MAGI-2 scaffold protein is critical for kidney barrier function

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MAGUK Inverted 2 (MAGI-2) is a PTEN-interacting scaffold protein implicated in cancer on the basis of rare, recurrent genomic translocations and deletions in various tumors. In the renal glomerulus, MAGI-2 is exclusively expressed in podocytes, specialized cells forming part of the glomerular filter, where it interacts with the slit diaphragm protein nephrin. To further explore MAGI-2 function, we generated Magi-2–KO mice through homologous recombination by targeting an exon common to all three alternative splice variants. Magi-2 null mice presented with progressive proteinuria as in human prostate tumors (21), and genetic deletion and inactivation are viable and born at expected Mendelian ratios. However, they present with early-onset, progressive proteinuria and severe noninflammatory, proliferative glomerulosclerosis (8, 9).

MAGUK Inverted 2 (MAGI-2) is a scaffold protein with a putative tumor-suppressor role and also interacts with nephrin in the glomerular slit diaphragm protein complex. To gain insight into its function, we generated Magi-2–KO mice and found that loss of MAGI-2 expression leads to slit diaphragm disruption, podocyte foot process effacement, and severe podocyte loss. Magi-2–null mice develop diffuse glomerular extracapillary epithelial cell proliferations, and died of renal failure by 3 mo of age. As confirmed by immunohistochemical analysis, the proliferative cell populations in glomerular lesions were exclusively composed of activated parietal epithelial cells (PECs). Our results reveal that MAGI-2 is required for the integrity of the kidney filter and podocyte survival. Moreover, we demonstrate that PECs can be activated to form glomerular lesions resembling a noninflammatory glomerulopathy with extensive extracapillary proliferation, sometimes resembling crescents, following rapid and severe podocyte loss.

MAGI-2/S-SCAM | glomerulosclerosis

Significance

MAGUK Inverted 2 (MAGI-2) is a scaffold protein with a putative tumor-suppressor role and also interacts with nephrin in the glomerular slit diaphragm protein complex. To gain insight into its function, we generated Magi-2–KO mice and found that loss of MAGI-2 expression leads to slit diaphragm disruption, podocyte foot process effacement, and severe podocyte loss. Magi-2–null mice develop rapidly progressive glomerular disease and renal failure. Our findings suggest that MAGI-2 is essential for kidney filter function and podocyte survival, thereby providing insights into the pathogenesis of proteinuric kidney disease. Moreover, Magi-2–null mice can serve as an excellent model system for studying glomerular disease progression and identification of new treatment targets for the difficult-to-treat spectrum of primary podocytopathies.

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a few weeks after birth. The onset of glomerular dysfunction in Magi-2Δ/Δ mice coincides with a decrease in protein abundance of nephrin and increase in protein abundance of CIN85, an adaptor protein that regulates turnover of the slit diaphragm complex (26). Ultrastructural analysis of glomeruli from MAGI-2–null mice revealed diffuse podocyte injury, followed by rapid and severe podocyte loss and decline in kidney function, resulting in death from renal failure by 3 mo of age. Our data support an essential role for MAGI-2 in the maintenance of the kidney filter by promoting podocyte survival.

Results

Generation of Magi-2-KO Mice. We disrupted the Magi-2 gene by homologous recombination in 129 LW1 ES cells by replacing a genomic fragment of exon 4 (Magi-2 transcript variant 1) with a neomycin-resistance cassette (Fig. L4). Exon 4 encodes a portion of the GUK domain common to all three MAGI-2 protein isoforms. By using an external probe, correct targeting was confirmed in 12 of 576 G418 resistant colonies by Southern blot (Fig. L8). ES cells were injected into C57BL/6 (B6) blastocysts, and resulting male chimera were crossed with B6 or 129/SvJ (129) females. Offspring were genotyped initially by Southern blot and subsequently by PCR (Fig. 1C). Heterozygous Magi-2Δ/+ mice were intercrossed to generate Magi-2Δ/Δ mice. Germ-line transmission was achieved with two distinct ES clones (no. 94 and no. 278) that were analyzed in parallel. Both lines displayed the same phenotype, with the data presented hereafter from line 94. Western blot analysis and quantitative RT-PCR (qRT-PCR) performed on brain protein lysates and RNA from littermates of each genotype confirmed the loss of protein and mRNA transcript expression in KO mice (Fig. 1D and Fig. S1).

