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Semaphorin3a signaling, podocyte shape and glomerular disease

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Abstract

Semaphorin3a (sema3a), a member of class 3 semaphorins, is a guidance protein that regulates angiogenesis, branching morphogenesis, axon growth and cell migration, and has pleiotropic roles on organogenesis, immune response and cancer. Sema3a is secreted by podocytes and is required for normal kidney patterning and glomerular filtration barrier development. We recently discovered that after completion of kidney development *Sema3a* gain-of-function in podocytes leads to proteinuric glomerular disease in mice. Excess sema3a causes foot process effacement, glomerular basement lamination and endothelial damage in vivo, and disrupts podocyte shape cell autonomously by down-regulating nephrin and inhibiting $\alpha v \beta 3$ integrin. We identified a novel direct interaction between nephrin and plexinA₁, the sema3a signaling receptor. Nephrin-plexinA₁ interaction links the slit-diaphragm signaling complex to extracellular sema3a signals. Hence, sema3a functions as an extracellular negative regulator of the structure and function of the glomerular filtration barrier.

Keywords

semaphorin3a; glomerular filtration barrier; podocyte signals; proteinuria; cell shape; integrin activity

Introduction

Semaphorins are phylogenetically conserved guidance proteins that regulate cellular function and shape (1). Semaphorins were initially identified as axon repellents during nervous system development, and later found to modulate heart, kidney, lung, bone and immune development [2-5]. More recently, semaphorins role has been evaluated in cardiovascular, cancer, osteoporosis and immune-mediated diseases. Excellent updated reviews analyze this large body of literature [6-8]. Human *SEMA3A* mutations have been detected in Kallman syndrome and Hirschprung's disease patients [9,10]. Semaphorins are classified as membrane bound, secreted or glycosylphosphatidyl-inositol linked proteins [1]. Class 3 semaphorins comprise seven secreted proteins (sema3A-3G). Several class 3 semaphorins and their receptors are expressed in the kidney [11,12]. Our group has examined the role of sema3a and sema3c in kidney development and defined their

importance in glomerular development and ureteric bud branching morphogenesis [4,13]. This review is focused on sema3a function in podocytes and glomerular disease.

Sema3a is a secreted guidance protein that plays important roles in axon pathfinding, cardiovascular, lung and kidney patterning during organogenesis [1,5,13]. *Sema3a* deficient mice have heart and bone abnormalities, as well as abnormal kidney vascular patterning, including excessive glomerular endothelial cells, and increased ureteric bud branching, suggesting that *Sema3a* inhibits endothelial cell migration and limits ureteric bud branching during kidney development [2,4,13,14]. Conversely, *Sema3a* gain-of-function during development induces glomerular hypoplasia, delayed podocyte differentiation and, strikingly, absence of slit-diaphragms [4]. Sema3a is expressed in podocytes and collecting ducts in the normal adult kidney [11]. Sema3a signals are mediated by a receptor complex consisting of neuropilin1 and plexinA₁, sema3a binding and signaling receptors, respectively [1,15-17]. Both receptors are known to interact with other signaling proteins, including VEGFR2, Fyn, Src and integrins in cell specific contexts, adding further complexity to the pathways involved in sema3a effects [1,8]. Neuropilins are single pass transmembrane proteins with a large extracellular domain encoding sema3a and VEGF₁₆₅ binding sites, a transmembrane domain and a short cytoplasmic tail [15]. Sema3a binding to neuropilin1 assembles the holoreceptor complex and releases plexin autoinhibition [16]. Plexins A-D include nine conserved transmembrane receptor proteins, among these sema3a binds plexinA₁ [8]. PlexinA downstream signaling is complex, involving integrins and PI3K/Akt inhibition, the latter prevents GSK3 inactivation, eliciting interaction with CRMPs and thereby regulates microtubule dynamics; as well as Fyn phosphorylation, Rho and Rac GTPases and MICAL, which regulate actin dynamics and de-polymerization, respectively [8,16-18]. Sema3a binding receptor neuropilin 1 is expressed in the kidney throughout life in podocytes, endothelial cells and ureteric bud derived cells [4,11,19]. PlexinA₁, the signaling receptor, is also expressed throughout ontogeny in the same cell types [13,19,20]. Sema3a induces downregulation of podocin, decreases the interactions between nephrin, podocin, and CD2AP in cultured podocytes [19]. Further, excess sema3a increases starvation-induced apoptosis in cultured podocytes and in the developing kidney in vivo [4,19].

