Immunopathogenesis of idiopathic nephrotic syndrome with relapse.

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Abstract:	Idiopathic change nephrotic syndrome (INS), the most frequent glomerular disease in children and young adults, is characterized by heavy proteinuria and a relapsing remitting course. Although the mechanisms underlying the pathophysiology of proteinuria remain unclear, clinical and experimental observations suggest that lymphocyte and podocyte disturbances are two sides of the disease. The current hypothesis suggests that immune cells release a putative factor, which alters podocyte function resulting in nephrotic proteinuria. Besides T cell abnormalities, recent evidence of B cell depletion efficacy in sustained remissions added a new challenge in understanding the immunological mechanisms of INS. In this review, we discuss recent insights related to podocyte disorders occurring in INS and their relevance in human diseases.

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Abstract

Idiopathic change nephrotic syndrome (INS), the most frequent glomerular disease in children and young adults, is characterized by heavy proteinuria and a relapsing remitting course. Although the mechanisms underlying the pathophysiology of proteinuria remain unclear, clinical and experimental observations suggest that lymphocyte and podocyte disturbances are two sides of the disease. The current hypothesis suggests that immune cells release a putative factor, which alters podocyte function resulting in nephrotic proteinuria. Besides T cell abnormalities, recent evidence of B cell depletion efficacy in sustained remissions added a new challenge in understanding the immunological mechanisms of INS. In this review, we discuss recent insights related to podocyte disorders occurring in INS and their relevance in human diseases.

Introduction

Idiopathic nephrotic syndrome (INS) is a primary glomerular disease, which mainly includes two histological variants, minimal change nephrotic syndrome (MCNS) and focal and segmental glomerulosclerosis (FSGS).

MCNS is a primary glomerular disease defined by massive proteinuria and hypoalbuminemia without inflammatory lesions or immune complex deposits. Glomerular changes consist mainly of podocyte foot-process effacement, assuming a flattened rather than a foot-like morphology. This aspect is non-specific since it is commonly observed in glomerular diseases with nephrotic proteinuria. MCNS is recognized as a chronic illness in childhood, accounting for 70% of idiopathic nephrotic syndrome (INS) in children and 25% of INS in adults [1, 2].

Focal and segmental glomerulosclerosis (FSGS), which is less frequent in young children (20%), is a distinct glomerular disease characterized by a nephrotic syndrome associated with histological lesions including segmental hyalinosis and sclerosis of the glomeruli [3]. Whereas MCNS seems to be a single entity, FSGS appears as a heterogeneous disease, with both immune and non-immune causes. Recent genetic approaches have elucidated some pathogenic aspects of FSGS by identifying several genes that play a critical role in podocyte function and glomerular filtration barrier [4-10]. However, a mechanistic explanation of the glomerular abnormalities in MCNS and idiopathic FSGS with relapse remains elusive. In this review, we will discuss the results of recent investigations that may contribute to our understanding of the pathophysiology of these glomerular diseases. Proteinuria in membranous nephropathy results also from podocyte dysfunction but this distinct glomerular disease deserves separate consideration.

The molecular relationship between MCNS and FSGS with relapse is unclear. Nearly 50% of patients with FSGS are steroid-sensitive but relapse. Given the infrequency of kidney biopsies in children with nephrotic syndromes, the relative prevalence of MCNS and FSGS at the time of presentation is unknown. The most critical parameter of prognosis is the response to steroid therapy: steroid-sensitive FSGS shares the same benign course as MCNS. Of those children with nephrotic syndromes, 80-90%, presumably MCNS, are steroid-responsive. Overall, 60-80% of steroid-responsive nephrotic children will relapse and about 60% of those will have five or more relapses [11]. Around 60% of MCNS children show steroid dependence. In these patients, anti-CD20 therapy by Rituximab has demonstrated its efficiency by inducing sustained remission even after switching off other immunosuppressive medications, while very rare side effects have been observed [12-14]. Thus, Rituximab

becomes an invaluable alternative therapy in patients with steroid and/or anti-calcineurin dependent nephrotic syndrome.

Over the past four decades, extensive research aiming to elucidate the pathogenesis of MCNS and FSGS with relapse yielded significant insight regarding both immune and podocyte disorders, although the exact origin of molecular mechanisms remain unclear.

Various alterations in cytokine production during MCNS have been described by a large number of studies, which, unfortunately, are not all in agreement [15]. This variation might result from the different immunogenetic backgrounds of the patients, or from the heterogeneity of studies on stimulated cells in environments that are far from physiological. It might also be due to the diversity of the dosage techniques, which do not always take soluble cytokine receptors into account. It can be deduced that TNF□ and IL13 levels often increase during relapse [16]. IL13 was found to increase transcellular ion transport in podocytes, which express IL13 receptor. IL13 transgenic rats develop nephrotic proteinuria and display a MCNS-like phenotype [17]. However, in many pathological conditions in which IL-13 is increased, such as asthma, psoriasis, and allergic dermatitis, proteinuria does not occur. Moreover, IL13 does not affect permeability to macromolecules [18]. These observations suggest that besides IL13, additional events are required to develop nephrosis in the human disease.

