Sidekick-1 Is Upregulated in Glomeruli in HIV-Associated Nephropathy

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Abstract. Infection of podocytes by HIV-1 induces unique changes in phenotype, which contribute to the pathogenesis of glomerular disease in HIV-associated nephropathy (HIVAN). The host genetic pathways altered by HIV-1 infection that are responsible for these phenotypic changes are largely unknown. For identifying such pathways, representational difference analysis was performed comparing cDNA from HIV-1 transgenic podocytes with nontransgenic controls. In this way, a gene named sidekick-1 (sdk-1) was cloned, a transmembrane protein of the Ig superfamily that is highly upregulated in HIV-1 transgenic podocytes.Sdk-1 and its ortholog, sidekick-2 (sdk-2), were recently shown to guide axonal terminals to specific synapses in developing neurons. Their presence and role in other organs, including the kidney, has not been described. The current study demonstrates developmental expression of both sdk-1 and sdk-2 and a tight spatial and temporal regulation of these genes in kidney. During nephrogenesis, sidekick expression was observed first in ureteric bud and ureteric bud–derived tissues in a pattern similar to other genes known to play important roles in branching morphogenesis. In adult murine renal tissue, sidekick proteins were seen in glomeruli at low levels, and expression of sdk-1 was greatly upregulated in diseased HIV-1 transgenic mouse kidneys. In a human HIVAN kidney biopsy, sidekick expression was increased in glomeruli in a pattern consistent with the mouse model. It is proposed that the dysregulation of sdk-1 protein may play an important role in HIVAN pathogenesis.

HIV-associated nephropathy (HIVAN) is the third leading cause of ESRD in black individuals between the ages of 20 and 64 in the United States (1,2). The clinical and pathologic findings of HIVAN include focal and segmental glomerulosclerosis, often of the collapsing variant, combined with microcystic tubulointerstitial disease (3,4). The disease untreated is rapidly progressive, often leading to renal failure within weeks to months.

HIVAN occurs almost exclusively in blacks and Hispanics (5); of all causes of ESRD, only sickle cell disease is more closely associated with black race than HIVAN (6). This striking racial predilection suggests that genetic factors play an important role in disease susceptibility. Moreover, recent studies in the HIV-1 transgenic mouse model suggest that host genetic responses likely determine both susceptibility and progression of HIVAN (7,8). Thus, events downstream of infection are critical. The goal of the current studies was to identify unique pathways induced by HIV-1 infection that could determine susceptibility to disease. Furthermore, as blacks are at much higher risk of developing all forms of renal disease compared with the general population, pathways that are important in HIVAN pathogenesis may also explain the strong racial predilection for renal disease in this population in general.

Recent evidence demonstrates that HIV-1 directly infects renal epithelium (9) and replicates within this unique compartment (10). In individuals with HIVAN, the impact of viral expression is manifested by a profound change in the cellular phenotype. The predominant glomerular target of HIV-1 is the glomerular visceral epithelial cell, or podocyte. Podocytes are highly specialized cells that are critical in forming the kidney’s ultrafiltration barrier. Instead of remaining in a relatively quiescent differentiated state, podocytes respond to HIV-1 infection by dedifferentiating (11,12) and proliferating (13,14). Such proliferation, which is unique to HIVAN and idiopathic collapsing glomerulopathy (CG), is manifested by increased podocyte number, expression of Ki-67 and cyclin A, and downregulation of cyclin-dependent kinase inhibitors such as p27 and p57 (12,14). Podocyte dedifferentiation, also uniquely characteristic of HIVAN and CG, is typified by loss of typical differentiation markers such as synaptopodin and WT-1 (11,12).