Magi-2Δ/Δ Mice Show Early-Onset Proteinuria and Impaired Survival. Magi-2Δ/Δ mice appeared normal at birth and were born at expected Mendelian ratios, but growth retardation became apparent within a few weeks in mice of the 129 background. The median survival of Magi-2Δ/Δ mice in the 129 background was 50 d (n = 11), and all mice died by 3 mo of age (Fig. 2A). Magi-2Δ/Δ mice on a 129 background developed progressive proteinuria by 3 wk of age (Fig. 2B and Fig. S2). Serum creatinine and blood urea nitrogen (BUN) levels were elevated in proteinuric Magi-2Δ/Δ mice (Fig. S3), thereby confirming the development of chronic kidney disease. In contrast, in the mixed (B6/129) background, the median survival of Magi-2Δ/Δ mice was 169 d (n = 41), and many of these mice lived beyond 1 y (Fig. S44). Correspondingly, urinalysis in mixed-background Magi-2Δ/Δ mice revealed lower-grade proteinuria with variable age of onset (Fig. S4B). No proteinuria or adverse survival was observed in heterozygous mice of either background (Fig. 2 and Fig. S4A). Because of the variability and delay in phenotype in the mixed B6/129 background Magi-2Δ/Δ mice, subsequent analyses were performed in 129 background mice.

Nephrin Protein Levels Are Decreased Early in Magi-2Δ/Δ Glomeruli. We confirmed MAGI-2 protein expression in isolated glomeruli from WT mice, and this expression was absent in glomeruli isolated from Magi-2Δ/Δ mice (Fig. 2C and D). Because of its proposed role as a scaffold protein, we investigated the effect of MAGI-2 deficiency on the expression of other podocyte proteins by Western blot analysis. Interestingly, nephrin protein levels were already profoundly decreased in glomeruli from Magi-2Δ/Δ mice 10 d postnatally, coinciding with the onset of proteinuria (Fig. 2C). This finding is consistent with early down-regulation of nephrin during disassembly of the slit diaphragm in many cases of glomerular disease without genetic disruption of NPHS1 (14). In contrast, other essential slit diaphragm-associated proteins, such as podocin (27), CD2AP (28), and α-actinin-4 (29) remained unchanged at this time point. We also investigated protein expression of the known MAGI-2 targets PTEN and pAKT(S473) (20), which showed no significant changes in Magi-2Δ/Δ mice within the first 18 d of life (Fig. 2C). These findings suggest that MAGI-2 may have a specific role in orchestrating slit diaphragm maintenance by regulating nephrin protein abundance in podocytes.

CIN85 Up-Regulation Coincides with Nephrin Reduction in Magi-2Δ/Δ Mice. At 3 wk of age, nephrin protein expression was almost undetectable in Magi-2Δ/Δ glomeruli, and we observed a reciprocal increase in protein abundance of the 70-kDa and 150–170-kDa isoforms of the adaptor protein CIN85 (30) (Fig. 2D). To further analyze these changes in nephrin and CIN85 expression, we performed qRT-PCR on cDNA generated from isolated glomeruli and found no significant difference in either mRNA transcript (Fig. S5), indicating posttranscriptional regulation of their expression. Previous work has demonstrated a role for CIN85 in slit diaphragm turnover in podocytes (26), and these data provide evidence that nephrin may be targeted for degradation by CIN85 in the absence of MAGI-2 expression. Of note, at 3 wk of age, a mild reduction in the abundance of podocyte proteins synaptopodin (31) and podocin was observed (Fig. 2D), consistent with more profound podocyte injury and/or loss.

