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Podocyte Differentiation and Glomerulogenesis

JORDAN A. KREIDBERG
Division of Nephrology, Department of Medicine, Children's Hospital, Department of Pediatrics, Harvard Medical School, Boston, Massachusetts.

This review will concentrate on the assembly of the glomerulus and the differentiation of the podocyte during that process. The differentiation of the vascular component of the glomerulus will not be emphasized, as it has been the subject of several recent reviews (1–4), except in discussing the influence of the podocyte on the development of the glomerular vasculature.

Glomerulogenesis

Our understanding of the morphologic features of glomerular assembly, and indeed of the development of the nephron and the entire kidney, owe much to the work of Edith Potter and her colleague Vitoon Osathanondh during the mid 1960s. In a seminal series of articles that are today overly neglected, they provided much of understanding of how the kidney as an entire organ is assembled (5–10). With regard to the glomerulus, it was shown how it emerges from one end of the S-shaped body during the development of the nephron (10). In understanding this process, and indeed understanding the development of the nephron in its entirety, it is essential to convert two-dimensional pictures into a three-dimensional understanding. This is especially difficult as the nephron is not a fully symmetrical structure on all axes. With these caveats in mind, the following is a description of glomerular development. As shown in Figures 1A and 1B, at one end of the S-shaped body a layer of columnar epithelial cells is present, which represents the future visceral epithelial cells or podocytes. The basal aspect of these cells rests on the future glomerular basement membrane (GBM); on the other side of this basement membrane is a cleft between the podocytes and the cells, which will contribute to the tubular portion of the nephron. The cells that will contribute to the glomerular capillaries, *i.e.*, endothelial and mesangial cells, migrate into this cleft. The origin of these cells is a subject of ongoing research and, as mentioned above, has been discussed in recent reviews (1–4). On the other side of this patch of future podocytes, overlying their apical surface, is a lining of thin cells that will become the parietal epithelium, also known as Bowman's capsule.

Corresondence to Dr. Jordan A. Kreidberg, Division of Nephrology, Department of Medicine, Children's Hospital, and Department of Pediatrics, Harvard Medical School, Boston, MA 02115. Phone: 617-247-5194; Fax: 617-232-4315; E-mail: Jordan.Kreidberg@tch.harvard.edu

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The development of the glomerulus is a dynamic process involving the expansion of the original capillary component into a plexus of six to eight individual loops, and the concomitant migration of the podocytes to be distributed around these loops (Figure 1, C and D). Although cause and effect relationships are not known, the capillary bundle displaces the layer of future podocytes as it expands. This layer of future podocytes, in turn, form a "pocket" surrounding the capillary bundle (Figure 1D), which maintains contact with the outside vasculature through an arterial and venous supply that will become part of the glomerular stalk.

As the primitive podocytes form this pocket, the GBM remains a constant barrier between the epithelial and capillary components. The podocytes themselves do not remain a columnar epithelium. As they form this pocket, they begin to lose their lateral cell-cell attachments to each other (Figure 1F), but they remain attached to the GBM such that they no longer resemble a traditional epithelial cell. During this time, they also begin to migrate around the capillary loops, so that they no longer form a continuous uniform patch of cells. By this time, the glomerulus can be recognized as a discrete structure apart from the remainder of the nephron. It is during this phase of glomerular development and podocyte maturation that foot processes begin to form (Figure 1E). Early pictures of foot process assembly suggest that it begins with the selective detachment of podocytes from the GBM. However, the appearance of mature podocytes, with foot processes extending a significant distance from the main cell body, are also suggestive of a process whereby cytoplasmic extensions resembling filopodia extend themselves as a scaffolding around the capillary loops.

Foot Process Assembly

The extent to which foot process assembly reflects selective cell detachment *versus* cell extension and migration is not known; indeed, it is difficult to determine, given our inability to observe this process in living cells. One informative observation bearing on this issue is that adjacent foot processes are derived from different podocytes. Cells that begin as adjacent epithelial cells end up as cells with isolated cell bodies but interdigitated foot processes. It is possible that this situation arises entirely by extension of foot processes from cells that have initially dissociated from each other. However, another possibility is suggested by recent work examining cell-cell contact in keratinocytes (11). In this case, which is not generalizable to all epithelial cells, cell-cell contact is initiated through the extension of interdigitated filopodia between two

