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Emerging Role of SH3BP2 as Regulator of Immune and Nonimmune Cells in Nephrotic Syndrome

Tarak Srivastava[a, b] Mukut Sharma[b, c]

[a]Section of Nephrology, Children's Mercy Hospital and University of Missouri at Kansas City, Kansas City, MO, USA; [b]Kansas City VA Medical Center, Kansas City, MO, USA; [c]Midwest Veterans' Biomedical Research Foundation (MVBRF), Kansas City, MO, USA

Keywords

Nephrotic syndrome · Immune activation · SH3BP2 · PLCG2 and VAV2

Abstract

Background: Minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) are major forms of nephrotic syndrome that remain difficult to treat. MCD and FSGS have distinct but also overlapping clinical, histological, metabolic, and molecular features. Effective use of immunosuppressive drugs, activated immune cells, altered cytokine profiles, and upregulated signaling pathways suggest a link between immune dysfunction and nephrotic syndrome, but the exact mechanism of immunopathogenesis is unclear. Immune dysfunction is an area of ongoing research for identifying novel molecular targets for treating nephrotic syndrome. However, the available animal models do not directly address the role of immune dysfunction in nephrotic syndrome. ***Summary:*** Genetic analysis indicates that heterogeneous genes related to the podocyte-specific proteins may indirectly cause damage to filtration barrier and influence the onset and progression of nephrotic syndrome. SH3BP2 protein regulates several pathways through its role as a scaffold for many signaling mediators and enzymes. SH3BP2 is expressed in immune as well as in nonimmune cells including podocytes. The role of SH3BP2 is discussed in the context of cells and molecules of adaptive and innate immune systems. Available information on the importance of SH3BP2 in diseases other than nephrotic syndrome and its role in the immunopathogenesis of human nephrotic syndrome are summarized. We outline the key features of a transgenic mouse strain with a gain-in-function mutation (*Sh3bp2*$^{KI/KI}$) as a potential model to study immunopathogenesis of nephrotic syndrome. ***Key Messages:*** Non-receptor, non-catalytic proteins such as SH3BP2 are a novel group of proteins that regulate the innate and adaptive immune responses in nephrotic syndrome. New evidence suggests a critical role of SH3BP2 in immunopathogenesis of nephrotic syndrome. Our recent results demonstrate that transgenic mice (*Sh3bp2*$^{KI/KI}$) with a gain-in-function mutation will likely be a unique model to study immunopathogenesis of nephrotic syndrome.

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Introduction

Idiopathic nephrotic syndrome (INS) is a condition characterized by heavy proteinuria, hypoalbuminemia, hypercholesterolemia, and edema. It has an incidence of 2.2 cases/100,000 children and 3.0 cases/100,000 adults

karger@karger.com
www.karger.com/gdz

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Correspondence to:
Tarak Srivastava, tsrivastava@cmh.edu

[1, 2]. The two most common histological findings on renal biopsy in INS are minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS). INS is characterized by the absence of inflammatory cells on light microscopy, negative immunofluorescence for antibody or complement deposition, and effacement of the foot processes and slit diaphragm of the podocyte (visceral epithelial cell) on electron microscopy, which indicates the breakdown of the glomerular filtration barrier in INS.

Patients with INS are classified as having either steroid-sensitive (SSNS) or steroid-resistant nephrotic syndrome (SRNS) based on their response to steroids. Additional immunosuppressive drugs are prescribed based on the clinical course of the disease [3]. While immunosuppressants effectively control the disease in many patients with nephrotic syndrome, they are associated with both acute and long-term side effects, including short stature, obesity, hypertension, bone mineral diseases, ocular complications, undesirable fertility outcomes, and potential malignancies [4, 5]. This presents a challenging situation for patients with nephrotic syndrome as they are treated with potent immunosuppressive agents while the underlying immunopathogenesis of the disease remains unclear.

It is uncommon for a child with MCD or FSGS to have a sibling, parent, or first-degree relative with nephrotic syndrome. In a recent review, more than 60 gene mutations reported in SRNS can be grouped into genes involved in the "podocyte slit diaphragm," "signaling to actin cytoskeleton," "signaling to the glomerular basement membrane," and "other podocyte mutations" [6]. Pathogenic genetic variants account for 10–30% of children with SRNS and rarely associate with SSNS [7]. These monogenic mutations in SRNS have improved our understanding of podocyte biology in nephrotic syndrome, although such mutations account for only a small subset of all patients with nephrotic syndrome.

In large population genome-wide association studies in children with SSNS, variants in the HLA-DR/DQ region (HLA-DQA1, HLA-DQB1, HLA-DRB1), genes associated with podocytes (NPHS1, KIRREL2), with immune regulation (PLCG2, BTNL2, CALHM6, TNFSF15, AHI1, CLEC16A), and with actin cytoskeleton (ELMO1) have been reported, but pathogenic gene mutations are extremely rare [8–13]. The association of SSNS with variants in HLA-DR/DQ that encode MHC class II molecules for antigen presentation supports a role for the immune system with an overlay of genetic susceptibility in SSNS. While monogenic causes are predominantly limited to SRNS, overlapping genetic risk factors in SSNS and non-monogenic SRNS suggest a shared common mechanism for immunopathogenesis of these conditions [14]. At the regulatory level, transcription factors with a known role in immune regulation have been shown to have an enriched binding to genetic loci of SSNS, suggesting genetic regulatory mechanism underlying SSNS, with podocytes as their secondary target [15]. Thus, a likely confluence of several gene variants, their interactions, and environmental factors contributes to SSNS. These lines of evidence are further strengthened by our recent analysis of the glomerular transcriptome data from the NEPTUNE consortium, which identified SH3BP2, a scaffold protein that regulates several signaling proteins involved in immune signaling in both immune cells and nonimmune cells [16].

