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Abstract

Fibrocytes are supposed to be a circulating connective tissue cell progenitor that consists of a novel population of peripheral blood cells. This distinct population of blood-borne cells shares markers of leukocytes as well as mesenchymal cells. Accumulating evidence indicates that fibrosis is characteristic of progressive chronic kidney diseases of any etiologies, resulting in kidney failure. We have uncovered that CCR7-positive fibrocytes migrate into the kidney in response to secondary lymphoid tissue chemokine (SLC/CCL21) and contribute to kidney fibrosis induced by unilateral ureteral obstruction in mice. In addition, the blockade of CCL21/CCR7 signaling by anti-CCL21 antibodies reduced kidney fibrosis, which was confirmed by a decrease in fibrosis in CCR7-null mice with concomitant reduction in macrophage recruitment along with reduced renal transcripts of monocyte chemoattractant protein-1 (MCP-1/CCL2). These findings suggest that fibrocytes dependent on CCL21/CCR7 signaling pathways contribute to the pathogenesis of kidney fibrosis, thereby providing that regulating fibrocytes may provide a novel therapeutic benefit 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 (1). Fibrocytes uniquely comprise a minor fraction of the circulating pool of leukocytes (less than 1%) and share the markers of leukocytes as well as mesenchymal cells (e.g., type I collagen) (2-3). Since both of fibrocytes and bone marrow stroma express CD34, fibrocytes were at first thought to consist of scaffold to support normal hematopoiesis (4). However, accumulating evidence suggests that fibrocytes are a strong candidate for participating in organ fibrosis associated with conditions such as pulmonary fibrosis, bronchial asthma, skin wounds and intimal hyperplasia, even though the intracellular mechanisms leading from fibrocytes to fibrosis remain unclear (5-11) (Table 1). In relation with human diseases, fibrocytes contribute to nephrogenic fibrosing dermopathy as well as burns (9-10). A recent study reveals that the impairment of fibrocytes caused by the loss in otospiralin leads to abnormal cochlear physiology and auditory function (12). In addition, fiborcytes are characterized as circulating adipocyte progenitors as well as fibroblasts and myofibroblasts (10, 13). Transforming growth factor (TGF)-β, serum amyloid P and aggregated IgG influence fibrocyte function and differentiation (10, 14, 15). However, the detection and role of fibrocytes in the progressive fibrosis in the kidney remains investigated.

Fibrosis is a characteristic pathological feature that determines the prognosis of diseases independent of their etiologies. Kidney diseases progress to end-stage failure, showing pathological characteristics including glomeruloscleorsis and interstitial fibrosis (1-2).

The histological picture of interstitial fibrosis is characterized by tubular atrophy and dilation, interstitial leukocyte infiltration, accumulation of fibroblasts, and increased interstitial matrix deposition (16). In this aspect, our recent study has uncovered the evidence that human peripheral CD14-positive monocytes/macrophages directly contribute to producing type I collagen, resulting in fibrogenesis, which are dependent on an amplification loop of monocyte chemoattractant protein-1 (MCP-1/CCL2)/CCR2 (17). These results may provide a key role of immune competent cells for their own promoting and escalating tissue fibrosis in addition to participating in the inflammatory cascade. Further, fibrocytes are capable of producing profibrotic molecules such as TGF-β as well as collagen (10). These results prompt us to investigate a distinct impact of fibrocytes on kidney tissue remodeling by secreting fibrogenic factors in the progression of kidney fibrosis. Here we focus on the pathophysiological role of fibrocytes dependent on chemokine system in kidney fibrosis.

