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INVITED REVIEW

Fibroblast heterogeneity and tertiary lymphoid tissues in the kidney

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Abstract

Fibroblasts reside in various organs and support tissue structure and homeostasis under physiological conditions. Phenotypic alterations of fibroblasts underlie the development of diverse pathological conditions, including organ fibrosis. Recent advances in single-cell biology have revealed that fibroblasts comprise heterogeneous subpopulations with distinct phenotypes, which exert both beneficial and detrimental effects on the host organs in a context-dependent manner. In the kidney, phenotypic alterations of resident fibroblasts provoke common pathological conditions of chronic kidney disease (CKD), such as renal anemia and peritubular capillary loss. Additionally, in aged injured kidneys, fibroblasts provide functional and structural supports for tertiary lymphoid tissues (TLTs), which serve as the ectopic site of acquired immune reactions in various clinical contexts. TLTs are closely associated with aging and CKD progression, and the developmental stages of TLTs reflect the severity of renal injury. In this review, we describe the current understanding of fibroblast heterogeneity both under physiological and pathological conditions, with special emphasis on fibroblast contribution to TLT formation in the kidney. Dissecting the heterogeneous characteristics of fibroblasts will provide a promising therapeutic option for fibroblast-related pathological conditions, including TLT formation.

KEYWORDS
chronic kidney disease, fibroblast, fibrosis, myofibroblast, tertiary lymphoid tissue

1 | INTRODUCTION

In the past, cellular identification in the tissue relied on morphological appearance under careful microscopic observation. Advancements in molecular biology led to the discovery of molecular markers specific to each cell type that can be utilized for cellular classification, and this contributed significantly to the exploration of diverse biological mechanisms. Recent advances in single-cell multi-omics analysis have unveiled the heterogeneous features of cell populations at unprecedented resolution and have dramatically reconstructed our understanding of biological phenomenon. Each cell category comprises various subpopulations with distinct functions, which characterize the tissue-specific microenvironment in both physiological and pathological contexts. The fibroblast research field is no exception, and tremendous evidence has accumulated suggesting their heterogeneous characteristics.

Fibroblasts are spindle-shaped cells located in the interstitial spaces of various organs, where they synthesize several structural

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proteins, such as collagens and fibronectins, to organize the extracellular matrix (ECM) and maintain organ homeostasis.[1] In addition to their common functions across organs, fibroblasts play organ-specific roles in concert with other resident cells. For example, a subpopulation of resident fibroblasts in the kidney produces erythropoietin (EPO), a principal regulator of erythropoiesis. Recent studies have revealed that fibroblasts in different organs exhibit high functional heterogeneity. Under pathological conditions, dramatic phenotypic alterations of fibroblasts occur and significantly contribute to the development of organ dysfunction. Fibroblasts transdifferentiate into myofibroblasts and promote the progression of organ fibrosis, which is the manifestation of maladaptive repair after injury.[2] In the kidney, fibroblasts play crucial roles in the development and progression of chronic kidney disease (CKD), which has high morbidity and mortality worldwide even in the modern era.[3] Fibroblasts drive several CKD-related pathological conditions, such as renal fibrosis and renal anemia, regardless of the etiology of CKD. Additionally, fibroblasts also play multifaceted roles in a context-dependent manner. In aged injured kidneys, for instance, fibroblasts play a crucial role in the formation of tertiary lymphoid tissue (TLT), an inducible ectopic lymphoid tissue triggered by chronic inflammation.[4] Clarifying the individual characteristics of fibroblasts will elucidate the underlying mechanisms of fibroblast-related pathological conditions and facilitate the development of effective therapeutics.

In this review, we first describe fibroblast heterogeneity in terms of its origin and function under physiological conditions. Subsequently, we discuss the functional heterogeneity of fibroblasts in various pathological conditions, including organ fibrosis, and their interactions with other cell types and the surrounding microenvironment. Special emphasis is placed on resident fibroblasts in the kidney, and their implication in CKD progression. Finally, the fibroblast contribution to TLT formation and its clinical significance will be discussed.

2 | THE CHARACTERISTICS OF FIBROBLASTS

2.1 | Fibroblast characteristics and functions under physiological conditions

Fibroblasts are generally identified by their morphology, localization, and representative marker expression, such as PDGFRβ and CD73.[1] These markers, however, are not absolutely specific for fibroblasts, so the absence of markers for other cell lineages (eg, CD45 for hematopoietic cells) is often utilized to identify fibroblasts. Under physiological conditions, fibroblasts contribute to the maintenance of normal tissue structure by supporting ECM turnover. In addition to these functions, resident fibroblasts also play tissue-specific roles. For example, in the heart, where fibroblasts comprise the largest cell population, fibroblasts sense mechanical stretch and interact with myocytes via the gap junction, which enables appropriate electrical conduction and maintains normal cardiac function.[5] Additionally, fibroblast depletion in bone marrow and skeletal muscle leads to anemia and cachexia,[6] suggesting their importance in normal tissue homeostasis throughout various organs.

The kidney is a unique organ in that multiple types of cells reside close to each other and function harmoniously to exert renal-specific roles, such as the removal of waste products and the maintenance of body fluid balance. Fibroblasts provide structural supports for nephrons, the basic functional unit of the kidney, and have direct interaction with other renal resident cells, such as proximal tubular cells.[7] As the specific function of renal resident fibroblasts, certain subpopulations of fibroblasts in the deep cortex and outer medulla produce EPO in response to hypoxia, which stimulates erythropoiesis in bone marrow.[8] EPO-producing cells possess neuron-like dendritic morphology and express neuronal markers, such as microtubule-associated protein 2 (MAP2) and neurofilament light polypeptide (NFL).[8] Interestingly, under severely anemic conditions, almost all resident fibroblasts, including those in the outer cortex, produce EPO, which suggests the high plasticity of fibroblast function.[9] The localization of EPO-producing cells is in sharp contrast to other hematopoietic growth factors, such as granulocyte colony-stimulating factor, which are synthesized in the proximity of their target cells and function in a paracrine manner. One explanation for this discrepancy is that the physiological oxygen concentration in the kidney is lower than in other organs, and renal blood flow accounts for approximately 20% of cardiac output, which renders the kidney the ideal biological sensor for hypoxia.[10] Kidneys can also immediately detect fluid status at the juxtamedullary region and regulate extracellular volume by adjusting the amount of sodium reabsorption. Together with EPO production, kidneys can set the plasma volume and red blood cell mass at favorable levels, serving as a "critmeter."[11,12] In addition, fibroblasts in the renal medulla express COX1 and COX2, which are key enzymes of prostaglandin (PG) synthesis.[13] Under physiological conditions, PGs have multiple functions in the kidney, such as the regulation of renal blood flow and renin release. Overall, resident fibroblasts in the kidney contribute to the maintenance of renal homeostasis by several mechanisms.

