Identification and characterization of a glomerular-specific promoter from the human nephrin gene

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Received 15 May 2000; accepted in final form 18 August 2000

PROGRESSIVE RENAL DAMAGE OCCURS in a variety of disease states, even when the initiating injury is removed. Glomerulosclerosis and secondary tubulointerstitial fibrosis is the most common pathologic lesion identified in progressive renal failure (4). Together, these observations suggest that there is a common final pathway for many kidney diseases.

A number of studies have implicated the podocyte as a key cell in the progression of renal disease toward glomerulosclerosis (15, 16). Podocytes are mesodermally derived cells that are highly specialized and found only in the renal glomerulus. They exhibit unique characteristics such as foot processes and slit diaphragms, which are critical for glomerular filtration (21, 33). When podocytes are damaged, the foot processes fuse and eventually detach from the underlying glomerular basement membrane, leaving fewer cells to cover the capillary loops (16). Terminally differentiated podocytes are believed to be unable to divide in the adult kidney. Instead, they respond to glomerular injury through hypertrophy. Kriz has proposed that eventual loss of these hypertrophied podocytes leads to direct apposition of glomerular capillary endothelial cells to the overlying parietal epithelium, and obliteration of the filtration space (15, 16). Careful morphological studies have demonstrated a strong correlation between podocyte number and progression of glomerulosclerosis in diabetes (20). Although it has been reported that “dysregulated” glomerular visceral epithelial cells (podocytes) can proliferate in specific conditions such as collapsing glomerulopathy (2), these results remain controversial (14).

Despite the clinical importance of podocytes, their biology is still poorly understood. Previously, developing appropriate model systems to study podocytes in vitro has been difficult, as glomerular epithelial cells dedifferentiate in culture and lose their podocyte-specific markers. Recently, the gene responsible for congenital Finnish nephropathy was identified and named nephrin (13). Nephrin is a 135-kDa protein with homology to the immunoglobulin superfamily of cell adhesion molecules and is specifically located in the slit diaphragms of podocytes. Children who have mutations in the nephrin gene develop massive proteinuria and renal failure before age 2 yr (32).

Shih et al. (32) reported proteinuria and glomerular damage in mice that are homozygous null mutant for CD2AP (CD2-associated protein). These investigators demonstrated that CD2AP is expressed in podocytes and can associate with nephrin in vitro. The authors speculate that CD2AP links nephrin in the slit diaphragm to the intracellular cytoskeleton.

The purpose of the present study was to identify and characterize the podocyte-specific elements of the nephrin promoter. This promoter will be a valuable tool to study podocyte biology in vivo, with the hope of understanding its role in the development of glomerulosclerosis and ultimately enabling repopulation of the damaged glomerulus.

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and in situ hybridization was performed as described elsewhere cryosections were cut on a Leica Cryostat (model CM3050), and frozen at sucrose overnight at 4°C, embedded in OCT compound (Tissue-Tek 4583), and frozen at 30% paraformaldehyde overnight at 4°C, cryopreserved in 30% sucrose overnight at 4°C, embedded in OCT compound (Tissue-Tek 4583), and frozen at −70°C. Twelve-micrometer cryosections were cut on a Leica Cryostat (model CM3050), and in situ hybridization was performed as described else-where (12, 31). A 2.3-kb fragment of the mouse nephrin gene from bp 1810 to 3728 (GenBank accession no. AF168466) in pBluescriptKS+ was used as a template for antisense- and sense digoxigenin-labeled RNA probes that were prepared according to manufacturer’s (Boehringer Mannheim) instructions.

β-Galactosidase staining of embryos and kidneys. Kidneys or whole embryos from 9.5 to 18.5 dpc were fixed in 4% paraformaldehyde and 1% glutaraldehyde and stained for β-galactosidase activity as described (25). Kidneys and a variety of other tissues (lung, heart, gut, etc.) were also dissected from postnatal day 0 and postnatal day 14 mice, cut into small pieces, and fixed and stained with β-galactosidase by whole mount as described above. Tissues were then embedded in paraffin, and 5-μm sections were cut.

Sequence analysis of the promoter region. The 1.25-kb fragment of nephrin DNA that was used to generate transgenic lines was analyzed for transcription-factor binding sites by using the TRANSFAC Web site and the internet browser9 (9).

RESULTS

LacZ expression is restricted to podocytes in transgenic lines. Six founder lines were identified by Southern blot analysis (Fig. 1). Three of these lines had a single chromosomal integration of the transgene, while three had two or more integrations of the transgene. Kidneys from one newborn founder male and embryos and kidneys from the offspring of the other five founder lines were dissected and stained for galactosidase activity. Offspring and kidneys from three of the six founder lines demonstrated lacZ expression. Of note, only one of the expressing lines had two sites of integration of the transgene; the other two lines had a single integration of the transgene. Offspring from the other three founder lines and two nontransgenic-control littermates exhibited no lacZ expression at any

3The nucleotide sequence for the murine nephrin cDNA can be accessed through the NCBI nucleotide database under NCBI accession no. AF168466.
4The TRANSFAC transcription factor site database can be accessed on: http://transfac.gbf.de/TRANSFAC (9).
stage of development. In the three positive lines, lacZ expression was found exclusively in podocytes of capillary-loop stage and mature glomeruli (Fig. 2). No staining was identified in podocyte precursors in S-shaped bodies or in any other tissues at any embryonic or postnatal day studied. In comparison, endogenous nephrin mRNA was detected in podocyte precursors in S-shaped bodies, in podocytes from capillary-loop stage glomeruli, and in mature podocytes (Fig. 3).