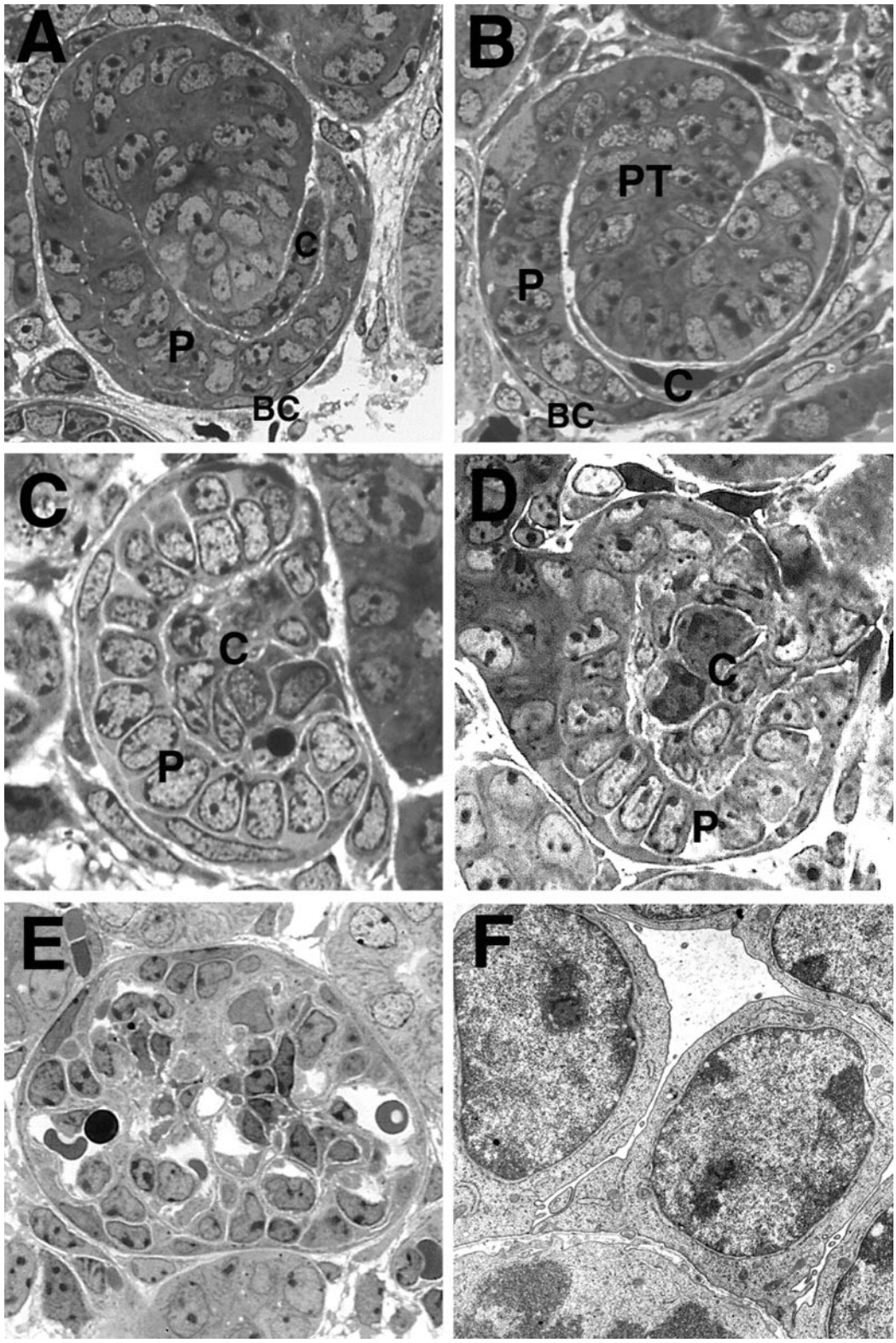

Figure 1. Development of the glomerulus. (A and B) show two different sections through an S-shaped body. In both cases, the early capillary loop is observed within the glomerular cleft and the thin lining of cells that will comprise Bowman's capsule is adjacent to the apical side of the podocytes. In panel B, the cells that will comprise the proximal tubule are also noted. Panels C and D show how the podocyte layer begins to form a "cup" around the capillary bundle and how the capillary bundle remains attached to the outside vasculature. Panel E shows a more mature glomerulus, by which time the podocytes have migrated around the capillary loops. The capillary loops are still wider than they will be in a fully mature glomerulus. Panel F is an electron micrograph showing the podocyte layer at the point where most lateral cell attachments have been lost, except at the basal end of the lateral membrane, where the slit-diaphragm complex will be assembled in mature podocytes. A thin GBM is present at this stage. P, podocyte; C, capillary loop; BC, Bowman's capsule; PT, proximal tubule.

cells (11). In the case of keratinocytes, these filopodia resolve into a conventional cell-cell junction mediated by cadherins. These findings in keratinocytes suggest two possible hypotheses for the assembly of foot processes. In one model, foot process assembly begins when podocytes are still maintaining cadherin-mediated adhesions along their lateral membranes (Figure 2B). In this case, adjacent cells would extend interdigitating filopodial-type extensions along their basal aspects, where they are attached to the GBM. Then, these filopodial extensions would be maintained as foot processes, while the remainder of the cell loses its cadherin-mediated adhesion such that appear as independent cell bodies. In the alternate model, podocytes first entirely dissociate from each other and then extend filopodial-like extensions that interdigitate to form foot processes (Figure 2A). Present observations appear to favor the model depicted in Figure 2B, as it is not normal to observe capillaries in developing glomeruli whose outer walls are not associated with an extension from a podocyte, implying that poodcytes or mesangial cells always fully encompass the capillary loops. However, the author is unaware of any studies that directly assess these hypotheses. A necessary component of both models is a phase in which there is partial dissociation of the initial cell body from the GBM, in places where it was originally fully attached along its entire basal membrane.

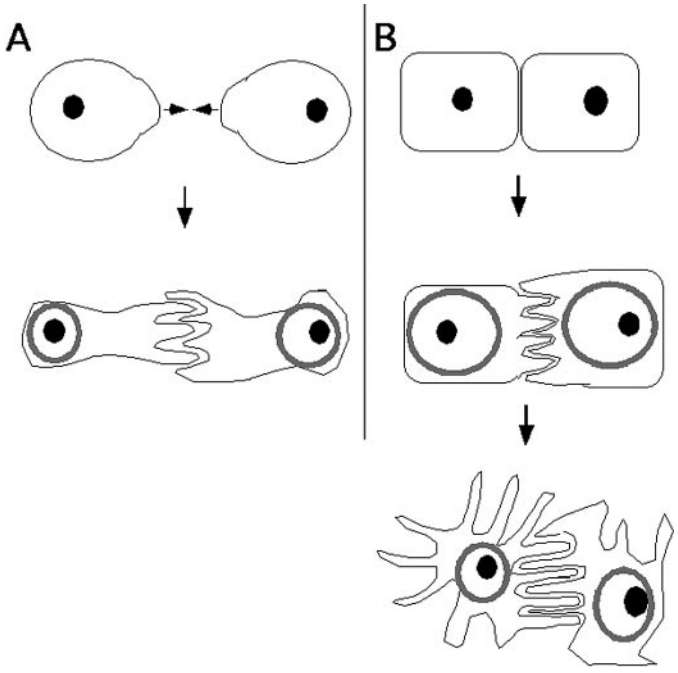

Figure 2. Two models of foot process formation. These are apical views from "on top" of the podocytes. For clarity, only two adjacent podocytes are depicted in both cases. (A) Two primitive podocytes that have separated from each other begin to extend processes that meet and become interdigitated. For simplicity, only one major extension is shown, although several would normally extend from the full circumference of the podocyte. (B) Two podocytes, which begin as columnar epithelial cells, always remain attached to each other along the basal end of their lateral membranes. Their common cell-cell junction is remodeled from a simple linear one to one that interdigitates and that contains the slit-diaphragm complex. The heavy lines represent the cell body of the podocyte, which loses attachment to other podocytes, except at the cell-cell junction between foot processes.

Maturation of the GBM

The maturation of the GBM has been the subject of several recent reviews (12,13) and is briefly dealt with here. The earliest epithelial cells of the nephron mainly express laminin-1. As soon as it is possible to define a nascent GBM, *i.e.*, the basal lamina underlying the layer of future podocytes, a shift in laminin expression to isoforms containing the α4 subunit is observed (14–16). Upon further maturation of the GBM, there is a second shift to the expression of α5 and β2 subunits, which are components of laminin 11, and this continues to be the major laminin isoform in the mature GBM (14–16). There is also a shift in the expression of type IV collagen (17). The early nephron mainly expresses the α1 (IV) and α2 (IV) subunits (17); upon maturation of the GBM, there is a shift to α3, α4, and α5 (IV) subunits. Other major components of the GBM are nidogen (entactin) and heparan sulfate proteoglycans, most notably agrin (13).

