Life and death in the kidney: prospects for future therapy

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Introduction

Cells may die by two distinct mechanisms. In adverse biological circumstances, such as severe ischaemia, cells may undergo necrosis: an inherently pro-inflammatory form of cell death secondary to the spillage of phlogistic intracellular contents into the local microenvironment. In general, however, cells prefer to die by undergoing apoptosis. Apoptosis is characterized by stereotypical morphological and biochemical changes including the activation of intracellular proteolytic enzymes, termed caspases, which seal the fate of the cell by cleaving numerous nuclear and cytoplasmic targets [1]. Apoptosis results in specific cell surface changes, such as the exposure of phosphatidylserine, and leads to the rapid uptake and degradation of apoptotic cells by local resident or infiltrating phagocytes without the release of pro-inflammatory mediators [2]. Indeed, the ingestion of apoptotic cells by pro-inflammatory macrophages is a ‘deactivating’ stimulus and therefore the clearance of apoptotic ‘corpses’ by macrophages may well promote the downregulation of inflammatory responses and subsequent tissue healing [3]. This brief article is intended to outline some of more important factors that affect apoptosis in the kidney and then focus upon interventions which may modulate renal cell apoptosis in renal disease.

Apoptosis in the kidney

One would expect fundamentally important biological processes to be involved in diverse physiological and pathological scenarios, and apoptosis is no exception. Apoptosis is prominent during nephrogenesis and has been well documented in both human renal diseases and experimental animal models of renal injury such as acute glomerulonephritis, acute tubular necrosis (ATN), obstructive nephropathy and toxic nephropathies as well as progressive renal scarring and cystic renal disease [4–6]. It is now apparent that in disease states apoptosis may be a ‘double-edged sword’ since it may be both beneficial and deleterious.

Benificial apoptosis

Acute inflammation such as acute glomerulonephritis is often characterized by leukocyte infiltration and proliferation of resident renal cells. Indeed, the removal of pro-inflammatory leukocytes and the restoration of a normal complement of resident cells is a prerequisite for tissue healing. It can therefore be appreciated that, in this context, the apoptotic demise and removal of leukocytes and excess numbers of mesangial cells, tubular epithelial cells or interstitial myofibroblasts is an important aspect of tissue remodelling following injury, and is therefore of benefit [7–9].

Deleterious apoptosis

Apoptosis also has a darker side: it is responsible for the undesirable widespread deletion of resident renal cells in conditions such as ischaemia-reperfusion injury and obstructive or toxic nephropathy. Furthermore, sustained low-grade levels of apoptosis may lead to a gradual and significant loss of resident renal cells, such as that which occurs during post-inflammatory scarring [6]. In these conditions apoptosis, in the absence of cell regeneration, leads to renal tubular atrophy and hypocellular fibrotic tubulointerstitial or glomerular scarring [6,10].

Life and death signals in the kidney

Cells constantly receive signals from the local microenvironment, many of which may be viewed as promoting either cell survival or cell death (Table 1). Indeed, it has been suggested that all somatic cells are destined to die by an apoptotic default mechanism
Table 1. Survival- and death-promoting signals received by cells from the local microenvironment

<table>
<thead>
<tr>
<th>Survival signals</th>
<th>Death signals</th>
</tr>
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<tbody>
<tr>
<td>Cytokines</td>
<td>Fas ligation</td>
</tr>
<tr>
<td>Growth factors, e.g. EGF, IGF-1, HGF and VEGF</td>
<td>Cytokines, e.g. TNFα, TGFβ</td>
</tr>
<tr>
<td>Hormones</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>Adhesion to cells or ECM</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>Cell cycle regulatory proteins, e.g. p53</td>
<td>Angiotensin II</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>p53</td>
</tr>
<tr>
<td>Anti-apoptotic members of the Bcl-2 family, e.g. Bcl-XL</td>
<td>Pro-apoptotic members of the Bcl-2 family, e.g. Bax, Bcl-XL</td>
</tr>
<tr>
<td>Oxygen</td>
<td>Loss of normal survival signals, e.g. hypoxia, disruption of cell-cell or cell-matrix adhesion</td>
</tr>
<tr>
<td>Nutrients</td>
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</tbody>
</table>

unless they are actively sustained by an adequate supply of survival signals [11]. In disease states, resident renal cells, including glomerular mesangial cells, tubular epithelial cells and the endothelial cells of both the glomerular and interstitial capillary networks, may undergo apoptosis. Cells may be actively killed or may die as a result of insufficient survival signals. The integration of conflicting life and death signals occurs at the level of individual cells, which then ‘decide’ whether it is appropriate to live or die.