Magi-2Δ/Δ Mice Develop Diffuse Podocyte Injury, Resulting in a Noninflammatory Glomerulopathy with Extracapillary Epithelial Proliferation. To assess histologic changes in the kidneys, we killed mice of each genotype at various time points, and analyzed periodic acid–Schiff (PAS)-stained kidney sections by light microscopy. The kidneys of WT (+/+ ) mice showed no morphologic abnormalities in glomeruli, tubulointerstitium, or vasculature at any age (Fig. 3A). As shown in Fig. 3B, Magi-2Δ/Δ kidneys displayed no light microscopic changes at 11 d of age; however, at 3 wk of age, there was a mild...
increase in extracellular matrix accumulation in the mesangium without glomerular hypercellularity, whereas the tubulointerstitium appeared normal. By 5.5 wk, the majority of glomeruli were severely injured, with a collapsed tuft and substantial epithelial cell proliferations (Magi-2\( ^{−/−} \): 49.78 ± 10.54%, n = 5, vs. Magi-2\(^{+/+}\): none, n = 5; P = 0.0015), in many cases resembling cellular or fibrocellular crescents (Magi-2\(^{−/−}\): 15.86 ± 3.69%, n = 5, vs. Magi-2\(^{+/+}\): none, n = 5; P = 0.0026), notably in the absence of glomerular inflammation or hypercellularity. In keeping with the observed severe proteinuria (Fig. 2B), tubular protein casts were present in all Magi-2\( ^{−/−} \) mice. Few glomeruli were globally hyalinized (Magi-2\(^{−/−}\): 4.2 ± 1.12%; n = 5, vs. Magi-2\(^{+/+}\): none, n = 5; P = 0.0056) or segmentally hyalinized (Magi-2\(^{−/−}\): 7.44 ± 0.69%; n = 5, vs. Magi-2\(^{+/+}\): none, n = 5; P < 0.0001), and the tubulointerstitium showed mild tubular atrophy and interstitial fibrosis (Magi-2\(^{−/−}\): 19 ± 4%; n = 5, vs. Magi-2\(^{+/+}\): none, n = 5; P = 0.0014). By 7 wk, the kidneys of Magi-2\(^{−/−}\) mice showed widespread chronic changes of the tubulointerstitium (Magi-2\(^{−/−}\): 70 ± 10%; n = 2, vs. Magi-2\(^{+/+}\): none, n = 2; P = 0.0198), and the vast majority of glomeruli were globally hyalinized or sclerosed (Magi-2\(^{−/−}\): 59.5 ± 15.5%; n = 2, vs. Magi-2\(^{+/+}\): none, n = 2; P = 0.0617) with focal residual fibrous or fibrocellular extracapillary lesions (Magi-2\(^{−/−}\): 7.00 ± 1.80%; n = 2, vs. Magi-2\(^{+/+}\): none, n = 2; P = 0.0198; Fig. 3B and Fig. S6).

Ultrastructural analysis by EM at 4 wk of age revealed profound podocyte injury with diffuse foot process effacement, microvillous transformation of the cell membrane, and dilation of the endoplasmic reticulum (Fig. 4). Of note, several podocytes appeared hypertrophic and showed long, thin extensions of their cell bodies. The endothelium showed mild loss of fenestrations, and the glomerular basement membrane appeared of normal thickness. Taken together, MAGI-2 deficiency causes a diffuse and progressive podocytopathy in mice.
Mice Elicits a Proliferative Podocyte Injury. Our data suggest that MAGI-2 acts as a molecular scaffold to maintain the nephrin multiprotein signaling complex. Magi-2 mice may serve as a clinically relevant model for slit diaphragm destruction and progressive proteinuria, in which nephrin reduction occurs before any apparent light microscopic abnormalities. Our findings suggest that loss of

