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Development of the renal glomerulus: good neighbors and good fences

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The glomerulus of the mammalian kidney is an intricate structure that contains an unusual filtration barrier that retains higher molecular weight proteins and blood cells in the circulation. Recent studies have changed our conception of the glomerulus from a relatively static structure to a dynamic one, whose integrity depends on signaling between the three major cell lineages: podocytes, endothelial and mesangial cells. Research into the signaling pathways that control glomerular development and then maintain glomerular integrity and function has recently identified several genes, such as the nephrin and Wilms' tumor 1 genes, that are mutated in human kidney disease.

Introduction

The glomerulus of the mammalian kidney is a highly developed vascular bed that acts as a filter, allowing a filtrate of small molecules, such as water, sugars, electrolytes and small proteins, to pass through a barrier that retains high molecular weight proteins and cells in the circulation. The proper development and preservation of this structure throughout life is essential to the prevention of serious disease. The past ten years have witnessed numerous advances in our understanding of glomerular development and function. Podocytes, the visceral epithelial cell of the glomerulus, are now recognized as being a key cell type, the injury of which can initiate glomerular scarring. Several genetic kidney disorders are caused by mutations in genes that encode proteins that appear to have highly specialized functions in podocytes, especially in the maintenance of the protein barrier, which prevents massive protein loss from the circulation (a condition known as nephrotic syndrome). The glomerular basement membrane and its receptors have also served as one of the key models for the study of how a basal lamina develops and interacts with adjacent epithelial cells. Moreover, glomerular research has added to our understanding of how signals between adjacent cell types are required for the proper development and maintenance of the structural integrity of an organ throughout life.

The de novo regeneration of an entire nephron or a whole glomerulus has never been documented in mammals. Indeed, aside from repairing proximal tubules damaged in acute situations, the kidney has a very limited ability to repair itself compared with many other organs. Because the glomerulus only develops in the context of the induction of an entire new nephron during kidney development, it is unlikely that we will learn how to regenerate glomeruli, except in the context of discovering how to regenerate entirely new nephrons. While this remains a long term goal of kidney development research, perhaps a more accessible therapeutic target will be the podocyte, where an ability to restore foot process architecture has the potential to reduce dramatically the morbidity and mortality that results from chronic kidney disease.

In this review, we focus on recent advances in glomerular development and biology, and relate them to the disease processes, possible avenues of treatment and the prevention of end-stage kidney disease that these advances have opened up.

Kidney development and glomerular formation

Nephron induction

Each human kidney contains approximately one million nephrons. The glomerulus is the most proximal component of the nephron (see Fig. 1). The segmentation of the nephron (Fig. 1C) presents a fascinating, but poorly understood, process. It is becoming clear that signaling via the Notch pathway, particularly through NOTCH2 and its ligands, is involved in this segmentation process (Cheng and Kopan, 2005; Cheng et al., 2003; Leimeister et al., 2003; McCright et al., 2002). Blocking Notch signaling in mouse embryonic kidney organ culture interferes with the development of the proximal components of the nephron, including the glomeruli and proximal tubules, although it does not block the differentiation of podocytes, a major cell type of the glomerulus, if they (or the glomerulus) have been specified prior to initiating the Notch blockade (Cheng and Kopan, 2005; Cheng et al., 2003). Moreover, the conditional deletion of *Notch2* from nephron progenitor cells in the developing mouse kidney results in a 'distal tubule only' phenotype, as the proximal tubules and glomeruli are absent in these mutants (Cheng et al., 2007). Thus, it appears that Notch signaling may be involved in establishing the major proximodistal axis of the nephron, but is less important in the specification of the glomerulus itself.

Glomerular formation: podocyte differentiation is the first determinant

The precursor structure of the glomerulus can be first appreciated in the 'S-shaped body', so-called because it is shaped like an 'S' when observed in histological sections. There are three major components to the early glomerulus (Fig. 2): the layer of primitive podocytes that begins as a columnar epithelium; the thin layer of Bowman's capsule, which appears to be nearly flat, similar to a squamous epithelium; and the capillary loop that first enters the glomerular cleft (Fig. 2A,B). How the podocytes extend themselves around the capillary loops remains unknown. Early in this process, both the podocytes and the capillary endothelial cells form their own basal lamina. As the glomerulus matures, these two basal lamina fuse to form a thick basement membrane, known as the glomerular basement membrane (GBM) (Fig. 2E). Concomitant with this fusion, which brings the podocytes and endothelial cells into close apposition with each other, the podocytes undergo a remarkable transformation, during which they acquire some mesenchymal-like characteristics, although they remain an atypical epithelial cell. The podocytes begin to lose their lateral cell attachments, except at a

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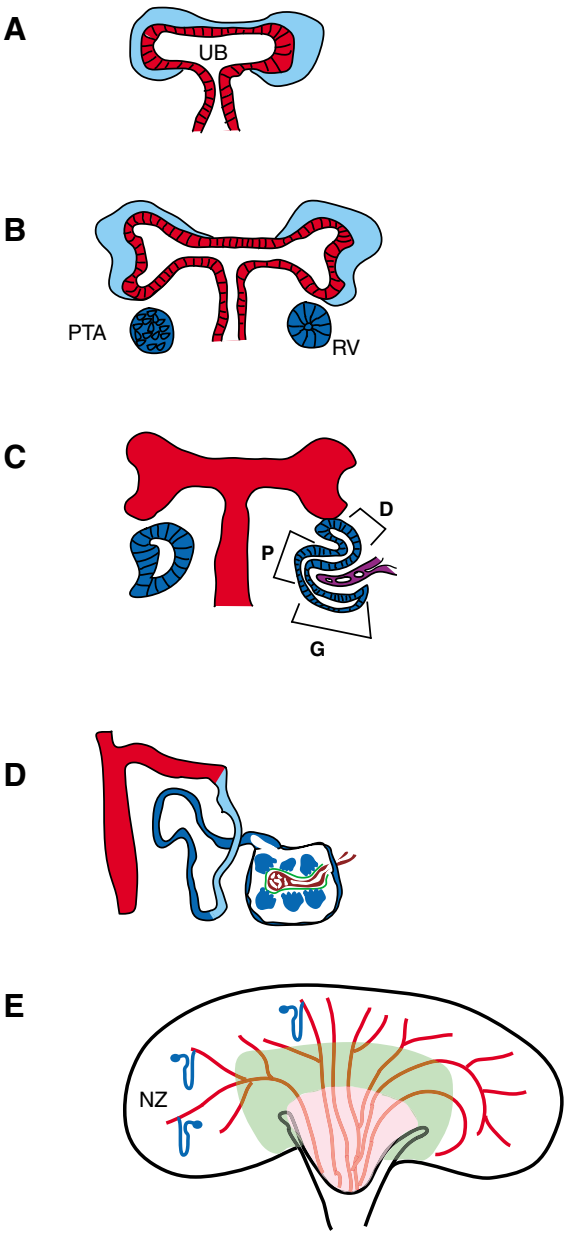

