Review Article

Fibrocyte: New participant in the pathogenesis of renal fibrosis

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Progressive fibrosis is a common pathological finding in various organs, resulting in organ failure. Renal fibrosis is a progressive disease caused by diverse clinical entities. Irrespective and independent of the primary lesion, it is the severity of renal fibrosis that correlates best with the loss of renal function and the risk for progression to renal failure. A circulating population of cells (termed fibrocytes) that display leukocyte markers such as CD45 and CD34 as well as mesenchymal markers including type I collagen has been described. Fibrocytes have been reported to rapidly enter sites of tissue injury and contribute to the pathogenesis of fibrotic conditions. Fibrocytes express CCR7 and migrate in response to secondary lymphoid tissue chemokine (SLC/CCL21). We hypothesized that CCR7-positive fibrocytes contribute to renal fibrosis. To investigate this idea, renal fibrosis was induced by unilateral ureteral obstruction in mice. CD45- and type I collagen-dual positive fibrocytes infiltrated in the fibrotic kidneys and the number of infiltrated fibrocytes increased with the progression of the extent of fibrosis in an experimental murine renal fibrosis model. Most infiltrated fibrocytes in the kidneys were positive for CCR7. In addition, CCL21 protein co-localized with high endothelial venule-like vessels in fibrotic kidneys. The blockade of CCL21/CCR7 signaling reduced the number of infiltrating fibrocytes as well as the extent of renal fibrosis, which was confirmed by the reduction of the amount of hydroxyproline and the levels of renal transcripts of pro-α1 chain of type I collagen and transforming growth factor-β1. These results suggest that CCR7-positive fibrocytes infiltrate into the kidney via CCL21-positive vessels, thereby, contributing to the pathogenesis of renal fibrosis. Thus, regulating the recruitment and activation of fibrocytes by modulation of CCL21/CCR7 signaling may provide a novel therapeutic approach for combatting organ fibrosis.
Progressive fibrosis is a common pathological finding in various organs, resulting in organ failure. Renal fibrosis is a common pathway leading to renal failure regardless of their etiologies\(^4\). Irrespective and independent of the primary lesion, it is the severity of renal fibrosis that correlates best with the loss of renal function and the risk for progression to renal failure\(^4\). The histological picture of renal fibrosis is characterized by tubular atrophy and dilation, interstitial leukocyte infiltration, accumulation of fibroblasts, and increased interstitial matrix deposition\(^5\). Thus far, resident fibroblasts, epithelial-mesenchymal transition (EMT)-derived fibroblasts/myofibroblasts and monocytes/macrophages have been thought to contribute to the pathogenesis of renal fibrosis\(^6\)-\(^9\).

In addition to these cells involved, a circulating bone marrow-derived population of fibroblast-like cells (termed fibrocytes), first identified a decade ago, has been thought to be new participant involved in organ fibrosis\(^10\). Fibrocytes comprise a minor fraction of the circulating pool of leukocytes (less than 1%) and share the markers of leukocytes (e.g., CD45, CD34) as well as mesenchymal cells (e.g., type I collagen, fibronectin) as shown in Table 1\(^11\)-\(^12\). In addition, fibrocytes are capable of producing various cytokines and growth factors (Table 1). Accumulating evidence suggests that fibrocytes are a strong candidate for participating in organ fibrosis associated with conditions such as pulmonary fibrosis, ischemic cardiomyopathy, liver fibrosis, and skin wounds, even though the intracellular mechanisms leading from fibrocytes to fibrosis remain unclear\(^13\)-\(^16\). Furthermore, fibrocytes are detected in human fibrosing diseases including nephrogenic fibrosing dermopathy and burns\(^17\)-\(^19\). Of note, fibrocytes express chemokine receptors such as CCR7, CXCR4 and CCR2\(^12\)-\(^13\). Recent studies demonstrate that chemokine/chemokine receptor systems on fibrocytes are involved in the recruitment of circulating fibrocytes to sites of fibrosis\(^12\)-\(^13\).

These findings prompt us to investigate a distinct impact of fibrocytes on renal fibrosis. Here we review the pathophysiological roles of fibrocytes in renal fibrosis and discuss their trafficking into diseased kidneys from circulation.

