REVIEW ARTICLE

Port J Nephrol Hypert 2018; 32(3): 279-282 • Advance Access publication 31 August 2018

Cellular origin and regulation of kidney fibrosis

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Received for publication: Sep 09, 2018
Accepted in revised form: Sep 22, 2018

ABSTRACT

Myofibroblasts take a key position as fibrosis driving, matrix secreting cells in kidney fibrosis and are thought to be important therapeutic targets in chronic kidney disease (CKD). However, their origin and activation pattern have been discussed for many years and are still partly unclear. Recently, Gli1+ cells, which reside in the perivascular niche, have been identified as progenitors of fibrosis-causing myofibroblasts. However, Gli1+ cells only account for about 50% of the myofibroblast population and are predominantly located in the kidney medulla. Nevertheless, the data suggests that Gli1+ cells are an important therapeutic target in kidney fibrosis since genetic ablation of these cells significantly ameliorates kidney fibrosis in rodents. Other potential sources of myofibroblasts in the kidney are circulating bone-marrow derived cells, endothelium and epithelium. The current review will discuss the cellular origin of myofibroblasts and potential mechanisms of myofibroblast activation driving fibrosis and CKD.

Keywords: kidney fibrosis, myofibroblasts, Gli1, Hedgehog.

INTRODUCTION

Kidney fibrosis is the final common pathway in chronic kidney disease (CKD), regardless of the primary kidney disease. While it has been known for decades that the amount of cortical interstitial fibrosis in kidney biopsies correlates better than any other structural change with impaired renal function[1], no approved drug or therapy for kidney fibrosis exists to date. Given that nearly 11% of the population in the western world suffers from CKD[2] with massively increased mortality and morbidity, it is of the utmost importance to decode the underlying molecular and cellular mechanisms and pathways for the development of new therapeutic options. One of the key characteristics of fibrosis across diverse organs is that after kidney injury, activated fibroblasts, known as myofibroblasts, start expanding and produce extracellular matrix (ECM). However, if the activation continues, the process, initially intended to contribute to repair, leads to destruction of kidney tissue and ultimately to irreversible loss of kidney function – End-Stage Renal Disease (ESRD).

In this review we will discuss the debated cellular origin of myofibroblasts and which signaling pathways contribute to their activation and in the end to scar formation in the kidney and functional decline.

CELLULAR ORIGIN OF MYOFIBROBLASTS

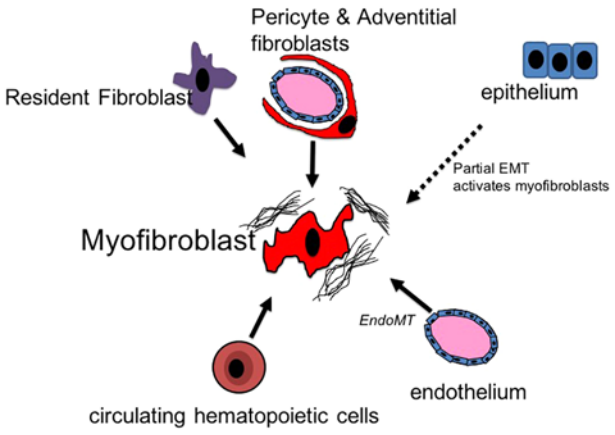
It has been accepted that in various organs, myofibroblasts are the key players in pathologic matrix-production after injury[3]. Myofibroblasts are of mesenchymal origin and display an extensive rough endoplasmic reticulum, which explains their capability to secrete high amounts of different forms of collagen and other ECM proteins all known to promote fibrosis and express

alpha-smooth muscle actin (α-SMA)[4]. Alpha SMA forms stress fibers, i.e. bundles of myofilaments, that mediate a contractile force and might be involved in scar contraction.