2.2 | Origin of fibroblasts

The developmental origins of fibroblasts are heterogeneous. In the kidney, we previously revealed that resident fibroblasts in the renal cortex and outer medulla are derived from *myelin protein zero (P0)-Cre* lineage-labeled extrarenal cells.[14] In the embryonic phase, P0 is expressed on neural crest cells.[15] *P0-Cre* lineage-labeled cells migrate from the neural crest into the embryonic kidney at embryonic day 13.5 and are localized along the outer capsule and ureter of the kidney. In the adult kidney, *P0-Cre* lineage-labeled cells are observed in the renal interstitium and express fibroblast markers such as PDGFRβ and CD73. Interestingly, the majority of EPO-producing fibroblasts are also lineage-labeled by *P0-Cre*, consistent with their features of neuron-like phenotypes described above.[8] These results are also supported by the observation that the major source of EPO in primitive erythropoiesis is neural and neural crest cells.[16]

In contrast, resident fibroblasts in the renal inner medulla are lineage-labeled by *Wnt4-Cre*,[17] exemplifying the regional heterogeneity of fibroblast origin in the kidney. In kidney development, Wnt4 expression is observed in the assembling nephrons and medullary stroma.[18,19] Wnt4 regulates the differentiation of medullary stromal cells to smooth muscle cells,[20] as well as mesenchymal-to-epithelial transition and tubulogenesis.[19] Indeed, Wnt4 depletion attenuates α-smooth muscle actin (αSMA) expression in the medullary stromal cells.[20]

In conjunction with the different origins of fibroblasts in the renal cortex and medulla, several phenotypic differences between cortical and medullary fibroblasts have also been reported. For example, while cortical fibroblasts express ecto-5'-nucleotidase (5'NT), most medullary fibroblasts do not.[21] In ultrastructural analysis, cortical fibroblasts tend to exhibit a dendritic structure to interact with adjacent cells whereas medullary fibroblasts contain lipid droplets.[7,22] Interestingly, these lipid droplets also appear in the cortical fibroblasts of anemic rat kidneys.[21] Future research is needed to determine whether these phenotypic differences are related to functional differences between cortical and medullary fibroblasts.

2.3 | Pericytes and fibroblasts

Pericytes are mural cells that encircle vascular endothelial cells and control microcirculation. Traditionally, pericytes are mainly defined by their anatomical location, their morphology, and typical marker expression.[23] Pericytes share common characteristics with resident fibroblasts, such as their mesenchymal origin and morphology. Pericytes express the same cellular markers as resident fibroblasts, such as PDGFRβ. Although several markers have been utilized to identify pericytes, such as NG-2, they are not absolutely specific for pericytes, and there is no definite marker that completely distinguishes pericytes from fibroblasts. Indeed, the tracing efficiency of renal pericytes by NG-2 is relatively low.[24] Although the differences in the anatomical localization under physiological conditions partly aid in the differentiation of pericytes from resident fibroblasts, some resident fibroblasts also reside in the perivascular area ("perivascular fibroblasts"), so the differentiation between pericytes and fibroblasts by anatomical localization is not sufficient.

Like fibroblasts, the origin of pericytes is heterogeneous. While pericytes in the central nervous system and thymus originate from the ectoderm-derived neural crest, pericytes in the heart and lung are derived from mesothelium.[23] In the kidney, pericytes originate from FoxD1-positive cortical mesenchymal progenitors.[25] FoxD1$^+$ cells are originally derived from Osr1-positive nephrogenic intermediate mesoderm progenitors in the embryonic phase.[26,27] FoxD1$^+$ cells serve as mesenchymal progenitors and differentiate into several cell types, such as pericytes, fibroblasts, vascular smooth muscle cells, and mesangial cells.[27] Interestingly, *FoxD1-Cre* lineage-labeled stromal cell populations largely overlap with *P0-Cre* lineage-labeled cells, except in the inner medulla.[14,25,27] This is confirmed by the observation that migrating neural crest cells in the embryonic phase express FoxD1,[28] and *P0-Cre* lineage-labeled cells entering the embryonic kidney transiently express FoxD1.[14] Consistently, EPO-producing cells are also lineage-labeled by *FoxD1-Cre*.[29] Hence, there is a large overlap between cell populations defined as "fibroblasts" and "pericytes" and, as such, pericytes and fibroblasts have often been discussed as a whole. In this review, we use the term "fibroblasts/pericytes" to define the cell population including both fibroblasts and pericytes. The contribution of FoxD1$^+$ mesenchymal progenitors to the myofibroblast pool and fibrosis development will be discussed in the next section.

Although it has been difficult to distinguish pericytes from fibroblasts, a recent study that conducted scRNAseq of fibroblasts and mural cells (vascular smooth muscle cells and pericytes) in muscular organs enables us to understand their differences at the transcriptional levels.[30] Muhl et al performed scRNAseq of cells derived from mouse heart, skeletal muscle, colon, and bladder. Although each cell type exhibits organ-dependent transcriptional heterogeneity, 90 gene subsets overlapping in all four organs were identified that could be utilized to discriminate between pericytes and fibroblasts. Although common fibroblast markers include many ECM genes (eg, Col1a1), CD34, and PDGFRα, mural cell markers comprise Mcam, Tagln, and Notch3. Although no single marker alone can completely define each cell type, the combination of these markers efficiently distinguishes pericytes from fibroblasts. Interestingly, pericytes exhibit organ-specific localization and marker expression compared with fibroblasts. For example, although colon pericytes are localized in the apex of the subepithelial capillary loops with strong expression of PDGFRβ and NG-2, bladder pericytes tend to express αSMA instead. The distinct transcriptional profiles of pericytes might explain the functional differences of pericytes among different organs.

3 | MYOFIBROBLAST: ORIGIN, FUNCTION, HETEROGENEITY, AND RELATED PATHOLOGICAL CONDITIONS

Fibrosis is the final common manifestation of organ dyshomeostasis and is defined as excessive ECM deposition with distorted tissue architectures. Fibrosis development is regulated by the intricate balance between ECM synthesis and its degradation.[31] Once developed, fibrosis is associated with poor prognosis in various organs, including the kidney, and is regarded as a reliable predictor of organ function decline. In the kidney, fibrosis develops concomitantly with renal anemia and peritubular capillary loss, both of which are hallmarks of CKD. In fibrosis development, myofibroblasts emerge de novo after injury, and actively proliferate and synthesize ECM components (Figure 1).

3.1 | The origin of myofibroblasts

Over the last decade, several cellular sources have been proposed as candidates of myofibroblast progenitors. Studies have revealed

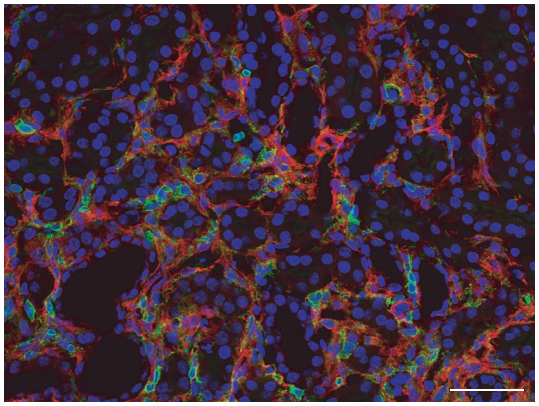

FIGURE 1 Renal fibrosis and inflammatory cell infiltration in murine injured kidney. αSMA-positive myofibroblast accumulation and inflammatory cell infiltration are observed in murine kidney 10 d after unilateral ureteral obstruction. Immunofluorescence of CD45 (green), αSMA (red), and 4',6-diamidino-2-phenylindole (DAPI, blue). Scale bar: 50 μm

that the majority of myofibroblasts are derived from resident fibroblasts, both in the kidney and other organs (eg, heart).[14,32,33] In the kidney, *P0-Cre* lineage-labeled resident fibroblasts transdifferentiate into myofibroblasts after injury, with concomitant loss of EPO production.[14] Consistently, myofibroblasts in injured kidneys are also lineage-labeled by *FoxD1-Cre*.[25] These results were confirmed by a subsequent study utilizing *EPO-Cre* mice under unilateral ureteral obstruction (UUO), a major in vivo experimental model of renal fibrosis.[9] In myofibroblasts, NFκB and Smad signaling are activated, and these underlie the phenotypic transition of myofibroblasts and decreased EPO production. Interestingly, EPO production in myofibroblasts is restored by administering neuroprotective agents, such as dexamethasone and neurotrophins,[14] or reversing UUO,[9] exemplifying the high functional plasticity of myofibroblasts.