Slit-Diaphragm

The slit-diaphragm (SD) has also been the subject of many recent reviews (18–20). This is a structure observed by electron microscopy between adjacent foot processes, which is a major component of the protein barrier between the circulation and Bowman's space. The SD has, in recent years, been the focus of intense study, as mutations in several genes encoding protein components of the SD have been found to be the cause of various forms of childhood kidney disease (21). The first of these was nephrin; mutations in the NPHS1 gene that encodes nephrin are responsible for the Finnish form of congenital nephrotic syndrome (22). Nephrin is a transmembrane protein localized to the SD (23–25) that was originally thought to provide the structural link between two foot processes, without which it is not possible to maintain the foot process structure. Recent work has suggested a more complicated model. First, mice containing a targeted mutation in the nephrin gene were surprisingly capable of assembling foot processes, although the SD structure itself was not apparent (26–28). These mice suffered from heavy proteinuria during the neonatal period and died within a few days, with foot process effacement observed before death. These results confirm that the SD itself is a major component of the protein barrier and that the GBM by itself is not sufficient. Second, they indicate that there must be other structural molecules besides nephrin responsible for the initial assembly of foot processes. Recently, additional members of the nephrin family, neph1 to neph3, have been identified (29,30). Neph1-deficient mice also develop heavy proteinuria (30), indicating that neph1 is also an important component of the protein barrier.

Two other proteins have also been identified as important components of the SD: podocin and CD2-AP (18,31–34). Podocin, the product of the NPHS2 locus, was identified as the product of a gene involved in autosomal recessive steroid-

resistant nephrotic syndrome (35). Podocin is a transmembrane protein that interacts with nephrin and neph1 (29,31). CD2-AP, originally identified as an important protein in lymphocytes, was found to also be important in the kidney when CD2-AP knockout mice developed glomerular disease (36). CD2AP is also associated with the SD. Podocin, CD2AP, and nephrin all appear to be present in a complex within lipid rafts (31,37), which may also be associated with the cytoskeleton (38).

Foot processes can be assembled in the absence of nephrin; it is therefore necessary to formulate new hypotheses about the role of the SD complex in foot process assembly. As alluded to above, it is possible that Neph1 is partially redundant with nephrin. Alternatively, it is possible that the driving forces responsible for the cytoskeletal reorganization involved in foot process assembly are entirely anchored at the adhesion apparatus linking the podocyte to the GBM. Finally, it is possible that there are additional molecules at the SD involved in cytoskeletal reorganization. Two additional adhesion molecules have been described at the SD: P-cadherin and a large proto-cadherin named FAT (39,40). Finding P-cadherin at the SD suggests that this structure or some subset of its components may bear some relationship to adherens junctions found in more traditional epithelial cells. However, the importance of P-cadherin at the SD is obscured by the failure to find significant renal dysfunction in P-cadherin knockout mice, although their kidneys have not been closely examined (41). FAT is a large proto-cadherin, containing a much larger extracellular domain than traditional cadherins (42,43). It was first defined as a tumor suppressor gene in drosophila (44). Its function in mammalian cells is unknown.

ZO-1 is a protein most commonly associated with tight junctions, but it is also sometimes associated with adherens junctions (45,46). ZO-1 is found at the cytoplasmic face of the SD (47), providing another indication that the SD complex may functionally resemble an epithelial cell-cell junction. The observation that cell junctional proteins are found at the SD may have implications for the assembly of foot processes. Podocytes begin as columnar epithelial cells, and adjacent foot processes are always from different podocytes; it is possible that the SD complex begins as a cell-cell junction between adjacent epithelial cells and is conserved as these cells otherwise dissociate from each other. However, this model does not explain how the extensive interdigitation would occur to achieve mature foot processes.

Podocyte Differentiation

Podocytes are distinguished as a separate population from other cells in the developing nephron during the transition from the S-shaped body to the distinct glomerulus. At this time, they are expressing podocyte-specific markers such as WT1 and nephrin (22,48,49), which are not expressed in other cells of the nephron (in the case of nephrin) or whose expression pattern becomes restricted to podocytes (*e.g.*, WT1). Little is known about the molecular genetic basis for segmentation of the nephron, particularly which genes act to define the podocyte *versus* tubular lineages. There is speculation that genes generally involved in segmentation, such as Hox genes or members of the Notch family, may be involved, but there is no published evidence of a role for any of these genes in segmentation of the mammalian nephron. However, in the development of the frog pronephros, inhibition of the Notch signaling pathway expands the domain of Wt1 expression (50), the latter usually associated with development of the glomus. Thus, this provides support for the involvement of the Notch signaling pathway in segmentation of the nephron. It should be noted that because of the extraordinary convolution of the mammalian nephron, analysis of segmentation will be a difficult proposition, as it is not straightforward to recognize the various nephronic segments within the nephrogenic zone of a developing kidney. It is therefore likely that our understanding of segmentation will require further studies in frogs and possibly zebrafish before this can be adequately approached in mammalian nephrons.

Transcription Factors in Podocytes

WT1. The transcription factors best studied in podocytes include WT1, Lmx1b, Pod1, and Kreisler. WT1 is a zinc finger transcription factor and RNA binding protein first identified as a tumor suppressor gene for Wilms tumor, a tumor of the kidney observed in young children (reviewed in reference 51). WT1 is expressed in the metanephric mesenchyme at the beginning of kidney development, but as the S-shaped tubules appear, expression of WT1 becomes restricted to the podocyte lineage, where it is maintained throughout adult life, albeit at lower levels than in developing glomeruli (48,52). Mouse embryos deficient in WT1 suffer from complete renal agenesis (53); thus the Wt1 knockout is not informative about a role for WT1 in podocyte differentiation or function. It must also be stated that, although there is copious literature on the molecular function of WT1, including the identification of many genes that are putative regulatory targets (51), much of this work has not been very informative of a role for WT1 in podocytes. Fortunately, recent studies have begun to shed more light on the role of WT1 in podocytes.

The WT1 gene has two alternatively spliced exons, producing four major splice forms of WT1 mRNA (54). There are several alternative translational start sites, such that at least 16 distinct peptides are possible (55), although there is little information on distinct functions of peptides with different start sites. The first alternatively spliced exon, exon 5, inserts 17 amino acids in between the proline-rich amino terminal domain of WT1 and the four zinc finger domains (54). Mice unable to express WT1 containing exon 5 are normal (56), suggesting that isoforms containing exon 5 do not have a major role in kidney development or function. The other alternative splice site is not a separate exon, but rather features two distinct splice donor sites at the end of exon 9 such that three amino acids, lysine-threonine-serine (KTS), can be alternatively spliced in between exons 9 and 10 (54). The KTS sequence changes the structure of the third zinc finger, altering its DNA binding capabilities (57).