Objectives

The following provides a brief description of the key molecular features and role of Src homology (SH) domain 3 binding protein 2 (SH3BP2) and its binding partners. Our ongoing work on how SH3BP2 regulates multiple pathways in INS is outlined, along with reports linking SH3BP2 to pathological conditions to show its connection to immune signaling. Available information on the role of SH3BP2 in adaptive and innate immune systems and its relevance to INS is summarized. The role of known cytokines and key signaling pathways downstream of SH3BP2-binding proteins in the pathogenesis of INS is highlighted. Additionally, data from a transgenic mouse overexpressing SH3BP2 will be summarized to evaluate its potential as a novel model to study the immunopathogenesis of nephrotic syndrome.

Src Homology Domains

Src Kinase Family

SH3BP2 is a key mediator of several signaling pathways largely due to its ability to bind many proteins containing SH3 domains that were first described in Src family kinases. Briefly, the Src kinase family consists of non-receptor protein tyrosine kinase proteins involved in various cellular processes such as angiogenesis, differentiation, proliferation, and migration. This family includes several subgroups: Src, Yes, Fyn, and Fgr form the SrcA subfamily, while Lck, Hck, Blk, and Lyn belong to the SrcB subfamily. Additionally, Brk, Frk, and Srm form the Brk family [17]. Apart from their well-studied role in oncogenesis, Src kinases play crucial roles in signaling

pathways related to acute kidney disease, chronic kidney disease of various causes, and kidney and lung fibrosis [18–20]. The significant role of SH phosphatases in diabetic nephropathy has also been recently reviewed [21]. Several low molecular weight compounds are being studied as potential inhibitors of Src kinase activity in CKD [19].

SRC Homology Domains

Src kinases possess regulatory domains that maintain low basal activity, guide localization, and facilitate interactions with substrates and other signaling proteins. Src homology domains 1–4 (SH1–4) are arranged from the C to N terminus. The SH1 domain is the catalytic domain, while SH2 and SH3 domains are non-catalytic and are involved in protein-protein interactions. The SH4 domain at the N-terminus is responsible for membrane binding. Between the SH4 and SH3 domains, each Src kinase contains a region of variable sequence that is unique to that kinase.

SH2 and SH3 Domains

Proteins lacking catalytic activity but containing SH2 and SH3 domains can recognize amino acid motifs containing phosphotyrosine and polyproline. These proteins act as adaptors, linking tyrosine kinases with target proteins to activate signaling pathways [22].

The SH2 domain, ~100 amino acids long, is located N-terminally to the SH1 (catalytic) domain in Src kinases. It is constituted by an N-terminal α-helix on each side of a 4-stranded antiparallel β sheet. A conserved arginine residue on the SH2 domain interacts with phosphotyrosine. Another pocket interacts with hydrophobic amino acid residues downstream of phosphotyrosine. By interacting with pYEEI motif of more than 100 target proteins, SH2 domain plays key role in recruitment and localization of substrates. Additionally, it is believed to stabilize several active non-receptor kinases through direct interactions [23].

The SH3 domain, ~60 amino acids in length, is a critical component of cell signaling, typically located at the C-terminal end. It adopts a globular structure composed of five or six β strands arranged in two tightly packed antiparallel β sheets. In humans, approximately 300 SH3 domains have been reported in 207 human proteins. Type I and type II SH3 domain ligands contain RPLPPLP and ØPPLPXR (Ø = a hydrophobic amino acid; P = proline; L = leucine; R = arginine; X = any amino acid) as consensus sequences, respectively. SH3 domains interact with adaptor/scaffold proteins and tyrosine kinases, and their binding is affected by phosphorylation or changes in amino acid sequences. However, SH3 domains interact with small peptides or non-proline with lower affinity. Protein interactions mediated by SH3 domains are highly dependent on the characteristics of SH3 host protein since these domains participate in intramolecular associations and module formation [22].

SH3BP2 Scaffold and Proteins Containing SH3 Domains

Scaffold proteins play a crucial role in facilitating cellular signaling by acting as platforms that spatially organize two or more proteins as functional units near the plasma membrane or intracellular locations. Such organization enhances the specificity and higher order regulation required for intracellular signaling. These non-receptor proteins, with or without catalytic activity, can tether proteins involved in signaling pathways. They can also position multiple proteins to specific areas within the cell, regulate signal transduction by coordinating positive and negative feedback signals, protect their ligands (signaling components) from deactivation, and minimize "false signals" by insulating signaling proteins from competing proteins. This ability to localize, organize, and orient signaling proteins enables scaffold proteins to play a unique regulatory function [24].

