Editorial Review

The renal (myo-)fibroblast: a heterogeneous group of cells

Peter Boor¹,²,³ and Jürgen Floege¹

¹Division of Nephrology, RWTH University of Aachen, Aachen, Germany, ²Institute of Pathology, RWTH University of Aachen, Aachen, Germany and ³Institute of Molecular Biomedicine, Comenius University, Bratislava, Slovakia

Correspondence and offprint requests to: Peter Boor; E-mail: boor@email.cz

Abstract
Renal fibrosis is a central pathological process in kidneys of patients with chronic kidney disease (CKD). Identification of effective treatments that halt or reverse fibrosis would be beneficial for most, if not all, CKD patients. Key to this is an understanding of fibrogenesis, including the principal responsible cells, the renal fibroblasts. It is in part due to their inconspicuous appearance that it was believed that there might not be much more to a fibroblast than a simple interstitial mesenchymal cell which makes up the organ stroma. The so-called ‘renal fibroblasts’ are a heterogeneous population of mesenchymal cells with various essential functions during kidney development and in adult life. Still, remarkable uncertainties exist in the nomenclature of renal mesenchymal cells—or renal fibroblasts—and molecular characterization remains poor. The embryonic origin of fibroblasts is unclear as well, although some studies point to a neural crest origin of these cells. The renal myofibroblasts appear de novo in renal fibrosis, originating from renal fibroblasts. Myofibroblasts most likely represent a stressed and dedifferentiated phenotype of fibroblasts. We have only just begun to appreciate that renal fibroblasts are anything but simple renal interstitial cells.

Keywords: kidney development; origin of fibroblasts; renal fibrosis; renal stroma; markers

Chronic kidney disease (CKD), defined as impaired renal function for 3 months or longer, is often asymptomatic, which delays the diagnosis of CKD until advanced stages of disease. The prevalence of CKD has reached pandemic proportions and some epidemiological studies have shown that up to 10% of the world’s population is affected [1]. With increases in the aged population, the number of CKD patients is expected to rise even further in the future. CKD is a strong and independent risk factor for cardiovascular morbidity and mortality and the risk increases with each CKD stage. In so-called developing countries, where access to renal replacement therapies is limited or does not exist at all, the final stage of CKD most often equates to death. It is estimated that >1 million patients die per year as a result of the final CKD stage, i.e. end-stage renal disease. In developed countries, both renal replacement modalities, dialysis and renal transplantation, are available, but they are extremely expensive and are associated with a substantial reduction in the quality of life and life expectancy of the patients. The worldwide cost of renal replacement therapy is estimated to be ~$1 trillion [1].

Essentially, all chronic renal diseases, but also repeated or serious acute insults, inevitably lead to renal fibrosis. The underlying and principal pathological finding in the kidneys of CKD patients is renal fibrosis [2–4]. The importance of fibrosis, be it glomerulosclerosis or tubulointerstitial fibrosis, is illustrated by its close correlation with the decline in renal function. It has been reported that interstitial fibrosis correlates more closely with the decline in renal function than glomerulosclerosis [5], but that may simply reflect our inability to accurately quantify glomerulosclerosis in a renal biopsy with limited numbers of glomeruli.

Fibrosis is a fundamental biological process, and an essential and beneficial step in the course of tissue repair and regeneration. It is possible that focal or initial fibrosis is a beneficial process in kidney disease, since it might support the mechanical stability of the injured organ and encapsulate injured nephrons [2]. Nevertheless, sustained and uncontrolled fibrosis becomes pathological, since the functional tissue is ultimately replaced by permanent scar tissue. Such scars disrupt normal organ structure and thereby hinder the possibility for regeneration and normal organ function. The key characteristics of renal fibrosis are the extensive deposition of the extracellular matrix (ECM) and expansion of the fibroblast population. Renal fibrosis is often, if not always, associated with monocytic inflammatory infiltrates and phenotypic alterations or loss of resident renal cells, e.g. tubular and capillary endothelial cells. The histological appearance of these changes is shown in Figure 1.