Detection of fibrocytes in progressive interstitial fibrosis in murine and human kidneys

In progressive kidney fibrosis induced by a ureteral ligation in mice, CD45- and type I collagen-dual positive fibrocytes ($CD45^+/ColI^+$) infiltrated the interstitium, especially the corticomedullary regions (18) (Figure 1a, b, c). The number of infiltrating fibrocytes increased with the progression of fibrosis after a ureteral ligation, reaching a peak on day 7 (Figure 1d). 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 fibrocytes was also observed in the interstitium and correlated

with disease progression as determined by CD45- and type I collagen-dual immunostainings (18). In addition to murine progressive intersitital fibrosis, $CD45^+/ColI^+$ positive fibrocytes infiltrate into human chronic kidney diseases. The number of infiltrating fibrocytes well correlates with the intensity of interstitial fibrosis in human various kidney diseases including diabetic nephropathy (manuscript in preparation). Further, CD34-positive spindle cells, detected in tubulointerstitial lesions in patients with glomerulonephritis, were closely related with interstitial volume, but not with kidney function (19)

Fibrocytes and chemokine system

Recent studies demonstrate that chemokine/chemokine receptor systems on fibrocytes are involved in the recruitment of circulating fibrocytes to sites of fibrosis (3, 5, 10). It is of note that fibrocytes, isolated from human and mice, express chemokine receptors such as CCR2, CCR3, CCR5, CCR7, and CXCR4 (3, 5, 10). Intradermal instillation of secondary lymphoid tissue chemokine (SLC/CCL21) was firstly described to induce the recruitment of fibrocytes at the injected site (7). The migration of fibrocytes to the lung has been demonstrated to be dependent on signaling pathways of CXCL12/CXCR4 or CCR2 (3, 5). CCR7-expressing fibrocytes, also positive for type I collagen ($CCR7^+/ColI^+$), were detected in diseased kidneys 7 days after a urtereral ligation in wild-type mice (18). 37.8% of infiltrating fibrocytes expressed CCR7 (number of $CCR7^+/ColI^+$ divided by the number of $CCR7^+$ or $CXCR4^+$ or $CCR2^+/ColI^+$). In wild-type mice, the ratio of $CCR7^+/ColI^+$ cells 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 fibrocytes 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 $CXCR4^-/CCR2^-$. In contrast, the percentage of CCR7-negative collagen-producing cells ($CCR7^-/ColI^+$) increased to 26.7% of the total isolated renal cells from obstructed kidneys.

A ligand for CCR7, secondary lymphoid tissue chemokine (SLC/CCL21), which is a member of the CC chemokine family, remains to be investigated in the progression of kidney fibrosis. CCL21 has been reported to act as a chemotactic stimulus for fibrocytes (18, 20). In humans as well as in mice, CCL21 is constitutively abundant in lymphoid tissues, particularly in the lymph nodes and spleen. It is of note that CCL21 is also expressed at lower levels in some non-lymphoid tissues, including the lung (21). CCL21 expression is relatively localized in high endothelial venules (HEVs) in lymph nodes under physiological conditions (21), as well as in non-lymphoid tissues under inflammatory conditions (22). In fact, CCL21- and MECA79-dual positive vessels were found in synovial tissues from patients with rheumatoid arthritis (23). The number of CCL21- and MECA79-dual positive HEV-like vessels as well as *in situ* expression of CCL21 mRNA increased with disease progression after ureteral ligation (18). HEVs express certain chemokines, such as EBI1-ligand chemokine/CCL19 (24) as well as CCL21 that can activate CCR7-expressing cells, even though CCL19 expression remained investigated in the previous report (18). Concomitantly, MECA79-positive HEV-like vessels located at the corticomedullary junction were detected and associated with interstitial leukocyte infiltration in human glomerulonephritis (25). These findings suggest that CCR7-expressing circulating fibrocytes infiltrate the kidney via CCL21-positive HEV-like vessels, resulting in the contribution to kidney fibrosis.

Blockade of CCL21/CCR7 signaling reduced interstitial fibrosis in the kidney

The impact of CCL21/CCR7 signaling on progressive kidney interstitial fibrosis was further examined. Mean interstitial fibrosis as well as the amount of hydroxyproline was 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 redution in CCR7-null mice (18) (Figure 2). Accordingly, based on the finding that treatment with anti-CCL21 antibodies or CCR7 deficiency resulted in over 50% reduction in the number of CD45- and type I collagen-dual positive fibrocytes, thereby CCL21/CCR7 signaling is thought to be the major pathway attracting fibrocytes into the kidney in this particular model. In addition, ureteral ligation enhanced the pro α1 chain of type I collagen mRNA expression as well as transcripts of TGF-β_1, which was also downregulated by the inhibition of CCL21/CCR7 signaling.