Fig. 1. A schematic of kidney development. (**A**) Cross section of an E11.5 mouse embryonic kidney at induction. Mesenchyme (blue) condenses around the two branches of the ureteric bud (UB, red), and receives an inductive signal from it, which includes Wnt9b (Carroll et al., 2005). (**B**) Some of the mesenchymal condensate forms a pre-tubular aggregate (PTA) adjacent to the underside of each branch of the UB. This aggregate undergoes a mesenchymal to epithelial transformation, under the influence of Wnt4 (Stark et al., 1994), to form a simple tubule called a renal vesicle (RV). (**C**) The RV then undergoes segmentation to form the nephron, which consists of the glomerulus (G) at the proximal end, and the tubular component [the proximal (P) tubule, the ascending and descending loops of Henle (not shown) and the distal (D) tubule]. The distal tubule connects to the UB, which itself transforms into collecting ducts that conduct urine out of the kidney. (**D**) A mature nephron (not to scale), showing capillary loops (red) inside the glomerulus, and the glomerular basement membrane (GBM, green) between podocytes (blue) and the capillaries (mesangial cells are not shown). The distal segment of the nephron (light blue) connects to a collecting duct (red) that is derived from the UB. (**E**) To form the mature kidney, the process shown in A-D is reiterated by continued branching of the UB and its derivatives (red). Each of the tips of the UB derivatives continue to induce new nephrons (blue) from a population of progenitor cells present at the periphery of the developing kidney, known as the nephrogenic zone (NZ). Nephrons are located in the cortex (unshaded; with some segments dipping into the medulla), whereas collecting ducts (red) derived from the UB extend from the cortex to the medulla (green) and the medullary papilla (pink), where they drain into the ureter. Reproduced, with permission, from Kreidberg (Kreidberg, 2006).

point immediately adjacent to the basal membrane (Fig. 3). They also extend themselves nearly completely around the capillary loops. Finally, mature podocyte cell bodies that have become isolated from each other, extend several large projections, each of which divides into intermediate branches, which then divide into many smaller ‘foot processes’ that interdigitate with the foot processes of adjacent podocytes (Fig. 2D).

Foot process assembly

The inter-digitation of podocyte foot processes around the capillaries is a unique aspect of glomerular development that is fundamental to the maintenance of renal function and the prevention of glomerular disease. The basal aspect of the foot processes adhere to the GBM, and, where they retain their cell-cell contacts, they form a cell-cell junction, called the slit diaphragm, which is discussed in detail below. The appearance of these cellular extensions and interdigitated foot processes is indicative of a process in which cell bodies that have become isolated from each other, extend processes towards each other, that then become interdigitated as they envelope the capillaries. Alternatively, they might remain attached at their lateral faces, as described above, and remodel their cell-cell junctions into the projections that acquire the appearance of foot processes (Fig. 3). This model suggests that the major and intermediate projections between the cell bodies and the foot processes arise passively as a consequence of the separation of podocytes, which only remain attached to each other where foot processes interdigitate. There is, at present, little understanding of how this might occur, although some insight may be gained from a study of keratinocytes, in which an intermediate step in the formation of a cell-cell junction was reported to involve the interdigitation of filopodial processes (Vasioukhin et al., 2000). Unfortunately, the small size of foot processes requires that they are studied by electron microscopy, and the lack of a suitable in vitro model that displays interdigitating foot processes makes the real-time analysis of foot process assembly very difficult, if not impossible, with currently available imaging technology.

Glomerular vascular development: mesangial and endothelial cells

Glomerular capillary development begins when a single capillary loop grows into the glomerular cleft, which is situated between the primitive podocytes and the proximal tubule of the S-shaped body (Figs 1, 2). As glomerular maturation proceeds, the capillary loop becomes divided into six to eight loops (Potter, 1965). The endothelial cells acquire a fenestrated morphology, such that there are slit-like openings on both sides of the GBM: on the podocyte side there are slits between adjacent foot processes, and on the endothelial side, the slits are actually through the endothelial cells themselves (Figs 2, 4).

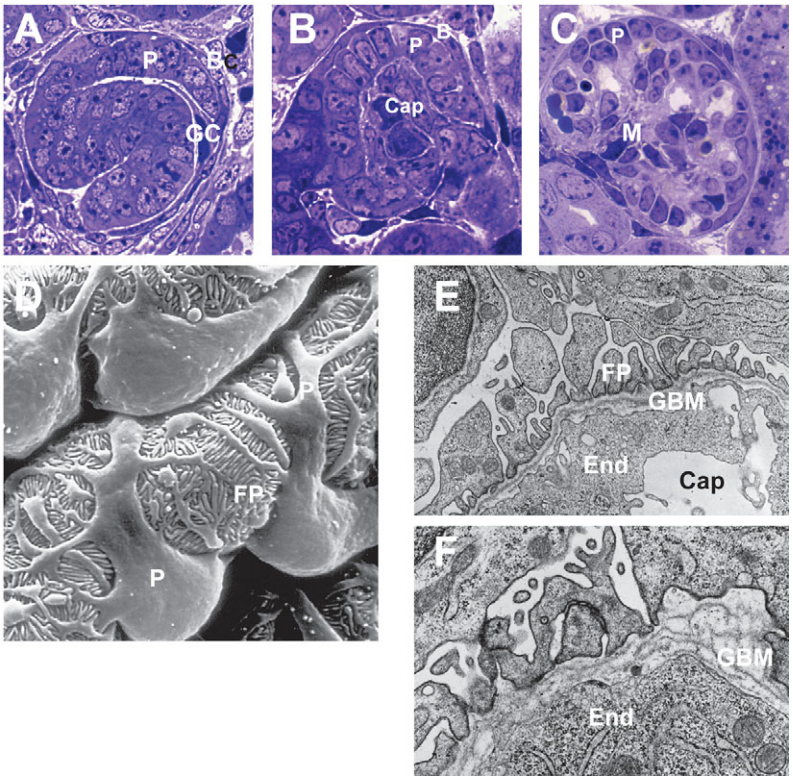

Fig. 2. Histology of glomerular development. (**A-C**) Toluidine blue-stained sections from newborn mouse kidneys. (A) S-shaped body. P, podocyte progenitors; GC, glomerular cleft. A capillary loop is present in the cleft. B, Bowman's capsule. (B) Immature glomerulus showing the 'bowl'-shaped arrangement of the podocytes. Cap, capillary loops. (C) Mature glomerulus. P, podocytes; M, mesangial cells. (**D**) Scanning electron micrograph of the interior of an adult rat glomerulus showing interdigitating foot processes (FP) encompassing capillary loops. P, podocyte cell body. (**E**) Transmission electron micrograph of a newborn mouse glomerulus. FP, foot processes; GBM, glomerular basement membrane; End, endothelial cell; Cap, capillary lumen. (**F**) Transmission electron micrograph from an α3 integrin mutant newborn mouse kidney, showing malformed foot processes and fragmented glomerular basement membrane. Image in D kindly provided by Wilhelm Kriz (Heidelberg).