Several protein ligands of the SH3 domain have been identified, including members of the SH3 domain-binding protein (SH3BP) family. This family includes SH3BP1, SH3BP2, SH3BP3 (ZNF106), SH3BP4, and SH3BP5 (BTK associated). The scaffold protein SH3 domain-binding protein 2 (SH3BP2) with a molecular weight of ~62 kDa and 561 amino acids (UniProt: P78314) was discovered as a ligand of the SH3 domain of the kinase Abl [25]. The SH3BP2 gene, located on chromosome 4, spans 48 kb and contains 13 exons (Gene ID: 6452; Ensembl ID: ENSG00000087266; NCBI Accession: NM_003023, NP_003014). The mouse *Sh3bp2* gene, located on chromosome 5, spans 34 kb and contains 17 exons (Gene ID: 24055; Ensembl ID: ENSMUSG00000054520; NCBI Accession: NM_001145858, NP_001139330). The mouse protein has a molecular weight of ~62 kDa and 559 amino acids (UniProt: Q06649). SH3BP2 is highly conserved, with 87% and 84% sequence homology at protein and nucleotide levels, respectively, between human and mouse [26]. Gene Ontology (GO) molecular functions assigned to SH3BP2 include modulation of (1) phosphotyrosine residue binding (GO: 0001784), (2) protein binding (GO: 0005515), and (3) SH3 domain binding (GO: 0017124).

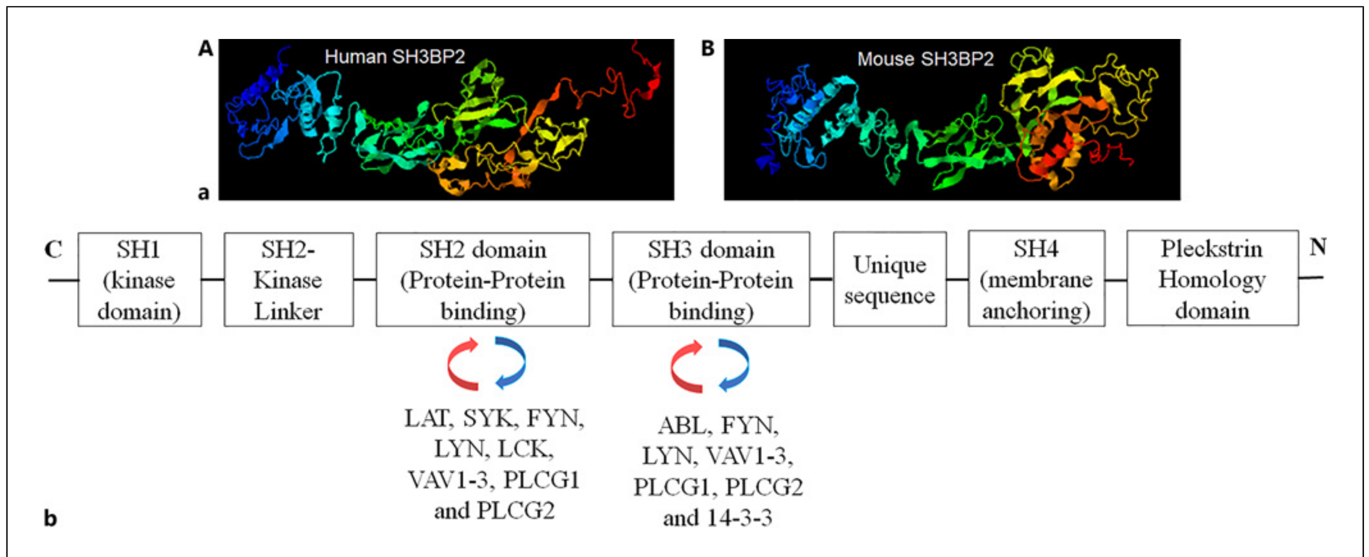

Fig. 1. a Ribbon models of human (**A**) and mouse (**B**) SH3BP2. **b** Arrangement of SH3BP2 domains from N to C terminus. Function of each domain is outlined.

SH3BP2 is a modular protein featuring an N-terminal pleckstrin homology domain that links membrane phosphatidyl inositol lipids with G proteins and protein kinase C, a proline-rich region that binds SH3 domains, and a C-terminal SH2 domain that recognizes phosphotyrosine (as shown in Fig. 1). Phosphorylation at Tyr, Ser, or Arg residues enables SH3BP2 to bind to partner/ligand proteins, thus facilitating downstream signaling pathways. For instance, phosphorylation at Tyr^{183}, Tyr^{446}, and Tyr^{448} enables interaction with VAV1, LCK, and activation of T cells, respectively. Phosphorylation at Ser^{225} and Ser^{277} allows interaction with 14-3-3 proteins. Phosphorylated SH3BP2 also links membrane phosphatidylinositols lipids to hematopoietic tyrosine kinase Fes (proto-oncogene *c-Fes/Fps*) [26]. The known binding partners of SH3BP2 are shown in Table 1. SH3BP2 is post-translationally modified by tankyrase through the addition of ADP-ribosyl chain, which marks it for E3-ubiquitin ligase RNF146 for proteasomal degradation [27, 28].

SH3BP2 and Disease Conditions

SH3BP2, a scaffold protein, forms a signaling complex (signalosome) with its binding partners and regulates immune signaling in both immune cells and nonimmune cells (as shown in Fig. 2). The gain-of-function mutation in *Sh3bp2* mice results in human phenotype of cherubism and nephrotic syndrome (vide infra) while loss-of-function mutation in *Sh3bp2* in mouse is not linked to a specific human disease [29, 30]. Mice lacking *Sh3bp2* show suboptimal activation of T and B cells [30–33]. *Sh3bp2*$^{-/-}$ mice are osteoporotic with impaired osteoblast and osteoclast function [27]. Thus, *Sh3bp2*$^{KI/KI}$ and *Sh3bp2*$^{-/-}$ mice along with the wild type *Sh3bp2*$^{+/+}$ mice (control) present a suitable model to investigate changes in signaling pathways in both immune and nonimmune cells.