Early and Rapid Podocyte Loss in Magi-2Δ/Δ Mice Elicits a Proliferative Parietal Epithelial Cell Response. Podocyte loss is a hallmark of progressive proteinuric kidney disease (16). Given the development of podocyte hypertrophy and the rapid progression of kidney disease, we sought to evaluate podocyte loss as a cause of glomerular disease. We performed immunohistochemistry for the podocyte marker synaptopodin in glomeruli of WT and Magi-2Δ/Δ mice in various stages of disease development at 10 d, 3 wk, 5.5 wk, and 7 wk of age (Fig. 5A). We found that synaptopodin expression was markedly reduced in 3-wk-old Magi-2Δ/Δ mice compared with WT littermates, and was absent by 5.5 wk in all glomeruli with extracapillary proliferations, indicating a complete loss of podocytes (Fig. 5B). As progressive podocyte loss elicits a parietal epithelial cell (PEC) reaction (32), we hypothesized that the extracapillary epithelial proliferations seen in the majority of glomeruli at 5.5 wk might contain activated PECs. Immunohistochemistry for the PEC marker PAX2 confirmed that all proliferative and crescentic lesions were exclusively composed of PECs (Fig. 5C). The majority of PECs within those lesions were positive for KI67, indicating cell cycle activation, whereas the macrophage marker CD68 was negative (Fig. S7). These data indicate that PEC activation and proliferation represents a response to rapid and severe podocyte loss in Magi-2Δ/Δ mice, leading to an early-onset proliferative and noninflammatory glomerulopathy that ultimately results in death from renal failure.

Discussion

Previous reports had documented MAGI-2 expression in glomerular podocytes (12, 13), but its function in these highly specialized cells remained unknown. In this study, we present in vivo evidence that MAGI-2 is essential for kidney filter maintenance and preservation of podocytes. Mice deficient for all three MAGI-2 protein isoforms develop progressive proteinuria as a result of a severe podocytopathy, and ultimately die of renal failure. We also show that nephrin, an essential slit diaphragm protein, is reduced early in glomeruli of Magi-2Δ/Δ mice. Our data suggest that MAGI-2 acts as a molecular scaffold to maintain the nephrin multiprotein signaling complex.

Nephrin is commonly reduced in patients with nephrotic syndrome without a specific nephrin (i.e., NPHS1) mutation (15). Therefore, Magi-2Δ/Δ mice may serve as a clinically relevant model for slit diaphragm destruction and progressive proteinuria, in which nephrin reduction occurs before any apparent light microscopic abnormalities. Our findings suggest that loss of
nephrin is an early causative event in the development of proteinuria in Magi-2 Δ/Δ mice. MAGI-2 has been shown to physically interact with nephrin (13, 33), raising the possibility that the interaction is required for nephrin localization or protein stability. Intriguingly, it has been previously shown that the adaptor protein CIN85 can bind to nephrin and contribute to the regulation of nephrin ubiquitination and turnover (26). Here we find an increase in CIN85 protein abundance in glomeruli of Magi-2 Δ/Δ mice, suggesting that MAGI-2 may directly regulate CIN85-mediated nephrin turnover in podocytes. The near-complete loss of podocytes and the rapid progression of glomerular disease in Magi-2 Δ/Δ mice are unusual and cannot be explained purely on the basis of nephrin reduction. These findings suggest an additional, nephrin-independent prosurvival role for MAGI-2 in podocytes, perhaps by blocking the proapoptotic signaling properties of dendrin (34), another MAGI-2 interacting protein (35).

Our results also provide evidence that primary podocyte injury, when severe enough, can lead to a fulminating proliferative glomerulopathy resembling a crescentic glomerulonephritis without an obvious inflammatory component. The cells composing the proliferative lesions in Magi-2 Δ/Δ mice are PAX2-positive and syntenopodin-negative, indicating their PEC origin. A tumor suppressor function of MAGI-2 in PECs seems unlikely, as there is no documented evidence of MAGI-2 expression in this cell type. However, similar activation and proliferation of PECs, although less pronounced, was recently described in murine Adriamycin-induced nephropathy following podocyte loss (32). Thus, we attributed the observed severity of the glomerular lesions in Magi-2 Δ/Δ mice to the particularly rapid and abundant podocyte loss, and, as such, would include a noninflammatory proliferative glomerulopathy at the extreme end of the spectrum of primary podocytopathies.