Survival signals

Survival signals are myriad and include growth factors, hormones, cytokines, members of the Bcl-2 family, adhesion to adjacent cells or extracellular matrix (ECM) as well an adequate supply of oxygen and nutrients. It is important to note that some survival signals may be cell lineage specific; for example, growth factors such as basic fibroblast growth factor (bFGF), epidermal growth factor (EGF) or vascular endothelial growth factor (VEGF) have markedly differing effects upon the survival of mesangial, tubular or endothelial cells and it may be important to take this into account when contemplating future therapeutic interventions [12]. The field is becoming increasingly complicated since there is now evidence that cell cycle regulatory proteins that had previously been thought to be primarily involved in regulating cell proliferation and hypertrophy can also modulate cell survival [13]. Survival factors, by their absence, are probably important in disease states. For example, the extracellular matrix protein osteopontin (OPN) is a tubular cell survival factor in vitro and a macrophage chemoattractant that is expressed at low levels in normal kidney but is rapidly upregulated in almost all forms of experimental renal injury. In experimental hydropnephrosis, mice targeted for the deletion of the OPN gene are protected from macrophage infiltration and collagen deposition compared with wild-type mice. However, despite significant amelioration of interstitial inflammation and scarring, OPN knockout mice develop increased levels of tubular cell apoptosis [14].

Death signals

Although signals that induce apoptosis are often thought to be specific ‘death stimuli’, it is important to note that significant levels of renal cell death may result simply from the absence of fundamental survival signals such as a supply of nutrients or oxygen. However, more specific ‘active’ death stimuli include ligation of the cell surface Fas death receptor by Fas ligand, cytokines such as tumour necrosis factor-α (TNFα), interleukin-1β (IL-1β) and transforming growth factor-β (TGFβ), reactive oxygen species (ROS), complement (C5b-9) attack, angiotensin II and genotoxic drugs such as cisplatin. A detailed discussion of the role of various individual death signals in renal disease is beyond the scope of this article, but many of these factors have been documented to play an important role in renal pathophysiology. It is also pertinent to note that some factors may have variable effects upon cells. For example, nitric oxide (NO) may be either a survival factor or a death signal depending upon the concentration of NO and the cells involved. Although resident renal cells can produce many such death factors, it is probably the infiltrating macrophage that is the most important source during inflammatory disease states.

Interventions to modulate renal cell apoptosis

The induction of apoptosis of infiltrating leukocytes or resident renal cells that are surplus to requirements may augment tissue remodelling. However, the administration of pro-apoptotic reagents, unless accurately directed to the target cells, is potentially hazardous since they may initiate renal damage [15]. There are several potential strategies, many of which are currently in clinical practice, which may ameliorate renal cell death either indirectly or directly.

Indirect inhibition of renal cell apoptosis

Anti-inflammatory treatment

Infiltrating leukocytes such as macrophages may induce renal cell apoptosis by release of TNFα, Fas ligand or NO, whilst neutrophils may release damaging ROS and degradative enzymes. Therefore, any treatment that effectively reduces renal leukocyte infiltration or inhibits the action of pro-apoptotic cytokines would be predicted to result in diminished levels of resident renal cell apoptosis. Similarly, inhibition of complement activation will be protective.

Anti-proteinuria treatment

Recent data indicates that, as well as promoting interstitial inflammation, proteinuria may induce tubular
cell apoptosis [16]. Therefore, treatments such as the administration of angiotensin-converting enzyme inhibitors that reduce proteinuria may also diminish tubular cell apoptosis.