Transcription factor binding sites in the podocyte-specific promoter. The DNA fragment capable of directing podocyte-specific expression in the glomerulus was searched for potential transcription factor binding sites (Fig. 4). Within this 1.25-kb fragment there is no TATA box, but GATA binding sites are seen. Of note, there are several E-box consensus sequences that are recognition sites for basic-helix-loop-helix proteins and a potential Pax-2 binding site. The transcription initiation site has been determined by primer extension and is reported to occur 156 bp upstream of the initiating codon (17).

DISCUSSION

Podocytes are an integral component of the glomerular filter and play a pivotal role in the progression of many renal diseases. In contrast to the mesangial cell, which is also found in the glomerulus but is not a structural component of the renal filter, the biological role of the podocyte in renal disease has not been studied extensively. In part, this has been due to the lack of cell-culture models. Although it has been possible to isolate glomerular epithelial cells from primary cultures, these cells lose their podocyte-specific characteristics after a few passages (23). Mundel et al. (22) have isolated SV40-transformed murine-podocyte cell lines that retain certain characteristics and markers of podocytes but cannot replace in vivo studies. As a first step in the development of molecular tools to study the podocyte in vivo, we report the first glomerular-specific promoter to be identified. In this paper, we demonstrate that a 1.25-kb fragment of genomic human DNA, which includes the predicted initiation codon and immediate 5′-flanking region of nephrin, directs podocyte-specific expression in vivo.

The expression pattern of our human lacZ transgene recapitulates the expression pattern of the endogenous murine nephrin gene with a few exceptions. In the mouse, nephrin mRNA is weakly expressed in podocyte precursors at the S-shaped body stage (Fig. 3A) and is strongly expressed in podocytes in capillary-loop stage and mature glomeruli (11; Fig. 3, B and C). In addition, Holzman et al. (11) describe nephrin expression in the

![Fig. 2. LacZ expression in transgenic mouse kidneys at embryonic day 18.5. Embryonic mouse kidneys from offspring of the founder line shown in Fig. 1 (lane 2) were dissected at day 18.5 and stained with β-galactosidase by whole mount. The kidneys were embedded in paraffin and sectioned. A: the 1.25-kb nephrin fragment directs expression of the lacZ reporter gene to glomeruli and specifically to po (B, C). pa, Parietal epithelial cells; me, mesangial cells.](http://ajprenal.physiology.org/)

![Fig. 3. mRNA expression of the endogenous murine nephrin gene. A digoxigenin-labeled antisense 2.3-kb probe from the 3′ end of the murine nephrin cDNA was used for in situ analysis of embryonic day 18.5 mouse kidneys. Murine nephrin is weakly expressed in po precursors of an S-shaped body (developing glomerulus, A), but is highly expressed in po in capillary-loop stage (B) and mature glomeruli (C). In contrast, no staining was seen in samples hybridized with the sense digoxigenin-labeled probe (not shown).](http://ajprenal.physiology.org/)
spleen, and we also see weak expression in developing pancreas (data not shown). In contrast, we did not observe any expression of the nephrin transgene outside the renal podocyte. Furthermore, we did not detect lacZ expression until the capillary-loop stage podocyte; no lacZ expression was detected in S-shaped bodies. This may represent sensitivity of the lacZ-expression assay, absence of a regulatory element in the transgene, and/or interspecies expression differences as the human promoter was used for the transgenic studies. Because three of the six transgenic founder lines did not demonstrate any lacZ expression, it follows that expression of the 1.25-kb promoter of human nephrin is influenced by the chromosomal integration site. Although we observed these stable position effects, we did not observe any heterocellular expression of the transgene (19).

Given the limited size (1.25 kb) of the podocyte-specific promoter, it is of interest to identify putative cis-binding elements. Of note, a putative Pax-2 binding element and multiple canonical E-box consensus sequences exist. Pax-2 is a member of the paired box family of transcription factors; it is expressed in renal vesicles and is specifically downregulated in podocyte precursors at the S-shaped-body stage (6). Studies have shown that Pax-2 can act as both a transcriptional activator and a repressor (5, 8). Thus one might speculate that Pax-2 actively represses transcription of the nephrin gene in epithelial cells of the renal vesicle prior to podocyte differentiation. In addition, we and others have identified a basic-helix-loop-helix transcription factor, Pod1/capsulin/epicardin (18, 27, 29), which is highly expressed in podocyte precursors and mature podocytes. Similar to other bHLH proteins, Pod1 can bind to E-box consensus sequences in vitro (18). Although podocytes fail to differentiate terminally beyond the capillary-loop stage in Pod1 mutant mice, nephrin is still expressed in the mutant podocytes. These results demonstrate that Pod1 is not required to activate transcription of the nephrin gene (28; data not shown).
Lenkkeri et al. (17) reported promoter deletion mutations in two patients with Finnish nephropathy. These occurred in the GA repeat sequence between bp −292 to −327 of the proximal promoter. One of these patients presented with an atypical course and did not require renal transplantation until age 5 yr (17). It will be of interest to determine whether mutations in this region affect expression of our transgene.

We have identified and begun to characterize the first glomerular-specific and podocyte-specific promoter. Identification and characterization of cis-acting elements in this promoter fragment will be useful to identify transcription factors required for podocyte-specific expression. Use of this transgene will allow genetic manipulation of the podocyte in vivo, characterization of its biological role in renal function and disease, and ultimately, testing of the hypothesis that the podocyte plays a pivotal role in the progression of renal injury toward glomerulosclerosis.

We thank Johanne Pellerin and Lois Schwartz for expert technical assistance.

S. E. Quaggin is the recipient of a Clinician Scientist Award from the Medical Research Council of Canada, the Carl Gottschalk Scholar Award from the American Society for Nephrology, and is a Canadian Foundation for Innovation Researcher. This work was supported by a Medical Research Council of Canada grant to S. E. Quaggin.

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