Contribution of inflammation-associated bone-marrow-derived cells to kidney fibrosis

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Chronic inflammation-associated kidney fibrosis leads to progressive kidney dysfunction. Cell sources of matrix-producing cells in diseased kidneys include activated resident stromal cells (e.g., fibroblasts and pericytes), cells derived from epithelial-mesenchymal transition/endothelial-mesenchymal transition, and infiltrating bone-marrow-derived cells (e.g., fibrocytes, T cells, and monocytes/macrophages). Recent studies show that bone-marrow-derived cells are recruited to diseased kidneys, interact with renal resident cells, and produce chemokines/cytokines, growth factors, and collagens, thereby promoting and escalating chronic inflammatory processes and eventually leading to kidney fibrosis.

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Introduction

The number of dialysis patients due to chronic kidney diseases (CKD) is on the rise. In addition, CKD is an independent risk factor for cardiovascular diseases and has a large influence on all-cause mortality. Thus, improving the prognosis of CKD is an important issue based on medical, social, and health economical aspects¹. Kidney fibrosis is a common process of progressive kidney diseases that lead to renal failure, regardless of its etiologies. The histological characteristics of kidney interstitial fibrosis include tu-
bular atrophy and dilation, interstitial leukocyte infiltration, fibroblast accumulation, and increased interstitial matrix
deposition\(^2\). Among these characteristic changes, interstitial
matrix deposition is key step. Cell sources of matrix-
producing cells in diseased kidneys include activated resid-
et stromal cells (e.g., fibroblasts and pericytes), cells
derived from epithelial-mesenchymal transition (EMT), and
endothelial-mesenchymal transition (EndMT), and infiltrat-
ing bone-marrow-derived fibrocytes\(^3,5\).

In 1994, Bucala et al. identified fibrocytes as a circulating
bone-marrow–derived CD34\(^+\) cell population of fibroblast-
like cells that infiltrate from inflammatory exudates into
subcutaneously implanted wound chambers\(^6\). Accumu-
lating evidence proposes that fibrocytes occupy 0.1-0.5% of
peripheral blood leukocytes and that these cells are can-
didate participants in organ fibrosis in the lungs, skin, heart,
liver, and kidneys\(^7,8\). Originally, fibrocytes were identified
by the coexpression of CD34 and type 1 collagen. In addi-
tion, fibrocytes were identified by dual positivity of CD34 or
CD45 and type 1 collagen or type 1 procollagen\(^6,7\). A re-
cent study revealed that other marker (e.g., CD45RO, 25F9,
or S100A8/A9) can distinguish fibrocytes from monocytes/
macrophages or fibroblasts\(^8\). In this review, we focus on
the involvement of bone-marrow-derived cells and their
interaction to renal resident cells in the process of kidney
fibrosis.

**Involvement of T cells and monocytes/macrophages in kidney fibrosis**

Tapmeier et al. investigated the role of different T-cell
populations in kidney fibrosis in a mouse model of UUO
and found that CD4\(^+\) T cells are critical in the pathogenesis
of kidney fibrosis\(^9\). Nikolic-Paterson speculated three func-
tions of T cells during kidney fibrosis: 1) T cells may oper-
ate directly on fibroblasts and pericytes to promote their
migration, proliferation, and differentiation, resulting in
myofibroblasts accumulation; 2) T cells may induce a
profibrotic phenotype in the infiltrating macrophage popu-
lation, which secretes profibrotic and pro-proliferative
cytokines and growth factors; 3) T cells may affect directly
tubular epithelial cells to induce secretion of cytokines and
growth factors that, in turn, act on fibroblasts\(^1\). However,
precise functions of T cells during kidney fibrosis are un-
clear so far.