Given its almost universal appearance in CKD, renal fibrosis, in particular interstitial fibrosis, is an excellent treatment target. Within the fibrotic process, a prime target for therapy is the principal cell responsible for the exaggerated production and deposition of ECM, i.e. the renal fibroblast.
Fig. 1. Histological features of renal fibrosis in rats. The kidney tubulointerstitium of a healthy rat is depicted in (A). Note the narrow interstitial space with capillaries and very few inconspicuous cells (arrows). In (B) a rat kidney 5 days after UUO, a widely used model of renal fibrosis is shown. The altered tubulointerstitium shows all of the typical features observed in renal fibrosis: expansion of ECM (asterisk), altered tubular phenotype (arrowhead points to a mitosis in a tubular cell of a dilated and partly atrophied tubule) and inflammatory infiltrates (arrow). These changes closely resemble the fibrosis observed in renal biopsies of patients with kidney diseases. Exaggerated deposition of ECM is shown on the example of type I collagen immunohistochemistry in healthy (C) and fibrotic rat kidneys (D). In normal kidneys, very fine focal expression is found in the interstitium (arrowhead in C) and around arterioles (a, arrow in C), whereas its expression is significantly increased during fibrosis (arrow and arrowhead in D). In UUO, no expression of collagen I is found in the glomeruli (glo). Expression of a widely used marker for myofibroblasts, α-SMA, is shown for normal (E) and fibrotic kidneys (F). In normal kidneys, α-SMA is expressed only by VSMCs of arteries and arterioles (a). In fibrosis, a striking de novo expression in the interstitium is found (arrowhead in F). Note that tubular cells do not express α-SMA (arrows in F) and no expression in glomeruli (glo) is found. A marker for mesenchymal cells, vimentin, is shown for normal (G) and fibrotic kidneys (H). In normal kidneys, vimentin is strongly expressed in glomeruli (glo) by podocytes, mesangial cells and parietal epithelial cells as well as in arteries by VSMCs and arterioles (a) and interstitial fibroblasts (arrowhead in G) but not by tubular cells (arrow in G). During fibrosis, expansion of interstitial vimentin-positive cells is obvious (arrowhead in H). Note the de novo expression of vimentin in single and clusters of injured tubular cells (arrows in H). PAS, periodic acid-Schiff staining, original magnifications ×400.
What are fibroblasts and myofibroblasts?

At first glance, the term fibroblast seems a straightforward one. Fibroblasts are mesenchymal cells residing and embedded in the ECM or stroma of connective tissues or organs [6]. Several general definitions exist for fibroblasts, e.g. ‘a connective-tissue cell of mesenchymal origin that secretes proteins and especially molecular collagen from which the extracellular fibrillar matrix of connective tissue forms (http://www.merriam-webster.com/medical/fibroblast)’. Fibroblasts are characterized by light microscopy as elongated, spindle- or stellate-shaped cells with rather pale cytoplasm and oval (or round) nuclei. The first description of these cells dates back to the 1850–70s by Virchow [7, 8]. Ultrastructurally, renal fibroblasts have an abundant endoplasmatic reticulum, collagen-secreting granules and processes forming contacts to other cells and basement membranes, including tubular, endothelial and dendritic cells [9]. These cells can be isolated, cultured and expanded in vitro, where the cells show a morphology similar to the one in vivo.

The term fibroblast, although widely used, is not completely correct. A ‘blast’ denotes a cell with stem cell features or in an activated state. The correct term for a resting, differentiated fibroblast should be fibrocyte, as is the case for other mesenchymal cells, e.g. chondrocytes or osteocytes. However, the term ‘fibrocyte’ is not used to describe resting fibroblasts. Instead, it is used for circulating progenitors of fibroblasts expressing haematopoietic markers and collagen (e.g. CD45+, CD33+, coll1+) [10, 11]. For clarity, in this review, we will use the term fibroblast for both resting and activated fibroblasts, and the term fibrocyte for the circulating precursor of fibroblasts.

The term myofibroblast was first used some 100 years after the term fibroblast was introduced, when in 1971 Majno et al. [12] described phenotypic alterations of fibroblasts towards a smooth muscle-like phenotype in contracting dermal wound granulation tissue. This also remains the main definition of a myofibroblast today: ‘a fibroblast that has developed some of the functional and structural characteristics (as the presence of myofilaments) of smooth muscle cells (http://www.merriam-webster.com/medical/fibroblast)’. A fully developed myofibroblast is defined as a cell with a light-microscopic appearance similar to that of fibroblasts (i.e. spindle- or stellate-shaped cells with oval nuclei and pale cytoplasm) that expresses vimentin, α-smooth muscle actin (α-SMA) and extra-domain A of fibronectin (ED-A fibronectin) but not smooth muscle myosin or desmin [13, 14]. An important part of the definition, however, is based on the ultrastructure. A fully differentiated and mature myofibroblast should have a prominent endoplasmatic reticulum, prominent Golgi apparatus and collagen-secreting granules, peripheral myofila- ments and gap and fibronexus junctions (the latter being connections of intracellular myofilaments with extracellular fibronectin) [14]. As in most studies of renal fibrosis, in the following section, we will use the term myofibroblast for renal interstitial cells positive for α-SMA, i.e. not necessarily mature myofibroblasts.