Mesangial cells are found adjacent to endothelial cells on the opposite side of the GBM from podocytes (Figs 2, 4). Some studies indicate they originate from the mesenchymal precursors that contribute to the other cells of the nephron, whereas others suggest an extra-renal origin, perhaps from a component of the hematopoietic lineages (Abe et al., 2005; Masuya et al., 2003; Takeda et al., 2006). They are mostly found in the stalk of the glomerular tuft, where they possibly help to maintain the structure of the capillary loops. Mesangial cells share similarities with pericytes and smooth muscle cells, and thus, may help the glomerular vasculature respond to various physical stimuli (Schlondorff, 1987; Yamanaka, 1988). Moreover, in some forms of glomerular disease referred to as 'diffuse mesangial sclerosis' (DMS), there is an accumulation of extracellular matrix (ECM) on the vascular side of the GBM, of which mesangial cells are presumed to be the origin, which eventually forms scar tissue that can replace capillary loops and dramatically interfere with renal function. Interestingly, mutation of the Wilms' tumor 1 (WT1) gene, which is expressed in podocytes, is one of the most well-characterized situations that leads to DMS (Denys et al., 1967; Drash et al., 1970; Habib et al., 1985). This finding, and others discussed in the following sections, has led to the paradigm that interactions between podocytes and mesangial and endothelial cells are essential for maintaining normal glomerular structure and function throughout life.

When mature, the glomerular capillary 'tuft' (see Fig. 4) consists of several capillary loops with mesangial cells at their base, some of which extend into each branch of the capillary structure (Potter, 1965). The entire tuft is enveloped by the GBM, and podocytes extend their foot processes around these capillary loops.

The glomerular basement membrane

The GBM is a specialized basal lamina and is an important component of the protein barrier that prevents high molecular weight proteins from leaving the circulation while transiting the glomerular capillary bed. The major components of the GBM are type IV collagen, laminin, and the heparan sulfate proteoglycan agrin (Miner, 1999). The earliest epithelial cells of the nephron mainly express laminin 1 (α1β1γ1). As soon as it is possible to define a nascent GBM, a shift in laminin expression occurs to isoforms that contain the α4 subunit (laminin 8: α4β1γ1). Upon further maturation of the GBM in the S-shaped body, there is a second shift to the expression of laminin 10 (α5β1γ1). At the capillary loop stage, laminins 9 (α4β1γ1) and 11 (α5β2γ1) are found, but in the mature glomerulus, laminin 11 is the only laminin isoform present in the GBM (Abrahamson and St John, 1993; Durbeej et al., 1996; Ekblom et al., 1991; Miner, 1998; Miner et al., 1995; Miner and Yurchenco, 2004; Sorokin et al., 1997). There is also a shift in the expression of type IV collagen (Miner and Sanes, 1994). The early nephron mainly expresses the α1 (IV) and α2 (IV) collagen subunits and, upon maturation of the GBM, there is a shift to α3, α4 and α5 (IV) subunits (Miner and Sanes, 1994).

The slit diaphragm

The identification of nephrin as the product of the *NPHS1* gene, which is mutated in the Finnish form of Congenital Nephrotic Syndrome (Holthofer et al., 1999; Kestila et al., 1998; Lenkkeri et al., 1999), renewed attention on the slit diaphragm (SD) as a structure that is involved in maintaining normal renal function, the damage of which may be involved in the initiation and progression of glomerular disease, leading to dialysis and transplantation. The assembly of the SD is an important part of glomerular development, as it is integral to the assembly of correctly and interdigitated podocyte foot processes. The SD, which is only visible by high power electron microscopy, is a structure that connects adjacent foot processes. It consists of a complex of proteins that serves as a component of the protein barrier (Hamano et al., 2002; Tryggvason and Wartiovaara, 2001) (Fig. 5). The relative importance of the endothelial layer versus GBM versus the SD in preventing proteins

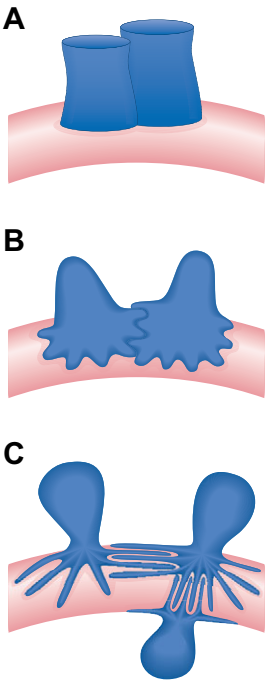

Fig. 3. A theoretical model of podocyte maturation and foot process assembly. (**A**) Two podocytes (blue) begin as discs of columnar epithelial cells, which are attached along their entire lateral membranes. (**B**) Podocytes lose their lateral cell attachments except at their base, and begin to interdigitate along the basal aspect of the lateral membrane. (**C**) Podocyte cell bodies become independent of each other, but remain attached through interdigitated foot processes.

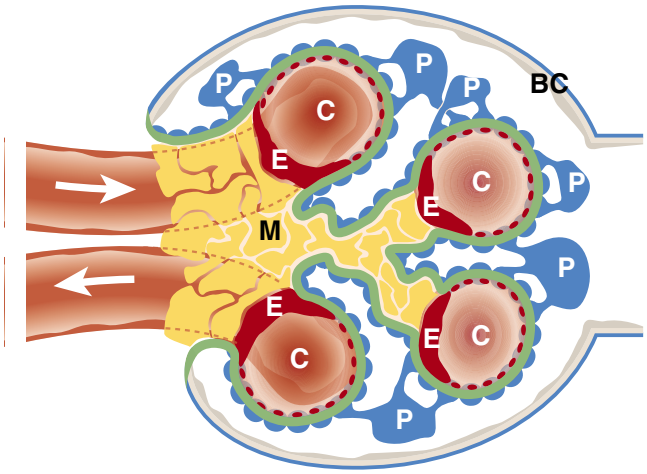

Fig. 4. Schematic of a mature glomerulus in cross section. Fewer capillary loops are shown than normal for clarity, and the size of cells are exaggerated in proportion to the overall size of the glomerulus. The four major cell types of the glomerulus are the Bowman's capsule (BC) or parietal epithelium (gray), podocytes (P, blue) or visceral epithelium, mesangial cells (M, orange) and endothelial cells (E, red). The mature glomerulus is encompassed by the Bowman's capsule. The glomerulus comprises a self-contained network of capillary loops (C, red), with mesangial cells forming a nexus at the base of the capillary network. The glomerular basement membrane (GBM, green) divides the glomerulus into two compartments, an inner one containing the capillaries and the mesangial cells, and an outer one containing podocytes and the space into which the filtrate passes. The glomerulus remains connected to the remainder of the nephron through an opening in the Bowman's capsule that connects the glomerulus to the proximal tubule, shown on the right. The arrows in the capillaries indicate the flow of blood in and out of the glomerulus. Also omitted for clarity is the branching of the single capillary loop into the multiple loops within each glomerulus.