Denys-Drash Syndrome (DDS) results from mutations that affect the DNA-binding ability of the WT1 zinc finger domains (58). DDS involves a diffuse mesangial sclerosis within the

glomeruli. Because the mutant forms of WT1 found in DDS presumably maintain the ability to interact with putative WT1-interacting proteins through the proline-rich amino terminal domain, it is hypothesized that the pathologic sequelae associated with DDS are due to a dominant-negative phenotype (59). A recent study from our laboratory involved the derivation of transgenic mice that expressed a DDS truncation mutation of WT1 specifically in podocytes (60). Podocyte differentiation, as judged from the expression of GBM, SD, and cytoskeletal proteins, appeared unaffected by this mutation, but there was abnormal development of the glomerular capillaries and decreased expression of PECAM on capillary endothelial cells (60). These results suggested that WT1 might mainly be responsible for directing expression of growth factors that regulate development of the glomerular vasculature, rather than regulating the intrinsic differentiation of the podocyte itself. A contrasting conclusion was obtained in a recent study using an immortalized kidney cell line, which suggested that WT1 may regulate expression of podocalyxin, a highly charged podocyte cell surface protein that is thought to be involved in maintaining separation between podocytes due to electrostatic charge repulsion (61). In the aforementioned transgenic study, podocalyxin expression appeared unchanged in glomeruli of transgenic mice (60).

A recent study by Hammes *et al.* (62) was more pertinent to Frasier syndrome, which also involves mutations in WT1, which lead to glomerulosclerosis. Frasier syndrome results from dominant splice donor site mutations that render an allele unable to express the +KTS form of WT1, thus altering the splice form ratio of WT1. Hammes *et al.* (62) targeted mutations to the murine Wt1 gene that resulted in the inability to express either the −KTS or +KTS forms of WT1. Both mutations resulted in abnormal glomerular development, although mice only able to express the +KTS isoform had a more severe phenotype, suggesting that there may be an earlier or broader requirement for the −KTS isoform during glomerular development (62). Mice containing one normal and one −KTS only allele reproduced the Frasier phenotype, with glomerulosclerosis developing several weeks after birth. Interestingly, the expression of nephrin, a component of the slit diaphragm, was decreased in these knockout mice (62), again in contrast to studies with the WT1 transgene (60). Together, these studies emphasize the complexity of the Wt1 gene, and the difficulty in making progress in achieving a full understanding of its function during podocyte differentiation.

LMX1B. LMX1B is a LIM-homeodomain transcription factor, mutations of which are responsible for nail-patella syndrome, a condition that may involve glomerular disease (63–66). LMX1B is expressed in podocytes, and recent studies involving LMX1B knockout mice demonstrated reduced assembly of podocyte foot processes and reduced expression of CD2AP, podocin, and the α3 and α4 chains of type IV collagen in LMX1B-deficient glomeruli (65,66). LMX1B binding sites have been found in the upstream regions of these target genes (65,66), indicating a major role for LMX1B in regulating podocyte-specific gene expression.

Pod1. Pod1 (also known as epicardin and capsulin) is a basic helix-loop-helix (bHLH) transcription factor expressed early in kidney development and subsequently in podocytes of S-shaped bodies (67,68). Expression persists in podocytes of adult kidneys. In Pod1 knockout mice, glomerular development appears arrested at the single capillary loop stage (68). In most Pod1-deficient glomeruli, 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 the requirement for Pod1 in podocyte differentiation and glomerular development is slightly before the time when podocytes would normally begin migrating around the capillary loops and assembling foot processes. At present, there is no information on possible Pod1 target genes in podocytes.

Kreisler. Kreisler (MafB) is a basic domain leucine zipper (bZip) transcription factor of the Maf subfamily, expressed in podocytes of capillary loop stage glomeruli (69). It has previously been shown to have an important role in segmentation of the hindbrain. Expression is maintained in mature podocytes. There is a failure of foot process formation in Kreisler-deficient podocytes, though similarly to Pod1 mutants, podocytes of Kreisler mutants have lost their lateral cell-cell attachments (69). Pod1 is expressed in Kreisler mutant podocytes, indicating that Kreisler is more likely acting either downstream or in a separate pathway from Pod1 (69). There is a small decrease in expression of podocin and nephrin in Kreisler mutants, although this probably does not, by itself, account for the glomerular phenotype.

Mf2. Mf2 (mesoderm/mesenchyme forkhead 2) encodes a forkhead/winged helix transcription factor expressed in the developing kidney, among other tissues in the developing embryo (70). It is expressed in the condensed mesenchyme; as the nephron matures, MF2 expression appears to be restricted to the podocytes, similarly to Wt1. Mf2-deficient mice are generally normal, but some experience renal hypoplasia (70). The morphology of podocytes in these hypoplastic kidneys has not been reported.

Podocyte Adhesion Molecules

Two major adhesion complexes are involved in interactions between the podocyte and the GBM, $\alpha3\beta1$ integrin, and the α,β dystroglycan complex. $\alpha3\beta1$ is the major integrin expressed by podocytes (71), although low levels of $\alpha6\beta4$ are also detected (72). The importance of $\alpha3\beta1$ integrin in podocyte differentiation first became apparent when the knockout of the $\alpha3$ integrin gene resulted in an inability to assemble foot processes, and $\alpha3\beta1$ integrin neonatal mice do not survive more than a few hours after birth, most likely due to glomerular dysfunction (73). The GBM itself is fragmented, suggesting a role for $\alpha3\beta1$ integrin in maintaining the structural organization of the GBM. Interestingly, the portion of the $\alpha3\beta1$ integrin-deficient podocyte in contact with the GBM appears fully adhered (73). Thus a simple role of an adhesion receptor is not appropriate for $\alpha3\beta1$, as this might suggest that podocyte should adhere more loosely to an adjacent extracellular matrix.

This is consistent with findings in other cells which suggest a regulatory role for $\alpha3\beta1$ integrin (74).

$\alpha3\beta1$ integrin is a receptor for laminin-5 and laminin-11 (75,76), the latter of which is a major component of the GBM (16). Older studies on $\alpha3\beta1$ had also ascribed roles as a receptor for laminin-1, collagen, and fibronectin, but it is currently thought that $\alpha3\beta1$ acts mainly as a receptor for these other isoforms of laminin (reviewed in reference 77). $\alpha3\beta1$ is expressed early in podocyte differentiation, probably before the transition from laminin-1 to laminin-11 (71). It is not known with what ligand $\alpha3\beta1$ integrin interacts before expression of laminin-11; it may interact weakly with laminin-1, or perhaps with small amounts of laminin-11 that are beginning to accumulate in the GBM. It should also be noted that the preferential binding of $\alpha3\beta1$ to laminin-11 is described in tissue culture studies; it is possible that the preference for laminin-11 is not as dramatic in the *in vivo* situation. CD151, a member of the tetraspanin family, is often if not always co-expressed with $\alpha3\beta1$ integrin, including in podocytes (78,79). A tight interaction between $\alpha3\beta1$ and CD151 has been defined, which also appears to recruit certain isoforms of PKC to a site on CD151 (79,80). In general, tetraspanins appear to have a role in cell-cell adhesion (81), rather than in cell-matrix adhesion; thus the functional consequence of the $\alpha3\beta1$:CD151 interaction in podocytes is not well understood.

