One hundred ways to kill a podocyte

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ABSTRACT

The podocyte is a highly specialized cell, forming within the developing glomerulus from a mesenchymal origin, acquiring some but not complete features of an epithelial cell as it matures. Once mature, this cell has the potential to receive signals from several different directions and sits within a dynamic microenvironment. By taking an overview of many lines of evidence, it is clear that we already know many signals that are tightly controlled in keeping the podocyte healthy. For example, vascular endothelial growth factor, insulin and integrins are all known to have bidirectional effects on podocyte functionality, depending on whether there is too much or too little. It is of little surprise therefore that disrupting this delicate balance can result in a dramatic loss of function, and manifestation of glomerular disease originating from many different primary insults. The cues directing podocyte phenotype and functionality for the purpose of this review will be divided into four main sources: (i) genetic, (ii) paracrine signals from endothelial and mesangial cells, (iii) direct contact signals to/from the glomerular basement membrane and (iv) signals from circulating plasma. Of course there are other influences, which we still know little about, such as flow and shear stresses, signals from the urinary space that should all be considered in the overall healthy environment.

Keywords: endothelin, endothelial, integrin, mesangial, nephrotic, podocyte

WHAT MAKES A PODOCYTE A PODOCYTE?

In order to approach the question of what kills a podocyte, we need to address the important elements of what keeps a podocyte alive, and phenotypically healthy in normal physiology (Figure 1). These influences are discussed in the following sections.

Genetic influences

A great deal has been learnt from genetic studies, both in mouse and man, about molecules that are crucial for podocyte health and survival. Podocyte-specific knockout (KO) models have allowed the testing of many proteins for their relevance in podocyte biology. Some of the deleterious results discovered are understandable in the context of disrupting important cell survival pathways (e.g. [1, 2]). However, landmark discoveries of podocyte-restricted proteins, in particular nephrin and...
podocin [3, 4], point to these molecules as essential mediators of the very specific phenotype and functionality of this unique cell. They are expressed at the podocyte slit diaphragm, and nephrin’s critical role is exemplified by the observation that mutations in this gene (NPHS1) result in the most severe forms of human congenital nephrotic syndrome.

Paracrine influences
Within the glomerulus, the podocyte is adjacent to endothelial cells across the glomerular basement membrane (GBM), and mesangial cells in the glomerular tuft. There is little information about critical signals deriving from mesangial cells, and it would seem intuitive that there is likely to be crucial cross-talk between those cell types to maintain glomerular health. More has been discovered about cross-talk between podocytes and glomerular endothelial cells, and the principle of soluble factors moving in both directions across the glomerular filtration barrier is now well established (e.g. [5, 6]). An intriguing recent player in this regard is the endothelin (ET) system. ET-1 is released by endothelial cells and has long been known to promote mesangial sclerosis [7], and intriguingly there are also now known to be functionally important receptors on podocytes [6].

Endothelial cells
Endothelial cells invade the developing podocyte tuft relatively late during podocyte maturation. Studies from zebrafish show that endothelial cells are not required for determination of the podocyte cell lineage because podocytes develop in ‘cloche’ mutants that have no endothelia [8]. However, differentiation of specialized podocyte features, such as slit diaphragms, was not described in these mutants. It is likely therefore that endothelial cells produce signals that are required for maintenance, survival and differentiation of podocytes.

Mesangial cells
The mesangium is the central stalk of the glomerulus, and has no direct contact with podocytes, although does have contact with glomerular endothelial cells. Podocyte injury frequently results in mesangial cell proliferation and hypertrophy, and mesangial cell injury can lead to podocyte effacement and proteinuria.

During development, mesangial cells migrate into the cleft of immature podocytes at the S-shaped body stage and are essential for capillary looping [9]. Mesangial cells are likely to be crucial for podocyte formation, particularly with respect to secreted factors including chemokines [10].

The most dramatic example is perhaps the platelet-derived growth factor receptor (PDGFR)-beta KO mouse. Platelet derived growth factor B is secreted by endothelial cells, with the receptor on mesangial cells. These KO mice display a complete loss of mesangial cells, and a consequent single balloon-like capillary loop [11]. Deletion of ephrinB2, a soluble ligand
for Eph receptor tyrosine kinases, from mouse mesangial cells at 14.5 day post coitum results in arrested glomerular development with a reduced number of capillary loops [12].

**Developmental pathway of a podocyte**

In order to understand both specialized functions of this unique cell, and the ways in which it might phenotypically alter during disease processes, it is crucial to understand its own developmental pathway.