from exiting the circulation is a matter of long-standing debate; most probably they all have an integral role. In the pediatric setting, in conditions such as Congenital Nephrotic Syndrome, the SD never develops properly (Holthofer et al., 1999; Ruotsalainen et al., 1999), and infants born with this condition require intensive support early in life, including dialysis. Similarly, mice with targeted mutations in the *Nphs1* gene encoding nephrin also fail to survive beyond the first day or two after birth (Hamano et al., 2002; Putaala et al., 2001; Rantanen et al., 2002). Three other genes that encode proteins with structural similarity to nephrin, *Neph1*, *Neph2* and *Neph3*, have been identified that appear to interact with other slit-diaphragm proteins similarly to nephrin (Donoviel et al., 2001; Gerke et al., 2005; Sellin et al., 2002). A gene trap mutation in *Neph1* leads to glomerular disease in mice (Donoviel et al., 2001). There is evidence of both homophilic interactions between nephrin molecules and heterophilic interactions between nephrin and members of the Neph family (Barletta et al., 2003; Gerke et al., 2005; Khoshnoodi et al., 2003; Liu et al., 2003). At least some portion of the SD can also be viewed as the cell-cell junction that is retained between adjacent podocytes (Reiser et al., 2000; Ruotsalainen et al., 2000). In support of this notion, P-cadherin and the protocadherin FAT1 have been discovered in the SD (Inoue et al., 2001; Reiser et al., 2000). Although mutation of the P-cadherin gene in mice does not result in any glomerular abnormalities (Radice et al., 1997), a null mutation in *Fat1* results in a failure to form foot processes (Ciani et al., 2003). Together, these results raise the question of whether some proteins, such as nephrin and associated proteins (discussed below), primarily serve as a protein barrier, possibly by acting as a repulsive force to maintain a small distance between adjacent foot processes, whereas others, such as P-cadherin and FAT1, serve as structural components of the cell-cell junction. In support of this is the observation that foot processes are not immediately lost in nephrin-deficient mice (Hamano et al., 2002; Putaala et al., 2001; Rantanen et al., 2002), although the SD is no longer detectable by electron microscopy.

Molecular regulation of podocyte function

The first marker of glomerular development in vertebrates is the restriction of *Wt1* expression to a subset of cells within the renal vesicle, as it is transforming through the comma to S-shaped tubule (Armstrong et al., 1993; Pelletier et al., 1991b). Several other transcription factors are expressed in the early podocytes within S-shaped tubules, including podocyte-expressed 1 (*Pod1*; also known as capsulin; *Tcf21* – Mouse Genome Informatics) (Quaggin et al., 1998b), forkhead box C2 (*Foxc2*) (Takemoto et al., 2006), kreisler (*Mafb*) (Sadl et al., 2002), the forkhead domain transcription factor *Mf2* (*Foxd2* – Mouse Genome Informatics) (Kume et al., 2000), and the Lim domain protein *Lmx1b* (Dreyer et al., 1998).

WT1

WT1 is probably the best studied of the transcription factors expressed in podocytes. *WT1* encodes a protein with four zinc fingers that can bind to both DNA and RNA (Call et al., 1990;

Caricasole et al., 1996; Drummond et al., 1994). Its loss in mice leads to complete renal and gonadal agenesis (Kreidberg et al., 1993), a phenotype that can be rescued in *Wt1*-YAC transgenic mice, but these mice are still predisposed to developing glomerular disease as adults (Guo et al., 2002; Menke et al., 2003; Moore et al., 1999; Moore et al., 1998; Patek et al., 2003; Schedl and Hastie, 1998). Whether the adult-onset glomerular disease observed in these mice reflects developmental abnormalities that are not obvious, but nevertheless lead to pathological changes, or whether WT1 regulates the expression of genes that are required to maintain normal glomeruli throughout life is a matter of investigation. The latter possibility is consistent with the emerging paradigm that glomeruli are structures that require an active 'maintenance' function throughout life.

The targets of and functions of WT1 remain an enigma, as there are four major splice forms of *WT1* mRNA (Haber et al., 1991), whose encoded proteins have differing abilities to bind DNA and RNA, and different translational start sites, indicating the possibility of many different peptides (Bruening and Pelletier, 1996). It is therefore likely that WT1 has several functions during early kidney and glomerular development. Numerous genes have been suggested to be its regulatory targets, but their validity remains unclear, either because they are not co-expressed with *Wt1* in vivo, or because mutations in these genes do not yield phenotypes that overlap with the *Wt1* mutant phenotypes in mice or humans, though it is conceivable that, if WT1 regulates many genes, no single phenotype resulting from mutation of a target gene would recapitulate the entire *Wt1* mutant phenotype.

With the recent strides in the ability to analyze gene expression within the context of chromatin, the study of transcription factors has undergone dramatic advancements that will impact the consideration of the past literature on the function of WT1. Many studies of the molecular function of WT1 were done using plasmids containing putative target sequences of WT1 (reviewed by Scharnhorst et al., 2001). Even though WT1 binds to DNA containing these sequences, in most instances they were not studied in the context of assembled chromatin in cells that would express *Wt1* in vivo. More recent studies are beginning to use chromatin immunoprecipitation to verify WT1 target genes (Kim et al., 2007). However, even these studies generally use immortalized cell lines, in which WT1 might bind to chromatin differently than it does in the developing kidney and other tissues. WT1 also binds to RNA, associates with spliceosomes and shuttles between the nucleus and cytoplasm, where it associates with polysomes (Niksic et al., 2004). A specific interaction with heterogeneous nuclear ribonuclear protein U has been suggested (Spraggon et al., 2007), findings that are consistent with a post-transcriptional function for WT1 (Larsson et al., 1995; Niksic et al., 2004). Thus it is possible that older publications identifying WT1 target genes might have been observing post-transcriptional effects of WT1 on putative target genes. A WT1 interacting protein (WTIP) has been shown to shuttle between the nucleus and the membrane (Rico et al., 2005; Srichai et al., 2004), suggesting WT1 may mediate signals from the extracellular environment that regulate gene expression, adding to the enigma of WT1.

In humans, *WT1* mutations that affect the zinc finger region, particularly in the vicinity of the third zinc finger (Barbosa et al., 1999; Kikuchi et al., 1998; Little and Wells, 1997; Pelletier et al., 1991a), are associated with two glomerulopathies, Deny-Drash Syndrome (DDS) (Denys et al., 1967; Drash et al., 1970), and Frasier syndrome, which can both present early in life and cause abnormal glomerular development. DDS is caused by mutations that eliminate or disrupt the zinc finger regions, whereas Frasier syndrome results from an inability to include an alternatively spliced lysine-threonine-serine (KTS) sequence after the third zinc finger. Mice genetically engineered to exclusively express only the +KTS or the –KTS versions of WT1 undergo normal metanephric kidney induction, but glomeruli are mal-developed in both mutants, suggesting that the different splice forms of WT1 may have more distinct roles in glomerular development than in the initiation of kidney development (Hammes et al., 2001).

