Editorial Comments

Kidney remodelling and scarring: the plasticity of cells

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Introduction

The progression of chronic kidney failure remains one of the main challenges in nephrology in view of the ever-increasing number of patients presenting every year with end-stage renal failure. It is predicted that the increased trend will continue unabated for at least another 10 years [1]. It is therefore imperative to improve our understanding of the underlying kidney scarring process in order to design imaginative therapeutic preventive approaches.

Mechanisms of kidney scarring

Glomerular sclerosis

It is assumed currently that progressive kidney scarring is due to the interactions between resident renal cells and infiltrating, mainly inflammatory, cells [2]. Within the glomeruli, injury is followed by potential damage to resident cell lines with, as a consequence, the release by endothelial cells of pro-inflammatory cytokines, chemokines and growth factors attracting inflammatory cells to the glomerular capillaries and initiating a microinflammatory process [2]. The infiltration of the glomerular capillaries by monocytes/macrophages leads to their interaction with all glomerular cell lines (endothelial, mesangial and epithelial) to stimulate the proliferation of some (endothelial and mesangial) and the synthesis of extracellular collagenous matrix (ECM) by most (endothelial, mesangial and epithelial). Such increased synthesis in the face of a presumed decreased breakdown would lead to irreversible glomerular sclerosis.

Plasticity of glomerular cells. Recent data suggest a much more fluid state of affairs, with significant changes in the phenotypic characteristic of glomerular cells playing a key role in the glomerular scarring process. First, the endothelial cells, once injured, lose their mature anticoagulant, anti-inflammatory and anti-proliferative phenotype and acquire new proagulant, pro-inflammatory and mitogenic characteristics [3]. Platelets and inflammatory cells are attracted by the release of a wide range of cytokines and chemokines by endothelial cells, which in turn provide a pro-aggregant surface expressing new cell adhesion molecules, facilitating the adherence of platelets and inflammatory cells alike [3].

Mesangial cells ‘trans’-differentiate in response to injury from a mature, adult, pericyte phenotype to an embryonic myoﬁbroblastic one (the ‘mesangioblast’) characterized by proliferation and contraction as well as the expression of cytoskeletal cell markers such as smooth muscle actin (α-SMA) [4,5]. This mesangioblastic phenotype observed in experimental and clinical nephropathies is associated with proliferative and sclerotic changes [5]. In particular, the mesangioblast is capable of releasing interstitial collagens type I and III not normally detected within healthy glomeruli [5]. The deposition of collagens I and III is irreversible as glomeruli are devoid of collagenases (metalloproteinases (MMPs)) capable of breaking down such collagens. In other words, the trans-differentiation of adult mesangial cells to embryonic mesangioblasts is characteristic of mesangial injury and is the forerunner of mesangial and glomerular sclerosis.

Changes in mesangial phenotype and the associated qualitative changes in glomerular ECM are likely to accentuate glomerular sclerosis further by affecting mesangial cell survival. The expression of α1β1 integrin by mesangial cells appears to be a critical determinant of mesangial cell phenotype, growth and collagen remodelling capacity [6]. This integrin is a mesangial collagen receptor that has been implicated in collagen
remodelling after injury. Collagen IV and laminin, the normal constituents of the mesangial and glomerular ECM, have mesangial survival-promoting properties through a β(1) integrin-mediated, but Arg-Gly-Asp (RGD)-independent, mechanism [7]. On the other hand, collagen I/III and fibronectin, which are overexpressed in diseased glomeruli, do not promote rat mesangial cell survival, thus potentially contributing to their apoptosis and depletion during the course of glomerular sclerosis [7]. These observations demonstrate that glomerular mesangial cell phenotype and survival are tightly regulated by changes in the underlying ECM. They imply a vicious circle of mesangial de-/trans-differentiation leading to qualitative ECM changes which in turn further affect mesangial phenotype and survival.

Phenotypic changes also take place within the epithelial cells in response to injury. Glomerular epithelial cells respond to injury by acquiring an embryonic phenotype similar to that of the mesangioblast/myofibroblast with the expression of α-SMA [8]. Glomerular parietal epithelial cells acquire an intracellular network of stress fibres and the morphological ultrastructural appearance of myofibroblasts [8]. These changes are also associated with crescent formation and excessive production of ECM.