Cherubism

The binding of tankyrase to SH3BP2 results in proteasomal degradation of SH3BP2 [27, 28]. As mentioned, tankyrase induces a post-translational modification by adding ADP-ribosyl chain as a marker for E3-ubiquitin ligase RNF146 for proteasomal degradation. In humans with cherubism, a mutation in tankyrase binding sites at Pro418 (to Leu, Arg, or His), Arg415 (to Pro or Gln), and Gly420 (to Glu or Arg) result in a marked increase in SH3BP2 activity due to decreased degradation [34]. A homozygous transgenic knock-in mouse (*Sh3bp2*$^{KI/KI}$) with a proline-to-arginine (P416R) mutation in the SH3BP2 gene develops a mouse equivalent of human cherubism [29]. Cherubism is characterized by abnormal bone tissue growth in the lower face, particularly the jaw and cheekbones, giving affected individuals a rounded, "cherub-like" facial appearance. It typically manifests in early childhood and may cause facial swelling, dental

Table 1. Binding partners of SH3BP2 as available on the Human Protein Atlas (www.humanproteinatlas.org)

Name	Description	Total interactions in all IntAct
ABL1	Tyrosine-protein kinase ABL1	448
ARHGAP10	Rho GTPase-activating protein 10	25
ccsb orf id: 71178	The center for cancer systems biology open reading frame id: 71178	141
CD244	Natural killer cell receptor 2B4	82
CTTN	Src substrate cortactin	10
DBNL	Drebrin-like protein	132
ERBB2	Receptor tyrosine-protein kinase erbB-2	933
HCLS1	Hematopoietic lineage cell-specific protein	56
KIT	Mast/stem cell growth factor receptor kit	385
LCK	Tyrosine-protein kinase Lck	341
MET	Hepatocyte growth factor receptor	433
MYO1F	Unconventional myosin-If	31
NLRP12	NACHT, LRR, and PYD domains-containing protein 12	85
PDLIM7	PDZ and LIM domain protein 7	399
PLCG1	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-1	405
PLCG2	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-2	122
PTPN6	Tyrosine-protein phosphatase non-receptor type 6	253
SH3BP2	SH3 domain-binding protein 2	63
SH3KBP1	SH3 domain-containing kinase-binding protein 1	438
TF	Serotransferrin	232
TNKS2	Poly [ADP-ribose] polymerase tankyrase-2	149
VAV1	Proto-oncogene vav	219
VAV2	Guanine nucleotide exchange factor VAV2	87
VAV3	Guanine nucleotide exchange factor VAV3	39
YWHAQ	14-3-3 protein theta	1,644

The protein-protein interaction data are selected in which the protein pairs have at least one interaction classified as direct interaction or physical association in the Human Protein Atlas.

abnormalities, and bone expansion. While cherubism often stabilizes or regresses after puberty, it can vary widely in severity and its impact on facial appearance and dental health. Mice with a gain-of-function mutation in *Sh3bp2* (referred to as "cherubism" mice) exhibit lymphocyte-independent trabecular bone loss, TNF-alpha-dependent inflammation, and cortical bone erosion. Mutant myeloid cells, similar to myeloid cells with overexpressing wild-type SH3BP2, show increased responses to M-CSF and RANKL stimulation, leading to the formation of large osteoclasts. These findings indicate that the mutant phenotype is a result of gain-of-function mutation of *Sh3bp2* [29].

Lupus Erythematosus

Studies using mouse models of lupus erythematosus have highlighted the functions of SH3BP2 in immunopathogenesis. In one study, $Fas^{lpr/lpr}$ mice were utilized as a lupus model. The deficiency of *Sh3bp2* in these mice was found to be associated with the amelioration of the lupus phenotype. Specifically, the deficiency of *Sh3bp2* significantly reduced splenomegaly, renal abnormalities, serum anti-dsDNA antibody levels, and the presence of B220+ CD4− CD8− T cells in the spleen. Importantly, these effects were independent of T and B cells [35]. In another model of lupus erythematosus, double-mutant