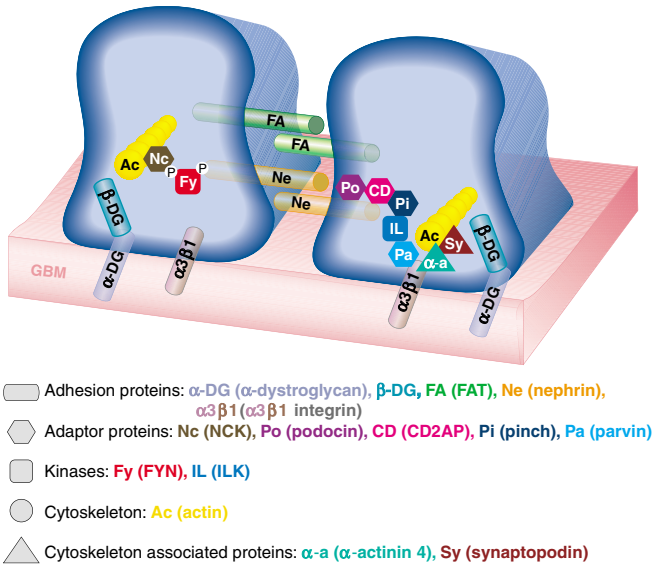

Fig. 5. Schematic of the slit diaphragm and other important proteins involved in maintaining foot process assembly. Many proteins are omitted to emphasize the two major complexes of proteins discussed in the text. For clarity, one complex is shown within the left foot process, the other in the right foot process. Two adjacent foot processes are shown. In the left foot process is the nephrin-FYN-NCK complex, associated with an actin filament. In the right foot process, the nephrin-podocin-CD2AP complex and the integrin-linked kinase (ILK)-parvin-pinch complex associated with both α3β1 integrin and nephrin are shown. Synaptopodin and α-actinin 4 are also shown associated with the cytoskeleton, the latter also with integrins. Nephrin and FAT are shown as two major proteins that bridge the space between adjacent foot processes, although, as noted in the text, nephrin also associates with Neph proteins. The integrin and dystroglycan complexes are shown in each foot process. P, phosphorylation (see key for other abbreviations used in the figure).

WT1 mutations in humans are also associated with diffuse mesangial sclerosis (DMS), which is characterized by an increase in ECM deposition on the vascular side of the GBM. Since *WT1* is expressed in podocytes, on the opposite side of the GBM from the ECM deposition, this pathological process highlights the important regulatory interactions that occur between podocytes, on one side of the GBM, and endothelial and mesangial cells on the other. Why excess ECM deposition is the response to presumed aberrant gene expression in podocytes is not known, but WT1 and other transcription factors may regulate the signals that control the ECM protein expression that maintains a normal GBM and the matrix that surrounds mesangial cells; mutant forms of WT1 might thus lead to either excessive or insufficient signals, triggering mesangial cells to overexpress ECM proteins. In the most severe instances, neonates with DDS are identified at birth, indicating that DMS may be considered a case of abnormal development.

WT1 might also regulate the expression of factors that affect glomerular vascular development. Indirect evidence suggests that WT1 may regulate VEGFA in the metanephric mesenchyme during early mouse kidney development, but there is as yet no evidence that this occurs in podocytes (Gao et al., 2005). Transgenic mice that express a truncated version of *Wt1* develop abnormal glomeruli with dilated capillaries (Natoli et al., 2002).

Nephrin, a major component of the slit diaphragm complex might also be a target of WT1, as WT1 can bind a sequence in the promoter of *NPHS1* and regulate the expression of a reporter gene placed downstream of the *NPHS1* promoter (Guo et al., 2004; Wagner et al., 2004). Podocalyxin, a highly charged transmembrane protein that may confer the repulsive effects that keep podocyte cell bodies separated, is also a possible WT1 target (Palmer et al., 2001).

LMX1B

LMX1B encodes a Lim-domain protein and is mutated in Nail-Patella syndrome (Chen et al., 1998; Dreyer et al., 1998). LMX1B binding sites are found in the promoter region of several genes expressed by podocytes, including *NPHS2*, which encodes podocin, *CD2AP* (see below), and *COL4A3* and *COLA4*, which respectively encode the α3 and α4 subunits of type IV collagen (Miner et al., 2002; Morello et al., 2001).

POD1 and kreisler

POD1 (also known as epicardin and capsulin) encodes a basic helix-loop-helix (bHLH) transcription factor that is expressed early in mouse kidney development, and subsequently in the primitive podocytes of S-shaped bodies (Quaggin et al., 1999; Quaggin et al., 1998a). Kreisler (MAFB) encodes a basic domain leucine zipper (bZip) transcription factor of the MAF subfamily and is expressed in mouse podocytes of capillary loop-stage glomeruli (Sadl et al., 2002). It also has an important role in hindbrain segmentation (Sadl et al., 2003). *Pod1* and kreisler mutations in mice result in similar phenotypes: glomerular development is arrested at the single capillary loop stage (Quaggin et al., 1999; Sadl et al., 2002), and the podocytes remain as columnar-shaped cells that have lost their lateral cell-cell attachments but remain fully adhered to the GBM without any foot processes. Thus, *Pod1* and kreisler are required just prior to the time when podocytes would normally begin migrating around the capillary loops and assembling foot processes. *Pod1* is expressed in kreisler mutant podocytes, indicating that kreisler is likely to act either downstream or in a separate pathway from *Pod1* (Sadl et al., 2002).

Foxc2

Foxc2 was identified during a screen for genes with enriched expression in mouse glomeruli (Takemoto et al., 2006). It belongs to the forkhead-domain family of putative transcription factors and is expressed in podocytes. In *Foxc2* mutant mouse kidneys, mesangial cells cluster at the base of the glomerular stalk, podocyte foot processes and endothelial fenestrations are absent, and dilated capillaries are observed, similar to the other phenotypes discussed above (Takemoto et al., 2006).

Regulatory interactions within the glomerulus

Is the SD a signaling complex?

Nephrin is an essential component of the protein barrier and also has an important function in signal transduction (summarized in Fig. 5). Nephrin has multiple tyrosine residues that are targets for phosphorylation by, for example, the Src family kinase FYN (Verma et al., 2006; Verma et al., 2003). Phosphorylation at these tyrosine residues occurs transiently during glomerular development, concomitant with the formation of mature foot processes (Jones et al., 2006; Verma et al., 2006). Nephrin phosphorylation results in the recruitment of the adaptor protein NCK and cytoskeletal reorganization in podocytes (Jones et al., 2006; Verma et al., 2006; Verma et al., 2003), supporting the hypothesis that NCK-mediated cytoskeletal organization is related to foot process formation. The conditional mutation of *Nck1* and *Nck2* in podocytes in mice results in an inability to assemble normal foot processes (Jones et al., 2006). Interestingly, the phosphorylation of nephrin also occurs during podocyte damage, when foot processes disassemble; this may reflect the onset of a repair process aimed at restoring foot process structure (Verma et al., 2006). Further work is needed to determine whether additional adaptor proteins also bind nephrin at other tyrosines and whether it is a substrate for other kinases, in addition to FYN.