It is therefore apparent that the regression of mature glomerular cells into an embryonic phenotype is a feature of glomerular response to injury (remodelling) with the excessive production of ECM, an undesirable effect that leads to irreversible fibrosis. In addition, the qualitative changes in glomerular ECM further destabilize mesangial cells through integrin-mediated signalling, triggering cell death and apoptosis. Glomerular cell deletion, along with excessive ECM deposition, is the hallmark of glomerular sclerosis and obsolescence.

The phenotypic changes affecting glomerular cells have been labelled trans-differentiation as well as de-differentiation. However, these changes represent a regression of the adult phenotype to a mesenchymal embryonic phenotype reminiscent of the metanephric phenotype [9].

**Haematopoietic stem cells (HSC).** A new twist in the glomerular phenotypic changes characteristic of the response to injury is the possibility of the migration into injured glomeruli of haematopoietic progenitor cells suggestive of stem cells. These appear to be involved in the normal turnover of mesangial cells [10] and in the response of the mesangium to injury. Following experimental mesangiolysis, it appears that a significant percentage of cells repopulating the mesangium are derived from a pool of progenitor cells located at the vascular pole [11] and apparently haematopoietically derived [12]. The migration of these cells into the injured glomerulus seems to require platelet-derived growth factor (PDGF) as well as basic fibroblast growth factor (bFGF) [13]. Whilst PDGF plays an important role in the migration of the progenitor cells into the glomerulus, bFGF has been linked to the proliferation of repopulating mesangial cells [13]. This sequence of events is reminiscent of the role played by PDGF B chain in the migration of mesangial precursors into nascent glomeruli during embryogenesis [14]. In PDGF B-null mice, the absence of PDGF B leads to a failure of mesangial cell migration into the glomerular tuft, and the formation of collapsing glomeruli with a limited number of mesangial cells [14].

Bone marrow transplantation experiments in rodents have demonstrated that the development of glomerulosclerosis is dependent on the bone marrow phenotype rather than that of the kidney itself. When mice resistant to glomerulosclerosis (GS) (ROP +/+) were transplanted with bone marrow from GS-prone congenic ROP Os/+ mice, they developed progressive glomerulosclerosis [15]. In another set of experiments, the transplantation of bone marrow from normal mice into those producing high circulating IgA levels and prone to IgA nephropathy (HIGA mice) led to a resolution of the glomerular sclerotic changes [16]. These experimental data clearly show that beside phenotypic changes of resident mesangial cells, there is a migration of bone marrow-derived haematopoietic precursors into injured glomeruli contributing to their remodelling. Healing may depend on whether these cells acquire a mature phenotype (adult mesangial cells), while scarring may be due to the persistence of the embryonic phenotype (mesangioblast) with the inherent propensity to sclerosis.

**Tubulo-interstitial scarring**

As with glomerular sclerosis, it is assumed that the interactions between resident cells (tubulo-epithelial and fibroblastic) and infiltrating inflammatory cells (lympho-monocytic) lead to the initiation and progression of tubulo-interstitial scarring [17].

**Plasticity of proximal tubular cells.** As with glomerular cells, tubular epithelial cells have the capacity to regress from an adult, mature phenotype to an embryonic one in response to injurious stimuli. This so-called trans-differentiation has been reported in response to a variety of growth factors [transforming growth factor-β1 (TGF-β1), epidermal growth factor (EGF) and interleukin-1 (IL-1)] in vitro where the proximal tubular epithelial cells (PTCs) lose their adult phenotype and markers (cytokeratins) and acquire myofibroblastic ones such as α-SMA and vimentin [18]. This epithelial–mesenchymal trans-differentiation (EMT) is merely the regression of the adult phenotype to the embryonic one in response to injury. As PTCs are derived from the same metanephric mesenchyme as the renal fibroblasts [9], both cell lines regress to such an embryonic mesenchymal phenotype in response to activation and injury. Interstitial fibrosis is also characterized by the regression of interstitial renal fibroblasts to a myofibroblastic phenotype, with the expression of a variety of cytoskeletal markers including α-SMA, vimentin and desmin [5].
As with glomerular cellular integrity, the architecture of the basement membrane appears to play an important role in the maintenance of tubular epithelial phenotype. Changes in basement membrane architecture may lead to the upregulation of tubular epithelial release of TGF-β1. TGF-β1 is thought to be the most potent promoter of EMT. It was also demonstrated that fibroblast growth factor-2 (FGF-2) made an important contribution to the mechanisms of EMT by stimulating microenvironment proteases (MMPs 2 and 9) essential for basement membrane disintegration and facilitating tubular epithelial cell motility [19]. On the other hand, hepatocyte growth factor (HGF) has been shown to inhibit EMT, thus explaining some of its anti-fibrotic potential [20].