**Anti-fibrotic treatment**

Components of normal ECM such as collagen IV and laminin provide important integrin-mediated survival signals to resident renal cells such as mesangial cells. Interestingly, collagen I and fibronectin, which are present in diseased glomeruli, do not support mesangial cell survival [17]. The logical extrapolation from these *in vitro* studies is that the scarring kidney may become a ‘hostile’ environment by not providing an adequate supply of ECM-derived survival signals, resulting in low-grade cell ‘dropout’ by apoptosis. Therefore, all treatment strategies with significant anti-fibrotic consequences would be expected to inhibit renal cell apoptosis.

**Anti-ischaemia treatment**

Attention has recently been drawn to the role of renal ischaemia and hypoxia upon the progression of tubulo-interstitial disease [18]. In a murine model of chronic renal failure, apoptosis of tubular cells was found to colocalize with areas of hypoxia, with hypoxia-induced upregulation of Fas expression being proposed as a potential mechanism of tubular cell death [19]. VEGF promotes both the proliferation and survival of endothelial cells, and recent, as yet unpublished, data indicates that the exogenous administration of VEGF significantly ameliorates renal disease induced by cyclosporin A, subtotal nephrectomy or the administration of complement fixing antibodies directed against the endothelium (R. J. Johnson, personal communication). In these experimental models, the protective effect is presumably at least partly mediated by the inhibition of endothelial cell apoptosis. The resultant preservation of the critically important microvascular ‘skeleton’ of the kidney, with improved renal tissue blood flow and oxygenation, would be expected to inhibit apoptosis of non-endothelial renal cells.

**Direct inhibition of renal cell apoptosis**

**Inhibition of downstream caspase activation**

Caspase activation during apoptosis results in the cleavage of numerous intracellular target substrates. Reagents are now available that can specifically inhibit these enzymes and are therefore of potential therapeutic interest. Indeed, a recent study has indicated that the widespread renal cell apoptosis characteristic of murine renal ischaemia/reperfusion injury is markedly inhibited by exogenous administration of caspase inhibitors [20].

**Administration of growth factors that inhibit apoptosis by stimulation of intracellular survival pathways**

Growth factors such as insulin-like growth factor 1 (IGF-1), hepatocyte growth factor (HGF) and EGF may support cell survival even in the presence of pro-apoptotic factors [12,21]. These growth factors inhibit apoptosis by downregulating pro-apoptotic intracellular pathways and stimulating survival promoting pathways, such as that involving phosphoinositide 3’-hydroxykinase (PI3K) and the protein kinase Akt [21,22]. Administration of growth factors *in vivo* may be very efficacious. For example, IGF-1 administration in murine renal ischaemia/reperfusion injury has comparable effects on apoptosis as caspase inhibition [20]. Furthermore, IGF-1 and EGF inhibit apoptosis and improve outcome in obstructive nephropathy [23,24]. In addition, HGF, EGF and IGF-1 significantly ameliorate experimental models of toxic renal injury, an effect likely to involve inhibition of renal cell death as well as stimulation of renal regeneration and repair [25–27].

**Potential pitfalls**

There are potential problems with attempts to promote or inhibit apoptosis in the kidney. For example, beneficial and detrimental renal cell apoptosis may occur concurrently and the inhibition of apoptosis associated with tissue remodelling and restoration of normal tissue architecture is obviously undesirable. In addition, in immunological renal diseases, systemic anti-apoptosis treatments such as caspase inhibition may also reduce the apoptotic deletion of autoreactive T cells, thereby prolonging the autoimmune response. Finally, the inhibition of low levels of apoptosis over a prolonged period, such as that associated with slowly progressive scarring, is difficult unless treatment can be targeted accurately to the kidney.

**Conclusion**

The fate of resident renal cells during inflammation and scarring is determined by the balance between antagonistic survival and death signals. It should now be appreciated that treatment strategies currently in practice undoubtedly affect levels of renal cell apoptosis. However, the future holds promise that, using techniques such as gene targeting, it should become possible to direct more accurately factors that promote either survival or death. Such interventions will promote the resolution of renal inflammation and aid renal repair, thereby preserving renal tissue and function.

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References