HIV-1 infection seems to induce a characteristic proliferative response in podocytes, and we are just beginning to understand the mechanisms responsible. We now know, for example, that expression of HIV-1 as a transgene (13,15) or infection of podocytes by HIV-1 is required to induce these changes. In addition, recent studies have defined the Nef gene as the likely gene product responsible (16,17). More specifically, an interaction between the proline-rich domain of Nef
with Src kinases seems to initiate the cascade of events that leads to cellular proliferation. What is less well understood, however, are the host cellular and genetic responses to expression of HIV-1 in podocytes. Recently, we identified a novel small leucine-rich protein that is dramatically increased in infected podocytes (18). This protein, named podocan, seems to localize to the glomerular basement membrane and is greatly increased as a component of sclerotic glomerular lesions in experimental HIVAN.

In the current studies, we used representational difference analysis (RDA) of cDNA to isolate differentially expressed genes in HIV-1 transgenic podocytes. In this way, we identified a recently cloned gene that responds to HIV-1 infection named sidekick-1 (sdk-1). Sidekick was initially identified in Drosophila melanogaster as being important in specifying photoreceptor cell fate in the retina (19). More recently, sdk-1 and its ortholog, sidekick-2 (sdk-2), were shown to be important adhesion molecules in directing synapse formation in developing chicken retinas by guiding axon terminals to specific lamalae (20). However, the potential role for sidekicks in the development of other organ systems, including the kidney, is completely unknown. In the current article, we describe the regulation and expression of sidekicks in developing renal epithelium and demonstrate a dramatic upregulation of sdk-1 in the setting of HIV-1 infection. We propose that sdk-1 upregulation may play an important role in podocyte dysfunction in HIVAN.

Materials and Methods

Representational Difference Analysis of Podocyte cDNA

Temperature-sensitive conditionally immortalized HIV-1 transgenic and wild-type podocyte cell lines were previously established by breeding heterozygous HIV-1 transgenic mice (Tg26; FVB/N, pNL4-3-d1443 [-gag.-pol]) with H-2K\(^{b}\) –tsA58 Immortomice: Charles River Laboratories, Wilmington, MA) (13). Murine podocytes were isolated from the HIV-1 transgenic mice and nontransgenic littermates at the onset of detectable disease in the HIV-1 transgenic mice as described previously (13). Before the RDA was performed, these wild-type and HIV-1 transgenic podocytes were cultured for 2 wk under “nonpermissive” conditions to induce degradation of T antigen and allow for maximal differentiation. RDA of cDNA was then performed using the original protocol described by Hubank and Schatz (21) and as previously reported by Ross et al. (18). RDA was carried out through the generation of the second difference product.

For performing multifluid analysis of sdk-1 and sdk-2, organs were harvested from the E17 embryos and 4-mo-old adult mice. For analyzing the temporal regulation of sdk-1 and -2, kidneys were isolated from embryos and harvested at embryonic days 12 to 18 and from postnatal mice ages 0 to 64 d at various intervals. Northern blot analysis was performed as above using 12 \(\mu\)g (multitissue northern) and 15 \(\mu\)g (temporal northern) of total RNA per sample. Full-length sdk-1 and the N-terminal 3.2 kb of sdk-2 were used as the probes.

Analysis of Sidekick RNA Expression

Total RNA was extracted from cultured podocytes using TriReagent (Sigma). Messenger RNA was purified using the FastTrack mRNA Isolation Kit (Invitrogen). Two micrograms of mRNA was separated by agarose gel electrophoresis, transferred to a 0.45-\(\mu\)m Biodyne A nylon membrane (Pall Gelman Laboratory), and probed with the P\(^{32}\)-labeled 1.0-kb difference product.

For performing multifluid analysis of sdk-1 and sdk-2, organs were harvested from both E17 embryos and 4-mo-old adult mice. For analyzing the temporal regulation of sdk-1 and -2, kidneys were isolated from embryos and harvested at embryonic days 12 to 18 and from postnatal mice ages 0 to 64 d at various intervals. Northern blot analysis was performed as above using 12 \(\mu\)g (multitissue northern) and 15 \(\mu\)g (temporal northern) of total RNA per sample. Full-length sdk-1 and the N-terminal 3.2 kb of sdk-2 were used as the probes.