Unlike cortical myofibroblasts, medullary myofibroblasts originate from $Wnt4^+$ resident fibroblasts in the inner medulla.[17] A recent single-cell transcriptional analysis also supports the possibility that cortical and medullary (myo)fibroblasts might exhibit distinct phenotypes. snRNAseq on murine kidney injury models revealed that fibroblast subpopulations are subdivided into four groups according to their cortical or medullary site of origin.[34] Interestingly, cortical fibroblasts transiently express Acta2 and Col1a1 only in the acute phase of IRI whereas medullary fibroblasts exhibit sustained expression of these myofibroblast markers, even at 6 weeks after IRI. These phenotypic differences between cortical myofibroblasts and medullary myofibroblasts might stem from the different origin of fibroblasts, as described above.

A recently published comprehensive study provided convincing evidence that the majority of myofibroblasts originate from resident fibroblasts/pericytes not only in murine kidneys, but also in human kidneys. Kuppe et al analyzed human and mouse fibrotic kidney by scRNAseq with very high resolution.[35] Most myofibroblasts are differentiated from fibroblasts and pericytes, with a minor contribution from other cell types such as de-differentiated proximal tubular cells. In this study, myofibroblasts were defined as the cells expressing the most ECM genes, instead of αSMA expression, and it was revealed that myofibroblasts are more efficiently marked by periostin (Postn). Interestingly, fibroblast/pericyte to myofibroblast transition is characterized by the early expression of genes related to cell cycle cessation, such as activator protein-1 (AP1). The expression of AP1 is followed by the expression of integrins and TGFβ, both of which are major drivers of renal fibrosis. They also identified Nkd2 as the selective marker for mature myofibroblasts. Nkd2 expression is positively correlated with Postn and ECM expression, but negatively with genes associated with fibroblasts and pericytes. Knockdown of Nkd2 on kidney organoids suppressed Col1a1 expression, suggesting that Nkd2 might play a role in fibrosis development. Although further validation is needed in vivo, there is a possibility that Nkd2 can serve as a therapeutic target for renal fibrosis.

Although the contribution of fibroblasts/pericytes to the myofibroblast pool is the most significant, the contribution of fibrocytes has also been reported. Fibrocytes are bone marrow-derived cells with positive hematopoietic markers, which produce collagens and ECM components. Fibrocytes migrate into the kidney as predifferentiated collagen-producing cells and contribute to fibrosis development in the kidney.[36] Early studies reported conflicting results concerning the overall contribution of fibrocytes to myofibroblasts,[24,37] mainly due to the differences in the reliability of transgenic reporters. Recent studies utilizing parabiosis models and scRNAseq demonstrated that most myofibroblasts are derived from fibroblasts/pericytes, but not from hematopoietic lineage-derived cells.[38] scRNAseq of human kidney also revealed similar results,[35] suggesting that fibrocyte contribution to the myofibroblast pool is relatively small compared with fibroblasts/pericytes.

A previous study argued that epithelial cells transdifferentiate into myofibroblasts by epithelial-to-mesenchymal transition (EMT),[39] mainly based on the colocalization of epithelial and mesenchymal markers. Detailed cell fate tracing studies questioned the major contribution of EMT in vivo, however, and demonstrated that EMT accounts for only a small fraction of the myofibroblast pool, if any.[24,25,35] Endothelial-to-mesenchymal transition (EndoMT) is another biological process in which endothelial cells transdifferentiate into myofibroblasts. Although some studies have revealed that EndoMT contributes to renal fibrosis,[40-42] the contribution of EndoMT to overall myofibroblast populations appears to be less significant than fibroblasts/pericytes or fibrocytes.[24,35]

3.2 | Redefining myofibroblasts

Traditionally, myofibroblasts have been defined as cells possessing characteristics of both fibroblasts and smooth muscle cells, and expressing contractile protein αSMA.[1] However, several studies have suggested that, in addition to fibroblasts, myofibroblasts also exhibit high heterogeneity, and αSMA is an inconsistent marker for myofibroblasts. As previously described, in the recently conducted

scRNAseq analysis of fibrotic kidneys, myofibroblasts were defined as the cells with the most ECM expression, and Postn was utilized to identify myofibroblasts instead of αSMA.[35] Indeed, Sun et al utilized Col-EGF/αSMA-RFP dual reporter mice and revealed that only a small fraction of collagen-producing cells express αSMA in renal and lung fibrosis models.[43] Another study also indicated that smooth muscle cells and pericytes express higher levels of αSMA than myofibroblasts, rendering αSMA an inconsistent marker for myofibroblasts, at least in lung fibrosis.[44] Interestingly, myofibroblasts are subdivided into five clusters by scRNAseq, and pseudotime analysis suggests that each of them represents different stages of myofibroblast transition from fibroblasts.[35] Further validation study is needed to confirm the functional heterogeneity of different myofibroblast subtypes.

3.3 | Functional heterogeneity of (myo)fibroblasts

Under pathological conditions, fibroblasts acquire distinct phenotypes and play diverse roles depending on their microenvironment and clinical context. In the lung, high functional heterogeneity of resident fibroblasts has been reported, which contributes to lung fibrosis via distinct mechanisms.[44-46] There are distinct subpopulations of lung fibroblasts residing in different anatomical locations (alveolar, adventitial, and peribronchial fibroblasts), and each of them exhibits different transcriptional profiles in scRNAseq.[44] Among them, a unique fibroblast subpopulation with high ECM production was identified by the expression of collagen triple helix repeat containing 1 (Cthrc1). Cthrc1-positive fibroblasts are differentiated from alveolar fibroblasts, and mainly observed in the fibroblastic foci, the central site of fibrogenesis. They exhibit high migratory and colonizing capacity after intratracheal transfer, suggesting their pathological functions. These results indicate that distinct fibroblast subpopulations occupy different anatomical locations in the lung, and Cthrc1 marks detrimental fibroblasts with high ECM expression. Another study revealed that phenotypic alterations of aged myofibroblasts in the lung underlie the impaired capacity of lung fibrosis resolution in aged individuals.[47] Aged myofibroblasts exhibit higher expression of NADPH-oxidase 4 (Nox4) whereas NFE2-related factor 2 (Nrf2), which is the primary regulator of the anti-oxidative stress response, is downregulated. The redox imbalance between Nox4 and Nrf2 triggers the acquisition of anti-apoptotic and senescent phenotypes in aged fibroblasts, which promotes persistent fibrosis. Indeed, in vivo blockade of Nox4 activity by siRNA or small-molecule inhibitor restores the capacity of fibrosis resolution in aged mice, with decreased senescent and anti-apoptosis phenotypes in myofibroblasts.