During development, podocytes are derived from undifferentiated mesenchyme, which is induced by the ureteric bud [13]. The earliest identifiable podocytes are detected in the S-shaped bodies, where cells are seen to express podocyte-specific markers, such as nephrin and podocin. These early podocytes also synthesize vascular endothelial growth factor (VEGF), which initiates the recruitment of endothelial cells into the cleft of the S-shape. The podocytes at this stage are columnar epithelium, but do not stay this way. They start to lose lateral cell–cell attachments, and they begin to migrate around the capillary loops, so that they no longer form a continuous uniform patch of cells. At this point foot processes begin to form. This is suggestive of a process whereby extensions resembling filopodia extend themselves as a scaffolding around the capillary loops [14]. The final mature phenotype has a mixture of epithelial and mesenchymal features [15], which is important in understanding ‘dedifferentiation’ during certain disease processes. The mature podocyte loses many epithelial features and displays features of specialized mesenchymal cells such as smooth muscle, as well as neuronal features [16, 17].

**THE KILLING FIELDS—PATHWAYS TO PODOCYTE DEMISE**

**Hanging on for dear life—attachment to the glomerular basement membrane**

Podocytes attach to the GBM, a uniquely composed matrix [18, 19], with complex two-way signalling between the two. This is predominantly via integrins, dimeric cell surface adhesion receptors formed from one of 18 α and 8 β subunits. Integrins work alongside other receptors to mediate cell–cell and cell–matrix interaction. Cell–matrix adhesion receptors expressed by podocytes include the integrins α2β1 and αvβ3, dystroglycan, syndecan-4 and type XVII collagen [20].

Binding to GBM, otherwise termed podocyte adhesion, permits a bi-directional transmission of force across the plasma membrane [21]. This relies on linkage to the intracellular cytoskeleton. Integrins also transmit chemical signals into the cell (outside-in signalling), providing information on its local environment, adhesive state and surrounding matrix [22], determining appropriate migration and motility, aspects of podocyte behaviour increasingly being recognized as key disease drivers [23]. Integrins can undergo conformational changes in their extracellular domains in response to signals that impinge upon the integrin cytoplasmic tails, and this increases their affinity for ligands—a process that is termed inside-out signalling or ‘activation’ [21]. These concepts have gained importance in recent years in glomerular biology, with the discovery of specific integrins that are activated in experimental or human disease.

Much glomerular interest has focused on two particular integrins—β1 and β3. α3β1 integrin is the most highly expressed integrin in podocytes and binds to laminin in the GBM. Both the α3, and β1 integrin podocyte-specific KO mice develop proteinuria [24], resulting from podocyte loss. It has been shown that β1-integrin activation can be induced by upregulation of podocyte B7.1 (CD80) [25], and that therapeutically targeting B7.1 can attenuate proteinuria in certain forms of focal segmental glomerulosclerosis (FSGS), by means of downregulating β1 activation [26]. Additional evidence of the importance of β1 integrin comes from a recent study that loss of RAP1GAP (which inhibits RAP1 activation) activates β1 in the podocyte, and thereby maintains podocyte adhesion [27].

Other laminin-related proteins causing both human FSGS when mutated and glomerular disease in mouse models are tetratraspin (CD151, which binds to α3β1) [28] and laminin β2 (Pierson syndrome) [29].

The α5 integrins bind to RGD (arginylglycylaspartic acid) domains in vitronectin/fibronectin [30], and α5β3 integrin has been implied in damage pathways leading to proteinuria, particularly as a mediator of the urokinase receptor, uPAR [31], and its soluble version suPAR [32]. This is via activation of b3, shown by protection from uPAR-mediated proteinuria in mice lacking β3 integrin [31].

The cytoskeletal protein talin is another example of the complexity of integrin activation. Talin links integrins to the actin cytoskeleton, as well as having a central role in integrin activation [33]. Podocyte-specific KO of talin results in modest effects on β1-integrin activation, but dramatic changes in actin morphology, and severe proteinuria [34]. Additionally, mouse models of glomerular injury result in cleavage of talin-1, which is mediated by the intracellular protease calpain, and this injury can be attenuated by administration of a calpain inhibitor.

Thus modulation of integrin behaviour in podocytes is an exciting area for targeting therapy to limit podocyte loss.

**The engines can’t take it!—damaging the mitochondria**

Podocytes are highly energy dependent cells, needing to constantly adapt to counteract capillary pressures, and display glucose utility kinetics analogous to smooth muscle cells [15, 35]. Therefore it is no great surprise that damaging mitochondrial pathways is deleterious to podocytes. The most revealing clue to this is in patients withmutations in genes encoding the COQ10 (co-enzyme Q10) biosynthesis pathway. There are at least eight proteins in the pathway leading to COQ10 synthesis, and of these four have been found to cause steroid-resistant nephrotic syndrome (SRNS) if mutated [36–39]. This particular pathway is therefore clearly important in podocyte homeostasis, and the important clinical lesson here is that in patients with these mutations, supplementation with COQ10 can result in significant reversal of both renal and neurological features [40].

