Just a decade ago, our knowledge of podocyte injury in animal models was rudimentary. We recognized 2 major paradigms of irreversible podocyte injury leading to focal segmental glomerulosclerosis (FSGS). The first is direct podocyte injury due to exposure to such cell toxins as puromycin aminonucleoside and adriamycin/dauxorubicin (1). These toxic models fostered the concept of primary podocyte injury in the pathogenesis of foot process effacement and glomerulosclerosis. By contrast, renal ablation models pointed to glomerular hypertension (elevated glomerular capillary pressures and flow rates) as the primary pathophysiologic process in the course of adaptive responses to reduced number of functioning nephrons or other glomerular stress, in turn causing secondary podocyte injury (2). These basic paradigms form the conceptual dichotomy between “primary” and “secondary” (post-adaptive) FSGS. Only in the past few years has the molecular basis for these pathologic alterations been elucidated through a greater understanding of podocyte biology.

**ACT 1: SEEING IS BELIEVING**

**ULTRASTRUCTURAL STUDIES PROVIDE MECHANISTIC INSIGHTS**

Initial insights into the podocyte injury in proteinuric conditions came from meticulous 3-dimensional ultrastructural observations using scanning electron microscopy. Studies by Inokuchi et al. in puromycin nephropathy elucidated that foot process effacement consists of a distinctive and predictable change in podocyte shape (3). The foot processes, which are analogous to specialized lamellipodia in other biologic systems, spread and fan out, incriminating the actin cytoskeleton in the effacement process. This simple, but elegant, observation laid the groundwork for elucidation of specific actin-associated podocyte proteins that may be operant in foot process maintenance.

By contrast, seminal ultrastructural observations by Nagata and Kriz in an ablation model of uninephrectomy in the young rat showed remarkably different podocyte alterations (4). As the tuft hypertrophied in response to the reduced number of functioning nephrons, podocyte cell number did not increase. Podocyte cell bodies were forced to stretch to cover a much larger surface area and serve many more glomerular capillaries. Cell bodies hypertrophy and become attenuated into cytoplasmic sheets. Primary processes thin out and extend to remote capillaries. Filtrate is now delivered into the subcellbody space, causing bulging of the cytoplasmic sheets and formation of pseudocysts, under which foot processes are largely preserved. The formation of denuded patches of GBM owing to podocyte detachment and the apposition of distended podocyte cell bodies to Bowman’s capsule form the nidus of the segmental sclerotic lesion. In this model, podocyte insufficiency and maladaptive responses develop into irreversible structural lesions.
ACT 2: GOING, GOING, GONE…
THE ROLE OF PODOCYTE DEPLETION

In both toxic and adaptive models of FSGS, a central role for podocyte loss has been proposed. Podocyte depletion has been identified in many human glomerular diseases, including diabetic nephropathy, focal segmental glomerulosclerosis and IgA nephropathy (5, 6). In human disease, podocytes can be detected in the urine and the reduction in podocyte cell number correlates with the degree of proteinuria and severity of sclerosis (7).

This mechanism has been validated in several ingenious models of targeted podocyte cell death (8-10). Using a transgenic rat strain in which the human diphtheria toxin (DT) receptor is specifically expressed in podocytes driven by the podocin promoter, Wiggins and coworkers were able to produce different stages of glomerular injury depending on the percentage of podocytes depleted after injection of DT, consistent with a dose response (8). Over 40% podocyte depletion produced segmental and global glomeruloclerosis with high grade proteinuria and reduced renal function. In analogous experiments, Ichikawa and colleagues engineered a mouse model of glomerular sclerosis by selectively expressing human CD25 in podocytes (9,10). Injection of anti-Tac (Fv)-PE38 (LMB2) immunotoxin induced progressive proteinuria and glomerulosclerosis in a dose dependent fashion. By permanently labeling the podocyte lineage with lacZ, the investigators could determine their fate. The number of lacZ stained podocytes progressively declined as parietal epithelial cells avidly proliferated to cover the denuded tuft, resembling collapsing FSGS. These studies demonstrate the central role of podocyte depletion in the process of glomerular sclerosis. If the initial insult is of sufficient impact, there may be spreading of sclerosis to adjacent segments after the insult has been withdrawn. This process suggests a vicious cycle of local spreading of sclerosis, incriminating toxic substances secreted in a paracrine or autocrine fashion (such as TGFβ, AII, MIF) or reduction in survival factors (such as VEGF) (11).