Sema3a gain-of-function causes glomerular disease

Administration of sema3a to adult mice induces foot process effacement and proteinuria, which are transient and reversible, similar to the changes caused by protamine sulfate [21,22]. Adult mice with inducible *Sema3a* gain-of-function in podocytes develop proteinuria, associated with mesangial matrix expansion and mild hypercellularity on light microscopy and remarkable abnormalities on transmission electron microscopy (TEM) involving the three layers of the glomerular filtration barrier [20]. Indeed, excess sema3a induces endothelial cell swelling and interdigitation in the glomeruli, thickening and lamination of the glomerular basement membrane lamina rara interna (GBM), and significant podocyte foot process effacement. This phenotype indicates that *Sema3a* overexpression induces glomerular endothelial cell and podocyte injury, disrupting the GBM. Notably, sema3a-induced albuminuria, foot process effacement, GBM and endothelial damage are reversible upon withdrawal of the transgene induction, providing

proof of principle of the cause-effect relationship of excess sema3a and glomerular disease, and suggesting that this pathway is amenable to therapeutic targeting.

Sema3a gain-of-function down-regulates nephrin

To understand the molecular basis of the glomerular phenotype induced by excess podocyte sema3a in adult mice we examined the expression of slit-diaphragm and GBM proteins. Sema3a-induced GBM phenotype in adult kidneys results from increased MMP9 expression and subtle changes in collagen and laminin chain composition, as assessed by quantitative PCR, immunoblotting and immunohistochemistry [20]. Sema3a excess causes reversible nephrin downregulation, while podocin expression level is not altered, suggesting that decreased nephrin expression is not due to podocyte loss. This was confirmed by similar WT1⁺ nuclei counts, which indicate the number of podocytes present in each glomerular section. Taken together, the concomitant reversibility of podocyte foot process effacement, proteinuria and nephrin downregulation without podocyte loss establish the pathogenic mechanism induced by excess sema3a: nephrin loss disrupts slit-diaphragm integrity leading to foot process effacement and increased glomerular filtration barrier permeability (Figure).

PlexinA₁ interacts with nephrin

To understand the molecular mechanism of the crosstalk between sema3a and nephrin signaling we tested the relationship between sema3a signaling receptor plexinA₁ and nephrin, since both proteins are expressed in the podocyte cell membrane and known to form multi-protein holoreceptor complexes [8,23]. Notably, we discovered that plexinA₁ and nephrin colocalize and physically associate in podocytes [20]. Additional experiments using transfected cells and purified proteins confirmed that plexinA₁-nephrin interaction is direct [20]. These experiments document for the first time a direct link between extracellular sema3a signals and the slit-diaphragm signaling complex. We determined that sema3a influences podocyte shape in an autocrine fashion, inducing time and dose-dependent decrease in cell size and shape changes due to podocyte contraction and F-actin collapse [20].

Sema3a inhibits glomerular α v β 3 integrin activity and alters podocyte shape

Previous studies have shown that sema3a signaling inhibits integrin activity and thereby negatively regulates endothelial cell adhesion and motility [1,24,25]. Integrins (α 3, β 1) and their interacting proteins (*CD151*) facilitate podocyte adhesion to the GBM and are required for capillary loop and GBM assembly [26-28]. Therefore, we tested whether excess sema3a regulates α v β 3 and β 1 integrin expression and activity in the adult kidney. In the normal kidney α v β 3 integrin is expressed by the glomerular endothelium and by podocytes, the target cells for sema3a paracrine [13,25] and autocrine effects [4,11,16]. Using antibodies that specifically recognize total or active integrins we found that *Sema3a* gain-of-function decreases glomerular α v β 3 integrin expression and activity ~ 50% in vivo, suggesting that low α v β 3 activity contributes to the observed glomerular phenotype. Remarkably, we detected similar downregulation of glomerular α v β 3 integrin activity and similar glomerular phenotype in mice harboring *Vegf-a* knockdown in podocytes [29]. VEGF-A, an angiogenic