Moreover, blockade of CCL21/CCR7 signaling reduced the number of CCR7-expressing fibrocytes as well as CCR2-expressing fibrocytes in immunohistochemical studies. A recent study reported that CCL2/CCR2 signaling mediated recruitment of CCR2-expressing fibrocytes to the alveolar space after administration of fluorescein isothiocyanate, resulting in pulmonary fibrosis (3). Fibrocytes have been considered to be capable of producing CCL2 under pathological fibrotic conditions (10). Therefore, inhibiting the infiltration of CCR7-expressing fibrocytes appears to decrease the infiltration of CCR2-expressing fibrocytes through suppression of CCL2 production, thereby contributing to more effective protection from kidney fibrosis (26-27) (Figure 3). In contrast, the infiltration of CXCR4-positive fibrocytes was not reduced by the

blockade of CCL21/CCR7. Therefore, further studies will be required to elucidate the precise mechanisms that other chemokine/chemokine receptor pathways may also be involved in the recruitment and activation of fibrocytes, resulting in progressive fibrosis.

Fbrocytes and epithelial-mesenchymal transition

Detailed molecular mechanisms involved in progressive organ fibrosis are not fully uncovered. Currently, resident fibroblasts, epithelial-mesenchymal transition (EMT)-derived fibroblasts/myofibroblasts, and monocytes/macrophages are thought to be participants in the pathogenesis of kidney fibrosis (28-29). In addition, Iwano et al. reported that 15% were derived from CD34-negative fibroblasts in bone marrow, whereas 36% of renal fibroblasts found in a UUO model were derived from EMT (8). Interestingly, circulating fibrocytes express CD34, however, fibrocytes reduce the expression of CD34 on the surface as they become more specialized (10). Moreover, the stimulation of TGF-β1 decreases the expression of cell surface CD34. However, the relation of fibrocytes with EMT-derived fibloblasts and CD34-negative fibroblasts remains investigated. Recent reports raise the possibility that these participants may interact with each other. TGF-β1 may play a key role in this aspect. TGF-β1, which could be produced by fibrocytes (2), is a well-characterized inducer of EMT in tubular epithelial cells (30). Concomitantly, fibrocytes further differentiate to contractile myofibroblasts co-existant with TGF-β1 (10). Alternatively, a very recent study revealed that an average of 32% of all interstitial α-smooth muscle actin-positive myofibroblasts were derived from the bone marrow. Functional bone marrow-derived

myofibroblasts infiltrate in the postischemic renal interstitium and are involved in extracellular matrix (31). Therefore, further study would be required to examine the fundamental properties, differentiation potential and mutual interactions of participants in progressive organ fibrosis in depth (Figure 3).

Fibrocytes in other diseases

Fibrocytes are now considered to be involved in various conditions including tumor biology, immunostimulatory properties, infection, and scleroderma (10) (Table 1). Dual positive spindle shaped cells for CD34 and procollagen are present at a thickened dermins in a patient with nephrogenic systemic fibrosis, which is closely related to renal insufficiency (32). A more recent study revealed a population of small spindle-shaped fibroblasts that were highly proliferative and expressed collagen I and alpha-smooth muscle actin (myofibroblast markers), CD34 (a precursor marker), and CD45 (a hematopoietic marker) in ischemic cardiomyopathy in mice. Importantly, serum amyloid P may be an important regulator in the linkage between inflammation and nonadaptive fibrosis through the modulation of proliferative spindle-shaped fibroblasts in the heart (33).

Conclusion

An insight of fibrocytes dependent on CCL21/CCR7 signaling pathways through HEV-like vessels has shed light on the novel pathogenesis of progressive organ fibrosis including kidney fibrosis. The most compelling part in our previous study was that the inhibition of CCL21/CCR7 signaling ameliorated progressive kidney fibrosis by almost 50% (18). In addition to inhibiting chemokine system, our recent unpublished data

suggest that renin angiotensin aldosterone system (RAAS) plays a role in the activation of fibrocytes, suggesting the blockade of RAAS might provide a beneficial therapeutic approach, at least in part, via the inhibition of fibrocyte activation. Deeper knowledge of signals for the recruitment and activation of fibrocytes during the progression of fibrosis may provide a key to better therapeutic benefit for combating fibrosis in humans.