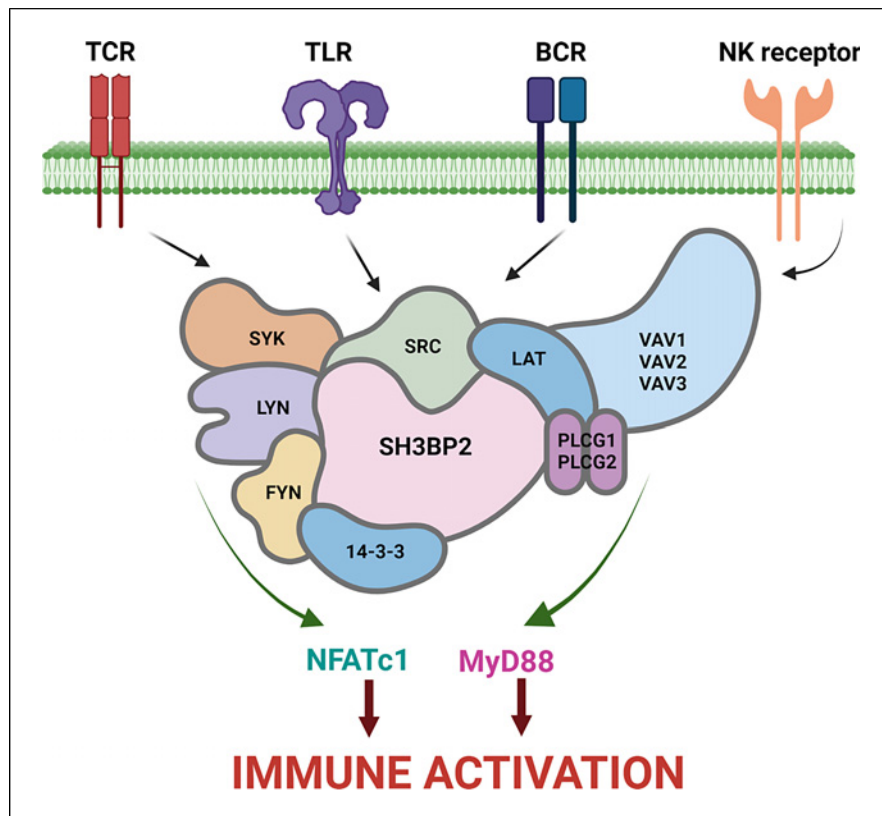

Fig. 2. SH3BP2 "signalosome." SH3BP2, a cytoplasmic scaffold protein, integrates multiple signaling pathways in T cells, B cells, macrophages, natural killer (NK) cells, etc., by complexing with Src family kinases (LYN and FYN), Syk family kinases, Rho-guanine nucleotide exchange factor VAV (VAV1, VAV2, and VAV3), linker for activation of T cells family member (LAT), phospholipase C gamma (PLCγ1 and PLCγ2), and 14-3-3 group of proteins following activation of T-cell receptor (TCR), B-cell receptor (BCR), TLRs, or NK receptor. The protein binding with SH3BP2 is dependent on the cell type. The SH3BP2-mediated downstream activation of NFATc1 and MyD88 results in immune activation (used with permission [16]).

mice ($Sh3bp2^{KI/+}Fas^{lpr/lpr}$) were studied. These mice carried a gain-of-function mutation in *Sh3bp2*, which led to amelioration of clinical and immunological phenotypes. The gain-of-function mutation in *Sh3bp2* improved survival and reduced glomerulosclerosis, proteinuria, and serum levels of anti-dsDNA antibody [36].

Other Diseases

The *SH3BP2* gene has been linked to several other conditions, including differential hypermethylated regions in patients or models of atherosclerosis [37], metabolic-associated fatty liver diseases [38], recurrent pericarditis [39], leukemia [40], gastrointestinal tumors [41], giant cell granulomas [42], bladder cancer [43], and arthritis [44]. Overall, studies on the role of SH3BP2 in human cherubism have provided important insights into this protein and its potential role in other diseases.

Nephrotic Syndrome

We reported an upregulated expression of SH3BP2, its associated signalosome proteins, and downstream signaling pathways in the glomerular transcriptome using kidney biopsies of adults and children with MCD and FSGS (NEPTUNE consortium data). Our results showed a significant increase in *SH3BP2* expression in MCD (p = 0.001) and FSGS ($p < 0.001$). Similarly, the SH3BP2-signalosome score and downstream MYD88, TRIF, and NFATc1 were also upregulated in MCD and FSGS. Immune pathway activation scores for toll-like receptors (TLRs), cytokine-cytokine receptor interaction, and NOD-like receptors were elevated in FSGS [16]. These findings suggest a role for SH3BP2 in both immune and nonimmune cells, including podocytes and mesangial cells. The pathway network map generated using *SH3BP2* as the seed gene in glomerular transcriptome (NEPTUNE cohort) shows that SH3BP2-mediated signaling involves PLCγ2 and VAV2 [16].

Transgenic Mice with a Gain-in-Function Mutation (*$Sh3bp2^{KI/KI}$*) and Nephrotic Syndrome

Several animal models of nephrotic syndrome involve administering chemicals or antibodies that have toxic effects on the glomerular filtration barrier, leading to proteinuria. Such compounds include polycations (e.g., protamine sulfate), antibiotics (e.g., puromycin, adriamycin), bacterial toxin lipopolysaccharide (LPS), and

podocyte-specific antibodies (anti-Fx1A). Additionally, targeting genes specific to podocytes or glomerular basement membrane proteins (such as podocin, nephrin, CD2AP, TRPC6, podocalyxin, laminin β2, collagen α3, etc.) has been an effective approach to induce proteinuria in animal models [45]. A recent review summarizes studies on the deletion of additional genes resulting in proteinuria [46]. However, there is no model to study immune dysfunction leading to nephrotic syndrome. With growing interest in the immunopathogenesis of nephrotic syndrome, such animal models will be highly valuable.

We used the observed upregulation of *SH3BP2* and its binding partners in human kidney biopsies to study the renal phenotype in transgenic mice with a gain-of-function mutation (*$Sh3bp2^{KI/KI}$*). The *$Sh3bp2^{KI/KI}$* transgenic mice showed significant albuminuria starting at 4 weeks, with a marked increase by 12 weeks of age. These mice also developed decreased serum albumin without changes in serum creatinine by 12 weeks of age. Kidney morphology in *$Sh3bp2^{KI/KI}$* mice appeared normal except for increased mesangial cellularity and foot process fusion without electron-dense deposits [16]. The "cherubism" phenotype of *$Sh3bp2^{KI/KI}$* animals can be rescued when crossed with $TNF\alpha^{-/-}$ or $MyD88^{-/-}$, but not with $Rag1^{-/-}$ mice, which lack T cells and B cells [29]. Further, crossing *$Sh3bp2^{KI/KI}$* mice with $TLR2^{-/-}$, $TLR4^{-/-}$, and $TLR2^{-/-}/TLR4^{-/-}$, but not with $IL1\beta^{-/-}$ mice, rescued the cherubism phenotype [47].