Until now, no markers have been found that are expressed specifically and exclusively on fibroblasts or that would label all fibroblasts (Table 1). This is not a specific problem of fibroblasts but of mesenchymal cell biology in general. For example, a definition of mesenchymal stem cells involves the expression and lack of expression of a number of surface markers (e.g. CD29, CD44, CD73, CD90, CD105 and CD31, CD33, CD11b, CD45, respectively) rather than any specific markers [15]. Mesenchymal stem cells, also denoted mesenchymal stromal cells, indeed bear some similarities to fibroblasts. Some studies suggested that these cells reside not only in bone marrow but also in other organs, especially in the perivascular space (or niche) [16]. Furthermore, fibroblasts

<table>
<thead>
<tr>
<th>Marker (human gene symbol)</th>
<th>Expression</th>
<th>Function</th>
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<tbody>
<tr>
<td>Vimentin (Vim)</td>
<td>VSMCs</td>
<td>Component of cytoskeleton (class III intermediate filament of the desmin group)</td>
</tr>
<tr>
<td>Desmin (Des)</td>
<td>VSMCs, Pericytes, Injured podocytes</td>
<td>Component of cytoskeleton (muscle-specific class III intermediate filament of the desmin group)</td>
</tr>
<tr>
<td>α-SMA</td>
<td>VSMCs, Myofibroblasts, Pericytes, Injured mesangial cells</td>
<td>Major actin isoform of the contractile cell apparatus</td>
</tr>
<tr>
<td>PDGFR-β-platelet-derived growth factor receptor-β (PDGFRβ)</td>
<td>VSMCs, Fibroblasts, Myofibroblasts, Pericytes, MSCs, Mesangial cells, Macrophages</td>
<td>Cell membrane receptor for platelet-derived growth factor (PDGF)—BB and—DD</td>
</tr>
<tr>
<td>S100A4/S100 calcium-binding protein A4 (S100/A4) also termed fibroblast-specific protein 1 (FSP-1)</td>
<td>VSMCs, Fibroblasts, Myofibroblasts, Inflammatory cells, Tubular cells</td>
<td>Cytoplasmic and nuclear protein, member of the S100 family of proteins with calcium-binding motifs and broad cellular functions</td>
</tr>
<tr>
<td>CD73 ecto-5′-nucleotidase [5′-NT] (Nt5c)</td>
<td>Fibroblasts, Myofibroblasts, Pericytes, T-lymphocytes, Tubular cells</td>
<td>Cell membrane enzyme involved in conversion of extracellular nucleotides to membrane-permeable nucleosides</td>
</tr>
</tbody>
</table>

As can be seen, using one single marker does not allow a distinction between various cell types. It should be mentioned that it is not yet clear whether these markers are expressed in all cells of a certain type (i.e. fibroblasts or pericytes) and to what extent the expression might change during phenotypic alteration. See also Figure 1.
isolated from fibrotic kidneys were shown to be heterogeneous and a certain population exhibited some stem cell features, such as clonal expansion or formation of 3D structures [17]. It is possible that some of the renal fibroblasts, in particular perivascular fibroblasts, might be resident mesenchymal stem cells. To distinguish between fibroblasts, fibrocytes, monocytes and macrophages in human tissue, their histological localization together with the analysis of at least three to four markers was necessary [18].

In healthy kidney cortex, apart from vascular smooth muscle cells (VSMCs), virtually no expression of α-SMA can be found. In renal fibrosis, a striking up-regulation is observed (Figure 1). This marker seems to be specific enough to distinguish myofibroblasts from other mesenchymal cells if analysed strictly in the peritubular space (Table 1 and Figure 1). However, α-SMA is not expressed by all myofibroblasts and some α-SMA-negative cells can be myofibroblasts [19]. The expression of other markers of myofibroblasts, e.g. of ED-A fibronectin, has not yet been used extensively, although it is expressed in an almost de novo fashion in human interstitial fibrosis [20]. S100A4, also termed fibroblast-specific protein 1 (FSP-1), was shown to be expressed by leukocytes, which limits its use as a marker for fibroblasts in renal fibrosis (Table 1) [9]. Other markers for renal fibroblasts include cadherin 9 [21] while some still need to be examined, e.g. fibroblast activation protein. The lack of specific markers led to a definition per exclusion. A renal fibroblast should not express markers of epithelial (e.g. cytokeratins), endothelial (e.g. von Willebrand factor), vascular (e.g. SM-22) and inflammatory cells (e.g. CD45), a definition especially relevant for cell characterization in in vitro studies. Such a definition obviously does not differentiate between the various populations of renal mesenchymal cells (Table 1).

The identification and characterization of mesenchymal cells using immunohistological markers remains poor, as was shown for myofibroblasts and pericytes [13, 22]. At present, the best way to distinguish between these cell types is via ultrastructural analyses [13, 22]. This is, however, not feasible in our ‘daily’ experimental studies.