ACT 3: PROOF OF CONCEPT:
ANIMAL MODELS AND THE GENETIC BASIS OF FSGS

Great advances have been made in our understanding of the genetic basis for MCD and FSGS. A number of critical podocyte proteins have been identified to be mutated or deficient in human forms of congenital nephrotic syndrome or inherited FSGS (12-24). Many of these proteins were identified first by positional cloning in affected families. Others were identified serendipitously while studying other disease systems. The critical role of these proteins in the mediation of FSGS was later validated in experimental models, including knock-out models or transgenic models expressing mutant proteins (25-31). The animal models provided proof of concept that deletion of a particular gene was sufficient to cause proteinuria or FSGS. A number of other podocyte proteins produce FSGS in null mice or conditional knock-outs, although a role in human disease has not yet been identified (32-35). The responsible genes encode proteins that are located in various subcellular domains of the podocyte, including membrane-associated (slit diaphragm, basal membrane), nuclear (transcription factors and chromatin bundling proteins), and cytosolic (associated with the actin cytoskeleton or cell energetics). These genes are listed in the table below. An asterisk marks the human disease genes that have been validated in animal models.
A unifying concept in all these models is the central role of the actin cytoskeleton in coordinating cell signaling from the various membrane compartments. Interference with cell signaling may promote the stereotypic response of foot process effacement common to all these conditions (36). This hypothesis is supported by evidence that the slit diaphragm is a mechanosensor that serves as a platform for signal transduction (37).

<table>
<thead>
<tr>
<th>HUMAN GENE PRODUCTS</th>
<th>GENE</th>
<th>INHERITANCE</th>
<th>CHROMOSOME</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Slit Diaphragm proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nephrin*</td>
<td>NPHS1</td>
<td>AR</td>
<td>19q13.1</td>
</tr>
<tr>
<td>Podocin*</td>
<td>NPHS2</td>
<td>AR</td>
<td>1q25-31</td>
</tr>
<tr>
<td>CD2 associated protein*</td>
<td>CD2AP</td>
<td>AD</td>
<td>6p12</td>
</tr>
<tr>
<td>Transient Receptor Cation 6</td>
<td>TRPC6</td>
<td>AD</td>
<td>11q21-22</td>
</tr>
<tr>
<td><strong>Cytosolic proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-actinin 4*</td>
<td>ACTN4</td>
<td>AD</td>
<td>19q13</td>
</tr>
<tr>
<td>Phospholipase C ε1</td>
<td>PLCE1</td>
<td>AR</td>
<td>10q23-24</td>
</tr>
<tr>
<td><strong>Basal Membrane proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laminin β2*</td>
<td>LAMB2</td>
<td>AR (Pierson syndrome)</td>
<td>3p21</td>
</tr>
<tr>
<td>Beta 4 integrin*</td>
<td>ITGB4</td>
<td>AR (Epidermolysis bullosa)</td>
<td>17q11</td>
</tr>
<tr>
<td><strong>Nuclear Proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wilm’s tumor 1</td>
<td>WT1</td>
<td>AD (DMS, Frasier syndrome)</td>
<td>11p13</td>
</tr>
<tr>
<td>Chromatin bundling protein</td>
<td>SMARCAL1</td>
<td>AD (Schimke syndrome)</td>
<td>2q34-36</td>
</tr>
<tr>
<td><strong>Mitochondrial Products</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondrial tRNAleu</td>
<td>mtDNA-A3243G</td>
<td>Maternal</td>
<td>mtDNA</td>
</tr>
</tbody>
</table>