Production and Characterization of Anti-Sidekick Antibodies

The Stal-EcoNI fragment of sdk-1 cDNA was subjected to Klenow polymerase fill in reaction and then cloned into the Apal site of the PGEX-4T2 bacterial expression vector (Amersham Pharmacia). The resulting glutathione S-transferase fusion protein was produced in BL21 cells and purified using B-Per GST Protein Purification Kit (Pierce) as per the manufacturer’s protocol. The purified fusion protein was then provided to Research Genetics (Invitrogen) for production of rabbit anti-sidekick polyclonal antisera.

The BamHI-BamHI fragment of murine sdk-1 was cloned into the BamHI site of pcDNA3.1 (Invitrogen), producing a full-length sdk-1 expression vector. The EcoRV-EcoRI fragment of murine sdk-2 was cloned into the KpnI (blunted with T4 DNA polymerase) and EcoRI sites of pcDNA4/HisMax (Invitrogen), resulting in a carboxyl termi-
nal (900 bases) sdk-2 expression vector. Sdk-1, partial sdk-2, and corresponding control vectors were transfeeted into HEK-293T cells using Lipofectamine 2000 (Invitrogen). After 48 h, the cells were washed three times in 1× PBS and lysed in Bug Buster (Novagen) supplemented with 1× complete protease inhibitor cocktail (Roche) and 1 µl/ml nuclease (Novagen). The samples were separated by SDS-PAGE and transferred to Immobilon-P transfer membranes (Millipore). Western blots were performed using 1:500 dilutions of anti-sidekick and preimmune sera and a 1:10,000 dilution of horse-radish peroxidase (HRP)-labeled goat anti-rabbit IgG (Kirkegaard and Perry).

**Immunolocalization of Sidekicks in Kidney**

Kidneys were isolated from E12.5 and E15.5 mice, fixed for 2 h in 4% paraformaldehyde in 1× PBS, and then paraffin-embedded. Paraffin-embedded human fetal kidney (24 wk) sections were similarly prepared. After dewaxing and rehydration through graded ethanols, endogenous peroxidase activity was blocked in 7.5% H₂O₂ for 10 min. Anti-sidekick and preimmune sera were used at 1:500 dilutions of a 1-h incubation at room temperature. A biotinylated goat anti-rabbit antibody (Kirkegaard and Perry) was used as the secondary antibody at a 1:200 dilution. Streptavidin/biotin-HRP complex was introduced using Vectastain Elite ABC Kit (Vector Labs). Color reaction was created using AEC Substrate Kit for Horse-radish Peroxidase (Vector Labs).

Kidneys from adult HIV-1 transgenic and age-matched wild-type mice were harvested and immediately frozen in OCT Compound (Tissue-Tek). Thin sections (5 µm) were cut using the CryoJane Tape-Transfer System (Instrumedics) and fixed for 7 min in ice-cold acetone. Endogenous peroxide was blocked by incubation in 7.5% H₂O₂ for 10 min. Tissue staining was performed using the TSA Cy3 System (Perkin Elmer) as per the manufacturer’s protocol. The anti-sidekick and preimmune sera were used at 1:500 dilution for 2 h at room temperature.

Frozen kidney sections (8 µm) were prepared from an adult patient with biopsy-confirmed HIVAN and from a block of adult normal kidney. Endogenous peroxide was blocked in 0.3% H₂O₂ in methanol for 60 min. Endogenous biotin was also blocked with Avidin-Biotin Blocking Kit (Vector Labs). Tissue staining was performed using the TSA HRP System (Perkin Elmer) as per the manufacturer’s protocol at 1:500 dilutions of primary antibody. Color reaction was generated using AEC Substrate Kit (Vector Labs).