A unique fibroblast phenotype is also reported in IgG4-related disease (IgG4-RD), which is characterized by IgG4-positive plasma cell infiltration and storiform fibrosis.[48] Although the majority of patients with IgG4-RD respond well to glucocorticoid treatment, the underlying rationale for the therapeutic response was unclear. We revealed that the expression of glucocorticoid receptor (GR) is significantly upregulated in the affected organs of patients, such as the submandibular glands, retroperitoneum, and kidneys.[49] Interestingly, GR is mainly expressed on fibroblasts as well as leukocytes of the affected organ, which partly explains why glucocorticoid administration attenuates fibrosis development in IgG4-RD. Although fibroblasts' contribution to the development of IgG4-RD should be further validated, these results exemplify the functional heterogeneity of fibroblasts in the development of various pathological conditions.

3.4 | Renal anemia and peritubular capillary loss: two common pathological conditions driven by fibroblast dysfunction in the kidney

Fibrosis is the final common pathway of CKD, regardless of its underlying etiology. Indeed, fibrosis in the renal cortex is regarded as the best histologic predictor of renal dysfunction in CKD.[50] This is partly because fibrosis is closely related to other pathological conditions common in CKD, such as renal anemia and peritubular capillary loss. Several factors trigger fibroblast transition into myofibroblasts and fibrosis development in the kidney, such as proximal tubular injury.[51,52] As described in the previous chapter, EPO-producing fibroblasts/pericytes transdifferentiate into myofibroblasts in response to injury, with concomitant loss of EPO production.[14,29] EPO production is induced in the hypoxic condition and regulated by hypoxia-inducible factors (HIFs). Under normoxic conditions, HIFs are rapidly hydroxylated by HIF-prolyl hydroxylase domain-containing proteins (PHDs) that promote the proteosomal degradation of HIFs. In the kidney, HIF activation by PHD inhibition restores EPO production in myofibroblasts, and the PHD2-HIF2α axis is the principal regulatory cascade.[53] Interestingly, EPO production in *FoxD1-Cre* lineage-labeled fibroblasts is upregulated by the inactivation of PHD2, but not by the inactivation of PHD1 or PHD3, suggesting that EPO production in these cells is regulated by unique mechanisms.[53] Although the current standard therapy for renal anemia is the administration of recombinant human EPO (rhEPO), exogeneous rhEPO administration is associated with several adverse effects, such as hypertension and thrombotic events.[54,55] In order to overcome these disadvantages of rhEPO, PHD inhibitors that upregulate hypoxia-induced genes, including EPO, have been developed and utilized for the treatment of renal anemia.[56] Although further studies are required regarding the long-term adverse effects of PHD inhibitors, targeting myofibroblasts to induce endogenous EPO production could be a promising and favorable therapeutic strategy for renal anemia in CKD.

Peritubular capillary loss and capillary rarefaction also develop in conjunction with fibrosis, and the functional alteration of pericytes underlies these manifestations.[57-59] In the kidney, peritubular capillaries are surrounded by pericytes, which support capillary structure and function under physiological conditions. After injury, these pericytes detach themselves from peritubular capillaries and migrate to injured tubules.[53,60] In this process, peritubular capillaries lose the mechanical support of pericytes, leading to

capillary regression and rarefaction.[57,58] In the UUO model, endothelial cell apoptosis and peritubular capillary loss develop in concert with the progression of fibrosis.[61] In addition, the peritubular capillaries of fibrotic kidneys exhibit morphological abnormalities, such as caveolae formation and vacuolization, and their permeability is elevated, as evident in the increased extravasation in two-photon microscopic imaging.[62] Indeed, the decreased renal blood supply induced by peritubular capillary loss and rarefaction is detected in CKD mouse models by contrast-enhanced microcomputed tomography.[63] Together with fibrosis development and relative EPO deficiency, peritubular capillary loss contributes to the decrease in oxygen supply to renal tubules and interstitium, which exacerbates renal injury and forms a vicious cycle of CKD progression.[64]

4 | FUNCTIONAL HETEROGENEITY OF FIBROBLASTS BEYOND FIBROSIS

Recent studies employing scRNAseq analysis have revealed the phenotypic heterogeneity of fibroblasts under pathological conditions in various organs and consequently demonstrated the versatile functions of fibroblasts. Fibroblasts not only exert detrimental effects, but also have beneficial functions in a context-dependent manner. In this section, we review the current understanding of the diverse functions of fibroblasts, other than fibrosis development.

4.1 | Inflammatory functions

Fibroblasts promote tissue inflammation in several contexts.[65] For example, in the mouse model of myocardial infarction, resident fibroblasts drive not only cardiac fibrosis, but also local inflammation to deteriorate cardiac function. After myocardial infarction, cardiac fibroblasts in the fibrotic area upregulate Sox9,[66,67] which is a transcription factor responsible for the deposition of ECM in chondrocytes.[68] The fibroblast-specific deletion of Sox9 in vivo alleviates cardiac inflammation and fibrosis in the chronic phase after myocardial infarction, leading to ameliorated left ventricular dysfunction and myocardial scarring. RNAseq of cardiac scar tissue revealed that Sox9 deletion in fibroblasts downregulates proinflammatory genes, such as IL-6, as well as collagen genes. ChIPseq on mammalian chondrocytes revealed that some of these proinflammatory genes are directly bound by Sox9 at the enhancer lesion.[69] Taken together, although upstream signals for SOX9 upregulation should be further investigated, these findings suggest that Sox9 in cardiac fibroblasts regulates both fibrosis and inflammation after myocardial infarction, and could be regarded as a novel therapeutic target.

The proinflammatory phenotypes of fibroblast subpopulations have also been intensively investigated in rheumatoid arthritis (RA). Croft et al investigated mouse models of resolving and persistent arthritis, and revealed that fibroblast activation protein-a (FAPa)-positive fibroblasts accumulate in the inflamed joint.[70] The deletion of $FAPa^+$ fibroblasts attenuates local inflammation and joint deformity, suggesting their pathological functions. Interestingly, $FAPa^+$ fibroblasts are subdivided into two distinct subpopulations by scRNAseq: $FAPa^+THY1^+$ fibroblasts and $FAPa^+THY1^-$ fibroblasts. $FAPa^+THY1^+$ fibroblasts are localized in the synovial sublining layer whereas $FAPa^+THY1^-$ fibroblasts reside in the synovial lining layer. In addition to their spatial difference, these fibroblasts are functionally distinct; the adaptive transfer of $FAPa^+THY1^+$ fibroblasts into the joint induces severe local inflammation with increased leukocyte infiltration whereas the transfer of $FAPa^+THY1^-$ fibroblasts results in increased osteoclast activity and joint deformity without aggravating inflammation. These functionally distinct fibroblasts are also observed in human joints with RA. Indeed, $FAPa^+THY1^+$ fibroblasts are more abundant in the joints of RA patients than osteoarthritis (OA) patients,[70] exemplifying their inflammatory signature. Another study investigated human synovial tissues by bulk RNAseq and scRNAseq and identified three major fibroblast subsets with distinct surface protein expression.[71] Among them, $Pdpn^+THY1^+CD34^-$ fibroblasts expand in the perivascular zone of the inflamed synovium and are positively correlated with the severity of synovial inflammation in RA. $Pdpn^+THY1^+CD34^-$ fibroblasts exhibit high migratory and proliferative profiles in vitro. Interestingly, most of these $Pdpn^+THY1^+CD34^-$ fibroblasts express cadherin-11, which underlies the pathological characteristics of murine synovial fibroblasts.[72] The underlying mechanism to regulate proinflammatory fibroblasts in RA has also been investigated. Wei et al revealed that Notch3 signaling governs the differentiation of $THY1^+$ sublining fibroblasts that underlies the synovial inflammation of RA.[73] Genetic depletion or pharmacological blockade of Notch3 signaling attenuates joint inflammation and bone erosion in the mouse model of RA. Overall, these findings indicate that specifically targeting inflammatory fibroblasts can be a promising therapeutic strategy in RA.