**MOUSE GENE MUTANTS**
(Role in human disease unknown)

| Slit Diaphragm Proteins            |        |             |            |
| Neph1                             |        |             |            |
| Fyn                               |        |             |            |
| FAT1                              |        |             |            |

**Actin Associated Proteins**

Nek 1/2

**ACT 4: MORE IS NOT NECESSARILY BETTER:**
PODOCYTE PROLIFERATION AND DYSREGULATION IN COLLAPSING FSGS

The mature podocyte is a post-mitotic cell. Podocytes can undergo DNA synthesis to a limited degree but do not proliferate because they arrest in the G2/M phase of the cell cycle (38). Findings in human collapsing FSGS suggest that podocytes may exhibit rare replicative capacity under select conditions, producing the glomerular pseudocrescents typical of this variant (39). This has been difficult to prove without lineage specific markers, and there is increasing evidence that parietal epithelial cells contribute to the glomerular epithelial cell proliferation (40). In collapsing FSGS, the podocytes downregulate their mature podocyte markers (WT-1,
synaptopodin, podocalyxin, GLEPP-1), express KI-67 and enter the cell cycle (39, 41). A similar podocyte phenotype has been identified in primary collapsing glomerulopathy and human HIV-associated nephropathy, as well as an HIV transgenic model (39, 42). A decrease in the CDK inhibitor, p27 and increase in cyclin D1 underlie the proliferative phenotype (43, 44).

Evidence in human HIVAN supports a direct viral infection of renal parenchymal cells, rather than a systemic or indirect immune dysregulation by the HIV virus. Cohen et al in 1989 were the first to report the detection of HIV-1 in renal epithelial cells by DNA in situ hybridization (45). In 2000, Bruggeman et al. detected HIV-1 in renal epithelial cells of patients with HIVAN by RNA in situ hybridization (46). These findings were confirmed using DNA in situ hybridization and by application of riboprobes specific for both the nef and gag genes. Virus was identified in renal tubular cells, often involving many contiguous cells in individual tubular profiles, as well as podocytes, parietal epithelial cells, and some interstitial leukocytes (46). In one particularly illustrative case treated with highly active anti-retroviral therapy (HAART), virus persisted in the tubular epithelium as determined by RNA in situ hybridization, even after viral load in the peripheral blood had become undetectable and renal histology had improved (47). The ability of the kidney to serve as a reservoir for HIV-1 was later confirmed by Marras et al using laser capture microdissection to characterize the HIV-1 quasi-species present in tubular epithelium (48). Comparison of the envelope sequences from renal tubular epithelial cells and peripheral blood leukocytes in individual patients showed variations in the HIV-1 envelope sequences in tubular epithelium compared to blood, indicating that the kidney is able to support viral replication and quasispecies evolution as a separate compartment.

Several animal models have provided insights into disease pathogenesis. One of the first models, Tg26, was established in transgenic mice containing a replication-defective HIV-1 construct that lacks the *gag* and *pol* genes and is expressed under the control of the long terminal repeat (LTR) viral promoter (49). In this model, which closely recapitulates the morphologic features of the human HIVAN, viral transgene was expressed in glomerular and tubular epithelial cells (50). Cross-transplantation of kidneys between Tg26 and WT mice showed that renal transgene expression was required for the development of nephropathy (50). A similar model has been established in rats using the same transgene construct (51). Hanna et al. generated several different transgenic lines with mutations in one or more of the HIV genes, and found that nef was necessary and sufficient to produce the renal phenotype (52). In vitro studies in cultured podocytes suggest that nef-induced activation of Stat3 and Ras-MAPK1,2 via Src-dependent pathways is responsible for podocyte proliferation and dedifferentiation (53).