Using collagen Iζ1 promoter-driven green fluorescent protein (GFP)-expressing reporter mice, it was found that in fibrosis induced by unilateral ureter obstruction (UUO) ~75% of the GFP-positive interstitial cells also expressed α-SMA [23]. Similarly, other studies showed that, depending on the stage of experimental fibrosis, renal interstitial fibroblasts vary in their phenotype when assessed by vimentin and α-SMA (co-) expression [23]. These studies suggested that, especially in renal fibrosis, various phenotypes or subtypes of fibroblasts and myofibroblasts exist. This is well in line with the notion that fibroblasts are a heterogeneous population of cells not only between different organs but also within a given organ [24–27].

What do renal fibroblasts do?

The best established and most likely major role of fibroblasts is ECM production. Fibroblasts are essential in shaping the structure of organs, providing structural and mechanical support. On the basis of localization and ultrastructure, it seems plausible that renal fibroblasts are involved in the production of basement membranes of tubules and capillaries and transmit mechanical and biochemical signals between these structures. This might indicate a pericyte-like and neuronal-like function of fibroblasts (regarding the latter point, see the section concerning the embryonic origin of fibroblasts). A role of fibroblasts in the control of microcirculation is also supported by the expression of certain molecules affecting haemodynamics, such as ecto-5′-nucleotidase (CD73) or soluble guanylyl cyclase (cGC) [9].

Renal fibroblasts are the major producers of erythropoietin (EPO) [28–30]. Supporting the idea of renal fibroblasts as a heterogeneous group of cells, a recent study showed that ~20% of renal fibroblasts produce EPO [31]. It is not yet clear why a certain subset of interstitial fibroblasts is the major source of EPO and thereby the major regulator of erythropoiesis for the whole organism. In renal fibrosis, fibroblasts reduce or even lose their ability to produce EPO, which is the major cause of renal anaemia [31]. In vitro fibroblasts from healthy kidneys express EPO mRNA, whereas myofibroblasts do not. Stimulation of these myofibroblasts in vitro with various factors, e.g. neurotrophins, brain-derived neurotrophic factor, hepatocyte growth factor or lower concentrations of dexamethasone is able to restore EPO production [31].

The localization of fibroblasts in the interstitium, i.e. the space in which the transport of fluids and solutes between tubules and capillaries takes place, implicates a potential role in this process as well. In particular, this was suggested for medullary fibroblasts. These cells can contract in response to vasoactive substances and peptides, e.g. prostaglandin E2 or atrial natriuretic peptide [32, 33].

As in other organs like dermis or lymph nodes [24, 34], fibroblasts interact with immune cells, in particular with resident dendritic cells and macrophages [35]. In renal fibrosis, the interaction between the expanded population of myofibroblasts and infiltrating inflammatory cells is most likely reciprocal influenced by each other. For example, infiltrating macrophages in fibrosis express platelet-derived growth factor (PDGF)-C, which induces chemokine expression in fibroblasts, thereby further augmenting inflammatory infiltrates [36]. To date, there exist only few data on the interaction of immune cells and fibroblasts in healthy and diseased kidneys. This field deserves further attention, given the potential immunomodulatory function of fibroblasts [37].

It is possible that renal fibroblasts also have other functions, which are not yet known. A subset of medullary interstitial fibroblasts has lipid inclusions (vitamin A-storing granules). These cells bear some similarities to hepatic stellate cells, but the relevance of these cells in the kidney is unclear [38, 39]. It has been suggested that these particular cells express a protein specific for activated hepatic stellate cells, the cytoglobinin/stellate-cell activation-associated protein (Cypg/STAP) [39]. Cypg/STAP-expressing cells significantly accumulate in experimental renal fibrosis but do not fully co-localize with α-SMA [39].
The functions of the various renal fibroblast subsets still remain to be characterized.

### What is the embryonic source of renal fibroblast?

During mammalian embryogenesis, the later kidney, termed metanephros, is formed from the ureteric bud and metanephric mesenchyme (also termed metanephrogenic mesenchyme or metanephrogenic blastema), both deriving from the intermediate mesoderm [40] (Figure 2). The structures induce each other, i.e. the ureteric bud induces the condensation of the metanephric mesenchyme and induces glomerulo- and tubulogenesis. On the other hand, the metanephric mesenchyme induces branching of the ureteric bud and the formation of the collecting ducts. The origin and development of the renal interstitium, often called renal stroma, is less well understood. In the following section, we will often use the term interstitium (or stroma) referring to mesenchymal stromal cells but not other stromal elements such as capillaries, vasculature or resident inflammatory cells [41]. Some uncertainties in the definitions exist in the embryonic kidney as well; the precise definition of what all comprises the term ‘renal stroma’ is not completely clear [42].