Fibroblasts also act as innate immune cells. Pericytes/fibroblasts express several pattern recognition receptors, such as Toll-like receptors (TLR), and promote the local inflammatory cascade. Leaf et al revealed that renal pericytes sense damage-associated molecular patterns (DAMPs) released from injured cells in a TLR- and MyD88-dependent manner.[74] In the same manner as classic immune cells, pericytes activate the NLRP3 inflammasome, which leads to IL-1β and IL-18 secretion. These findings demonstrate that the interstitial localization of pericytes/fibroblasts enables them to immediately sense initial injury and function as the propagator of local inflammation. As another mechanism to propagate local inflammation, intravital microscopic observation revealed that pericytes change their morphology during inflammation and form gaps between adjacent pericytes, which facilitates neutrophil transmigration and crawling.[75]

Fibroblasts also contribute to the formation of TLTs in aged injured kidneys.[4,76] By secreting homeostatic chemokines such as CXCL13 and CCL19, fibroblasts residing within TLTs recruit

lymphocytes and provide a functional scaffold for TLT, as discussed in the following section.

4.2 | Regulation of tumor progression

In the tumor microenvironment (TME), cancer-associated fibroblasts (CAFs) comprise the largest cellular component in the stroma of TME and play an important role in cancer pathogenesis.[77] CAFs promote cancer progression via multiple mechanisms, such as ECM remodeling, and the secretion of growth factors and proinflammatory cytokines. CAFs also regulate the recruitment of immune cells and suppress the anti-tumor immune reaction.[77,78] CAFs exhibit high functional and regional diversity depending on their residing organs. For example, in pancreatic ductal adenocarcinoma (PDAC), two distinct subtypes of CAFs are identified: one subset of CAFs resides adjacent to tumor cells with elevated expression of αSMA whereas the other subset is located distant from tumor cells and secretes inflammatory cytokines, such as IL-6.[79] In breast cancer, CAFs are classified into three subpopulations according to their origins, each of which has independent prognostic capabilities.[80] Interestingly, studies have suggested that these heterogeneous subpopulations of CAFs could be subdivided into two functionally distinct subtypes: cancer-promoting CAFs (pCAFs) and cancer-restraining CAFs (rCAFs).[77,81] The existence of rCAFs has been suggested by the observation that the genetic depletion or functional intervention of proliferating CAFs does not inhibit, but rather promotes, cancer progression.[82-84] Although the specific marker for rCAFs has yet to be determined, a recent study revealed that rCAFs in PDAC are characterized by the expression of Meflin,[85] which was first identified as a glycosylphosphatidylinositol-anchored protein specifically marking mesenchymal stomal/stem cells.[86] In patients with PDAC, the infiltration of Meflin$^+$ CAFs is positively correlated with favorable outcome. In the analysis on a subcutaneous xenograft mouse model, lentivirus-induced Meflin transfer to tumor stromal cells attenuated tumor growth and αSMA$^+$ CAF infiltration. The stroma of the tumors of Meflin knockout mice exhibit straighter and wider collagen structures, and the level of Meflin expression in human PDAC is associated with altered collagen structures. Given that cancer progression is closely associated with matrix remodeling,[87] these findings suggest that Meflin$^+$ CAFs suppress tumor progression by inhibiting collagen remodeling. Although further study is needed on the underlying mechanism of Meflin$^+$ CAF development, the regulation of rCAFs could be a novel therapeutic strategy in anti-cancer therapy.

4.3 | Regenerative functions

Although the detrimental effects of fibroblasts have attracted research interest as described in the previous section, fibroblasts also promote tissue regeneration from injury. Indeed, inhibiting fibroblast function does not necessarily lead to better outcome. For example, the deletion of Postn$^+$ myofibroblasts in the heart hinders appropriate scar formation, leading to ventricular rupture and higher mortality.[32]

Recent studies have revealed that, in tissue injury and repair, a specific subpopulation of fibroblasts exerts regenerative effects in multiple mechanisms. In skin, fibroblasts are subdivided into two categories by localization: papillary fibroblasts in the upper dermis and reticular fibroblasts in the lower dermis.[88] After injury, reticular fibroblasts are recruited to the lesion prior to papillary fibroblast migration and excrete collagens responsible for scar formation. In contrast, papillary fibroblasts, which are recruited at a later time point after injury, contribute to hair follicle regeneration. These findings indicate that spatially and functionally distinct fibroblast subtypes contribute to skin wound regeneration in a coordinated manner. A recent scRNAseq analysis also revealed that fibroblasts in skin wounds exhibit high heterogeneity, and can be classified into twelve subclusters.[89] Pseudotime and RNA velocity analysis demonstrated that some of these clusters represent differentiation states toward contractile phenotypes while others acquire regenerative phenotypes. Interestingly, a unique subpopulation of skin fibroblasts specialized in tissue repair from injury has been identified. After skin injury, fibroblasts residing in the fascia, which is a gelatinous membranous structure separating the skin and the rigid structure below, ascend to the skin surface.[90] In response to deep injuries, fascia fibroblasts steer their surrounding composite matrix into the wound and form a provisional matrix. The majority of these "fascia fibroblasts" are labeled by engrailed 1 (En1), which was first identified as the marker of fibrogenic lineage cells in the dorsal skin of mice.[91] The abundance of fascia fibroblasts in the wound is positively correlated with the depth of the scar. Indeed, the genetic depletion of fascia fibroblasts prevents matrix steering into the wound and leads to defective scarring. The implantation of an impermeable film beneath the skin prevents fascia fibroblast migration and results in an open scar. The contribution of another unique fibroblast subset, adipocyte precursor cells (APs), has also been reported; APs interact with CD301b$^+$ macrophages and contribute to skin regeneration after injury.[92] Overall, these findings suggest that heterogeneous subpopulations of fibroblasts with specialized functions contribute to skin regeneration after injury.

