collagen were present in the interstitium, especially of the corticomedullary regions in wild-type mice fibrotic kidneys after UUO (Fig.1a-c)[13]. In addition, the number of infiltrating fibrocytes increased with the progression of fibrosis after a ureteral ligation (Fig.2). Thus far, it has been reported that expression of certain chemokine

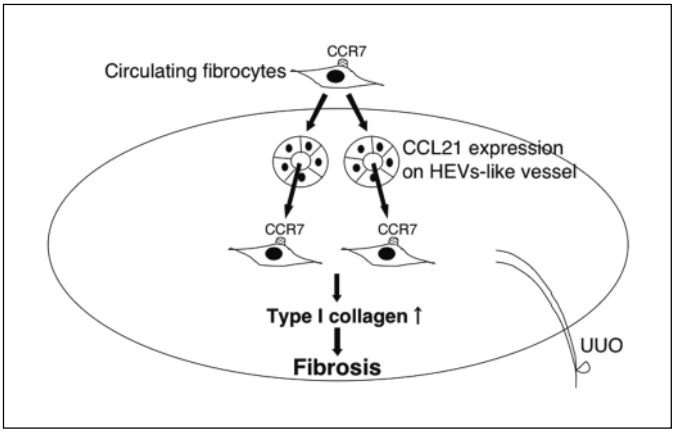

Fig.4 Schema for CCL21/CCR7-dependent renal fibrosis

receptors, such as CCR7, CXCR4 and CCR2, is detectable on fibrocytes isolated from humans and mice[7-8]. These findings prompted us to perform flow cytometry analyses to characterize the infiltrating fibrocytes based on expressions of chemokine receptors. In wild-type mice, 37.8% of the infiltrating fibrocytes expressed CCR7 following ureteral ligation[13]. Among these CCR7-expressing fibrocytes, 66.5% of cells were positive for both CXCR4 and CCR2, and 21.1% of cells were positive for either CXCR4 or CCR2[13].

2) CCL21/CCR7 signaling regulates fibrocyte infiltration and renal fibrosis

Secondary lymphoid tissue chemokine (SLC/CCL21), a ligand for CCR7, is a member of the CC chemokine family. The first two cysteine residues of which are adjacent to each other. CCL21 contains six cysteines and is a potent chemoattractant for T cells, B cells, and dendritic cells[14-16]. In addition, CCL21 also acts as a chemotactic stimulus for fibrocytes[10]. Our study demonstrated that treatment with anti-CCL21 antibodies or CCR7 deficiency in gene-targeted mice resulted in over 50% reduction in the number of CD45- and type I collagen-dual positive fibrocytes (Fig.2)[13]. It was also noted that the number of CCR7-expressing fibrocytes was also decreased in mice treated with anti-CCL21 antibodies compared with that in wild-type mice 7 days after UUO. Based on these findings, CCL21/CCR7 signaling is thought to be the major pathway attracting fibrocytes into the kidney in this model. Furthermore, the extent of renal fibrosis estimated by computer-assisted measurement as well as the amount of hydroxyproline was reduced by 50% in mice treated with anti-CCL21 antibodies and in CCR7-null mice compared with those in wild-type mice 7 days after UUO (Fig.3)[13]. Ureteral ligation enhanced the expression of pro $\alpha 1$ chain of type I collagen (COL1A1) mRNA as well as transforming growth factor (TGF)-$\beta 1$ mRNA in wild-type mice, which were significantly reduced by blockade of CCL21/CCR7 signaling[13]. These findings suggest that fibrocytes contribute to renal fibrosis by the production of type I collagen and that this process requires CCL21/CCR7 signaling. In contrast, the infiltration of CXCR4-positive fibrocytes was not reduced by the blockade of CCL21/CCR7[13]. In this aspect, CXCR4-positive fibrocytes are reported to migrate in response to CXCL12, a ligand for CXCR4, and trafficked to the lungs in a murine model of bleomycin-induced pulmonary fibrosis[8]. Further, treatment of bleomycin-exposed animals with specific neutralizing anti-CXCL12 antibodies inhibited infiltration of CXCR4-positive fibrocytes and attenuated lung fibrosis[8]. Therefore, these findings suggest that other chemokine/chemokine receptor pathways may be also involved in the recruitment and activation of fibrocytes, resulting in progressive fibrosis. Further studies will be required to elucidate the precise mechanisms of fibrocyte trafficking into fibrotic kidneys.