For performing semiquantitative RT-PCR, total kidney RNA was extracted from age-matched HIV-1 transgenic and wild-type adult mice using Tri-Reagent (Sigma). RT (Superscript; Invitrogen) was extracted from age-matched HIV-1 transgenic and wild-type adult mice using Tri-Reagent (Sigma). RT (Superscript; Invitrogen) was performed on 1 µg of both RNA samples using an oligo-dT primer. The use of consecutive dilutions of cDNA as templates, input RT was normalized using primers specific for the housekeeping gene glyceraldehyde-3 phosphate dehydrogenase. PCR amplification of sdk-1 and sdk-2 was then performed using the normalized samples. Primers were designed to amplify either sdk-1 or sdk-2 specifically (sdk-1 primers, CCCACCCAGTCAACCTTCTA and CTACGGGT-TGCGTTGTAAT; sdk-2 primers, GCCCCAAGATGATGTACCGCC and AGAGGGTGCTCTGCTGACTC). PCR without added RT product (RT−) was performed as a negative control.

**Results**

**Identification and Cloning of Mouse Sidekicks**

Representational difference analysis was performed on cDNA from conditionally immortalized HIV-1 transgenic and nontransgenic podocyte cell lines (13) (see Materials and Methods). More than 250 difference products were characterized corresponding to 29 differentially expressed genes between the two cell populations. The differentially expressed genes included known extracellular matrix components, adhesion molecules, transcription factors, and previously uncharacterized genes. Among the cDNA predicted to be most dramatically upregulated in HIV-1 transgenic podocytes compared with controls was a 1-kb difference product that was predicted to encode a protein bearing homology to the Drosophila melanogaster sidekick gene. To confirm the results of the RDA, we used this 1-kb difference product as a probe for Northern blot analysis of messenger RNA isolated from the transgenic and wild-type podocytes (Figure 1). Three distinct transcripts (6.5, 8, and 10 kb) were each highly expressed in the HIV transgenic podocytes compared with wild-type controls, thus verifying the differential expression.

We then cloned the 6.5- and 8-kb transcripts by combining cDNA phage library screening and 5′ RACE PCR. The novel gene, named mouse sidekick-1 (sdk-1), is 35% homologous at the amino acid level to the Drosophila sidekick gene and 80% identical to the more recently described chicken sdk-1. The cloned 8-kb cDNA encodes a putative type I transmembrane protein that contains a large extracellular domain composed of 6 Ig motifs followed by 13 fibronectin type III repeats, a short transmembrane domain, and a cytoplasmic tail of ~200 amino acids (Figure 2). This sequence is consistent with the predicted

![Figure 1. Expression of sidekick-1 (sdk-1) RNA in cultured podocytes. Northern blot analysis of sdk-1 mRNA demonstrates expression of sdk-1 in HIV-1 transgenic podocytes but not in nontransgenic control podocytes. The 1-kb difference product isolated from the representational difference analysis was used as the probe. Sdk-1 seems to be expressed as three distinct transcripts ranging in size from 6.5 to 10 kb. Hybridization with glyceraldehyde-3 phosphate dehydrogenase (GAPDH) was performed to demonstrate equivalent loading of RNA.](https://example.com/image1.png)
Figure 2. Alignment of mouse sdk-1 and sdk-2. Amino acid sequences of mouse sdk-1 (8-kb transcript) and sdk-2 as predicted from cDNA sequences. Identical residues are shown by dots. The predicted signal sequences are underlined, Ig C2 domains are shown in gray, FN-III repeats are shown in yellow, the transmembrane domain is green, and the conserved C-terminal PDZ binding domain is red. Sequence corresponding to the 6.5-kb transcript of sdk-1 starts at the red arrow, preceded by the unique peptide sequence MDRSG. The 6.5-kb transcript contains a unique upstream noncoding sequence (not shown).
domain organization of other sidekicks in other species. The coding region of the 6.5-kb transcript is virtually identical to that of the 8-kb transcript except that it contains a truncation of the terminal two-Ig domains and includes five unique amino acids at its amino terminus. The four- and six-Ig variants each contain entirely distinct upstream noncoding sequences derived from different exons. The six-Ig but not the four-Ig transcript contains a predicted signal sequence in this upstream region. Of note, the extreme carboxyl sequence GFSSFV constitutes a potential PDZ binding domain and is 100% conserved across all known sidekicks from all species.