Less is known about the role of the dystroglycan complex in podocytes. The dystroglycan knockout results in early embryonic lethality (82), making it impossible to study dystroglycan-deficient podocytes. However, conditionally mutant dystroglycan mice have now been published (83) that may be crossed with mice expressing Cre recombinase in podocytes (84,85) to address this question.

Other Cell Surface Proteins

Podocalyxin is a highly sulfated cell surface sialomucin-charged protein found on the cell-surface of podocytes (86). It is hypothesized to have a role in maintaining podocyte cell separation through charge repulsion, and expression of podocalyxin in MDCK cells leads to cell separation, consistent with this possibility (87). Mice deficient in podocalyxin are unable to assemble foot processes or slit diaphragms and are anuric, succumbing during the first 24 h after birth (88).

GLEPP1is a receptor tyrosine phosphatase present on the apical cell surface of podocytes (89). GLEPP1-deficient mice are viable but have reduced filtration and are more susceptible to hypertension in a unilateral nephrectomy model (90).

Podocyte Cytoskeletal Proteins

It is assumed that specialized cytoskeletal organization is the basis for foot process assembly, although our understanding of this process is at its earliest stages. Synaptopodin is an actin-associated protein found in podocytes and in a restricted population of cells in the nervous system (91). How synaptopodin might function in foot process assembly or other aspects of podocyte cytoskeletal organization is not known.

α-actinin 4 is an isoform of α-actinin, an actin-binding protein, expressed in podocytes (92). α-actinin 4 first came to prominence in the field of podocyte biology when mutations in the ACTN4 gene were identified in a familial form of focal segmental glomerulosclerosis (92). It is not understood why these mutations allow normal podocyte development, but then lead to disease later in life.

Podocytes and the Expression of Signaling Molecules

Podocytes express growth factors that have the potential to affect glomerular development. Most notably, they express VEGF-A and angiopoietin-1, which may affect the development of the glomerular capillaries (4,93,94). VEGF is generally involved in stimulating capillary growth, and angiopoietin-1 with the remodeling and maturation of capillaries (94,95). There is a growing body of evidence suggesting that VEGF is not only involved in the formation, but also in the maintenance of glomerular capillaries throughout life, and that failure to properly maintain the capillary environment may be a major component of glomerular disease (96,97). Thus this area warrants additional study.

Notch2 is a member of the Notch family family of signaling molecules that are involved in cell fate specification. Notch2 is expressed in podocytes; in Notch2-deficient mice, there is a failure to develop the normal glomerular vasculature (98). Jagged 1, a ligand of Notch2, was expressed in the adjacent endothelial and/or mesangial cells, providing additional support for the concept that glomerular vascular development depends on signals from podocytes (98).

Future Directions

There is much yet to be learned about podocytes and their interactions with adjacent cells. Although there are many genetic and pathologic models in which foot process assembly is diminished or prevented, our understanding of the molecular processes that establish foot processes is still in its infancy. In particular, it will be important to establish the relative roles of the SD complex and GBM adhesion apparatus in foot process assembly.

It will also be important to identify additional targets of the transcription factors expressed in podocytes, as well as whether there are additional transcription factors regulating podocyte differentiation that are yet to be identified. Potential targets whose regulated expression pattern may affect podocyte differentiation include structural molecules involved in foot process formation, as well as growth factors that affect capillary development. Another area of investigation important in podocyte differentiation as it relates to the segmentation of the nephron, *i.e.*, during the formation of the comma and S-shaped tubules, is what regulatory events determine the identity of particular glomerular cells and distinguish them from the proximal tubule, as well as those events that segment the tubule itself. The recent derivation of mouse strains that allow podocyte-specific gene targeting (84,85) and immortalized podocyte cells lines (84,85,99) will greatly aid future efforts.