These "regenerative fibroblasts" are also observed in the kidney. Ultramicroscopic observation revealed that myofibroblasts migrate around injured tubules and structurally support them in the renal injury model, suggesting that myofibroblasts might facilitate tubular cell regeneration.[93] By utilizing *P0-Cre*:iDTR mice, in which diphtheria toxin (DT) administration depletes *P0-Cre* lineage-labeled resident fibroblasts in the kidney, we revealed that fibroblast depletion at the early phase of injury attenuates tubular regeneration and aggravates renal injury.[94] Interestingly, in response to injury, fibroblasts upregulate the expression of retinaldehyde dehydrogenase 2 (RALDH2), the rate-limiting enzyme of retinoic acid synthesis. Retinoic acid receptor (RAR) γ is expressed on proximal tubular cells, and αB-crystallin, the product of RAR target genes, is upregulated exclusively in injured proximal tubular cells. An inverse agonist of RARs attenuates tubular

cell proliferation in vitro. Given that the retinoic acid secreted by the stromal mesenchyme in embryonic kidneys is essential for the development of the ureteric bud,[95,96] these findings suggest that RALDH2$^+$ myofibroblasts might play an important role in the regeneration of injured proximal tubules via retinoic acid signaling in the early phase of injury.

5 | FIBROBLASTS AND AGING

In general, aged individuals exhibit impaired tissue regeneration capacity and aggravated fibrosis compared with young individuals. Although the vulnerability to injury and fibrosis in aged individuals is attributable to multiple factors, such as chronic inflammation (called "inflammaging"), several age-dependent phenotypic alterations of fibroblasts are also involved.[97,98]

Interestingly, a study investigating the alterations of cardiac fibroblast phenotypes throughout the life span of mice reported that the proliferation rates or collagen gene expression levels in fibroblasts did not change with aging.[99] These findings suggest that increased fibroblast proliferation rates or collagen synthesis do not directly explain the increased fibrosis or declined organ functions in aged individuals, and other mechanisms might be involved. One of these underlying mechanisms is assumed to be the disruption of paracrine interactions between fibroblasts and endothelial cells. scRNAseq analysis of the hearts of young mice (12 weeks of age) and aged mice (1.5 years of age) revealed that aged fibroblasts exhibit the most significant transcriptional changes among cardiac cell subpopulations, which include the genes related to inflammation, ECM organization, angiogenesis, and osteogenesis.[100] Among them, angiogenesis-related genes with paracrine functions are dysregulated in aged fibroblasts. In particular, aged fibroblasts upregulate Serpine 1 and Serpine 2, both of which inhibit endothelial cell angiogenesis in a paracrine manner. Aged fibroblasts in the epicardial layer express higher levels of osteoblast genes, which might be associated with increased calcification of the epicardial layer in aged individuals. These findings suggest that phenotypic alterations of aged fibroblasts might contribute to declined organ function in aged individuals.

A recent study provided direct evidence that the functional heterogeneity of fibroblasts underlies different responses to injury in aged individuals. Multi-omics analysis of fibroblasts isolated from aged and young mice revealed that fibroblasts from aged mice exhibit secretory inflammatory phenotypes.[101] These activated fibroblasts are marked by the higher expression of THY1 and PDGFRα, and secrete several inflammatory cytokines, such as IL-1β, IL-6, and TNF. Importantly, in vitro, the proportion of activated fibroblasts and the ratio of inflammatory cytokines is positively correlated with the reprogramming efficiency in the culture. In the analysis of aged mice with different skin wound healing rates, distinct activated fibroblast subpopulations with different inflammatory cytokine profiles were observed, which may be associated with the increased variability of wound healing trajectories. Although genetic and environmental effects also play a role in humans, these findings suggest that the functional alterations of fibroblasts induced by aging underlie different tissue repair responses. Future study is needed to determine whether the diverse functions of aged fibroblasts among different organs are defined by the intrinsic mechanisms of fibroblasts or the extrinsic influences from adjacent cells or the microenvironment around them.

6 | FIBROBLASTS AND TERTIARY LYMPHOID TISSUES

6.1 | Characteristics of TLTs and their implication in various clinical contexts

Fibroblasts also promote tissue inflammation by organizing TLTs. TLTs are ectopic lymphoid structures that are induced in non-lymphoid organs by several stimuli, including autoimmunity, infection, and aging.[98,102,103] Like secondary lymphoid organs (SLOs), TLTs have the potential to initiate adaptive immune responses. TLTs are mainly composed of T cells and B cells, and are both structurally and functionally supported by fibroblasts with unique phenotypes (Figure 2). The effects of TLTs on host organs depend on the clinical context and underlying etiologies. In autoimmune diseases, such as RA and Sjogren's syndrome, TLTs are induced in the affected organ

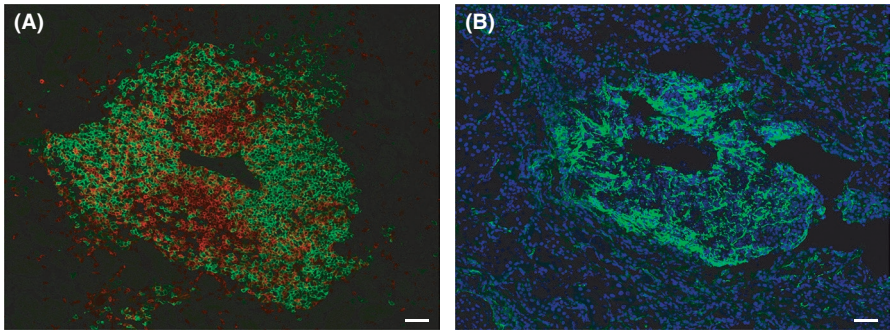

FIGURE 2 Tertiary lymphoid tissue in aged murine injured kidney. Tertiary lymphoid tissue (TLT) is composed mainly of T and B lymphocytes, which are supported by fibroblasts both structurally and functionally. Immunofluorescence of (A) CD3ε (green) and B220 (red), (B) p75 neurotrophin receptor (p75NTR, green) and DAPI (blue). Scale bar: 50 μm

and promote the accumulation of autoreactive B cells, which produce autoantibodies and exacerbate organ inflammation.[104-106] In lupus nephritis, the clonal restriction of immunoglobulin and somatic hypermutation is observed within TLTs, and the presence of TLTs is strongly associated with the immune complex deposition on glomerular basement membranes.[107] Unlike SLOs, autoreactive B cells are not excluded from the germinal center of autoimmune disease-associated TLTs,[108] and these B cells are thus able to produce disease-specific autoantibodies. TLTs are also frequently observed in chronically rejected allografts.[109] In the rejected allograft organs, an excessive humoral immune reaction with B cell maturation is induced within TLTs.[110] The presence of B cells and plasmablasts within renal allografts is associated with reduced graft survival,[111] and naïve T cell activation is also observed in the murine model of transplantation-related TLTs.[112] Although these results suggest the detrimental effects of TLTs on allografts, other studies indicate that intragraft TLTs are associated with allograft tolerance rather than rejection.[113,114] Although the precise mechanism explaining this discrepancy has yet to be elucidated, under certain conditions, TLTs may play a beneficial role by modulating the immunologic landscape within allografts.