EMT is also likely to be influenced by changes in the underlying ECM substrate, as the behaviour of epithelial cells is strongly influenced, through the action of integrins, by the underlying ECM. Recent experimental data have shown that type IV collagen contributes to the maintenance of the epithelial phenotype of proximal tubular epithelial cells, whereas type I collagen promotes EMT [21]. Incubation with recombinant human α1NC1 collagen domain inhibits assembly and deposition of type IV collagen and facilitates EMT in vitro [21]. Inhibition of type IV collagen assembly by the α1NC1 domain also upregulates the production of TGF-β1 in proximal tubular epithelial cells [21].

These observations suggest close interactions between tubular epithelial cells and the underlying ECM through integrin-mediated release of growth factors and MMPs.

Haematopoietic stem cells. As with the remodelling glomeruli, tubulo-epithelial injury recently has been shown to be associated with an influx into the kidneys of haematopoietic stem cells. This has been observed in experimental models [22] and in humans [23], where bone marrow-derived cells have been identified within regenerating tubules. In a model of ischaemia/reperfusion injury in rats, we have observed a significant infiltration of regenerating tubules by CD34+ haematopoietic cells [24]. It is possible, however, that the expression of CD34 may be an expression of the trans-/de-differentiation of injured tubulo-epithelial cells into a mesenchymal embryonic metanephric phenotype. However, experiments relying on bone marrow transplantation of male bone marrow into female rodents have confirmed the bone marrow-derived origin of some of these cells [22,23]. It is of relevance that clinical kidney transplantation of females with male kidneys leads to a significant number of donor cells (carrying the Y chromosome) repopulating the allograft tubules [23].

Preliminary data from our laboratory [25] and that of others [26] have also implied a contribution by bone marrow-derived mesenchymal cells (MSC) to the renal interstitial fibroblastic pool. Thus, stem cells along with quiescent renal interstitial fibroblasts, adventitia pericytes and proximal tubular epithelial cells may all contribute to the pool of interstitial myofibroblasts characteristic of interstitial renal fibrosis.

Therapeutic implications

A better understanding of the plasticity of renal cells and their response to injury will allow us in the future to manipulate such a response in order to promote healing and prevent scarring. It is becoming apparent that EMT and the associated regression to an embryonic phenotype by mature renal mesangial and tubulo-epithelial cells is associated with a significant activation of intracellular signalling pathways [18]. The inhibition of some of these enzymatic pathways involving Smads (Smad 2 and 3) has already led to the inhibition of EMT [27]. Conversely, the overexpression of some protective/inhibitory Smads (Smad 6 and 7) appears to inhibit EMT [28].

The putative role of HSC in renal remodelling may also have therapeutic implications, with the stimulation of the repopulation of injured glomeruli and tubules by injections of the relevant stem cells.

In conclusion, the renal remodelling process is an extremely dynamic process involving a constant differentiation and de-/trans-differentiation of intrinsic renal cells as well as those infiltrating the damaged kidney. Improved understanding of the mechanisms regulating these processes may lead to more innovative and original interventions favouring healing and preventing scarring.

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References

Macrophage heterogeneity in renal inflammation

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Introduction

Macrophages evolved to maintain and restore tissue integrity. They originate from bone marrow-derived precursors and traffic through tissues where they have essential roles in remodelling during fetal development; in host defence against infection and tumours; and in wound healing [1]. Macrophages also mediate injury in immune-mediated diseases including glomerulonephritis, and this aspect of their function together with their role in combating infection have tended to overshadow their involvement in tissue repair. Understanding how macrophage function adapts to the needs of particular microenvironments is a principal challenge for inflammatory cell biologists. Importantly, learning to manipulate macrophage function to promote their reparative properties would be a powerful therapeutic tool, a point emphasized by the recognition that many viruses, parasites and tumour cells have evolved to redirect macrophage function to promote their survival [2]. Our purpose here is to review briefly what is known about the role of macrophages in renal injury; to describe recent advances in understanding of macrophage activation; and to show that manipulation of macrophage function can have profound effects on the intensity of glomerular inflammation.

Macrophages and renal injury

In 1972, Shigamatsu identified macrophages in inflamed glomeruli by electron microscopy in patients

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