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References

1. Robert B, Abrahamson DR: Control of glomerular capillary development by growth factor/receptor kinases. *Pediatr Nephrol* 16: 294–301, 2001
2. Abrahamson DR: Glomerular endothelial cell development. *Kidney Int* 56: 1597–1598, 1999
3. Woolf AS, Loughna S: Origin of glomerular capillaries: Is the verdict in? *Exp Nephrol* 6: 17–21, 1998
4. Abrahamson DR, Robert B, Hyink DP, St John PL, Daniel TO: Origins and formation of microvasculature in the developing kidney. *Kidney Int Suppl* 67: S7–S11, 1998
5. Osathanondh V, Potter EL: Development of the human kidney as shown by microdissection II. Renal pelvis, calyces, and papillae. *Arch Pathol* 76: 53–65, 1963
6. Osathanondh V, Potter EL: Development of the human kidney as shown by microdissection III. Formation and interrelationship of collecting tubules and nephrons. *Arch Pathol* 76: 66–78, 1963
7. Osathanondh V, Potter EL: Development of the human kidney as shown by microdissection I. Preparation of tissue with reasons for possible misinterpretations of observations. *Arch Pathol* 1963: 47–52, 1963
8. Osathanondh V, Potter EL: Development of the human kidney as shown by microdissection IV. Development of tubular portions of nephrons. *Arch Pathol* 82: 391–402, 1966
9. Osathanondh V, Potter EL: Development of the human kidney as shown by microdissection V. Development of vascular pattern of the glomerulus. *Arch Pathol* 82: 403–411, 1966
10. Potter EL: Development of the human glomerulus. *Arch Pathol* 80: 241–255, 1965
11. Vasioukhin V, Bauer C, Yin M, Fuchs E: Directed actin polymerization is the driving force for epithelial cell-cell adhesion. *Cell* 100: 209–219, 2000
12. St John PL, Abrahamson DR: Glomerular endothelial cells and podocytes jointly synthesize laminin-1 and -11 chains. *Kidney Int* 60: 1037–46, 2001
13. Miner JH: Developmental biology of glomerular basement membrane components. *Curr Opin Nephrol Hypertens* 7: 13–9, 1998
14. Miner JH, Sanes JR: Molecular and functional defects in kidneys of mice lacking collagen alpha 3(IV): implications for Alport syndrome. *J Cell Biol* 135: 1403–1413, 1996
15. Miner JH: Renal basement membrane components. *Kidney Int* 56: 2016–2024, 1999
16. Miner JH, Patton BL: Laminin-11. *Int J Biochem Cell Biol* 31: 811–816, 1999
17. Miner JH, Sanes JR: Collagen IV alpha 3, alpha 4, and alpha 5 chains in rodent basal laminae: Sequence, distribution, association with laminins, and developmental switches. *J Cell Biol* 127: 879–891, 1994
18. Miner JH: Focusing on the glomerular slit diaphragm: podocin enters the picture. *Am J Pathol* 160: 3–5, 2002
19. Tryggvason K, Wartiovaara J: Molecular basis of glomerular permselectivity. *Curr Opin Nephrol Hypertens* 10: 543–549, 2001
20. Deen WM, Lazzara MJ, Myers BD: Structural determinants of glomerular permeability. *Am J Physiol Renal Physiol* 281: F579–F596, 2001
21. Jalanko H, Patrakka J, Tryggvason K, Holmberg C: Genetic kidney diseases disclose the pathogenesis of proteinuria. *Ann Med* 33: 526–533, 2001
22. Kestila M, Lenkkeri U, Mannikko M, Lamerdin J, McCready P, Putaala H, Ruotsalainen V, Morita T, Nissinen M, Herva R, Kashtan CE, Peltonen L, Holmberg C, Olsen A, Tryggvason K: Positionally cloned gene for a novel glomerular protein–nephrin–is mutated in congenital nephrotic syndrome. *Mol Cell* 1: 575–582, 1998
23. Holzman LB, St John PL, Kovari IA, Verma R, Holthofer H, Abrahamson DR: Nephrin localizes to the slit pore of the glomerular epithelial cell. *Kidney Int* 56: 1481–1491, 1999
24. Holthofer H, Ahola H, Solin ML, Wang S, Palmen T, Luimula P, Miettinen A, Kerjaschki D: Nephrin localizes at the podocyte filtration slit area and is characteristically spliced in the human kidney. *Am J Pathol* 155: 1681–1687, 1999
25. Ruotsalainen V, Ljungberg P, Wartiovaara J, Lenkkeri U, Kestila M, Jalanko H, Holmberg C, Tryggvason K: Nephrin is specifically located at the slit diaphragm of glomerular podocytes. *Proc Natl Acad Sci USA* 96: 7962–7967, 1999
26. Putaala H, Soininen R, Kilpelainen P, Wartiovaara J, Tryggvason K: The murine nephrin gene is specifically expressed in kidney, brain and pancreas: Inactivation of the gene leads to massive proteinuria and neonatal death. *Hum Mol Genet* 10: 1–8, 2001
27. Hamano Y, Grunkemeyer JA, Sudhakar A, Zeisberg M, Cosgrove D, Morello R, Lee B, Sugimoto H, Kalluri R: Determinants of vascular permeability in the kidney glomerulus. *J Biol Chem* 277: 31154–31162, 2002
28. Rantanen M, Palmen T, Patari A, Ahola H, Lehtonen S, Astrom E, Floss T, Vauti F, Wurst W, Ruiz P, Kerjaschki D, Holthofer H: Nephrin TRAP mice lack slit diaphragms and show fibrotic glomeruli and cystic tubular lesions. *J Am Soc Nephrol* 13: 1586–1594, 2002
29. Sellin L, Huber TB, Gerke P, Quack I, Pavenstadt H, Walz G: NEPH1 defines a novel family of podocin-interacting proteins. *FASEB J* 2002, in press
30. Donoviel DB, Freed DD, Vogel H, Potter DG, Hawkins E, Barrish JP, Mathur BN, Turner CA, Geske R, Montgomery CA, Starbuck M, Brandt M, Gupta A, Ramirez-Solis R, Zambrowicz BP, DR Powell: Proteinuria and perinatal lethality in mice lacking NEPH1, a novel protein with homology to NEPHRIN. *Mol Cell Biol* 21: 4829–4836, 2001
31. Schwarz K, Simons M, Reiser J, Saleem MA, Faul C, Kriz W, Shaw AS, Holzman LB, Mundel P: Podocin, a raft-associated component of the glomerular slit diaphragm, interacts with CD2AP and nephrin. *J Clin Invest* 108: 1621–1629, 2001
32. Shih NY, Li J, Cotran R, Mundel P, Miner JH, Shaw AS: CD2AP localizes to the slit diaphragm and binds to nephrin via a novel C-terminal domain. *Am J Pathol* 159: 2303–2308, 2001
33. Li C, Ruotsalainen V, Tryggvason K, Shaw AS, Miner JH: CD2AP is expressed with nephrin in developing podocytes and is found widely in mature kidney and elsewhere. *Am J Physiol Renal Physiol* 279: F785–F792, 2000
34. Roselli S, Gribouval O, Boute N, Sich M, Benessy F, Attie T, Gubler MC, Antignac C: Podocin localizes in the kidney to the slit diaphragm area. *Am J Pathol* 160: 131–139, 2002
35. Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fuchshuber A, Dahan K, Gubler MC, Niaudet P, Antignac C: NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet* 24: 349–354, 2000