Other cytoplasmic proteins that associate with the SD, probably through the cytoplasmic domain of nephrin, include podocin (encoded by the *NPHS2* gene) and CD2AP, an SH3 domain-containing protein (Boute et al., 2000; Li et al., 2000). Podocin and CD2AP are both required for proper SD assembly and link the SD to the cytoskeleton (Lehtonen et al., 2002; Roselli et al., 2002; Schwarz et al., 2001; Shih et al., 2001; Yuan et al., 2002). Mice deficient in these proteins do not develop normal foot process structure, as in nephrin mouse mutants (Roselli et al., 2004; Shih et al., 1999). A *Caenorhabditis elegans* homolog of podocin (*mec-2*) plays a role in mechanosensation, indicating that the SD might also have a mechanosensory function (Huang et al., 1995). Future work should determine whether adaptor proteins, such as NCK, also interact with SD complex proteins, such as Podocin and CD2AP, to form a larger signaling complex.

Recently, phospholipase C epsilon (*PLCE1*) has been identified as an important gene for podocyte development. Mutations in this gene were identified in humans with end-stage kidney disease, and the knockdown of its homolog in zebrafish leads to abnormal glomerular development within the pronephros (Hinkes et al., 2006). However, there is no obvious glomerular defect in mice that carry a targeted mutation of this gene (Tadano et al., 2005); whether this is due to compensation from other phospholipases is not known. PLCE1 has a cytoplasmic distribution in podocytes that begins early in nephron development, suggesting that signaling pathways that use this enzyme may play an important part in podocyte differentiation. Whether it is involved in transducing signals from adhesion complexes or SD complexes remains to be determined.

The physiology and biochemistry of ion channels that are expressed in the tubules of the kidney have been studied extensively. By contrast, the importance of ion channels in the glomerulus has received, until recently, little to no attention. This has changed with the surprising discovery that a mutation in the *TRPC6* gene accounts for a portion of human familial glomerular disease (Reiser et al., 2005; Winn et al., 2005). TRPC6, a member of the TRP family of cation channels, is a calcium channel located at the SD. Although *TRPC6* is not a 'developmental' gene per se, this finding hints at the possibility that ion channels may be of significant importance in glomerular function and perhaps in their development.

Adhesion molecules in glomerulogenesis

By immunoelectron microscopy, β1 integrin can be detected along the basal aspect of the foot process (Kerjaschki et al., 1989), as expected for a GBM receptor, a finding that is inconsistent with α3β1 integrin also being a component of the SD. Nevertheless, the SD and the basal aspect of the foot process are in such close proximity that a role for α3β1 integrin in regulating signaling at the

SD should not be excluded. Thus, discussing the role of adhesion molecules separately from those of the SD may be creating an artificial distinction. Clearly the foot process is an extremely small structure, and the distance between its lateral components such as nephrin, podocin and CD2AP, and the molecules that associate basally with integrin cytoplasmic domains, such as talin and α-actinin, could probably be spanned by a small number of proteins, depending, of course, on their particular size and shape. Moreover, as our knowledge of the protein-protein interactions that are involved in cytoskeletal assembly and signal transduction expands, it is becoming increasingly apparent that the same molecules are associated with integrin receptors for the GBM and with components of the SD complex (e.g. integrin-linked kinase).

Integrins are heterodimeric transmembrane proteins that serve as receptors for the ECM and that, by forming complexes with cytoplasmic proteins, form a structural link between the ECM and the cytoskeleton. Integrins are also involved in signaling through multiple pathways. An emerging paradigm is that coordinated signaling that involves integrins and receptor tyrosine kinases (RTKs) integrates information from secreted growth factors and the ECM (Comoglio et al., 2003). α3β1 integrin binds many ligands but is thought to function most effectively as a receptor for certain laminin isoforms, including laminins 5, 10 and 11 (Kikkawa et al., 1998; Kreidberg, 2000). Podocytes express some of the highest levels of α3β1 integrin observed among all tissues (Korhonen et al., 1990). α3β1 integrin is an early marker of podocyte differentiation, and continues to be highly expressed in mature podocytes (Korhonen et al., 1990). Mesangial cells (discussed below) also express α3β1 integrin, although not as highly as podocytes (Korhonen et al., 1990). α3β1 integrin is expressed before the podocyte shifts from laminin 1 expression to its preferred ligands, laminin 10 and 11, but whether this shift evokes signaling through α3β1 integrin that is related to podocyte or foot process maturation is a matter for investigation. Podocytes cannot assemble mature foot processes in mice with a null mutation in the α3 integrin or α5 laminin-encoding genes (Kreidberg et al., 1996; Miner and Li, 2000). However, α3β1 integrin appears not to function simply as an adhesion receptor, because, in its absence, podocytes do not detach from the GBM, but become flattened against a fragmented GBM (Kreidberg et al., 1996). Perhaps α-dystroglycan (Raats et al., 2000), another laminin receptor expressed by podocytes, functions redundantly to α3β1 integrin by also adhering podocytes to laminin in the GBM, such that foot processes do not detach from the GBM as long as either integrins or dystroglycan are present. Alternatively, α3β1 integrin might act primarily to transduce signals that mediate the cytoskeletal organization that is involved in forming mature foot processes. Whether these signals are induced by changes in laminin isoform expression is not known but it remains an intriguing possibility.

α3β1 integrin is also a component of the E-cadherin-based adherens junction. In immortalized collecting duct epithelial cells, it is reported to stimulate cadherin-mediated cell-cell adhesion. α3β1 integrin forms a complex that includes the tetraspanin CD151, PTPμ (a transmembrane receptor tyrosine phosphatase) and PKCβII (Chattopadhyay et al., 2003). This complex appears to be involved in maintaining low levels of tyrosine phosphorylation of β-catenin. It is not clear whether α3β1 integrin fulfills this function in podocytes, and, indeed, a major role for cadherins or β-catenin as a component of SD assembly has not been demonstrated, suggesting that this cell-cell junction may differ significantly from more typical cell-cell junctions, especially as it acquires its specific function as a protein barrier. However, podocytes do lose their cell-cell attachments more readily in the absence of α3β1 integrin (Fig. 6), and acquire a highly abnormal morphology that is probably incompatible with normal glomerular development and function (Kreidberg et al., 1996).