These findings demonstrate that immune activation in *$Sh3bp2^{KI/KI}$* mice is mediated by the innate immune system (TLR2/4-Syk-MyD88-NFκB-TNFα pathway) and is independent of T and B cells. The nephrotic syndrome phenotype observed in this model provides an advantage for studying the role of immune activation, particularly innate immune activation, in nephrotic syndrome, in contrast to other animal models used for this purpose.

Adaptive and Innate Immunity in Nephrotic Syndrome

The adaptive and innate arms of the immune system do not function in isolation but constantly interact with each other. Both adaptive and innate immune systems have been implicated in nephrotic syndrome as the molecular markers of T and B cells as well as those of macrophages are upregulated [48]. We will briefly discuss what is known about adaptive and innate immune systems in nephrotic syndrome and how SH3BP2 could play a role in development of nephrotic syndrome.

T Cells in Nephrotic Syndrome

In 1974, Shalhoub first implicated T cells in nephrotic syndrome [49]. However, a definitive T-cell subset has not yet been identified [50]. Early studies in nephrotic syndrome focused on the secretory products of T cells. Supernatant from T-lymphocyte cultures of children with nephrotic syndrome caused proteinuria and podocyte changes in rats, suggesting a role for cytokines and other soluble factors that remain unidentified [51]. Cytokines such as IL-2 are significantly elevated in the serum of children with MCD during relapse, with trends for elevated levels of sIL-2R and IFN-γ [52]. Similarly, increased production of IL-2 and IL-4 from T cells is observed in supernatants from children with MCD in relapse [53]. Elevated mRNA for IL-13 is reported in both CD4+ and CD8+ T cells in children with MCD during relapse [54]. We demonstrated that children in relapse exhibit a significant increase in serum IL-2, an increase in IL-2 in supernatant from monocytes, and an increase in CD3+ CD4+ cells expressing IL-2 by flow cytometry, while the levels of IL-4 and IFN-γ were not statistically significant (abstract presentation: Srivastava et al. Pediatr Nephrol. 2001;16:C129).

Engagement of the T-cell receptor (TCR) triggers tyrosine phosphorylation of SH3BP2, resulting in its recruitment to cytoplasmic lipid rafts and the formation of a signaling complex. SH3BP2 phosphorylation at Tyr448 in response to TCR engagement activates calcineurin-dependent increased transcriptional activity of nuclear factor activated in T cells (NFAT) [31, 32]. Suppression of SH3BP2 expression by siRNA inhibits TCR-mediated activation of NFAT [32]. Subsequent studies show that SH3BP2 creates a time-dependent positive feedback loop that sustains ERK and NFAT activity following T-cell activation and also regulates effector cell and memory cell differentiation [33]. Phosphorylation at Tyr183 is required for SH3BP2 interaction with Vav1 (a guanine nucleotide exchange factor of Rac1), Tyr446 for binding to Lck, and Arg486 for binding to ZAP-70, to LAT for NFAT activation in T cells [32]. SHP-1, a phosphatase, specifically recruits SH3BP2 leading to its dephosphorylation and terminating T-cell signaling [31]. Phosphorylation of SH3BP2 by upstream kinases is required for 14-3-3 binding, which negatively regulates SH3BP2 function in T cells [55]. *These observations suggest a potential role for SH3BP2 and its binding partners in T-cell activation and the response to calcineurin inhibitors seen in* nephrotic syndrome.

B Cells in Nephrotic Syndrome

The role of B-cell dysfunction in nephrotic syndrome has been reinforced by the effectiveness of rituximab, a B-cell-targeting therapy, in both children and adults with this condition [56]. The observed increase in Epstein-Barr virus