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References

1. Bucala R, Spiegel L, Chesney J, et al. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. Mol Med 1994; 1: 71-81.

2. Chesney J, Metz C. Stavitsky AB, et al. Regulated production of type I collagen and inflammatory cytokines by peripheral blood fibrocytes. J Immunol 1998; 160: 419-425.

3. Moore BB, Kolodsick JE, Thannickal VJ, et al. CCR2-mediated recruitment of fibrocytes to the alveolar space after fibrotic injury. Am J Pathol 2005; 166: 675-684.

4. Brown J, Greaves M, Molgaard H, et al. The gene encoding the stem cell antigen, CD34, is conserved in mouse and expressed in hematopoietic progenitor cell lines, brain, and embryonic fobroblasts. Int Immunol 1991; 3: 175-184.

5. Phillips RJ, Burdick MD, Hong K, et al. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. J Clin Invest 2004; 114: 438-446.

6. Schmidt M, Sun G, Stacey MA, et al. Identification of circulating fibrocytes as precursors of bronchial myofibroblasts in asthma. J Immunol 2003; 171: 380-389.

7. Abe R, Donnelly SC, Peng T, et al. Peripheral blood fibrocytes: Differentiation pathway and migration to wound sites. J Immunol 2001; 166: 7556-7562.

8. Yang L, Scott PG, Giuffre J, et al. Peripheral blood fibrocytes from burn patients: Identification and quantification of fibrocytes in adherent cells cultured from peripheral blood mononuclear cells. Lab Invest 2002; 82: 1183-1192.

9. Quan TE, Cowper S, Wu SP, et al. Circulating fibrocytes:collagen-secreting cells of the peripheral blood. Int J Biochem Cell Biol 2004; 36: 598-606.

10. Bucala R: Fibrocytes: discovery of a circulating connective tissue cell progenitor. New insights into tissue repair and systemic fibroses, Ed. Bucala R, (World Scientific, Singapore), in press

11. Varcoe RL, Mikhail M, Guiffre AK, et al. The role of the fibrocyte in intimal hyperplasia. J Thromb Haemost 2006; 4: 1125-1133.

12. Delprat B, Ruel J, Guitton MJ, et al. Deafness and cochlear fibrocyte alterations in mice deficient for the inner ear protein otospiralin. Mol Cell Biol 2005; 25: 847-853.

13. Hong KM, Burdick MD, Phillips RJ, et al. Characterization of human fibrocytes as circulating adipocyte progenitors and the formation of human adipose tissue in SCID mice. FASEB J 2005; 19: 2029-2031.

14. Pilling D, Buckley CD, Salmon M, et al. Inhibition of fibrocyte differentiation by serum amyloid P. J Immunol 2003; 171: 5537-5546.

15. Pilling D, Tucker NM, Gomer RH. Aggregated IgG inhibits the differentiation of human fibrocytes. J Leukoc Biol 2006; 79: 1242-1251.

16. Strutz F, Zeisberg M. Renal fibroblasts and myofibroblasts in chronic kidney disease. J Am Soc Nephrol 2006; 17: 2992-2998.

17. Sakai N, Wada T, Furuichi K, et al. MCP-1/CCR2-dependent loop for fibrogenesis in human peripheral CD14-positive monocytes. J Leukoc Biol 2006; 79: 555-563

18. Sakai N, Wada T, Yokoyama H, et al. Secondary lymphoid tissue chemokine (SLC/CCL21)/CCR7 signaling regulates fibrocytes in renal fibrosis. Proc Natl Acad Sci USA 2006; 103: 14098-14103.

19. Okon K, Szumera A, Kuzniewski M: Are CD34+ cells found in renal interstitial fibrosis? Am J Nephrol 2003; 23: 409-414.

20. Abe R, Donnelly SC, Peng T, et al. Peripheral blood fibrocytes: Differentiation pathway and migration to wound sites. J Immunol 2001; 166: 7556-7562.