Mouse sdk-2, 86% homologous to chicken sdk-2 at the amino acid level, was cloned via a PCR-based approach using embryonic kidney cDNA as a template and primers designed from both mouse genomic sequences and a predicted EST. Mouse sdk-1 and sdk-2 are 57% identical at the amino acid level and are similar in domain organization (Figure 2). Sdk-2 is expressed as a single transcript of 8 kb encoding a protein of similar topology to the 8-kb transcript of sdk-1. By Northern blot analysis, it was difficult to detect the presence of sdk-2 in both wild-type and HIV-1 transgenic podocytes, suggesting that unlike for sdk-1, sdk-2 expression is not upregulated by HIV-1 gene expression (data not shown). The nucleotide sequences of mouse sdk-1 and sdk-2 cDNA have been deposited in the GeneBank database under Genbank Accession Numbers AY 353236 and AY 351699.

Sidekick Expression and Regulation during Kidney Development

Multitissue Northern blot analysis of total RNA from both adult and fetal (E17) mouse organs revealed nearly identical
expression patterns for sdk-1 and sdk-2. Both genes are highly expressed in many fetal tissues, including kidney (Figure 3A), but show markedly lower expression levels in adult organs (data not shown). To look more closely at the developmental regulation of these genes in kidney, we evaluated expression of sdk-1 and sdk-2 mRNA at multiple time points during nephrogenesis (Figure 3B). This revealed sdk-1 and sdk-2 gene expression to be high throughout development with maximal expression occurring near birth. Although sdk-1 and sdk-2 RNA expression in adult tissue seems minimal in Figure 3, prolonged exposures clearly demonstrate their presence.

**Sidekicks Are Expressed in the Ureteric Bud during Nephrogenesis**

To localize sidekick protein, we generated polyclonal antibodies to a glutathione S-transferase fusion protein containing the carboxyl terminal 83 amino acids of sdk-1. To characterize the antiserum, we performed Western blot analysis on HEK293T cells that were transfected with full-length sdk-1 or partial-length sdk-2 expression vectors (Figure 4). We found single predominant bands corresponding to appropriate predicted molecular weights in transfected cells for both of these expression vectors. No reactivity was seen in control-transfected lysates or when using preimmune serum in both transfected and control-transfected cells. These results show that our antiserum is specific for sidekick proteins but cannot differentiate between sdk-1 and sdk-2.

We used the antiserum to perform immunohistochemistry on paraffin-embedded sections of mouse kidneys at various stages of nephrogenesis (Figure 5, A, B, D, and E). We found sidekick protein to be expressed abundantly in the ureteric bud and collecting ducts, which are the principle derivatives of the ureteric bud. At E12.5, sidekicks were expressed specifically throughout the entire ureteric bud, including both its trunk and its distal tips (Figure 5A). The proteins were not detected in induced mesenchymal tissue. As predicted from its amino acid sequence, the protein localizes to cell membranes (Figure 5B). By E15.5, however, expression in the tips of the ureteric bud is diminished, whereas prominent expression remains in the medullary ureteric bud and collecting ducts. Of note, at E15.5, sidekicks were not detected in developing glomeruli, although we cannot rule out some expression in developing tubules. No staining was detected using the preimmune serum from the animal in which the anti-sidekick antibodies were raised (Figure 5, C and F).

Homology between the human and mouse sdk-1 proteins is predicted to be ~86% at the amino acid level. As a result, our antiserum is also capable of recognizing human antigens in immunohistochemistry. In human fetal tissue at 24 wk of gestation (Figure 6), expression parallels what is seen at earlier stages in the mouse fetus; namely, sidekicks are expressed in the developing collecting ducts with significantly higher levels in the renal medulla relative to cortex. Expression was greatest in ureteric bud–derived tissue, whereas sidekicks were undetectable in glomeruli. Furthermore, sidekick expression seemed to be present equally on all ductal membrane segments (apical,
basolateral, and cell to cell junctions). Again, all preimmune controls were negative (data not shown).