Bacterial and viral infection also promotes TLT formation in affected organs. TLTs induced by infections generate anti-pathogen immune responses and play a protective role in host defense mechanisms. For example, bronchus-associated lymphoid tissues induced by influenza infection support lymphocyte proliferation and cytokine production in situ, which contributes to the early recovery from influenza infection.[115] TLTs also play beneficial roles in most types of cancer,[116-122] with a few exceptions, such as clear-cell renal cell carcinoma (RCC).[123] Several mechanisms underlying the beneficial effects of TLTs in cancer have been reported, such as activation of effector B cell responses and antibody production[118-120] and T-helper 1 cell skewing.[121] In breast cancer, the presence of TLTs in the lesion that regresses after treatment is associated with the treatment response to neoadjuvant anti-PD1 therapy.[124,125] Although rCAFs suppress tumor progression as discussed in the previous section, their function in tumor-associated TLTs has yet to be elucidated. The underlying mechanism and clinical implication of tumor-related TLTs have been comprehensively reviewed in the literature.[126,127]

6.2 | Fibroblast contribution to TLT formation

The initiation and development of TLTs largely resemble that of SLOs, and stromal cell populations provide a functional scaffold for the development of both TLTs and SLOs.[128] In SLO development, the interaction between stromal mesenchymal cells and hematopoietic lymphoid tissue inducer cells (LTi cells) plays a crucial role.[103,129] Stromal mesenchymal cells, also known as lymphoid tissue organizer cells (LTo cells), produce IL7 and RANKL to promote lymphotoxin αβ expression on LTi cells. LTi cells, in turn, activate LTo cells via lymphotoxin signaling, which promotes homeostatic chemokine release from LTo cells and HEV development. scRNAseq of SLOs revealed that there is vast heterogeneity in stromal cell populations,[130] each of which exhibits distinct phenotypes and possesses functional interaction with other cell types. For example, fibroblastic reticular cells (FRCs) in the T cell zone of SLOs govern T cell activation and turnover by producing several cytokines, such as CCL19, CCL21, and IL7.[131,132] In contrast, follicular dendritic cells (FDCs) secrete CXCL13 and BAFF, which facilitate B cell survival and migration.[133] Overall, these distinct stromal cell populations support SLO development and adequate immune reactions. Likewise, fibroblasts with unique phenotypes underlie the development of TLTs. TLT-driving fibroblasts possess some similarities with fibroblasts in SLOs, such as their differentiation mechanisms and interaction with adjacent cells via chemokine production.[128] In TLTs induced by pulmonary inflammation or infection during neonatal periods, IL-17 produced by $CD4^+$ T cells promotes the lymphotoxin-independent expression of homeostatic chemokine CXCL13 and CCL19 in fibroblasts.[134] Homeostatic chemokines are essential for TLT formation, as demonstrated by the fact that the ectopic overexpression of homeostatic chemokine alone is sufficient to drive TLT formation.[135]

A recent study suggests that the activation of $Pdpn^+$ fibroblasts plays a pivotal role in the early phase of TLT formation in the salivary glands of patients with primary Sjogren's syndrome.[76] By scRNAseq, $Pdpn^+$ fibroblasts were subdivided into two subpopulations according to CD34 expression. Interestingly, $Pdpn^+$ $CD34^-$ fibroblasts express CXCL13 and CCL19 whereas $Pdpn^+$ $CD34^+$ fibroblasts express IL7 and BAFF, both of which support the survival of lymphocytes. These results indicate that two distinct subpopulations of $Pdpn^+$ fibroblasts respectively contribute to the formation of TLT formation in different mechanisms. More than 85% of Pdpn+fibroblasts in TLTs express FAP, so the researchers utilized Dm2 mice in which the coding sequence of DTR is inserted under the FAP promotor.[6] The depletion of $Pdpn^+/FAP^+$ fibroblasts by DT administration in Dm2 mice significantly attenuated TLT formation and immune responses,[76] indicating that these fibroblasts are essential for TLT formation. Interestingly, the early priming of these unique fibroblasts is mainly regulated by IL13 whereas their proliferation after lymphocyte recruitment is dependent on IL22/IL22R signaling.[76] Overall, fibroblasts in TLTs could be regarded as a novel therapeutic target for TLT-associated diseases.

7 | TERTIARY LYMPHOID TISSUES IN THE KIDNEY AND THEIR CLINICAL IMPLICATION

TLTs are observed in the kidney with various pathological conditions, such as lupus and anti-neutrophil cytoplasmic antibody (ANCA)-associated nephritis,[136] IgA nephropathy,[137] allograft rejection,[111] and pyelonephritis.[138] In addition to the underlying pathological conditions, aging also plays an important role in renal TLT development. We previously revealed that 1-year-old aged mice, but not young mice, develop TLTs in the kidney after acute kidney injury (AKI).[4] In aged injured kidneys, fibroblasts with distinct phenotypes

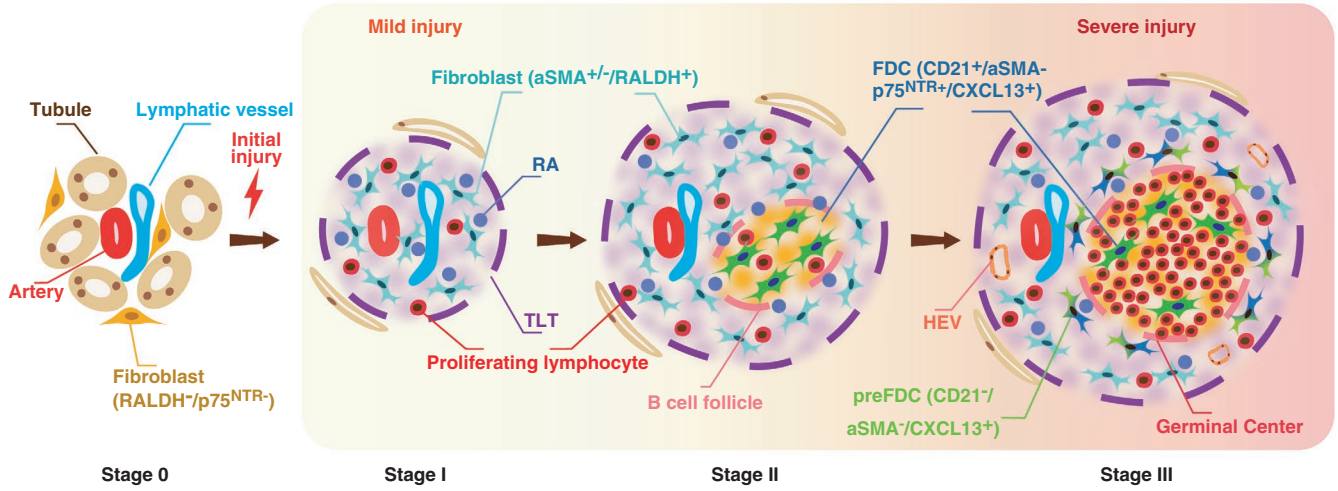

FIGURE 3 Heterogeneous characteristics of tertiary lymphoid tissue in the kidney. Fibroblasts acquire heterogeneous phenotypes in the development of tertiary lymphoid tissue (TLT) in the kidney. Renal TLTs are classified into three stages by the presence of follicular dendritic cells (FDCs) and germinal centers, and these TLT stages correlate with the severity of renal injury. HEV, high endothelial venule; p75NTR, p75 neurotrophin receptor; RA, retinoic acid; RALDH, retinaldehyde dehydrogenases 2. Figure modified from reference 138

orchestrate TLT formation (Figure 3). Some fibroblasts within TLTs produce homeostatic chemokines CXCL13 and CCL19, both of which are essential for TLT formation. Especially in the early phase of TLT formation, while fibroblasts outside TLTs strongly express RALDH2, those inside TLTs are negative for RALDH2 and instead express p75 neurotrophin receptor (p75NTR), a marker of the neural crest.[139] In vitro research revealed that fibroblasts upregulate p75NTR expression in response to retinoic acid in a dose-dependent manner, suggesting the paracrine interactions between fibroblasts inside and outside TLTs. These findings may correspond to the essential roles of retinoic acid in lymph node development.[140] In the later phase of TLT formation, $CD21^+$ FDCs develop as a part of the fibroblastic network. Most TLT-associated fibroblasts are lineage-labeled by *P0-Cre*, indicating that resident fibroblasts transdifferentiate into TLT-associated fibroblasts. Furthermore, the depletion of $CD4^+$ cells by anti-CD4 monoclonal antibody prevents renal TLT formation and, importantly, leads to improved renal inflammation and fibrosis. Overall, these results suggest that age-dependent TLT formation is maladaptive, and explains why AKI in the elderly tends to develop into advanced CKD or end-stage renal disease.[141-143]