The embryonic origin of the renal stroma, i.e. renal fibroblasts, is still largely unknown. Several possible sources exist (reviewed in [33, 42, 43]): first, it might arise from the metanephric mesenchyme itself along with tubulogenesis. Second, it might arise from the uninduced intermediate mesenchyme in which the developing structures are embedded. Third, it might be derived from different structures, e.g. the neural crest. And finally, given the heterogeneous nature of fibroblasts, it is possible that these cells might be derived from more than one of the aforementioned origins (Figure 2). The primary interstitium of the developing kidney was shown to be distinct from the condensed metanephric mesenchyme (and of course of the ureteric bud). These differences are both morphological and molecular. Compared with condensed metanephric mesenchyme, the primary interstitium was shown to express tenasin, glycolipid disialoganglioside G_{D_3} and, in particular, the winged helix transcription factor Foxd1 (formerly termed BF-2) [33, 41, 44, 45]. As all structures develop, the primary interstitium forms the progenitor population for the mature cortical and medullary interstitium. The latter is characterized by the appearance of interstitial cells with lipid inclusions (vitamin A-storing cells) [33, 42].

Descriptive studies showed that embryonic stromal cells express neuronal markers [46, 47]. This suggested an extrarenal neural crest origin of renal fibroblasts, recently supported by an intriguing study using reporter mice [31]. What is the neural crest? Arising from the neural tube, the neural crest is a multipotent and migratory cell population that appears transiently during embryogenesis. Various cells and tissues develop from the neural crest, e.g. smooth muscle cells, melanocytes or peripheral glial cells and neurons. In the aforementioned study, to mark and follow the cells of neural crest origin, the promoter for myelin protein zero (P0) was used [31]. P0 is expressed in the neural crest but not in the developing or adult kidney [31]. The marked cells were interstitial, showed no expression of inflammatory, dendritic or endothelial cell markers, overlapped nearly completely with PDGFR-β expression and they also partly co-expressed Foxd1, i.e. they appeared to be renal fibroblasts. In renal fibrosis, these cells were the main, if not the only, source of myofibroblasts. Using other neural crest reporter mice (Wnt-1 promoter), migration of neural crest cells to the developing kidney was also observed. However, these cells remained in a capsule-like fashion in the early stages of the metanephric kidney and largely disappeared thereafter with only very few positive intrarenal cells remaining [48]. In mice with a neural crest defect (Splotch-deficient mice) no expression of neural crest markers in metanephros was observed and histologically these kidneys and their interstitium were normal-appearing [48]. This might suggest that there are various embryonic sources of renal stroma. It should be mentioned that in contrast to the aforementioned study [31], the expression of P0 in mature murine podocytes and interstitial endothelial cells was observed [49]. Taken together, the origin of renal fibroblasts still remains to be clarified. But the possible neuronal origin of these cells could facilitate further studies of biology and present novel roles of renal fibroblasts.

What is the role of the stroma cells in kidney development? Similar to the reciprocal interactions between the ureteric bud and metanephric mesenchyme, reciprocal interactions between interstitial cells and the ureteric bud and evolving tubules have been suggested [33, 42, 50]. The retinoic acid receptors (RARα and RARβ2, involved in vitamin A signalling), fibroblast growth factor 7 (FGF-7) and bone morphogenetic protein 4 (BMP-4) are all expressed in embryonic stromal cells and are essential for normal ureteric bud development and branching [42]. Stromal cells have also been suggested to play a role in nephrogenesis [33, 42]. Foxd1-deficient mice lacking the stromal progenitors (Figure 2) develop only very few nephrons compared with wild-type littermates [45].

The molecules driving the development of renal stromal cells are largely unknown. To date, only some of the factors have been described to be important in the development and survival of embryonic stroma. These include PDGF receptor α ligands, PDGF-A and PDGF-C, BMP-7 and molecules of the renin-angiotensin system [33, 41, 42, 51].

Future studies focusing on the origin and clues for the development of renal stroma, i.e. of fibroblasts, could introduce new tools for the specific manipulation of this cell population, such as the Foxd1 mice [52].