36. Shih NY, Li J, Karpitskii V, Nguyen A, Dustin ML, Kanagawa O, Miner JH, Shaw AS: Congenital nephrotic syndrome in mice lacking CD2-associated protein. *Science* 286: 312–315, 1999
37. Simons M, Schwarz K, Kriz W, Miettinen A, Reiser J, Mundel P, Holthofer H: Involvement of lipid rafts in nephrin phosphorylation and organization of the glomerular slit diaphragm. *Am J Pathol* 159: 1069–1077, 2001
38. Yuan H, Takeuchi E, Salant DJ: Podocyte slit-diaphragm protein nephrin is linked to the actin cytoskeleton. *Am J Physiol Renal Physiol* 282: F585–F591, 2002
39. Reiser J, Kriz W, Kretzler M, Mundel P: The glomerular slit diaphragm is a modified adherens junction. *J Am Soc Nephrol* 11: 1–8, 2000
40. Inoue T, Yaoita E, Kurihara H, Shimizu F, Sakai T, Kobayashi T, Ohshiro K, Kawachi H, Okada H, Suzuki H, Kihara I, Yamamoto T: FAT is a component of glomerular slit diaphragms. *Kidney Int* 59: 1003–1012, 2001
41. Radice GL, Ferreira-Cornwell MC, Robinson SD, Rayburn H, Chodosh LA, Takeichi M, Hynes RO: Precocious mammary gland development in P-cadherin-deficient mice. *J Cell Biol* 139: 1025–1032, 1997
42. Cox B, Hadjantonakis AK, Collins JE, Magee AI: Cloning and expression throughout mouse development of mfat1, a homologue of the Drosophila tumour suppressor gene fat. *Dev Dyn* 217: 233–240, 2000
43. Ponassi M, Jacques TS, Ciani L, ffrench Constant C: Expression of the rat homologue of the Drosophila fat tumour suppressor gene. *Mech Dev* 80: 207–212, 1999
44. Mahoney PA, Weber U, Onofrechuk P, Biessmann H, Bryant PJ, Goodman CS: The fat tumor suppressor gene in Drosophila encodes a novel member of the cadherin gene superfamily. *Cell* 67: 853–868, 1991
45. Itoh M, Nagafuchi A, Moroi S, Tsukita S: Involvement of ZO-1 in cadherin-based cell adhesion through its direct binding to alpha catenin and actin filaments. *J Cell Biol* 138: 181–192, 1997
46. Gonzalez-Mariscal L, Betanzos A, Avila-Flores A: MAGUK proteins: Structure and role in the tight junction. *Semin Cell Dev Biol* 11: 315–324, 2000
47. Schnabel E, Anderson JM, Farquhar MG: The tight junction protein ZO-1 is concentrated along slit diaphragms of the glomerular epithelium. *J Cell Biol* 111: 1255–1263, 1990
48. Armstrong JF, Pritchard-Jones K, Bickmore WA, Hastie ND, Bard JB: The expression of the Wilms' tumour gene. *WT* 1: in the developing mammalian embryo. *Mech Dev* 40: 85–97, 1993
49. Putaala H, Sainio K, Sariola H, Tryggvason K: Primary structure of mouse and rat nephrin cDNA and structure and expression of the mouse gene. *J Am Soc Nephrol* 11: 991–1001, 2000
50. McLaughlin KA, Rones MS, Mercola M: Notch regulates cell fate in the developing pronephros. *Dev Biol* 227: 567–580, 2000
51. Scharnhorst V, van der Eb AJ, Jochemsen AG: WT1 proteins: Functions in growth and differentiation. *Gene* 273: 141–161, 2001
52. Pelletier J, Schalling M, Buckler AJ, Rogers A, Haber DA, Housman D: Expression of the Wilms' tumor gene WT1 in the murine urogenital system. *Genes Dev* 5: 1345–1356, 1991
53. Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Housman D, Jaenisch R: WT-1 is required for early kidney development. *Cell* 74: 679–691, 1993
54. Haber DA, Sohn RL, Buckler AJ, Pelletier J, Call KM, Housman DE: Alternative splicing and genomic structure of the Wilms tumor gene WT1. *Proc Natl Acad Sci USA* 88: 9618–9622, 1991
55. Bruening W, Pelletier J: A non-AUG translational initiation event generates novel WT1 isoforms. *J Biol Chem* 271: 8646–8654, 1996
56. Natoli TA, McDonald A, Alberta JA, Taglienti ME, Housman DE, Kreidberg JA: A mammalian-specific exon of WT1 is not required for development or fertility. Molecular and Cellular Biology. *Mol Cell Biol* 2002, in press
57. Drummond IA, Rupprecht HD, Rohwer-Nutter P, Lopez-Guisa JM, Madden SL, Rauscher FR, Sukhatme VP: DNA recognition by splicing variants of the Wilms' tumor suppressor, WT1. *Mol Cell Biol* 14: 3800–3809, 1994
58. Pelletier J, Bruening W, Kashtan CE, Mauer SM, Manivel JC, Striegel JE, Houghton DC, Junien C, Habib R, L Fouser L, Fine RN, Silverman BL, Haber DA, Housman D: Germline mutations in the Wilms' tumor suppressor gene are associated with abnormal urogenital development in Denys-Drash syndrome. *Cell* 67: 437–447, 1991
59. Little MH, Williamson KA, Mannens M, Kelsey A, Gosden C, Hastie ND, van Heyningen V: Evidence that WT1 mutations in Denys-Drash syndrome patients may act in a dominant-negative fashion. *Hum Mol Genet* 2: 259–264, 1993
60. Natoli TA, Liu J, Eremina V, Hodgens K, Li C, Hamano Y, Mundel P, Kalluri R, Miner J, Quaggin S, JA Kreidberg: A mutant form of the Wilms' tumor suppressor gene WT1 observed in Denys-Drash Syndrome interferes with glomerular capillary development. *J Am Soc Nephrol* 13: 2058–2067, 2002
61. Palmer RE, Kotsianti A, Cadman B, Boyd T, Gerald W, Haber DA: WT1 regulates the expression of the major glomerular podocyte membrane protein Podocalyxin. *Curr Biol* 11: 1805–1809, 2001
62. Hammes A, Guo JK, Lutsch G, Leheste JR, Landrock D, Ziegler U, Gubler MC, Schedl A: Two splice variants of the Wilms' tumor 1 gene have distinct functions during sex determination and nephron formation. *Cell* 106: 319–329, 2001
63. Dreyer SD, Morello R, German MS, Zabel B, Winterpacht A, Lunstrum GP, Horton WA, Oberg KC, Lee B: LMX1B transactivation and expression in nail-patella syndrome. *Hum Mol Genet* 9: 1067–1074, 2000
64. Hamlington JD, Jones C,. McIntosh I: Twenty-two novel LMX1B mutations identified in nail patella syndrome (NPS) patients. *Hum Mutat* 18: 458, 2001
65. Rohr C, Prestel J, Heidet L, Hosser H, Kriz W, Johnson RL, Antignac C, Witzgall R: The LIM-homeodomain transcription factor Lmx1b plays a crucial role in podocytes. *J Clin Invest* 109: 1073–1082, 2002
66. Miner JH, Morello R, Andrews KL, Li C, Antignac C, Shaw AS, Lee B: Transcriptional induction of slit diaphragm genes by Lmx1b is required in podocyte differentiation. *J Clin Invest* 109: 1065–1072, 2002
67. Quaggin SE, Vanden Heuvel GB, Igarashi P: Pod-1: A mesoderm-specific basic-helix-loop-helix protein expressed in mesenchymal and glomerular epithelial cells in the developing kidney. *Mech Dev* 71: 37–48, 1998
68. Quaggin SE, Schwartz L, Cui S, Igarashi P, Deimling J, Post M, Rossant J: The basic-helix-loop-helix protein pod1 is critically important for kidney and lung organogenesis. *Development* 126: 5771–5783, 1999
69. Sadl V, Jin F, Yu J, Cui S, Holmyard D, Quaggin S, Barsh G, Cordes S: The mouse Kreisler (Krml1/MafB) segmentation gene is required for differentiation of glomerular visceral epithelial cells. *Dev Biol* 249: 16–29, 2002