Recent studies show a diverse range of macrophage re-
sponses to the microenvironment, suggesting their role in
kidney injury\(^1\). Colony-stimulating factor-1 promotes re-
nal repair in mice after ischemia-reperfusion injury by re-
cruiting and resulting macrophage function\(^1\). Thus, mac-
rophages mediate tissue repair rather than drive inflam-
mation. On the other hand, we observed that human pe-
ripheral CD14-positive monocytes/macrophages directly
make a contribution to producing type 1 collagen, which is
dependent on MCP-1/CCL2-CCR2 signaling\(^2\). Addition-
ally, the presence of MCP-1/CCL2 expression is sug-

tive of a chronic stage of disease. Moreover, the measure-
ment of urinary MCP-1/CCL2 expression is a useful clini-
cal tool for monitoring disease activity and progression of
kidney fibrosis in inflammatory kidney diseases, including
diabetic nephropathy\(^14-18\). These findings were supported
by the fact that blockade of MCP-1/CCL2 prevents leuko-
cyte migration to the kidney, urinary protein excretion, and
TGF-β expression, thereby preventing glomerulosclero-
sis and interstitial fibrosis\(^16,19-21\). Besides MCP-1/CCL2,
blockade of fractalkine-CX3CR1 also reduced kidney fi-
brosis, along with reduction in macrophage infiltration\(^22,23\).
Glomerular podocytes express CCR2 receptor, suggest-
ing that MCP-1/CCL2 activation of CCR2 on podocytes
may underlie induction of MMP-12, leading to glomerular
basement membrane damage and urinary protein excre-
tion\(^24\). Furthermore, there were significant interrelation
between the numbers of CD45\(^+\)/proCol1\(^+\) cells and mac-
rophages in human kidneys, as well as urinary levels of
MCP-1/CCL2, indicating the close relationship between
CD45\(^+\)/proCol1\(^+\) cells and macrophages. Based on these
results, we consider that the MCP-1/CCL2-CCR2 signal-
ing recruits and activates bone-marrow–derived cells, es-
pecially macrophages, and mediates kidney fibrosis, regard-
less renal etiologies.

**Identification of cells positive for CD34 or CD45 and type 1 collagen in kidney fibrosis**

The signification of fibrocytes in kidney fibrosis remains
to be established. Using immunostaining and flow cyto-
metry, we observed CD45 and type 1 collagen dual-positive
(CD45\(^+\)/Col1\(^+\)) cells infiltrating the kidney interstitium, es-
pecially the corticomedullary regions, in a mouse model of
progressive kidney fibrosis induced by unilateral ureteral
obstruction (UUO)\(^25\). Additionally, the number of infiltrat-
ing CD45\(^+\)/Col1\(^+\) cells increased with fibrotic progression
after UUO, peaking on day 7. These findings prompted us
next to investigate the presence of CD45 and type 1 procollagen dual-positive (CD45+/proCol1+) cells infiltrating human diseased kidneys, particularly in patients with diabetic nephropathy. The number of infiltrating CD45+/proCol1+ cells in the interstitium positively correlated with the severity of interstitial fibrosis, the number of CD68-positive macrophages, and the levels of urinary monocyte chemokine (C-C motif) ligand 2 (CCL2) in patients with CKD. On the other hand, a negative correlation was observed between the estimated glomerular filtration rate and 24 hour creatinine clearance. Consistent with the reduction of disease activity after glucocorticoid therapy, the number of interstitial CD45+/proCol1+ cells and macrophages, as well as urinary MCP-1/CCL2 levels, significantly decreased40. These findings suggest that CD45+/proCol1+ cells could be involved in the pathogenesis of kidney fibrosis through interaction with macrophages and MCP-1/CCL2.