The cellular source of myofibroblasts has been controversial for many years due to the fact that they do not exist in healthy tissues and various experimental evidence points towards different cellular sources (Figure 1). Proximal tubular epithelium, endothelium, circulating cells such as macrophages and fibrocytes and resident mesenchymal cell populations such as pericytes and fibroblasts are among the hotly debated precursor candidates of myofibroblasts. One of the first hypotheses, first formulated about 20 years ago, says that tubular epithelial cells undergo a so-called epithelial-to-mesenchymal transition (EMT) after renal injury[5]. Various genetic fate tracing experiments have reported mixed results, ranging from no influence to major influence on fibrosis[6-8]. Recent work shows that tubular epithelial cells do not contribute to the direct production of collagen I[9] and inducible genetic fate tracing indicates no contribution of proximal tubular epithelium cells to the myofibroblast pool in kidney fibrosis[10]. However, tubular epithelium is certainly an important driver of kidney fibrosis and tubular epithelium dedifferentiates in response to injury and acquires many markers of mesenchymal cells in a process that has been recently termed partial EMT[11,12]. It is now thought that these injured tubular epithelial cells secrete various signals that mediate myofibroblast activation and inflammation. However, the cells do not leave the epithelial basement membrane to become an interstitial myofibroblast[10].

Figure 1

Potential sources of myofibroblasts in kidney fibrosis



The proportion of circulating cells contributing to the myofibroblast pool has been discussed for years[13]. In the past, up to one third of the myofibroblast pool was thought to derive from bone marrow-derived mesenchymal stem cells (MSCs)[8]. Most of these investigations used bone-marrow transplantation techniques, which is tricky since only hematopoietic stem cells engraft whereas other bone marrow cells, such as MSCs, do not engraft well[14,15]. Bone-marrow derived progenitor cells have been proposed to enter the circulation and home to sites of injury[16]. However, recent data using state of the art techniques such as inducible genetic fate tracing and single-cell RNA sequencing indicate that only a very small fraction of myofibroblasts derives from circulating cells, i.e. monocytes[10]. However, the data also indicates that there is strong cross-talk between resident myofibroblasts and circulating monocytes, with many receptor ligand interaction pairs expressed by both cell-populations. A recent paper by Buchtler et al. suggests that hematopoietic bone marrow derived cells contribute to almost 50% of collagen deposition in the kidney[9]. Thus the debate on whether hematopoietic cells contribute to collagen deposition and the myofibroblast pool is ongoing and further studies are needed to answer their exact contribution.

Recently, perivascular Gli1+ MSC-like cells were identified as a major cellular origin of kidney fibrosis. Gli1+ cells reside in the perivascular niche across various organs and express the mesenchymal marker platelet derived growth factor receptor beta (PDGFRβ). Using inducible genetic fate tracing in bigenic Gli1CreER;tdTomato mice, we demonstrated that Gli1+ cells expand after injury and transform to fibrosis driving myofibroblasts. Furthermore, ablation of these cells ameliorated fibrosis and restored kidney function[15]. However, as genetic fate tracing experiments indicate that Gli1+ cells only contribute to a subfraction of myofibroblasts (<50%), further studies are needed to dissect the cellular origin and heterogeneity of all kidney myofibroblasts.

PERICYTES AND CAPILLARY RAREFACTION IN KIDNEY FIBROSIS

The term “capillary rarefaction” describes the reduction of vascular density along with the functional consequences – hypoxia and impaired hemodynamic and sodium regulatory responses[17]. Interstitial capillary loss correlates with the degree of interstitial fibrosis[18,19]. In progressive kidney disease, peritubular capillaries

exhibit significant ultrastructural and functional changes independent of the underlying injury[20]. In diverse experimental sceneries, like unilateral ureteral obstruction[21], remnant kidney model[22] or experimental glomerulonephritis[23] peritubular capillary rarefaction goes along with interstitial fibrosis und tubular atrophy[17,20] – which led to the conclusion that regardless of the underlying injury, loss of the peritubular capillary network facilitates fibrosis. However, the mechanisms which connect peritubular capillary loss to kidney fibrosis are still in some part elusive. A longstanding hypothesis suggested that detachment of pericytes from peritubular capillaries drives capillary loss after kidney injury. We were recently able to provide experimental in vivo fate tracing data that indeed supports this hypothesis. Genetic fate tracing indicated that Gli1+ cells indeed detach from peritubular capillaries after kidney injury and differentiate into myofibroblasts. Furthermore, our data show that genetic ablation of Gli1+ cells in healthy kidneys results in capillary loss, hypoxia and subsequent tubular epithelial injury[24]. These data suggest that the activation of pericytes and their myofibroblast differentiation initiates a vicious circle that results in fibrosis, capillary loss and tubular damage driving kidney functional decline.