### What is the source of myofibroblasts in renal fibrosis?

Fibrosis is characterized by exaggerated ECM deposition and a striking accumulation of myofibroblasts (Figure 1). The role and origin of myofibroblasts are therefore of great interest. This is also one of the most controversially
discussed problems in the nephrological research field, as obvious from a plethora of critical reviews on this matter (for example, see [53–58]). We believe in the validity of Occam’s razor, i.e. that the simplest explanation making the fewest new assumptions is the most plausible one. In our opinion, the simplest explanation is that renal fibroblasts are the main, if not the only, source of myofibroblasts in renal fibrosis. This has indeed been recently shown in renal fibrosis in mice [31]. By renal fibroblasts, we mean the broad term encompassing renal interstitial mesenchymal cells (above). It is likely that in the future ‘renal fibroblasts’ will comprise several distinct cell groups, perhaps with different names, such as cells with pericyte-like functions, perivascular fibroblasts (or even mesenchymal stem cells), cells with neuronal-like functions, EPO-producing cells and vitamin A-storing cells. We also believe that if there is any epithelial or endothelial source of myofibroblasts in renal fibrosis, this will not be a major one. Studies analyzing the epithelial- and endothelial-to-mesenchymal transition using genetic cell fate tracking are summarized in Table 2 and were reviewed in detail elsewhere [53–58]. Most recent studies from various groups that specifically addressed this issue using different methodologies did not find any indication of an epithelial or endothelial source of myofibroblasts [23, 31, 52, 59–62]. This does not mean that phenotypic alteration of endothelial or tubular epithelial cells does not contribute to fibrosis. Phenotypic alteration in the course of epithelial (or endothelial)-to-mesenchymal transition or cell-cycle arrest might lead to changes in their paracrine cell signalling which drives fibroblast proliferation and ECM production and induces a myofibroblast phenotype. The role of circulating fibrocytes as a contributor to renal fibroblast and myofibroblast formation remains controversial as well (Table 3). We have previously reviewed these issues in more detail [2].

Some recent studies suggested that the major source of myofibroblasts in renal fibrosis is pericytes [23, 52, 55, 63]. By using marker expression such as PDGFR-β, it has been suggested that, in the peritubular interstitium, the mesenchymal cells are pericytes and not fibroblasts [23, 52, 55, 63]. There is currently no single marker that would be able to discriminate a pericyte from other mesenchymal cells [22] (Table 1). Other groups using ultrastructural analysis did not describe pericytes in the renal interstitium [9]. The simple definition of a pericyte is ‘a cell of the connective tissue about capillaries or other small blood vessels (http://www.merriam-webster.com/medical/pericyte)’. Especially in the healthy renal interstitium, such a definition could easily categorize most cells

Fig. 2. The origin of the renal stroma (interstitium). The nephrogenesis of metanephros is initiated when the metanephric mesenchyme (or metanephric blastema) induces the outpouching of the ureteric bud from the Wolffian duct (A). The outgrowing ureteric bud induces condensation of some of the metanephric mesenchyme, forming condensed mesenchyme or cap cells (B). These cells are Pax-2 positive and represent the nephron progenitor cell population. Surrounding the cap cells are a more loose and morphologically distinct cell population of mesenchymal Foxd1-positive cells. These cells are the progenitor population for the renal stroma. These cells might originate from metanephric mesenchyme but possibly also from the neural crest (grey arrows). At later stages, with branching of the ureteric bud (C) the condensed mesenchyme undergoes mesenchymal-to-epithelial induction to form renal vesicles from which glomeruli and tubules develop. At this stage, the Foxd1 cells migrate along the developing nephron and form the primary interstitium. The primary interstitium then differentiates to the mature medullary and cortical interstitium. Whether stromal cells arise directly from the metanephric mesenchyme, uninduced intermediate mesenchyme, neural crest or combination thereof is not clear. Representative PAS-stained section of human embryonic kidney at 20 weeks of gestation is shown in D (original magnification ×200). The schematics (A–C) were adopted from [42, 43].
Tracing endothelial cells
α(Tie2-Cre/ROSA26-stop-EYFP) FSP-1,
Tracing endothelial cells
β(P0-Cre/ROSA26-tdRFP) PDGFR-
α(Six2-GFP-Cre/ROSA26LacZ or Z/Red) PDGFR-
β
Tracing interstitial mesenchymal cells
FoxD1-GFP-Cre-ER/ROSA26LacZ or Z/Red) PDGFR-
β
Tracing tubular cells
UUO, unilateral I/R [52]
(Ksp-Cre/ROSA26-EYFP) FSP-1,
α
Tracing tubular cells
47; IHC, immunohistochemistry; I/R, ischemia/reperfusion injury model; STZ, streptozotocin; UUO, unilateral ureteral obstruction.
[22], in which a mature pericyte is a cell completely or is currently possible only using ultrastructural analyses [22, 64]. Pericytes have processes that form contacts with endothelial cells. These are of different types, e.g. peg and socket, adhesion plaques and gap junctions as pericytes. However, the true identification of pericytes is currently possible only using ultrastructural analyses [22], in which a mature pericycle is a cell completely or partially embedded within the vascular basement membrane [22, 64]. Pericytes have processes that form contacts with endothelial cells. These are of different types, e.g. peg and socket, adhesion plaques and gap junctions

### Table 2. Overview of studies using genetic cell fate tracking to analyze epithelial and endothelial source of renal (myo-)fibroblasts