Transgenic expression of nonstructural HIV-1 genes (*vif, vpr, nef, spliced forms of tat and rev, but not vpu*) selectively in podocytes using the nephrin promoter in mice with FVB/N genetic background results in podocyte injury, glomerulosclerosis and tubular microcyst formation (54). Podocyte specific expression of nef and vpr in a double-transgenic murine model recapitulated the severe morphologic and functional features of human HIVAN, suggesting a synergistic interaction of these proteins (55). In the Tg26 model of HIVAN, inhibition of podocyte proliferation using a CDK2 inhibitor reduced proteinuria and glomerulosclerosis (56).

In HIVAN and idiopathic collapsing glomerulopathy, the proliferating podocytes have an undifferentiated phenotype, leading to functional podocyte insufficiency, defective podocyte adhesion, and shedding of podocytes into the urine. Over the long-term, collapsing glomerulopathy is likely to lead to progressive podocyte depletion, as in other models of FSGS.
ACT 5: THE MISSING LINK
HOW PODOCYTE INJURY PROMOTES SCLEROSIS

Conventional wisdom suggests that foot process effacement, if reversed can lead to restoration of glomerular architecture (as in steroid responsive MCD). The failure of reparative mechanisms promotes persistent proteinuria and the development of glomerulosclerosis. Precisely how podocyte injury promotes glomerulosclerosis is poorly understood, but podocyte loss is emerging as a central pathomechanism. Evidence from animal models suggests that critical perturbations in the balance between pro-apoptotic and anti-apoptotic factors promote podocyte depletion and progressive glomerulosclerosis (Reviewed in 38). For example, toxins such as puromycin and adriamycin induce podocyte production of ROS, leading to podocyte DNA damage, apoptosis, and GBM protein peroxidation. Mechanical stretch can promote podocyte hypertrophy and apoptosis (57). Excessive protein trafficking through the podocyte itself generates ER stress and podocyte injury (58). Many of the pro-apoptotic factors listed below (such as All and TGFβ) also possess prosclerotic properties, providing a link to sclerosis.

The TGFβ1 transgenic mouse is a particularly valuable model that has shed mechanistic insights into the inter-relationship between podocyte apoptosis and glomerulosclerosis. (59). Podocytes expressing TGFβ1 undergo apoptosis associated with marked upregulation of Smad7. TGFβ1 and Smad7 promote podocyte apoptosis through different mechanisms. TGFβ1 induces apoptosis by activation of mitogen-activating protein (MAP) kinase p38 and classic effector caspase-3, whereas TGFβ-inducible Smad7 inhibits signaling by the cell survival factor NF-kB. In this model, podocyte depletion through apoptotic pathways leads to progressive FSGS.

### Balance of Factors Influencing Podocyte Depletion (adapted from Shankland, ref 38)

<table>
<thead>
<tr>
<th>Podocyte pro-apoptotic factors</th>
<th>Pro-survival (anti-apoptotic) factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II</td>
<td>Cyclin 1</td>
</tr>
<tr>
<td>AT1 Receptor</td>
<td>Nephrin</td>
</tr>
<tr>
<td>TGF-β</td>
<td>CD2AP</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>Dexamethasone</td>
</tr>
<tr>
<td>SMAD7</td>
<td>Bel-2</td>
</tr>
<tr>
<td>ROS</td>
<td>Cell-cell contact</td>
</tr>
<tr>
<td>Detachment</td>
<td>VEGF</td>
</tr>
<tr>
<td>↓ p21</td>
<td>Collagen via Ras-ERK signaling</td>
</tr>
<tr>
<td>↓ p27</td>
<td>Focal adhesion kinase</td>
</tr>
<tr>
<td>Stress-tension</td>
<td>Hepatocyte growth factor</td>
</tr>
<tr>
<td>bFGF</td>
<td>Insulin-like growth factor</td>
</tr>
<tr>
<td>Lytic C5b-9</td>
<td></td>
</tr>
<tr>
<td>p53</td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td></td>
</tr>
</tbody>
</table>
References