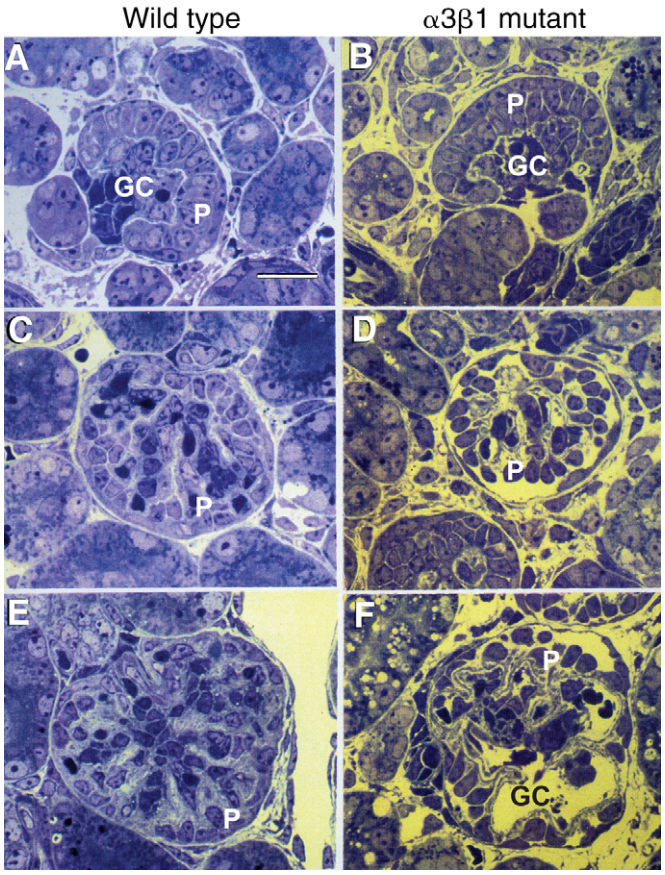

Fig. 6. A comparison of normal and α3 integrin mutant mouse glomerular development. (A,C,E) Successive stages of normal mouse glomerular development. (**A**) Capillary loop stage, at which time the podocytes (P) still resemble a columnar epithelium and are forming a 'bowl'-shaped sheet into which capillaries are beginning to branch from a single loop into multiple loops. Scale bar: 8 μm. (**B**) The capillary loop stage is relatively normal in the absence of α3β1 integrin. (**C**) Intermediate stage of glomerular development, where podocytes have begun to lose their cell-cell attachments and migrate around capillary loops. Mesangial cells (darker nuclei) are present in the middle of the glomerulus, where podocytes are beginning to encompass capillary loops. (**D**) In the absence of α3β1 integrin, podocytes have completely lost cell-cell attachments, and their cell bodies appear to be connected by a thin 'neck' to the basement membrane. (**E**) Mature normal glomerulus. (**F**) In the absence of α3β1 integrin, abnormally wide capillary loops are present, and podocytes are mainly situated in the peripherly of the glomerulus. GC, glomerular cleft. [Reproduced from Kreidberg et al. (Kreidberg et al.,1996).]

Integrin-linked kinase (ILK) is an important molecule that might link integrins and associated proteins to the SD complex. ILK has serine-threonine kinase activity (Hannigan et al., 1996), although whether the kinase activity is required for its in vivo functions is unknown. Recently, ILK has been shown in podocytes to associate with parvin and pinch (Yang et al., 2005), two adaptor proteins. This larger complex associates with actin-binding proteins through parvin and possibly with RTKs through an interaction between pinch and the adaptor proteins NCK1 and NCK2 (Xu et al., 2005). In podocytes, ILK also associates with a complex that includes nephrin

and α-actinin4 (the latter belongs to the α-actinin family of actin-binding proteins that interact with integrin-associated complexes) (Dai et al., 2006). Point mutations in the *ACTN4* gene in humans and mice lead to glomerular disease (Kaplan et al., 2000; Kos et al., 2003). These observations suggest that the ILK-pinch-parvin complex may be part of a bridge between the SD and integrins, where it could be involved in mediating or regulating their attachment to the cytoskeleton (Dai et al., 2006). Interestingly, the conditional inactivation of the ILK gene in the podocytes of mice does not cause developmental abnormalities, but postnatally mice develop glomerular disease beginning with loss of the foot process architecture (El-Aouni et al., 2006). Whether this is due to progressive gene inactivation as mice age, or is indicative of a more important role for ILK in foot process maintenance or repair than for their initial assembly, is not known.

Stroma in glomerular development

During kidney development, developing nephrons are surrounded by stromal or interstitial cells. In the mature kidney, only a small number of these cells remain, with the kidney consisting almost entirely of nephrons, with little apparent stroma. Nevertheless, the stroma plays a crucial part in overall kidney development (Hatini et al., 1996). Its role in glomerular development is most notable in chimeric mice consisting of wild-type and *Pod1*$^{-/-}$ cells, the latter carrying a *lacZ* marker, which shows that *Pod1*$^{-/-}$ cells can contribute to the glomerulus but not to the stroma (Cui et al., 2003). Most notably, the presence of wild-type cells in the stroma appears to rescue the POD1 mutant glomerular phenotype, suggesting that there is a cell non-autonomous role for POD1 in the stroma that is crucial for glomerular development (Cui et al., 2003).

Mesangial cells and vascular development

Platelet-derived growth factor (PDGF) signaling is required for the proper assembly of the glomerular capillary loops, and mesangial cells are the main kidney cell type through which PDGF exerts its effects (Bjarnegard et al., 2004; Leveen et al., 1994; Soriano, 1994). Mesangial cells express PDGF receptors, and, in either *Pdgfb* or *Pdgfrb* mutant mice, glomerular development is very abnormal. A single large capillary loop fills the glomerular capsule, and mesangial cells are not present (Alpers et al., 1992; Betsholtz and Raines, 1997; Bjarnegard et al., 2004; Leveen et al., 1994; Lindahl et al., 1998; Soriano, 1994). In chimeric mice that may contain a mix of wild-type and *Pdgfrb*-null cells, the *Pdgfrb*-null cells do not contribute to the mesangial lineage (Lindahl et al., 1998).

The multiple capillary loops present in mature glomeruli appear to originate from a single loop that invades the glomerular cleft. Whether podocytes or mesangial cells, or possibly both, provide the crucial signals or mechanical events that drive the establishment of the glomerular capillary network is unclear. For example, fewer, wider-than-normal capillary loops are present in the glomeruli of α3 integrin (Fig. 6) or α5 laminin mutant mice (Kreidberg et al., 1996; Miner and Li, 2000). A recent study of mice that express mutant forms of the α5 laminin subunit found that mesangial cells, but not podocytes, detach from the GBM (Kikkawa et al., 2003). [Consistent with this result, podocytes also remain attached to the GBM in α3 integrin mutant kidneys (Fig. 2) (Kreidberg et al., 1996).] This led Kikkawa et al. to hypothesize that the failure to form normal glomerular capillary loops was due to the inability of mesangial cells to adequately orient these loops because of their inability to securely attach to the GBM, possibly through α3β1 integrin (Kikkawa et al., 2003).

Signaling from podocytes

Development of the podocyte lineage is tightly linked to the differentiation and maturation of the two other major cell compartments in the glomerulus, the fenestrated endothelial and mesangial cells. The glomerulus is a highly specialized capillary bed, in which podocytes function as vasculature support cells. Podocytes produce various vascular growth factors, including VEGFA (vascular endothelial growth factor A), VEGFC, angiopoietin 1, and ephrin B2 (Eremina et al., 2003; Partanen et al., 2000; Satchell et al., 2004; Satchell et al., 2002; Takahashi et al., 2001), whereas the adjacent endothelial cells express the respective receptors for these ligands.