SIGNALING PATHWAYS REGULATING PERICYTE FATE AND ACTIVATION

A key characteristic of fibrosis consists of the reactivation of developmental signaling pathways, which are involved in various processes of kidney fibrosis such as extracellular matrix (ECM) production and myofibroblast differentiation and proliferation, among others. It is beyond the scope of this review to discuss all developmental pathways that have been studied in kidney injury and repair so we will only summarize some recent findings in the Hedgehog, Wnt and Notch signaling pathways.

Since these signaling pathways are activated after injury of the kidney, crosstalk between them seems highly likely. However, probably due to the complexity of their interplay, only a handful of studies which report direct interaction exists[25]. Better understanding of the interaction between these pathways may facilitate the development of new treatments in kidney fibrosis and CKD.

Hedgehog (Hh) signaling has been extensively studied in various cancers such as basal cell carcinoma as well as medulloblastoma and glioma, among others. Dysregulated Hh signaling has been identified as one of the drivers of cancer progression which lead to the development of smoothened (Smo) antagonists such as vismodegip, which is the first approved canonical Hh inhibitor for advanced basal cell carcinoma[26]. Recent data, including our own, indicates that Hh signaling is not only a critical driver of cancer progression but also drives fibrotic disease. During canonical Hh signaling, one of the three ligands Indian (Ihh), sonic (shh) or desert (dhh) Hh bind to the receptor patched (ptch1). Upon ligand binding, ptch1 releases its tonic inhibition of the transmembrane protein Smo. Smo then activates the nuclear translocation of the Gli family transcription factor into the nucleus, which results in increased expression of various Hh target genes that partly drive cell-proliferation. Three different Gli proteins, i.e. Gli1, Gli2 and Gli3, have been identified in vertebrates[27]. While Gli2 responds first to the binding of the Hh ligand, Gli1 serves primarily as an signal amplifier and Gli3 as an repressor[28]. It has been reported that after kidney injury, tubular epithelial cells (TECs) upregulate the expression of two Hh ligands, namely Ihh and Shh, which result in expression of Hh target genes in myofibroblasts and development of kidney fibrosis[29-31]. Using various genetic and pharmacologic models, we have recently shown that Gli2 is an important driver of myofibroblast expansion and fibrosis.[32] Furthermore, our work indicates that Hh target genes are also upregulated in human kidney fibrosis[32].

Wnt signaling is a highly conserved pathway which plays a central role in diverse biologic processes from embryogenesis to proliferation and carcinogenesis, and there is various evidence that Wnt signaling also drives kidney fibrosis[33-35]. Similar to Hh signaling, Wnt ligands are expressed by TECs upon injury and drive activation of pericytes and fibrosis[36].

Notch signaling describes a cell-cell communication during embryogenesis, which is usually quiescent in adult tissue. However, during fibrosis, Notch signaling is reactivated by binding of the ligands to the Notch receptor family (Notch1-4), leading to a signal cascade which, in the end, results in activation of Notch target genes. Several studies have reported central effects of Notch signaling in kidney fibrosis: Expression of Notch pathway proteins correlates with tubulointerstitial fibrosis and renal function[37]; human CKD samples showed increased cleaved Notch1 expression, and overexpression of cleaved Notch1 in tubular epithelial cells resulted in kidney fibrosis[38]. Thus, there are several lines of evidence showing that increased tubular epithelial Notch signaling induces proliferation of interstitial myofibroblasts and drives fibrosis[38-43].

CONCLUSION

The cellular origin of kidney fibrosis is still controversial. Most recent data suggest that resident mesenchymal cells such as pericytes are a major source of myofibroblasts in the kidney. The specific contribution of circulating hematopoietic cells to matrix secretion in the kidney is still unsolved but recent single cell RNA-sequencing data suggests that these cells primarily act through indirect mechanisms and activate resident mesenchymal cells. However, further studies are needed to dissect the heterogeneity, cellular origin and mechanism of activation of myofibroblast in mouse and human. The recent development of various single cell genomic tools will certainly help in understanding myofibroblast heterogeneity and crosstalk to other cell types driving CKD progression.

Disclosure of potential conflicts of interest: none declared.

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