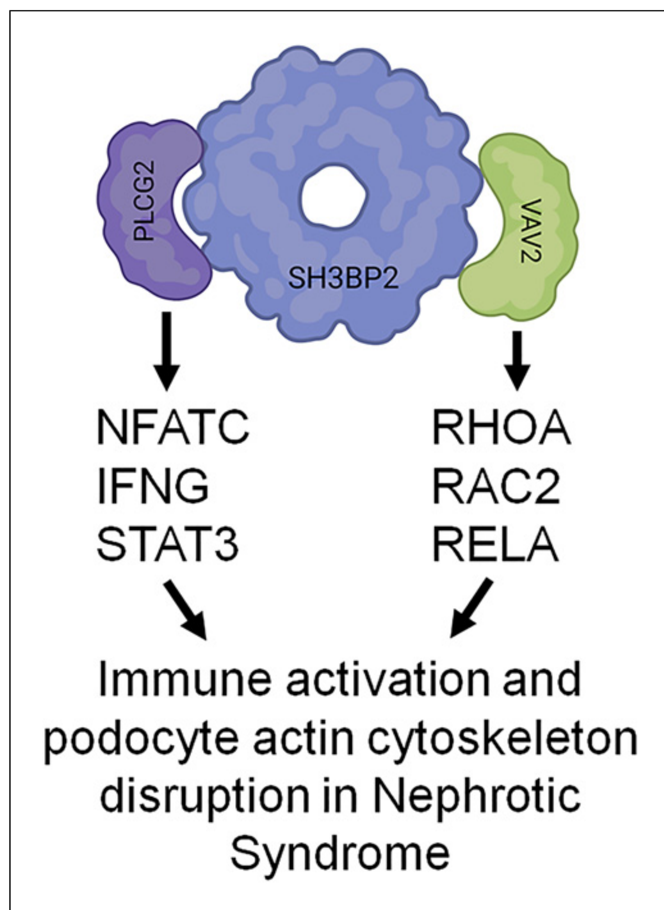

Fig. 3. PLCG2 and VAV2 are binding partners of SH3BP2 and coimmunoprecipitate in human podocyte lysates [16]. These are key components of immune signaling pathways leading to inflammation and actin cytoskeleton disruption in nephrotic syndrome. PLCG2 mediates activation of NFATC1, INFG, and STAT3 while VAV2 is involved in the RHOA-RAC2 interaction and RELA. NFATC1, nuclear factor of activated T cells, cytoplasmic 1; IFNG, interferon gamma; STAT3, signal transducer and activator of transcription 3; RHOA, Ras homolog family member A; RAC2, Ras-related C3 botulinum toxin substrate 2; RELA, RELA proto-oncogene, NF-KB subunit.

(EBV) DNA and EBV (VCA-IgM) antibodies, along with elevated memory B cells in children with nephrotic syndrome, has led to the formulation of the "EBV hypothesis" [57]. At the onset of nephrotic syndrome, children have significantly higher levels of circulating total CD19+ B cells and specific B-cell subsets (transitional, mature naïve, plasma cells, CD19+ CD27+, and atypical memory B cells), which is corrected with prednisone treatment [58]. Studies have also shown increased memory B cells (CD27+ CD19+) in children and increased plasmablasts (CD24− CD38^{high} CD27^{high}) in adult patients with nephrotic syndrome [59]. In some nephrotic syndrome biopsies, IgM deposits were initially observed, followed recently by the identification of circulating anti-actin, anti-ATP synthase beta chain, and anti-nephrin antibodies [60, 61]. A panel of antibodies to CD40, PTPRO, CGB5, FAS, P2RY11, SNRPB2, and APOL2 has been found to predict post-transplant FSGS recurrence [62].

Engagement of the B-cell receptor (BCR) triggers tyrosine phosphorylation of SH3BP2. SH3BP2, through its SH2 domain, binds to the CD19 signaling complex and is necessary for optimal Syk phosphorylation and calcium flux. B cells lacking SH3BP2 exhibit a proliferation defect and increased apoptosis in response to antigen receptor cross-linking [30]. Tyrosine phosphorylation at Tyr183 and the SH2 domain is required for SH3BP2-mediated BCR-induced activation of NFAT, and this is also regulated by Fyn, Syk, and Vav proteins [63]. *Sh3bp2*$^{-/-}$ animals show impaired BCR-mediated activation, including deficient B-cell proliferation, cell cycle progression, PLC-γ2 phosphorylation, calcium mobilization, and activation of NFAT, ERK, and JNK [30, 64]. Conversely, gain-of-function *Sh3bp2*$^{KI/KI}$ mice display enhanced BCR-mediated activation of NFAT, increased signaling complex formation with Syk, PLC-γ2, and Vav1, but no change in ERK and JNK activation [65]. SH3BP2 deficiency suppresses immunoglobulin production, especially autoantibody production in Fas$^{lpr/lpr}$ mice [35]. *These observations suggests a potential role for SH3BP2 and its binding partners in B-cell activation and the response to anti-CD20 antibodies therapies seen in* nephrotic syndrome.

Innate Immunity in Nephrotic Syndrome

Viral RNA/DNA and bacterial cell wall products activate the innate immune system through pattern-recognizing receptors such as TLRs, NOD-like receptors, RIG-I-like receptors (RLRs), complement system, etc., resulting in elevated levels of several serum cytokines. Viral infections are strongly associated with upper respiratory tract infections. These infections were shown to be temporally related to relapses (69%) and exacerbations (71%) in patients with nephrotic syndrome, leading the authors to conclude that a nonspecific host response is the primary reason for disease relapse/exacerbation [66]. Another study found that concomitant upper respiratory tract infection during relapse was a strong predictor of steroid dependence in nephrotic syndrome [67]. TLRs are sentinel receptors of the innate immunity. Viral RNA/DNA stimulates innate immunity via TLR signaling, leading to increased production of proinflammatory cytokines. TLRs activate two major pathways: the classical MyD88 dependent pathway and the non-MyD88 (or TRIF) pathway, which activate downstream transcription factors NFκB and IFN regulatory factors [68, 69]. LPS, a ligand for

TLR4, induces upregulation of TLR4 and disruption of the actin cytoskeleton in podocytes in vitro, as well as severe LPS-induced proteinuria in vivo in SCID mice (which lack T and B cells) [70]. Additionally, we reported functional TLRs and LPS-induced activation of the TLR4-MyD88-NFκB signaling pathway in human podocytes [71]. Nephrotic syndrome is consistently associated with elevated levels of serum IL-2, IL-2R, IFN-γ, and TNFα but normal levels of IL-1α, IL-1β, and IFN-α [52, 53]. Matsuguchi et al. [68] showed that cytokines such as IL-2, IL-1β, IL-15, IFN-γ, and TNFα can increase expression of TLR2 in vitro. Thus, the role of innate immune activation in nephrotic syndrome is evident by (i) frequent relapses due to viral or bacterial infection, (ii) LPS-induced proteinuria and podocyte foot process effacement in mice lacking T and B cells, (iii) the presence of functional TLRs in human podocytes that engage the TLR-MyD88-NFκB pathway, and (iv) the absence of inflammatory cells, complement proteins, or immune deposits (except occasional IgM) in MCD and FSGS.