**All in the family—Mendelian disease**

There is extensively reviewed literature on single gene mutations causing SRNS, either as isolated renal disease or as part...
of a syndrome (e.g. [38, 41]). The key lessons from an overview of the spectrum of genetic SRNS is that the most clinically relevant podocyte biological pathways are revealed by examining the function of those gene products. The main categories (with some overlap) would be slit diaphragm related/actin regulating/Rho GTPase family/developmental/GBM protein/mitochondrial pathways. There are interesting ‘outliers’ from this group, such as TRPC6 and PLCε1 [42, 43], which are known to be involved in calcium regulation, but nevertheless have likely functionality in actin regulation and cell motility. A new molecule is EMP2, found to be mutated in pedigrees with steroid-sensitive nephrotic syndrome, and the protein regulates caveolin-1 expression [44]. Caveolin-1 is expressed at the slit diaphragm of podocytes, but is also crucially important in the formation of endothelial cell fenestrations, and therefore may shed light on broader functional integration of the filtration barrier.

Developmental genes are also in the spotlight again recently. The transcription factor WT1 has long been known to be essential for renal development, yet is only expressed in the podocyte in the mature kidney [14]. Another important transcription factor in glomerular development, PAX2, interacts reciprocally with WT1 [45]. Mutations in WT1 usually cause severe, early onset glomerular disease [46], but until recently the phenotype of PAX2 mutations was limited to congenital anomalies (renal coloboma syndrome) [47]. Now, pedigrees of autosomal-dominant patients with FSGS have shown several novel variants in PAX2 causing adult-onset disease [48]. Moreover, mechanistically it appears that microRNAs (miRs) are important in regulation of WT1. Overexpression of miR193a in podocytes results in FSGS in mice, by downregulating WT1 expression [49], and resulting in a catastrophic collapse of the podocyte-stabilizing system. This demonstrates the central role of WT1 as a regulator of podocyte differentiation and function.

**Under attack from without—circulating factors**

The podocyte is constantly bathed in plasma components that make it through the endothelial cell and GBM layers. Circulating plasma factors are therefore likely to have an important influence on podocyte behaviour, both in homeostasis and disease. Perhaps the circulating factor story most are familiar with is that of idiopathic nephrotic syndrome, with the architecture of the disease. Complement-mediated disease

Another possible way to kill podocytes is by activation of the complement system. The complement cascade is well known, very tightly regulated, and its constituent proteins may be soluble (circulating) or membrane bound. Complement activation by the classical pathway (antigen–antibody complexes) results in cell death via the membrane attack complex (MAC). Immune complex glomerulonephritis works via this mechanism, and the diseases that result in subpodocyte immune complex deposits such as membranous nephropathy and class V lupus nephritis result in direct podocyte injury [57].

However, what is less well understood is the role of local complement protein expression, and how this may influence cell death in the context of these specific complement-activating diseases. Complement Factor H (CFH) is one of the most important negative regulators of complement activation and is serum based and predominantly produced in the liver. There is evidence that locally produced podocyte CFH is important to process immune complexes in the subepithelial space, where it also limits complement activation [58]. Thus, it will
be important to understand which regulatory complement proteins are locally produced, where they act and factors that influence their production.

**Future 'hot topics'**

There are many interesting directions in which podocyte biology is moving, both from the cell biology perspective and translational/therapeutic directions. Novel discoveries that have opened fresh areas of research include the study of miRs [49], cell polarity mechanisms in the podocyte [59], mitochondrial function [60], insulin signalling [54] and autophagy [61]. Translational success remains to be achieved, but is coming nearer, with the ability to target and test specific pathways using small compound screening, and the use of biologicals that may specifically target podocyte antigens [26].

**CONCLUSION**

Overall it is clear that the podocyte is a unique cell type, which cannot be classified into a clear phenotypic category. Functionally, it has a distinct role and has achieved this by evolutionary adaptation to cues from its microenvironment. This has resulted in a cell that requires dynamic homeostasis of many local elements to maintain its integrity and function, and an understanding of how disruptions to these signals occur gives us insight into how to prevent disruption and ultimate cell death.

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**CONFLICT OF INTEREST STATEMENT**

The author reports no conflicts of interests to declare.

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