### FIBROCYTES IN MURINE RENAL FIBROSIS MODEL

1) Detection of fibrocytes in fibrotic kidneys

One of the peculiar characteristics of fibrocytes is the dual expression of both leukocyte markers, such as CD45 and CD34, and type I collagen\(^15\). Therefore, the infiltration of fibrocytes dual positive for CD45 and type I collagen was examined in fibrotic kidneys. Renal fibrosis was induced by unilateral ureteral obstruction (UOO), which is an established renal fibrosis model in mice. Fibrocytes dual positive for CD45 and type I collagen were present in the interstitium, especially the cortico-medullary regions in wild-type mice fibrotic kidneys after UOO. In addition, the number of infiltrating fibrocytes increased with the progression of fibrosis after a ureteral ligation (Fig. 1a). To further verify the existence of fibrocytes, dual immunostainings for CD34 and type I collagen were also performed. CD34- and type I collagen-dual positive fibrocytes were also detected in fibrotic kidneys and correlated with disease progression as determined by CD45- and type I collagen-dual immunostainings.

#### Table 1 Fibrocyte markers and secreted mediators

<table>
<thead>
<tr>
<th>Markers</th>
<th>Secreted mediators</th>
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<tbody>
<tr>
<td>CD11a (LFA-1)</td>
<td>Type I collagen</td>
</tr>
<tr>
<td>CD11b (Mac-1)</td>
<td>Platelet-derived growth factor A</td>
</tr>
<tr>
<td>CD13</td>
<td>Transforming growth factor-α</td>
</tr>
<tr>
<td>CD18 (β2 integrin)</td>
<td>Transforming growth factor-β1</td>
</tr>
<tr>
<td>CD34</td>
<td>Basic fibroblast growth factor</td>
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<tr>
<td>CD45 (LCA)</td>
<td>Vascular endothelial cell growth factor</td>
</tr>
<tr>
<td>CD80 (B7.1)</td>
<td>Angiogenin</td>
</tr>
<tr>
<td>CD86 (B7.2)</td>
<td>Interleukin-1</td>
</tr>
<tr>
<td>MHC class II</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td>α-Smooth muscle actin</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Interleukin-8</td>
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<tr>
<td>Collagen I and III</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td>Type I procollagen</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td>Prolyl 4-hydroxylase</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td>CCR1</td>
<td>Macrophage inflammatory protein-1α</td>
</tr>
<tr>
<td>CCR2</td>
<td>Macrophage inflammatory protein-2</td>
</tr>
<tr>
<td>CCR3</td>
<td>Monocyte chemoattractant protein-1</td>
</tr>
<tr>
<td>CCR5</td>
<td>Granulocyte-macrophage-colony stimulating factor</td>
</tr>
<tr>
<td>CCR7</td>
<td>Granulocyte-macrophage-colony stimulating factor</td>
</tr>
<tr>
<td>CXCR1</td>
<td>Granulocyte-macrophage-colony stimulating factor</td>
</tr>
<tr>
<td>CXCR3</td>
<td>Granulocyte-macrophage-colony stimulating factor</td>
</tr>
<tr>
<td>CXCR4</td>
<td>Granulocyte-macrophage-colony stimulating factor</td>
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</tbody>
</table>

LCA: leukocyte common antigen

2) Characterization of infiltrated fibrocytes

Thus far, it has been reported that expression of certain chemokine receptors, such as CCR7, CXCR4 and CCR2, is expressed on fibrocytes isolated from humans and mice\(^2\)-\(^3\). Therefore, flow cytometry analyses were performed 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\(^19\). 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\(^20\).
3) Chemokine system regulates fibrocyte infiltration and renal fibrosis

Secondary lymphoid tissue chemokine (SLC/CCL21), a ligand for CCR7, is a member of the CC chemokine family. CCL21 contains six cysteines and is a potent chemoattractant for T cells, B cells, and dendritic cells. In addition, CCL21 also acts as a chemotactic stimulus for fibrocytes. Our study revealed that the number of infiltrating fibrocytes dual positive for CD45 and type I collagen was decreased by the inhibition of CCL21/CCR7 signaling with anti-CCL21 antibodies or CCR7 deficiency in gene-targeted mice (Fig.1a). It was also noted that the number of CCR7-expressing fibrocytes was also reduced in mice treated with anti-CCL21 antibodies compared with that in wild-type mice 7 days after UUO. Collectively, CCL21/CCR7 signaling is thought to be the major pathway recruiting 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.1b). The expression of pro-α1 chain of type I collagen (COL1A1) mRNA as well as transforming growth factor (TGF)-β1 mRNA in wild-type mice were enhanced by ureteral ligation, which were significantly reduced by blockade of CCL21/CCR7 signaling. These findings suggest that fibrocytes are involved in the pathogenesis of 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. In this aspect, CXCR4-positive fibrocytes have been 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. Furthermore, the treatment of bleomycin-exposed animals with specific neutralizing anti-CXCL12 antibodies reduced the number of infiltrated CXCR4-positive fibrocytes as well as the severity of lung fibrosis. Therefore, these findings suggest that other chemokine/chemokine receptor pathways may also be 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.