70. Kume T, Deng K, Hogan BL: Minimal phenotype of mice homozygous for a null mutation in the forkhead/winged helix gene, Mf2. *Mol Cell Biol* 20: 1419–1425, 2000
71. Korhonen M, Ylanne J, Laitinen L, Virtanen I: The α1-α6 subunits of integrins are characteristically expressed in distinct segments of developing and adult human nephron. *J Cell Biol* 111: 1245–1254, 1990
72. Kambham N, Tanji N, Seigle RL, Markowitz GS, Pulkkinen L, Uitto J, D'Agati VD: Congenital focal segmental glomerulosclerosis associated with beta4 integrin mutation and epidermolysis bullosa. *Am J Kidney Dis* 36: 190–196, 2000
73. Kreidberg JA, Donovan MJ, Goldstein SL, Rennke H, Shepherd K, Jones RC, Jaenisch R: Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. *Development* 122: 3537–3547, 1996
74. Hodivala-Dilke KM, DiPersio CM, Kreidberg JA, Hynes RO: Novel roles for alpha3beta1 integrin as a regulator of cytoskeletal assembly and as a trans-dominant inhibitor of integrin receptor function in mouse keratinocytes. *J Cell Biol* 142: 1357–1369, 1998
75. Delwel GO, de Melker AA, Hogervorst F, Jaspars LH, Fles DL, Kuikman I, Lindblom A, Paulsson M, Timpl R, Sonnenberg A: Distinct and overlapping ligand specificities of the alpha 3A beta 1 and alpha 6A beta 1 integrins: recognition of laminin isoforms. *Mol Biol Cell* 5: 203–215, 1994
76. Kikkawa Y, Sanzen N, Sekiguchi K: Isolation and characterization of laminin-10/11 secreted by human lung carcinoma cells. laminin-10/11 mediates cell adhesion through integrin alpha3 beta1. *J Biol Chem* 273: 15854–15859, 1998
77. Kreidberg JA: Functions of alpha3beta1 integrin. *Curr Opin Cell Biol* 12: 548–553, 2000
78. Sterk LM, Geuijen CA, van den Berg JG, Claessen N, Weening JJ, Sonnenberg A: Association of the tetraspanin CD151 with the laminin-binding integrins alpha3beta1, alpha6beta1, alpha6beta4 and alpha7beta1 in cells in culture and in vivo. *J Cell Sci* 115: 1161–1173, 2002
79. Yauch RL, Berditchevski F, Harler MB, Reichner J, Hemler ME: Highly stoichiometric, stable, and specific association of integrin alpha3beta1 with CD151 provides a major link to phosphatidylinositol 4-kinase, and may regulate cell migration. *Mol Biol Cell* 9: 2751–2765, 1998
80. Zhang XA, Bontrager AL, Hemler ME: Transmembrane-4 superfamily proteins associate with activated protein kinase C (PKC) and link PKC to specific beta(1) integrins. *J Biol Chem* 276: 25005–25013, 2001
81. Berditchevski F, Odintsova E: Characterization of integrin-tetraspanin adhesion complexes: role of tetraspanins in integrin signaling. *J Cell Biol* 146: 477–492, 1999
82. Williamson RA, Henry MD, Daniels KJ, Hrstka RF, Lee JC, Sunada Y, Ibraghimov-Beskrovnaya O, Campbell KP: Dystroglycan is essential for early embryonic development: disruption of Reichert's membrane in Dag1-null mice. *Hum Mol Genet* 6: 831–841, 1997
83. Moore SA, Saito F, Chen J, Michele DE, Henry MD, Messing A, Cohn RD, Ross-Barta SE, Westra S, Williamson RA, Hoshi T, Campbell KP: Deletion of brain dystroglycan recapitulates aspects of congenital muscular dystrophy. *Nature* 418: 422–425, 2002
84. Moeller MJ, Sanden SK, Soofi A, Wiggins RC, Holzman LB: Two gene fragments that direct podocyte-specific expression in transgenic mice. *J Am Soc Nephrol* 13: 1561–1567, 2002
85. Eremina V, Wong MA, Cui S, Schwartz L, Quaggin SE: Glomerular-specific gene excision in vivo. *J Am Soc Nephrol* 13: 788–793 2002
86. Kerjaschki D, Sharkey DJ, Farquhar MG: Identification and characterization of podocalyxin–the major sialoprotein of the renal glomerular epithelial cell. *J Cell Biol* 98: 1591–1596, 1984
87. Takeda T, Go WY, Orlando RA, Farquhar MG: Expression of podocalyxin inhibits cell-cell adhesion and modifies junctional properties in Madin-Darby canine kidney cells. *Mol Biol Cell* 11: 3219–3232, 2000
88. Doyonnas R, Kershaw DB, Duhme C, Merkens H, Chelliah S, Graf T, McNagny KM: Anuria, omphalocele, and perinatal lethality in mice lacking the CD34-related protein podocalyxin. *J Exp Med* 194: 13–27, 2001
89. Thomas PE, Wharram BL, Goyal M, Wiggins JE, Holzman LB, Wiggins RC: GLEPP1, a renal glomerular epithelial cell (podocyte) membrane protein-tyrosine phosphatase. Identification, molecular cloning, and characterization in rabbit. *J Biol Chem* 269: 19953–19962, 1994
90. Wharram BL, Goyal M, Gillespie PJ, Wiggins JE, Kershaw DB, Holzman LB, Dysko RC, Saunders TL, Samuelson LC, Wiggins RC: Altered podocyte structure in GLEPP1 (Ptpro)-deficient mice associated with hypertension and low glomerular filtration rate. *J Clin Invest* 106: 1281–1290, 2000
91. Mundel P, Heid HW, Mundel TM, Kruger M, Reiser J, Kriz W: Synaptopodin: An actin-associated protein in telencephalic dendrites and renal podocytes. *J Cell Biol* 139: 193–204, 1997
92. Kaplan JM, Kim SH, North KN, Rennke H, Correia LA, Tong HQ, Mathis BJ, Rodriguez-Perez JC, Allen PG, Beggs AH, Pollak MR: Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. *Nat Genet* 24: 251–256, 2000
93. Woolf AS, Yuan HT: Angiopoietin growth factors and Tie receptor tyrosine kinases in renal vascular development. *Pediatr Nephrol* 16: 177–184, 2001
94. Yuan HT, Suri C, Yancopoulos GD, Woolf AS: Expression of angiopoietin-1, angiopoietin-2, and the Tie-2 receptor tyrosine kinase during mouse kidney maturation. *J Am Soc Nephrol* 10: 1722–1736, 1999
95. Woolf AS, Cale CM: Roles of growth factors in renal development. *Curr Opin Nephrol Hypertens* 6: 10–14, 1997
96. Ostendorf T, Kunter U, Eitner F, Loos A, Regele H, Kerjaschki D, Henninger DD, Janjic N, Floege J: VEGF(165) mediates glomerular endothelial repair. *J Clin Invest* 104: 913–923, 1999
97. Kang DH, Hughes J, Mazzali M, Schreiner GF, Johnson RJ: Impaired angiogenesis in the remnant kidney model: II. Vascular endothelial growth factor administration reduces renal fibrosis and stabilizes renal function. *J Am Soc Nephrol* 12: 1448–1457, 2001
98. McCright B, Gao X, Shen L, Lozier J, Lan Y, Maguire M, Herzlinger D, Weinmaster G, Jiang R, Gridley T: Defects in development of the kidney, heart and eye vasculature in mice homozygous for a hypomorphic Notch2 mutation. *Development* 128: 491–502, 2001
99. Saleem MA, O'Hare MJ, Reiser J, Coward RJ, Inward CD, Farren T, Xing CY, Ni L, Mathieson W, Mundel P: A conditionally immortalized human podocyte cell line demonstrating nephrin and podocin expression. *J Am Soc Nephrol* 13: 630–638, 2002