To further elucidate the characteristics of TLTs in human kidneys, we recently examined surgically resected human kidney samples from patients with pyelonephritis,[138] because infection is a strong trigger for TLT formation, as discussed above. Multiple TLTs were observed in the kidneys with pyelonephritis, and their cellular components were equivalent to those in murine aged kidneys.[4] Interestingly, TLTs in the kidney are not randomly distributed but are instead located almost exclusively in the perivascular area of the renal cortex. Although tissue destruction was observed broadly throughout the kidney, TLTs were undetectable in the medulla. This is also true of human aged kidneys without pyelonephritis, suggesting that TLT formation is a common biological phenomenon throughout different pathological contexts. Notably, there is a vast phenotypic heterogeneity of TLTs, which reflects the developmental stages of TLTs. Renal TLTs are classified into three developmental stages based on the presence of FDCs and germinal centers (GCs).[138] Stage I TLTs comprise neither FDCs nor DCs whereas stage II TLTs harbor CD21-positive FDCs. Stage III TLTs are defined as those with both FDC and GC formation. Importantly, these TLT stages correlate with the severity of renal injury in murine kidneys, and dexamethasone treatment regresses the TLT stages and improves renal function. These results suggest that renal TLTs can serve as a reliable histological marker for kidney injury, and may be regarded as a novel therapeutic target for AKI-to-CKD transition.

8 | PERIVASCULAR FIBROBLASTS/PERICYTES AS A PROGENITOR FOR VARIOUS CELL TYPES

As described in the previous chapters, fibroblasts possess high plasticity and play diverse roles under both physiological and pathological conditions. Recently, a subpopulation of perivascular fibroblasts/pericytes that exhibit mesenchymal stem cell (MSC)-like phenotypes has received great attention, and these cell types significantly contribute to CKD development.

MSCs were first isolated as multipotent cells within bone marrow that possess stem cell characteristics and form mesenchymal tissues by transdifferentiating along distinct lineage pathways.[144,145] Studies have revealed that these perivascular fibroblasts/pericytes are significantly different from other resident fibroblasts/pericytes in that they have the capacity to differentiate into various mesenchymal cells. For example, perivascular fibroblasts/pericytes comprise MSCs with preserved myogenic potential,[146,147] and the postnatal ablation of perivascular fibroblasts/pericytes results in myofiber hypotrophy and impaired muscle stem cell quiescence.[148] Perivascular

fibroblasts/pericytes also provide adipocyte progenitors and play an important role in the beiging of white adipose tissue.[149] These findings indicate that perivascular fibroblasts/pericytes comprise unique cell populations with high potency.

Recently, Gli1-positive perivascular fibroblasts that possess MSC-like phenotypes have been identified. Gli1 is one of the target genes of the Hedgehog signaling pathway, and Gli1 protein functions as a transcriptional factor.[150] Studies have revealed that $Gli1^+$ cells provide progenitor cells with distinct lineages, such as cardiopulmonary mesoderm progenitors, osteogenic progenitors, and adventitial mesenchymal stem cells.[151-153] In CKD development, $Gli1^+$ adventitial MSCs migrate into the media and neointima, and differentiate into osteoblast-like cells to exacerbate vascular calcification.[153] The immunomodulatory function of $Gli1^+$ cells has also been reported, such as IL33 production to activate group 2 innate lymphoid cells,[154] suggesting their pathological implications in different mechanisms.

As an important function, $Gli1^+$ cells provide a myofibroblast pool and contribute to fibrosis development in various organs, such as the heart, lung, kidney, and bone marrow.[155,156] Under physiological conditions, $Gli1^+$ cells account for only 0.2% of $PDGFR\beta^+$ cells in the renal interstitium. After UUO, however, $Gli1^+$ cells significantly expand and transdifferentiate into myofibroblasts. Importantly, the depletion of $Gli1^+$ cells in the UUO model results in a more than 50% reduction of renal fibrosis, which demonstrates the function of $Gli1^+$ cells as myofibroblast precursors. The contribution of these perivascular fibroblasts/pericytes and $Gli1^+$ cells to TLT formation in the kidney has yet to be investigated.

9 | CONCLUSION AND FUTURE PERSPECTIVE

Fibroblasts acquire heterogeneous phenotypes in a context-dependent manner that underlies both physiological and pathological microenvironments in various organs, including the kidney. Further identification of heterogeneous fibroblast subpopulations and functions will contribute to our understanding of previously unrevealed biological mechanisms.

Although significant advances have been made in the field of fibroblast research, some important limitations and questions still remain. In order to therapeutically interfere with fibroblasts in humans, the characteristics of human fibroblasts should be investigated in more detail. As described, some subpopulations of fibroblasts not only exert detrimental effects, but also play beneficial roles, such as tissue regeneration after injury. Therefore, specifically targeting deleterious fibroblasts, instead of depleting all fibroblast populations, is a promising strategy. Second, although scRNAseq has revealed heterogeneous cellular characteristics at the single-cell level and enables speculation of the lineage trajectories of each cell type, it should be noted that scRNAseq does not necessarily reveal the true genetic lineages. Accordingly, results acquired by scRNAseq should be validated by other modalities, such as genetic lineage tracing analysis. Single-cell lineage tracing analysis, which combines features of both scRNAseq and lineage tracing analysis,[157] might also be helpful. Functional validations of each cell cluster are also necessary to confirm that the heterogeneous transcriptional profiles acquired by scRNAseq reflect functional heterogeneity in vivo. Third, the reason why fibroblasts in aged individuals are prone to transdifferentiate into TLT-associated fibroblasts remains unknown. Since aging affects the phenotypes of not only fibroblasts but also other cell types, alterations of the tissue microenvironment induced by aging may affect the development of TLTs. Further study is required to unravel the developmental mechanisms of TLTs and their clinical implications.

In conclusion, fibroblasts comprise heterogeneous subpopulations with distinct phenotypes that exert versatile functions in a context-dependent manner. Identifying the fibroblast subpopulations and their functions will provide important clues to novel therapeutic strategies against fibroblast-driven pathological conditions.

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CONFLICT OF INTEREST

YS is employed by the TMK Project, which is a collaboration project between Kyoto University and Mitsubishi Tanabe Pharma. MY receives research grants from Mitsubishi Tanabe Pharma and Boehringer Ingelheim. HA declares no competing interests.

AUTHOR CONTRIBUTION

HA drafted the manuscript, and YS and MY edited the manuscript.

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