<table>
<thead>
<tr>
<th>Genetic approach</th>
<th>Determination of transition (colocalization with)</th>
<th>Animal model Result</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracing tubular cells (β-glutamyl-transferase-driven Cre/ROSA26LacZ)</td>
<td>β-galactosidase/anti-β-galactosidase IHC with FSP-1, HSP-47 IHC</td>
<td>UUO, bone marrow chimeras</td>
<td>[80]</td>
</tr>
<tr>
<td>Tracing tubular cells (Pax8rtTA/LC1/ROSA26LacZ or Z/Red)</td>
<td>β-galactosidase/X-Gal with Collagen 1 IHC</td>
<td>Overexpression of active TGF-β in tubular cells (tetracyclin-induced)</td>
<td>[81]</td>
</tr>
<tr>
<td>Tracing tubular cells (Six2-GFP-Cre/ROSA26LacZ or Z/Red)</td>
<td>β-galactosidase/X-Gal, RFP with FSP-1, α-SMA IHC</td>
<td>No indication of tubular source of fibroblasts/EMT in any of the approaches</td>
<td>[52]</td>
</tr>
<tr>
<td>Tracing interstitial mesenchymal cells (FoxD1-GFP-Cre/ROSA26LacZ or Z/Red)</td>
<td>β-galactosidase/X-Gal, GFP with PDGFR-β, CD73, α-SMA IHC</td>
<td>Foxd1-traced interstitial cells form the majority of myofibroblasts</td>
<td>[31]</td>
</tr>
<tr>
<td>Tracing interstitial mesenchymal cells (P0-Cre/ROSA26ECFP or ROSA26-tdRFP)</td>
<td>EYFP with FSP-1, α-SMA, entactin IHC</td>
<td>UUO, unilateral I/R</td>
<td>[82]</td>
</tr>
<tr>
<td>Tracing endothelial cells (Tie2-Cre/ROSA26-stop-EYFP)</td>
<td>EYFP with FSP-1, α-SMA IHC</td>
<td>Some FSP-1 and fewer α-SMA-positive cells derived from Tie2 traced cells</td>
<td>[83]</td>
</tr>
<tr>
<td>Tracing endothelial cells (Tie2-Cre/EGFP)</td>
<td>EGFP with α-SMA IHC</td>
<td>STZ-induced diabetic nephropathy</td>
<td></td>
</tr>
</tbody>
</table>

α-SMA, α-smooth muscle actin; EMT, epithelial-to-mesenchymal transition; FSP-1, fibroblast specific protein 1 (S100A4); HSP-47, heat shock protein 47; IHC, immunohistochemistry; I/R, ischemia/reperfusion injury model; STZ, streptozotocin; UUO, unilateral ureteral obstruction.

### Table 3. Summary of the main conclusions of this review

Renal fibroblast is not a ‘simple mesenchymal spindle-shaped cell’, but rather a term including heterogeneous population of renal interstitial mesenchymal cells with various functions, e.g. EPO production. The nomenclature of mesenchymal cells in general is not clearly defined, mostly because an ultrastructural analysis is currently the only means of identification and differentiation between various mesenchymal cells and their transitional phenotypes. We lack specific molecular markers that could specifically differentiate renal mesenchymal cells and their different phenotypic subgroups. The embryonic source of renal fibroblasts is yet unclear, some studies suggesting a neural crest origin. Renal fibroblasts are the major, if not the only, source of renal myofibroblasts in renal fibrosis. Most studies refer to the appearance of the so-called renal myofibroblasts found in renal fibrosis, which in most studies is described solely by α-SMA expression, as ‘differentiated’ or ‘transformed’ cells. However, it is likely that these cells rather represent a ‘stressed’ or ‘dedifferentiated’ mesenchymal cells that have lost some of their (differentiated) functions, e.g. EPO production.

As described earlier, renal fibroblasts have been described to form connections with endothelial basement membrane and endothelial cells. To date, it is unknown how many of the ‘renal interstitial cells’ or ‘renal fibroblasts’ might indeed be mature pericytes. Detailed ultrastructural and immunogold studies that assess marker panels to characterize and quantify various phenotypes of renal mesenchymal cells are eagerly awaited and might solve this question [65].