**Sidekicks Are Upregulated in Glomeruli in HIVAN**

Initial attempts to localize sidekick proteins in paraffin-embedded adult tissue sections were unsuccessful despite continued, albeit lower, expression of RNA in adult kidneys. Therefore, immunohistochemistry was performed using frozen sections with a tyramide amplification protocol to allow for increased sensitivity. Under these conditions, sidekicks in wild-type adult kidney are detected in a pattern consistent with glomerular mesangial cells but few other cells (Figure 7A). Clearly, this represents a striking change from the expression pattern seen during kidney development. To determine whether glomerular expression of sidekicks can also be detected during nephrogenesis using this more sensitive staining technique, frozen embryonic (E15.5) kidney sections were also stained using tyramide amplification. Using this sensitive technique, we were unable to detect staining in developing glomeruli (data not shown). Thus, expression in glomeruli seems to be a late event in renal development and glomerular maturation. In diseased HIV-1 transgenic mouse kidneys, sidekick proteins seem to be increased overall, and they accumulate in sclerotic and collapsing glomeruli (Figure 7B). Thus, glomerular expression is increased in response to HIV-1 but is normally not present or is present only at very low levels in development. Control immunostaining with preimmune serum using an identical protocol was negative in glomeruli (Figure 7E).

To compare staining of sidekicks in HIV-1 transgenic and nontransgenic kidneys, we performed identical staining procedures in parallel and the sections were visualized by confocal microscopy performed with identical parameters (Figure 7, C and D). In diseased glomeruli, sidekicks were significantly overexpressed. Expression was seen in a diffuse glomerular pattern, which was no longer restricted to mesangial cells but seemed to include podocytes and parietal epithelial cells as well.

As previously stated, our antiserum does not differentiate between sdk-1 and sdk-2. Therefore, to determine whether the accumulation of sidekick proteins in HIV-1 transgenic kidney represents upregulation of sdk-1, sdk-2, or both, we performed semiquantitative RT-PCR to compare expression levels in HIV-1 transgenic and wild-type kidneys (Figure 7F). RT input was closely normalized to glyceraldehyde-3 phosphate dehydrogenase before proceeding to PCR with sdk-1– and sdk-2–specific primers. Whereas both sdk-1 and sdk-2 are detectable by PCR in adult kidney, only sdk-1 was significantly upregulated (at least fivefold) by HIV-1 expression. Sdk-2 expression was unchanged. These results are similar to what we observed in HIV-1 transgenic podocytes and suggest that the accumulation of sidekick protein induced by HIV-1 expression is largely that of sdk-1 rather than sdk-2.

To confirm that sidekick upregulation is truly relevant in human disease, we performed immunohistochemistry to compare sidekick expression in HIVAN and normal human control kidneys. We used a kidney biopsy specimen from HIVAN patient MS 111, whose pathology included CG, podocyte hypertrophy, and tubular microcystic disease. Immunohistochemistry was performed in parallel with identical parameters using tyramide amplification. Sidekicks were increased in expression...
Figure 7. Sidekicks are upregulated in adult glomeruli in HIV-associated nephropathy (HIVAN). (A and B) Frozen sections of adult wild-type and HIV-1–transgenic kidneys were harvested and immunostained for sidekick proteins. In adult wild-type kidney, sidekick is expressed in a pattern consistent with mesangial expression. In HIV-1–transgenic glomeruli, sidekick protein expression is increased throughout sclerotic or collapsed glomeruli. (C and D) Representative glomeruli (shown at ×100) from wild-type– and HIV-1–transgenic mice were immunostained for sidekick proteins. Identical staining procedures were performed in parallel and visualized by confocal microscopy using identical parameters. Sidekick staining in HIV-1–transgenic glomeruli is clearly increased when compared with nontransgenic controls, and sidekick expression in HIV-1–transgenic glomeruli is more diffuse and no longer limited to the mesangium. (E) An identical immunoassay using preimmune serum is negative. Gl, glomeruli. (F) Semiquantitative RT-PCR on kidney RNA extracted from age-matched adult wild-type– and HIV-1–transgenic mice using primers specific for sdk-1 and sdk-2. RT input was normalized using primers specific for GAPDH. Whereas both sdk-1 and sdk-2 are detectable by PCR in adult kidney, sdk-1 but not sdk-2 seems to be upregulated by the presence of HIV-1.
in many glomeruli in the HIVAN biopsy but were absent from glomeruli of normal kidney tissue (Figure 8). The pattern of glomerular sidekick expression was consistent with what was seen in the mouse model. Some staining in the renal interstitium was present in HIVAN sections when staining with either anti-sidekick or preimmune sera. This interstitial staining was also observed in normal kidney but was much less prominent. Preimmune serum on serial sections lacked any glomerular staining.