Despite high MAGI-2 expression in the adult brain, we found no evidence of morphological aberrations in the brains of Magi-2 Δ/Δ mice. A previous study found that mice with a genetic deletion of only the α-isomorph of MAGI-2/SCAM were born at normal Mendelian ratios but died within 24 h of birth (36). Iida et al. found no evidence of abnormal brain development or structure in the S-SCAM-α-null mice, but neurons cultured from these mice displayed abnormal elongation of dendritic spines as a result of NMDA-dependent RhoA activation (36). It is of particular interest that the α-isomorph displayed earlier lethality than we observe in mice lacking all three isoforms, raising the possibility that each MAGI-2 isomorph may have a distinct function in different tissues or that feedback regulation may occur between the different isoforms. We cannot formally exclude the possibility that strain differences also account for phenotypic differences between these mice.

Although we detected comparable pathologic and functional changes in the kidneys of Magi-2 Δ/Δ mice on the 129 and the mixed B6/129 background, the onset of kidney disease was significantly delayed and heterogeneous in the mixed background. This is comparable to findings in several mouse models of congenital nephrotic syndrome, in which the severity of the phenotype depended on the mouse strain. For example, KO alleles of the nephrin-interacting protein podocin (i.e., Nphs2) develop proteinuria and glomerulosclerosis in a B6/129 mixed background and in pure 129 mice, but onset is earlier in B6/129 mice (37). Characterization of mice null for the CD151 gene revealed strain-dependent phenotypes, with the development of severe glomerular disease in FVB strain mice, whereas B6 mice remained normal and healthy (38). Identification of genetic modifiers of renal phenotypes in different mouse strains may provide insights into genes that affect susceptibility to develop proteinuria in humans. In this context, a reduction in MAGI-2 copy number may confer genetic susceptibility to developing a podocytopathy following a triggering event or to a more rapid clinical progression.

We had previously identified MAGI-2 an interacting partner of the tumor suppressor PTEN, and presented data that MAGI-2 stabilizes PTEN protein expression, enhancing its phosphatase activity and tumor-suppressive function (20). Nephrin can also interact with the p85 subunit of PI3K and stimulate Akt signaling in cultured podocytes (39). We found no evidence of loss of PTEN protein stability or significant change in levels of phosphorylated Akt in the glomeruli of Magi-2 Δ/Δ mice (Fig. 2C), suggesting that aberrant PI3K/AKT signaling is unlikely to account for the observed phenotype. Further studies of potential MAGI-2 tumor suppressor function will require the generation of conditional alleles to avoid early death from renal failure. In conclusion, our results reveal that MAGI-2 in podocytes is required to prevent podocyte injury and progressive kidney disease and renal failure.

Materials and Methods

Magi-2 Δ/Δ mice. Magi-2 Δ/Δ mice were generated and genotyped as described in SI Materials and Methods. Mice were bred and maintained at the animal facilities at the University of California, Los Angeles, or Memorial Sloan–Kettering Cancer Center. All studies were approved by the institutional animal care and use committee at both universities. SI Materials and Methods provides detailed protocols of isolation of glomeruli, histology, immunohistochemistry, and transmission electron microscopy.

Antibodies, Western Blot, and qRT-PCR Analysis. Antibodies for MAGI-2 were generated in rabbits as described in SI Materials and Methods. Antibodies used were as follows: CD2AP (B11; Santa Cruz Biotechnology), nephrin (N2028-50; US Biological), CIN85 (H-300; Santa Cruz Biotechnology), synaptopodin (N-14; Santa Cruz Biotechnology), Neph1 (H-150; Santa Cruz Biotechnology), PTEN (A2B1; Santa Cruz Biotechnology), and pAkt-Ser473 (no. 9271; Cell Signaling Technologies). SI Materials and Methods provides details of Western blot and qRT-PCR analysis.

Urine Analysis of Proteinuria. Albumin and creatinine levels in mouse urine were measured by ELISA and the alkaline picrate method (Albuwell/Creati- nine Companion; Exocell), according to the manufacturer protocol. Alternatively, urine samples (2 μl) from each mouse were mixed with SDS-sample loading buffer, incubated at 95 °C for 5 min, and resolved by 10% SDS/PAGE. The gels were stained with Coomassie brilliant blue for 30 min, destained for 4 h, and then dried onto Whatman paper by using a gel dryer.

Note Added in Proof. A similar phenotype of MAGI-2 deletion was reported by Nishimori and coworkers (40) while our manuscript was under review.

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