According to the ultrastructural definition, mature myofibroblasts are tissue-contracting cells. They have important functions in wound closure but also in the initiation of revascularization and regeneration [66]. In organs such as the kidney, such functions are not entirely clear. It is possible, but not proven, that myofibroblasts normalize biomechanical forces in injured organs thereby supporting normal function in the uninjured areas and reducing further organ damage. It has been shown that biomechanical forces are crucial for correct cell differentiation and a major trigger to induce a myofibroblast phenotype [19]. In experimental renal fibrosis, alteration of renal intratubular hydrodynamic forces leads to phenotypic alterations of tubular cells towards a more profibrotic phenotype [67, 68]. Histology of human or experimental renal fibrosis shows tubular atrophy, dilatation and loss (Figure 1). It is well known that advanced fibrosis is characterized by shrinkage of the kidneys. These data suggest that in renal
fibrosis significant alterations of biomechanical forces take place, which might be a major signal for myofibroblast formation. This is supported by the observation that in reversible acute kidney injury myofibroblasts form only transiently during the period of tubular injury and dilatation [69]. Another major signal for the induction of a myofibroblast phenotype is paracrine signalling, including the profibrotic molecules like TGF-β, [70]. Other signals that may drive the myofibroblast induction in renal fibrosis are as yet unclear. The majority of interventions shown to be antifibrotic were also associated with a reduction of the number of α-SMA-expressing cells [3, 36, 70–73]. The molecules governing the phenotypic switch of fibroblasts are not yet well defined. In vitro, a wide variety of molecules were found to be involved in proliferation of (myo-)fibroblasts. These include TGF-β, PDGF, FGF-2, connective tissue growth factor (CTGF), tissue plasminogen activator (tPA), fibrinogen, potassium channel KCa3.1, high glucose or the anti-mitogenic EP4 and were reviewed previously in more detail [2–4, 70, 71, 74–76]. Some of the molecules involved in renal fibroblasts proliferation might also be involved in their phenotypic switch, and these processes might even be closely linked. One could argue that, from a clinical point of view, the source and precise phenotype of the expanded pool of interstitial mesenchymal cells in renal fibrosis is irrelevant as long as our intervention counteracts this process. However, we believe that a better characterization and understanding of cell biology of the various renal fibroblast subtypes might lead to more specific treatments or even targeting approaches for certain subtypes of these cells.

What does the myofibroblast phenotype in fibrotic kidneys represent?

Most of the studies of renal fibrosis describe the change of a renal fibroblast into a myofibroblast phenotype as ‘differentiation’, ‘transdifferentiation’, ‘transformation’ or ‘activation’. We hypothesize that most of the so-called myofibroblasts actually represent stressed cells that have lost their mature, differentiated phenotype. These cells lose their functions, alter their phenotype and proliferate, thereby resembling a non-mature state, i.e. they dedifferentiate to some degree. Such changes are well described in other mesenchymal cell types, e.g. the VSMCs but also in pericytes [77–79]. During injury, VSMCs lose the expression of proteins characteristic of their contractile function, thereby losing the ability to contract. Stress- or injury-induced alterations of cell phenotype that resembles the embryonic precursor were also proposed for renal tubular cells. Tubular cells develop embryonically via mesenchymal-to-epithelial transition from metanephric mesenchyme (Figure 2). In renal fibrosis re-expression of some mesenchymal markers in tubular cells has been observed and termed epithelial-to-mesenchymal transition (Figure 1) [2].

The same process most likely occurs in renal fibroblasts. In renal fibrosis, these cells lose their ability to produce EPO [31]. Using detailed ultrastructural analyses from nine patients with renal fibrosis, only one case presented with cells with features of mature myofibroblasts. The other patients showed many different phenotypes of myofibroblast-like cells [65]. Importantly, no typical mature fibroblasts were found in these biopsies either [65]. During embryonic development, the typical markers of myofibroblasts, including α-SMA, vimentin or fibronectin ectodomains, are expressed, but at later stages, they are down-regulated or lost as the cells differentiate [9, 19]. Furthermore, myofibroblasts, and especially their precursor forms, have also been characterized as ‘rather poor construction workers’ [19].

Thus, at least part of what we term renal myofibroblasts, i.e. α-SMA-expressing interstitial cells, might be dedifferentiated renal fibroblasts. Vice versa, only a fraction of so-called myofibroblast indeed meets the criteria of mature myofibroblasts. At first glance, this difference might be purely semantic. We think that by acknowledging this fact, we might move the research towards approaches that might translate into novel therapeutic options for renal fibrosis. For example, treatment that would re-establish mature fibroblast phenotype could also lead to re-expression of EPO, as was achieved in vitro [31].

What do we conclude and where do we go from here?

The simple, inconspicuous light-microscopic appearance of fibroblasts together with a lack of specific markers is most likely responsible for the current uncertainties in the nomenclature of renal fibroblasts, or more generally, of renal interstitial mesenchymal cells. We are beginning to understand that the term ‘fibroblast’, as currently used in the literature, comprises a large and functionally important population of differentiated and diverse mesenchymal cells.

Identification of specific markers for fibroblasts, which will allow their specific isolation and in vitro and in vivo characterization, are one of the essential goals for future research. Only a combination of anatomical localization, shape and protein expression together with ultrastructural analyses can distinguish between various mesenchymal cell types and their various phenotypes. Specific markers could also facilitate genetic animal studies allowing the specific targeting of these cells. The obvious question is whether there are such markers at all. Characterization of the embryonic origin and the molecules that drive their development are other issues deserving further studies.

The world of renal fibroblasts is open to be conquered.

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