21. Gunn MD, Tangemann DK, Tam C, et al. A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naïve T lymphocytes. Proc Natl Acad Sci USA 1998; 95: 258-263.

22. Kraal G, Mebius RE: High endothelial venules: lymphatic traffic control and controlled traffic. Adv Immunol 1997; 65: 347-395.

23. Drayton DL, Bonizzi G, Ying X, et al. I kappa B kinase complex alpha kinase activity controls chemokine and high endothelial venule gene expression in lymph nodes and nasal-associated lymphoid tissue. J Immunol 2004; 173:6161-6168.

24. Weninger W, Carlsen HS, Goodarzi M, et al. Naïve T cell recruitment to nonlymphoid tissues: A role for endothelium-expressed CC chemokine ligand 21 in autoimmune disease and lymphoid neogenesis. J Immunol 2003; 170: 4638-4648.

25. Segawa C, Wada T, Takaeda M, et al. In situ expression and soluble form of P-selectin in human glomerulonephritis. Kidney Int 1997; 52: 1054-1063.

26. Wada T, Furuichi K, Sakai N, et al. Gene therapy via blockade of monocyte chemoattractant protein-1 for renal fibrosis. J Am Soc Nephrol 2004; 15; 940-948.

27. Wada T, Yokoyama H, Furuichi K, et al. Intervention of crescentic glomerulonephritis by antibodies to monocyte chemotactic and activating factor (MCAF/MCP-1). FASEB J 1996; 12: 1418-1425.

28. Iwano M, Plieth D, Danoff TM, et al. Evidence that fibroblast derive from epithelium during tissue fibrosis. J Clin Invest 2002; 110: 341-350.

29. Kitagawa K, Wada T, Furuichi K, et al. Blockade of CCR2 ameliorates progressive fibrosis in kidney. Am J Pathol 2004; 165: 237-246.

30. Zeisberg M, Hanai J, Sugimoto H, et al. BMP-7 counteracts TGF-β1-induced epithelial-to-mesenchymal transition and reverse chronic renal injury. Nat Med 2003; 9: 964-968.

31. Martine B, Harmsen MC, van Luyn MJA, et al. Bone-marrow derived myofibroblasts contribute to the renal interstitial myofibroblast population and produce procollagen I after ischemia/reperfusion in rats. Am J Soc Nephrol 2007, 18: 165-175.

32. Cowper SE, Bucala R: Nephrogenic fibrosing dermopathy: suspect identified, motive unclear. Am J Dermatopathol 2003; 25: 358.

33. Haudek SB, Huebener P, Lee JM, et al. Bone marrow-derived fibroblast precursors mediate ischemic cardiomyopathy in mice. Proc Natl Acad Sci USA 2006; 103: 18284-18289.

Figure legends

Figure 1. Detection of fibrocytes in progressive fibrosis in kidney

In wild-type mice, CD45- and type I collagen-dual positive fibrocytes infiltrated the interstitium, especially the corticomedullary regions after ureteral ligation (a; CD45, b; merge, c; type I collagen, arrowheads; CD45- and type I collagen-dual positive fibrocytes; $CD45^+/ColI^+$). The number of infiltrating fibrocytes dual positive for CD45 and type I collagen was 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 (d). Dual positive cells for CCR7 and type I collagen were also reduced by the administration of anti-CCL21 antibodies (e). Values are the mean ±SEM.

Figure 2. Inhibition of CCL21/CCR7 signaling ameliorated progressive fibrosis in the kidney

CCL21/CCR7 signaling reduced the mean interstitial fibrosis (a) and the total tissue collagen content (hydroxyproline) (b) 7 days after ureteral ligation. Values are the mean ±SEM.

Figure 3. Schema for CCL21/CCR7-dependent fibrosis in the kidney

CCR7-expressing circulating fibrocytes, infiltrated in the kidney via CCL21-positive HEV-like vessels, take part in the pathogenesis of fibrosis not only by synthesizing collagen but also by regulating macrophages through CCL2 production and EMT through the production of TGF-β_1.