Sh3bp2$^{KI/KI}$ mice showed elevated levels of TNFα, IL-6, MCP-1, IFN-γ, IL-2, IL-17, and MIP-1α but not IL-1α and CXCL1 [16]. Results of crossing *Sh3bp2*$^{KI/KI}$ mice with other transgenic mice suggest that SH3BP2 plays a role in innate immunity but not as part of the IL-1β inflammasome. *Sh3bp2*$^{KI/KI}$ mice develop all the phenotypic characteristics of nephrotic syndrome. Macrophages in *Sh3bp2*$^{KI/KI}$ mice or those derived from myeloid progenitor cells in the presence of M-CSF become hyperactive, mediated by ERK, and differentiate into osteoclasts in the presence of RANKL mediated by Syk [29]. In *Sh3bp2*$^{KI/KI}$ mice, Syk deficiency in macrophages ameliorated inflammation [47]. In NK cells, CD244 (or 2B4) activation recruits SH3BP2 and Vav1, increasing NK cell cytotoxicity [72, 73]. The relationship between immune dysregulation, cytokines, and nephrotic syndrome is still unclear but appears to be critical, given the response of this disease to immunosuppressive drugs. *These observations suggest a potential role for SH3BP2 and its binding partners in innate immune activation (via either macrophages or NK cells) and the response to steroid therapy in* nephrotic syndrome.

Integrating the Role of SH3BP2 in Nephrotic Syndrome

Respective ligands of TCR in T cells, BCR in B cells, and TLR in macrophages are known to stimulate SH3BP2 based on the available data from Sh3bp2 loss-of-function and gain-of-function mice. Additionally, we have found increased expression of Sh3bp2 expression in the glomerular transcriptome of patients with FSGS and MCD. Other factors/pathways that stimulate SH3BP2 expression/activity include (a) SH3BP2-dependent regulation of RANKL-mediated activation of NFATc1 required for osteoclastogenesis, (b) SH3BP2-dependent regulation of bone marrow monocyte response to M-CSF, (c) CD244 ligation-induced SH3BP2 phosphorylation and CD244Tyr337 phosphorylation-dependent regulation of CD244-SH3BP2 interaction in NK cells, (d) stimulated autoinflammation by TLR-MYD88 signaling in SH3BP2 cherubism mice, and (e) tankyrase inhibitor-induced SH3BP2 accumulation leading to osteoclast formation and function. Overall, differential phosphorylation of SH3BP2 in accordance with its functions in various immune cells determines its binding with specific proteins, which, in turn, determines regulation of downstream signaling pathways. As discussed above, SH3BP2 is involved in immune activation of T cells and B cells of adaptive immunity and of macrophages and NK cells of innate immune system. We have shown SH3BP2 is also expressed in mesangial cells and podocytes [16]. We postulate three possibilities for the role of scaffold protein SH3BP2 in podocyte injury and disruption of the glomerular filtration barrier in nephrotic syndrome: (a) the direct involvement of SH3BP2 activation in podocytes, (b) a paracrine interaction between mesangial cells and podocytes, or (c) the influence of pro-inflammatory cytokines secreted by circulating immune cells. Bioinformatic analysis for in silico visualization of the pathways within the glomerular transcriptome from the NEPTUNE cohort for SH3BP2 showed SH3BP2 to engage with PLCG2 and VAV2. PLCG2 subsequently recruits NFATC1, IFNG, and STAT3, and VAV2 recruits RHOA, RAC2, and RELA for downstream signaling [16]. The activation and dysregulation of SH3BP2 may integrate all known observations of T-cell, B-cell, and innate immune activation, as well as treatment responses observed in nephrotic syndrome.

Future Directions

Nephrotic syndrome remains enigmatic due to several unresolved questions: (a) What is the underlying immune mechanism that causes podocyte injury? (b) How does immune activation lead to the reversible structural changes observed as foot process effacement in podocytes? (c) What triggers immune activation in nephrotic syndrome? We propose that SH3BP2 dysregulation may hold the key to these questions. Our hypothesis is that immune activation leading to podocyte injury may involve the interaction between SH3BP2 and PLCG2, while the structural changes in podocytes may involve the interaction between SH3BP2 and VAV2, as suggested by our preliminary data (shown in

Fig. 3). Furthermore, we speculate that viral infections could be the trigger for immune activation and that the regulation of SH3BP2 by SHP-1 and/or 14-3-3 might be inadequate in nephrotic syndrome. Further studies are essential to explore these possibilities and answer these potentially important questions.

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Conflict of Interest Statement

Authors do not have any competing financial interests to declare regarding the contents of the manuscript. The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs or the US government.

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Author Contributions

T.S. and M.S. drafted, revised, and finalized the manuscript. Authors approved the final version of the manuscript.

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