Podocytes begin to express all isoforms of the *Vegfa* gene in S-shape bodies and continue to express them in mature glomeruli (Kretzler et al., 1998). The major signaling receptor for VEGFA is VEGFR2 (also known as FLK1), which is expressed by endothelial cells as they migrate into the vascular cleft adjacent to the podocyte precursors (Robert et al., 1998). Conditional gene targeting experiments in mice have shown that VEGFA production by podocytes is essential for the formation of a functional glomerular filtration barrier and of the fenestrated endothelial capillary system (Eremina et al., 2003). Loss of the VEGFA gene from developing podocytes in mice results in arrested glomerular development and in the absence of glomerular endothelium (Eremina et al., 2003). Less severe reductions in *Vegfa* expression by podocytes, brought about by the conditional inactivation of a single *Vegfa* allele, also result in dramatic defects in the endothelial compartment that range from endotheliosis (swelling of the endothelium) to disappearance of the endothelium followed by rapid lysis of the mesangial cells (Eremina et al., 2006; Eremina et al., 2003). Together, these results demonstrate a fine dosage sensitivity to VEGFA production in the developing glomerulus and emphasize the role of paracrine signaling from the podocyte to the endothelial compartment. What is less clear is the role of juxtacrine or autocrine VEGF signaling loops within the developing glomerulus. Although podocytes do express the VEGFR1 (FLT1), neuropilin 1 and neuropilin 2 receptors (Guan et al., 2006; Villegas and Tufro, 2002), it is not known whether they also express VEGFR2, the major receptor believed to be responsible for VEGFA signaling. In vitro, inhibition of VEGF receptor function affects the survival of podocytes, consistent with an autocrine signaling loop (Foster et al., 2003; Foster et al., 2005). In the conditional *Vegfa* knockout models discussed above, mesangial cell migration and survival is also affected. Although mesangial cells express VEGF receptors in vitro and in diseased glomeruli, they do not appear to express these receptors in a healthy glomerulus. Thus, it is most likely that loss of VEGFA from podocytes affects the production of mesangial growth factors, such as PDGFB, by the glomerular endothelium with secondary effects on the mesangial compartment.

Multiple splice variants of the *Vegfa* gene give rise to a number of pro- and anti-angiogenic isoforms. As these isoforms exhibit different properties, they likely possess combined, as well as unique, functions. For each major VEGFA isoform there exists a 'b' isoform that arises from an alternative distal splice site in exon 8 (Bates et al., 2002). This results in isoforms of the same size but with a different carboxy terminus. Investigators have shown that the 165b isoform can inhibit VEGF165-mediated endothelial cell proliferation and migration (Bates et al., 2002). Intriguingly, glomerular maturation is associated with a downregulation of the VEGF165 isoform and coincident increase in the 165b isoform (Cui

et al., 2004). It has been suggested that failure to undergo this isoform switch may explain some of the glomerular dysgenesis observed in individuals with Denys-Drash syndrome caused by mutations in *WT1* (Schumacher et al., 2007).

Less is known about the signals that endothelial or mesangial cells may exert on podocyte differentiation. Studies in zebrafish show that endothelial cells are not required for the determination of the podocyte cell lineage, as podocytes develop in *cloche* mutants that have no endothelial cells (Majumdar and Drummond, 1999). However, the differentiation of specialized podocyte features, such as slit diaphragms, was not described in these mutants. Moreover, in zebrafish, in contrast to in mammals, podocytes and tubules are derived from distinct primordia, and caution must be used in extrapolating the results of studies of podocyte differentiation from zebrafish to mammals, although podocytes in zebrafish do express many of the same differentiation genes as in mammals.

Glomerular development versus damage and repair

Nephrons have a limited ability to undergo repair, confined mainly to the proximal tubules and the ability of podocytes to re-form foot processes in the reversible forms of glomerular disease. Podocytes have historically been regarded as a terminally differentiated cell, and it remains unclear whether podocytes undergo normal cell division in the mature kidney. Most studies indicate that they have a very limited ability to proliferate, except in certain pathological situations where podocyte proliferation replaces normal glomerular architecture. [See Shankland (Shankland, 2006) for a recent complete review on podocyte injury and repair.] This is supported by studies of experimental glomerular injury in mice with mutations in CDK (cyclin-dependent kinase) inhibitors, such as p21 and p27, which found that increased podocyte proliferation occurs in CDK mutant mice following renal injury (Shankland, 2006). However, this appears to correlate with a worsening glomerular function, rather than with a reparative process. Thus, CDK inhibitors appear to protect podocytes by maintaining them in a quiescent state, as a way of minimizing irreversible damage to them in glomerular disease.

Podocyte apoptosis is also observed in experimental models of glomerular injury (Shankland, 2006). The possibility has been raised that the SD complex is involved in transducing signals that affect podocyte survival. Supporting this possibility, podocyte apoptosis is increased in mice that carry a mutation in the CD2AP gene (Schiffer et al., 2004). In this case, podocyte apoptosis might be a response to decreased cell-cell adhesion, mediated through the SD.

Although podocytes do not appear to proliferate as part of a repair mechanism, the ability to regenerate foot process architecture is an important component of repair in glomerular disease. In some pathological situations, foot process architecture is lost, a process referred to as effacement. In cases where the initial damage was immune-mediated, treatment with anti-inflammatory drugs leads to foot process restoration. In other situations, the effacement is refractory to treatment and glomerular scarring (glomerulosclerosis) ensues, leading to chronic renal failure. Nearly all genetic disorders of glomerular development fall into this latter category, an exception being the recently described mutation in the *PLCE1* gene (Hinkes et al., 2006).

One final enigma of glomerular biology is that certain mutations in mice (or humans) that do not affect glomerular development can then lead to a loss of foot process architecture and kidney disease in older mice, for example, mutations in the mouse synaptopodin (*Synpo*) gene, which encodes an actin binding protein. The *Synpo* gene knockout does not affect initial foot process assembly, but does result in the decreased ability of podocytes to restore foot processes in models of transient glomerular injury, such as in the protamine sulfate/heparin model (Asanuma et al., 2005; Yanagida-Asanuma et al., 2007). In this situation, foot process effacement is rapidly induced over a matter of minutes by infusion of positively charged material (protamine) that probably acts by masking the interactions that occur between the highly negatively charged molecules that coat podocytes, such as podocalyxin. Foot process architecture can be rapidly restored by the subsequent infusion of heparin, which is negatively charged (Seiler et al., 1975). This demonstrates that, under experimental conditions, foot processes are dynamic structures, and suggests that these dynamic qualities may exist during normal in vivo function of the glomerulus, possibly as a mechanism to repair most glomerular injury that does not otherwise come to clinical attention. What these observations may be telling us is that the glomerulus has evolved to withstand stress brought on by immune or environmental injury, particularly with regard to foot process reassembly, and that there may be specific molecules, such as synaptopodin, whose function is more important in foot process reassembly than in their initial development. Moreover, even though it is pleasing to think that repair mimics development, molecular mechanisms might exist that are unique to glomerular repair. An alternate explanation is that there is functional redundancy between many of the molecular mechanisms that are involved in glomerular development.

Conclusion

Podocyte differentiation and damage has been the focus of much of the research into glomerular development and disease in recent years. The emerging paradigm that glomerular development and maintenance depends on crucial interactions between the three major cell types of the glomerulus will serve to re-focus future research from a podo-centric view back to one that examines the signals that pass between these cell types, as well as between the more distant cells within the nephron. Improved treatments of chronic and acute kidney disease will involve regenerative therapies that produce new nephrons, or pharmacological therapies that promote the repair of foot process architecture and prevent glomerular scarring. Developing these treatments will require further advancements in our understanding of the mechanisms of glomerular development and repair.

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