factor required for glomerular development and maintenance is, like sema3a, secreted by podocytes in vivo [30]. Both VEGF-A and sema3a bind neuropilin-1, which functions as a co-receptor for VEGF-A and a binding receptor for sema3a, in competitive fashion [1,15,25]. Moreover, neuropilin 1, VEGF receptor 2 and 3 integrin interact in podocytes and in endothelial cells [29,31]. Notably, this occurs downstream of both sema3a excess [20] or *Vegf-a* knockdown [29], suggesting that both proteins secreted by podocytes mediate opposite signals in the same pathway or in independent pathways that merge to downregulate integrin activity in vivo and in cultured cells [24,25]. Collectively, our findings suggest that sema3a excess or *Vegf*-knockdown - induced decrease in $\alpha v\beta 3$ integrin activity disrupts the integrity and function of the glomerular filtration barrier [20,29], causing glomerular phenotypes similar to integrin deletion models, except for the fact that they are dynamic and reversible (at least partially), mimicking pathophysiological changes in *Sema3a* or *Vegf-a* expression [20,29,32] (Figure).

Disregulation of integrin activity in podocytes has been implicated in glomerular disease, namely in nephrotic syndrome and focal segmental glomerulosclerosis, involving both β integrin activation and inactivation triggered by excess circulating suPAR or autocrine B7.1 upregulation, respectively [33,34]. Whether sema3a-induced $\alpha v\beta 3$ integrin decreased activity disrupts the podocyte cytoskeleton via the same actin binding proteins involved in foot process effacement caused by B7.1 remains to be determined. Evaluation of sema3a possible role in human nephrotic syndrome is ongoing.

Sema3a gene regulation in health and disease are poorly understood, and the majority of reports are focused on sema3a downstream signaling, developmental and pathological effects [1,6,7,13]. Our group reported increased sema3a protein in a mouse model of type 1 diabetes [32], and we observed sema3a increase in human renal biopsies with advanced diabetic nephropathy (Veron & Tufro, unpublished results), raising the possibility that excess sema3a may contribute to the pathogenesis of diabetic nephropathy, which we are actively investigating. Recently other groups reported sema3a dysregulation in patients with systemic lupus erythematosus [35,36], and that sema3a is increased early on in acute kidney injury [37,38], presumably due to sema3a upregulation in renal tubules. In addition, elegant studies provided evidence that sema3a functions as a negative regulator of immune responses [6], has osteoprotective [7], and anti-angiogenic effects [39], and suggested sema3a therapeutic potential for immune diseases, osteoporosis and cancer. Therefore, the off-target effects of sema3a, such as sema3a-induced glomerular disease should be carefully examined.

Conclusions

In summary, our studies demonstrate that tight sema3a regulation is essential for the maintenance of the glomerular filtration barrier structure and function in adult mice. Indeed, excess podocyte sema3a causes glomerular disease. We have uncovered the mechanisms whereby sema3a induces podocyte effacement, disrupts of the integrity of the GBM and causes endothelial injury, namely downregulation of nephrin via plexinA₁-nephrin interaction, F-actin cytoskeleton collapse, and decreased $\alpha v\beta 3$ integrin activity in glomerular podocytes and endothelial cells (Figure). Ongoing studies of sema3a signaling in

podocytes downstream from plexinA₁ may identify additional molecular and cellular pathogenic mechanisms and novel therapeutic targets for renal disease and diabetic nephropathy.

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Abbreviations

CD2AP	CD2-associated protein
GBM	glomerular basement membrane
MMP9	matrix metalloproteinase 9
sema3a	semaphorin3a
TEM	transmission electron microscopy
Vegf-a	mouse vascular endothelial growth factor A
WT1	Wilms tumor-1

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Figure. Model for sema3a-induced glomerular disease mechanism

Excess sema3a binds to neuropilin1 leading to activation of plexinA1, interaction with nephrin and integrin inhibition at the cell membrane, causing foot process effacement endothelial injury and glomerular basement membrane (GBM) lamination. Dashed lines indicate probable additional protein-protein interactions. *Vegf-a* knockdown also inhibits integrin activity and results in similar phenotype.