3) Infiltration routes of fibrocytes to fibrotic kidneys

High endothelial venules (HEVs) are specialized venules that allow rapid and selective lymphocyte trafficking from the blood into lymph nodes and Peyer's patches under physiological conditions[15]. HEVs express certain chemokines, such as CCL21[15] and EBI1-ligand chemokine/CCL19[17], that can activate CCR7-expressing cells. In contrast, HEV-like vessels, which are observed in chronically inflamed nonlymphoid tissues, are thought to play an important role in the pathogenesis of various inflammatory diseases, such as rheumatoid arthritis and Graves' disease[18-19]. In addition, CCL21-positive HEV-like vessels were found in synovial tissues from patients with rheumatoid arthritis[18]. With regard to human kidney diseases, HEV-like vessels are found at the corticomedullary junction and associated with interstitial leukocyte infiltration in human glomerulonephritis,

whereas HEV-like vessels are not detected in normal kidneys[20]. We observed that the expression of CCL21 mRNA in diseased kidneys was upregulated with the progression of fibrosis in wild-type mice after ureteral ligation[13]. Furthermore, CCL21 protein co-localized with HEV-like vessels in the corticomedullary regions in immunohistochemical studies. The increase in the number of CCL21-positive HEV-like vessels correlated with the progression of fibrosis after ureteral ligation. It was also noted that the number of infiltrating CCR7-positive fibrocytes was markedly reduced by the blockade of CCL21/CCR7 signaling. Taken together, these findings suggest that CCR7-expressing circulating fibrocytes infiltrate the kidney via CCL21-positive HEV-like vessels as illustrated in Figure 4, resulting in renal fibrosis.

4) Effect of blockade of CCL21/CCR7 signaling on expression of renal monocyte chemoattractant protein-1 (MCP-1/CCL2) and infiltration of macrophages

Progressive organ fibrosis is pathologically characterized by the presence of infiltrating macrophages and accumulation of extracellular matrix (ECM), including type I collagen[1]. Currently, macrophages are thought to be involved in the development of fibrosis by secreting various cytokines and growth factors including TGF-β [11]. Furthermore, recent studies reported that the CCL2/CCR2 signaling pathway is involved in the progression of fibrosis through the recruitment and activation of macrophages in various fibrotic diseases[5,21]. CCL2 is reported to be produced by tubular epithelial cells and infiltrating cells in fibrotic kidneys[22]. Recently, the expression of CCL2 mRNA was shown to be enhanced in fibrocytes under fibrotic circumstances[22]. In addition, we observed that renal expression of CCL2 mRNA and the infiltration of macrophages as well as CCR7-expressing fibrocytes were significantly reduced in mice treated with anti-CCL21 antibodies and in CCR7-null mice after ureteral ligation compared with those in UUO-treated wild-type mice[13]. Our previous reports demonstrated that monocytes/macrophages also contribute to renal fibrosis since the blockade of CCL2/CCR2 signaling resulted in a 30% reduction of renal fibrosis after ureteral ligation[5,21]. In contrast, fibrosis and infiltration of fibrocytes in the kidneys was reduced up to 50% by the inhibition of CCL21/CCR7 signaling[13]. Taken together, these findings suggest that CCR7-expressing fibrocytes are involved in the pathogenesis of fibrosis not only by secreting collagen but also by regulating the infiltration and activation of macrophages through CCL2 production.

Fibrocytes In Human Renal Diseases

Thus far, the precise role of fibrocytes in the pathogenesis of human renal diseases is still unclear. CD34-positive spindle cells are reported to be present in the interstitium in patients with glomerulonephritis[23]. In addition, the density of CD34-positive spindle cells showed a positive correlation with the interstitial volume, whereas that was not related to the kidney function parameters, such as serum creatinine and urea[23]. Circulating fibrocytes express CD34, whereas expression of CD34 by fibrocytes decreases over time under certain conditions[9-10]. TGF-β has been reported to induce a decrease in cell surface CD34 and an increase in α-smooth muscle actin, which is a characteristic marker of contractile myofibroblasts[9-10]. In contrast, additional cell surface markers, such as CD45, have been reported to be stably expressed on fibrocytes[13]. Thus far, we observed that fibrocytes dual positive for CD45 and type I collagen were present in the interstitium in patients with various renal diseases and that the number of infiltrating fibrocytes well correlated with the degree of renal fibrosis and renal function (unpublished data). Therefore, it is suggested that fibrocytes may be involved in the progression of human renal diseases, especially fibrotic lesions. Further studies will be needed to elucidate the precise roles of fibrocytes in human renal diseases.

Concluding remarks

In summary, fibrocytes are novel collagen-producing cells and contribute to the progressive renal fibrosis dependent on CCL21/CCR7 signaling. Regulating the recruitment and activation of fibrocytes may be a novel therapeutic strategy for renal fibrosis.

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