**CD45+/Col1+ cells and renin-angiotensin-aldosterone system in kidney fibrosis**

The renin-angiotensin-aldosterone system (RAAS) is a major pathway in the pathogenesis of fibrosis and depends on two major receptors, designated angiotensin II receptor type 1 (AT1R) and receptor type 2 (AT2R). Upregulation of RAAS was observed in UUO mice, in which plasma and intrarenal angiotensin II content were elevated27. Renal AT1 mRNA and receptor binding also increased in this model28. Aldosterone increased plasminogen activator inhibitor type 1 (PAI-1), a major inhibitor of extracellular matrix (ECM) degradation in rat fibroblasts. Aldosterone and TGF-β together produced dramatic synergistic effects on PAI-1 production and subsequent ECM accumulation29. We hypothesized that CD45+/Col1+ cells may depend on the RAAS for their contribution to kidney fibrosis. In a mouse model, the extent of kidney fibrosis in AT2R-KO mice was more evident, concomitant with the larger number of infiltrating CD45+/Col1+ cells in fibrotic kidneys30. CD45+/Col1+ cell numbers in bone marrow also increased in mice with UUO, especially in AT2R-KO mice. Pharmacologic inhibition of AT1R reduced the degree of kidney fibrosis, along with the decreased number of CD45+/Col1+ cells in the kidney and bone marrow. AT1R inhibition also decreased the angiotensin-II–stimulated expression of type 1 procollagen α1 mRNA in isolated human CD45+/proCol1+ cells, whereas an AT2R inhibitor augmented the expression of type 1 procollagen α1 mRNA. These results suggest that AT1R/AT2R signaling contributes to the pathogenesis of kidney fibrosis30.

**Stromal cell activation and kidney fibrosis**

Activation of local stromal cells (e.g., fibroblasts and pericytes) and generation of myofibroblasts from epithelial cells (via EMT) and endothelial cells (via EndMT) are associated with tubulointerstitial fibrosis31. Among these cells, tubular epithelial cells, glomerular podocytes and endothelial cells undergo transition after injury, and are involved in kidney fibrosis32-34.

In contrast to the cell transition, Duffield et al. reported that pericytes and perivascular fibroblasts were major sources of collagen-producing cells in the pathogenesis of kidney fibrosis35, 36. In addition to this finding, platelet-derived growth factor receptor activates pericytes in a mouse model of kidney fibrosis37. Pericytes were also reported as collagen-producing cell in hepatic fibrosis and spinal cord injury38, 39. These findings suggest that fibrosis by fibroblasts and pericytes would be principal and common pathways of organ fibrosis. In addition, Asada et al. reported that EPO-producing cells in healthy kidney and scar-producing myofibroblasts during fibrosis originate from the same P0-Cre lineage-labeled extrarenal cells, which enter the embryonic kidney at E13.5 to become renal fibroblasts and transit from one another depending on the condition of the kidney. They also demonstrated that almost all cortical fibroblasts in the kidney arise from P0-Cre—expressing precursors40. These findings are important to speculate different lineage of fibroblasts. However, the recent lineage tracing studies have excluded the role of EMT in experimental kidney and liver fibrosis35, 41. Moreover, Roufosse et al. revealed a minimal contribution of bone-marrow-derived cells to collagen production in experimental kidney fibrosis, using collagen promoter reporter mice42. Further studies are required to determine the origin and contribution of stromal cells in kidney fibrosis.

**Phospholipid mediators in kidney fibrosis**

There are a number of pro-fibrotic mediators. TGF-β could be a principal mediator, which stimulates the differentiation of fibroblasts into myofibroblasts and promotes extra cellular matrix deposition. Lysophosphatidic acid (LPA) is
a growth factor-like phospholipid, which is known to regulate several cellular processes including motility, proliferation, survival, and differentiation by acting LPA1-4 receptors. UUO induced tubulointerstitial fibrosis was significantly attenuated in LPA1-KO mice and, in LPA1 antagonist treated WT mice. Further, LPA induced proximal tubular cell secretion of platelet-derived growth factor-β and connective tissue growth factor through LPA2. Additionally, some studies revealed the interaction between bone-marrow-derived cells and phospholipid mediators. Maeda et al. investigated the involvement of sphingosine 1-phosphate (S1P) receptor subtypes in S1P-induced migration of CD4 T cells and bone marrow-derived dendritic cells in mice. However, further studies needed to understand the precise contribution of phospholipid mediators in kidney fibrosis.

**Conclusion and future directions**

Kidney fibrosis is caused by a complex network, consisting of various cell sources including infiltrating bone-marrow-derived cells, activated resident stromal cells, and cells derived from EMT/EndMT, and bioactive mediators, such as cytokines/chemokines, RAAS, and phospholipid mediators (Fig. 1). The interaction among fibrogenetic cells and mediators promote inflammatory processes, resulting in kidney fibrosis. Further studies are needed to clarify the contribution of cell types in bone-marrow-derived cells for kidney fibrosis.

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None

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