4) Fibrocytes migrate to fibrotic kidneys through high endothelial venules

High endothelial venules (HEVs) are specialized postcapillary venules that allow rapid and selective lymphocyte trafficking from the blood into lymph nodes and Peyer’s patches under physiological conditions. HEVs have plump endothelial cells and contain many vesicular structures including P-selectin enriched Weibel-Palade bodies. In addition, HEVs have been reported to express certain chemokines, such as CCL21 and EB11-ligand chemokine/CCL19, 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\(^2\)-\(^6\). In addition, CCL21-positive HEV-like vessels were found in synovial tissues from patients with rheumatoid arthritis\(^7\). With regard to human kidney diseases, HEV-like vessels are found at the cortico-medullary junction and associated with interstitial leukocyte infiltration in human glomerulonephritis, whereas HEV-like vessels are not detected in normal kidneys\(^9\). 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\(^9\). Furthermore, CCL21 protein co-localized with HEV-like vessels in the corticomedullary regions in immunohistochemical studies. The number of CCL21-positive HEV-like vessels increased 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 2, resulting in renal fibrosis.

5) Interaction of fibrocytes with macrophages in renal fibrosis

Progressive organ fibrosis is pathologically characterized by the accumulation of infiltrating macrophages as well as 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-\(\beta\)\(^^{10}\). Furthermore, recent studies reported that the monocyte chemoattractant protein-1 (MCP-1)/CCL2/CCR2 signaling pathway is involved in the progression of fibrosis through the recruitment and activation of macrophages in various fibrotic diseases\(^{9,30-35}\). CCL2 is reported to be produced by tubular epithelial cells and infiltrating cells in fibrotic kidneys\(^{12}\). Recently, fibrocytes are capable of producing various cytokines and chemokines including CCL2 (Table 1) and the expression of CCL2 mRNA in fibrocytes has been shown to be enhanced under fibrotic circumstances\(^{12}\). In addition, we observed that renal expression of CCL2 mRNA and the infiltration of F4/80-positive macrophages as well as CCR7-expressing fibrocytes were significantly reduced by the blockade of CCL2/CCR7 signaling after ureteral ligation compared with those in UUO-treated wild-type mice\(^9\). 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\(^{9,34}\). In contrast, fibrosis and infiltration of fibrocytes in the kidneys was reduced up to 50% by the inhibition of CCL2/CCR7 signaling\(^{9}\). Taken together, these findings suggest that fibrocytes may be 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 OTHER RENAL DISEASES**

Transient renal ischemia induces both inflammatory and fibrotic processes and is a major cause of acute and chronic renal insufficiency\(^{36}\). In addition, renal fibrosis after ischemia-reperfusion injury (IRI) has been identified as the chief cause of late graft failure after renal transplantation\(^{37}\). Recent report has revealed that bone marrow-derived myofibroblasts infiltrated in fibrotic kidneys after IRI in rats, and the contribution of bone marrow-derived myofibroblasts to the total renal myofibroblast population amounted to over 30%\(^{30}\). Fibrocytes may comprise a part of bone marrow-derived myofibroblast population. Further studies remain to be investigated.

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\(^9\). 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\(^9\). Circulating fibrocytes express CD34, whereas expression of CD34 by fibrocytes...
decreases over time under certain conditions\textsuperscript{(46)}. TGF-\(\beta\) has been reported to induce a decrease in cell surface CD34 and an increase in \(\alpha\)-smooth muscle cell actin, which is a characteristic marker of contractile myofibroblasts\textsuperscript{(46)}. In contrast, additional cell surface markers, such as CD45, have been reported to be stably expressed on fibrocytes\textsuperscript{(49)}. 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.

**FIBROCYTE REGULATION BY OTHER MECHANISMS**

Thus far, serum amyloid P (SAP), a member of the pentraxin family of proteins, has been demonstrated to have the ability to inhibit fibrocyte differentiation from peripheral blood \textit{in vitro}\textsuperscript{(40)}. In addition, aggregated IgG has also been reported to inhibit fibrocytes differentiation through Syk- and Src-related tyrosine kinases\textsuperscript{(41)}. A more recent study has revealed that fibrocyte differentiation regulated by SAP is critical to the development of ischemic cardiomyopathy as well as pulmonary fibrosis\textsuperscript{(14,42)}. Furthermore, fibrocytes have been reported to have the plasticity to differentiate into myofibroblast, osteoblast, and adipocyte through TGF-\(\beta\) and peroxisome proliferator-activated receptor (PPAR)-\(\gamma\) signaling\textsuperscript{(43)}. Further studies will be needed to elucidate the precise mechanisms of fibrocytes regulation.

**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.

**References**

3. Risdon RA, Sloper JC, de Wardener HE: Relationship be-