Discussion

In most chronic renal diseases, the inability of podocytes to undergo regenerative cell division is a key factor that leads to nephron loss. In HIVAN, however, podocytes become “dysregulated” such that they become dedifferentiated and are able to reenter the cell cycle (11–14). In the current work, we analyzed alterations in gene expression in HIV-1 transgenic podocytes in an effort to explore how HIV-1 induces these changes in host cells. We demonstrated that HIV-1 infection induces overexpression of sdk-1 and proposed sdk-1 as a candidate gene for contributing to the phenotypic changes seen in HIVAN. In contrast, sdk-2 is not significantly overexpressed either in HIV-1 transgenic podocytes or in HIV-1 transgenic mouse kidneys, suggesting that the regulation of sdk-1 and sdk-2 is different.

Sidekicks have been shown to play a role in directing synapse formation during neuronal development (20). Sdk-1 and sdk-2 localize specifically to the synaptic cleft in largely nonoverlapping subsets of retinal neurons. Sdk-1–expressing presynaptic cells form synapses with laminae that also express sdk-1; likewise, presynaptic cells that express sdk-2 target sdk-2–positive laminae. Ectopic expression of sdk-1 in sdk-negative cells redirected the processes of these cells to sdk-1–positive sublaminae. Furthermore, in vitro, sidekicks mediate homophilic adhesion such that each sidekick is able to bind only to its own kind. These data suggest that sidekick adhesion across the synapse plays an important role in determining synapse specificity.

In the current studies, we demonstrate that sidekick expression is by no means specific to the neuron but is in fact widespread during development. The function of sidekicks in nonneuronal cells is unknown, although, on the basis of the available functional data for these proteins, they likely mediate homophilic intercellular adhesion (20). In the fetal kidney at E12.5, we show that sidekicks are specifically expressed throughout the ureteric bud, including the stalk and tips, but by E15.5, expression is reduced in the tips. Furthermore, by postnatal day 1, expression is specific for medullary collecting ducts. This expression pattern correlates with several other genes known to have important roles in branching morphogenesis, including sonic hedgehog (22,23) and collagen XVIII (24). In general, the absence of high levels of expression in the nephrogenic zone is most consistent with sidekicks’ having a regulatory function in the complex cell division and migration required for branching tubulogenesis. Such a role in controlling cellular migration would be consistent with what is known about sidekick function in developing neurons. We are currently generating the reagents necessary to distinguish between sdk-1 and sdk-2 proteins so that their exact roles in kidney development can be better defined.

In normal adult kidney, sidekicks are expressed in a pattern consistent with glomerular mesangial cells, albeit at much lower levels than in the embryonic collecting system. The functional link between ureteric bud and adult mesangial cell expression is currently unclear. Consistent with in vitro data, wild-type podocytes do not seem to express sidekick proteins or do so at low levels. In HIVAN, however, sdk-1 expression not only is significantly upregulated in mesangial cells but also seems to be expressed in other cell types within the glomerulus, including parietal epithelial cells and podocytes. It is unclear whether the upregulation and/or ectopic expression of sdk-1 protein represents a
pathologic host response to HIV-1 infection or an attempt by podocytes to maintain the adhesion and structural integrity necessary to maintain the filtration barrier. Other groups have shown that podocyte injury is capable of inducing expression of genes that are not typically expressed in the podocyte, including desmin (11) and certain macrophage-specific markers (25). Ongoing experiments are focused on determining the exact role that sidekick dysregulation plays in the pathogenesis of HIVAN.

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