Angiopoietin-like 4 as potential soluble proteinuric factor in MCNS

Angiopoietin-like 4 (Angptl4) is a secreted glycoprotein, which was classified as an adipokine due to its predominant expression in adipose tissues and liver. Although it is involved in many functions including energy homoeostasis, wound repair, tumorigenesis, angiogenesis and redox regulation [19], its crucial role seems to be related to the regulation of lipid metabolism during fasting, in which Angptl4 induces hypertriglyceridemia through inhibition of lipoprotein lipase and stimulates intracellular adipocyte lipolysis [20]. The expression of Angptl4 is increased by various conditions and factors, including hypoxia, fasting, TGF□ and glucocorticoids, which are potent inducers of Angptl4 [21]. Transgenic mice overexpressing Angptl4 in liver did not exhibit overt phenotype or significant abnormalities but displayed high triglyceride serum levels [22].

Angptl4 has recently been reported as a possible mediator of MCNS, based on the finding that transgenic rats overproducing Angptl4 specifically in the podocyte, but not in adipose tissue, display heavy proteinuria with pathological features of MCNS [23]. The mechanism of proteinuria in this model was attributed to podocyte release of hyposialylated Angptl4, which interacts with negatively charged glycosaminoglycan chains, and promotes the loss of GBM charges, commonly observed in nephrotic proteinuria. Unfortunately, the serum and

urinary levels of Angptl4 and its derivatives in MCNS patients and related controls have not been reported.

CD80 and podocyte diseases

CD80, also named B7.1, is a transmembrane protein commonly expressed by antigen presenting cells (APC) and B cells, but not by normal podocyte. However, CD80 is induced in the latter by LPS and puromycin administration, as well as in human lupus nephritis, including proliferative and non-proliferative forms [24]. Further studies have shown that urinary CD80 levels are increased in MCNS but also in other glomerular diseases, suggesting that this phenomenon is common to proteinuric states [25-27]. In the absence of quantitative evaluation of podocyte CD80 abundance, the measurement of urinary CD80 deserves some caution because its podocyte origin is not ascertained.

The finding that LPS triggers a signaling pathway in podocyte miming APC through toll-like receptor 4 (TLR-4) is interesting in that it supports the role of podocyte not only as a target but also as an actor in the pathogenesis of glomerular diseases. The induction of CD80 by LPS trough TLR-4 signaling pathway is associated with cytoskeleton disorganization and foot process effacement, leading to proteinuria in wild-type but not in CD80-deficient podocytes [24]. Therefore, inhibition of CD80 could be of therapeutic interest in experimental models of nephrotic syndromes and in human glomerular diseases. This step has recently been achieved with success [28]. In this study, five patients with B7 positive podocyte biopsy-proven, four with FSGS recurrence and one with primary FSGS, were treated by Abatacept, a CD80 inhibitor (single dose, 10 mg/kg). Nephrotic syndrome was resolved in all patients with a sustained remission between 12 and 48 months. This study refines the mechanism of CD80-induced proteinuria. Using different *in vitro* and *in vivo* approaches, it was demonstrated that CD80 interferes with the binding of talin to integrin, thus preventing 1 integrin activation.

Expression of c-mip in glomerular diseases

C-mip was initially identified from MCNS T-cells by subtractive and differential cloning [29, 30]. The natural isoform of c-mip encodes an 86-kDa protein that does not belong to any known family. Its predicted structure includes an N-terminal region containing a pleckstrin homology domain (PH), a middle region characterized by the presence of several interacting docking sites including a 14-3-3 module, a PKC domain, an Erk domain, an SH3 domain similar to the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K), and a C-terminal region containing a leucine-rich repeat (LRR) domain (fig1). C-mip contains a nuclear localization site (NLS) near the C-terminus of the PH domain. Depending on physiological or pathological situations, c-mip can move

to the nucleus but its localization is not restricted to a particular subcompartment. In basal conditions, c-mip abundance is very low in most tissues examined, as well as in cell lines like Jurkat T or M15 ([31] and unpublished data). Unexpectedly, we found c-mip overproduced in the podocytes of patients with INS, including MCNS and FSGS with relapse [32]. A relevant argument supporting the implication of c-mip in INS comes from the observation that occurrence of INS in Hodgkin disease is closely associated with overproduction of c-mip in both Reed-Sternberg cells and podocytes [33, 34]. Several works summarized below point out a role of c-mip in the pathophysiology of immune and podocyte disturbances occurring in INS.

The expression of c-mip has been analyzed by both *in situ* hybridization, quantitative PCR on laser-microdissected glomeruli and Western-blots on glomerular protein lysates [32, 35]. Its basal expression is low or undetectable in humans, rats and mice, whereas in primary INS, as well as in mouse and rat models of adriamycin-induced nephrotic syndrome, c-mip is strongly increased ([32, 35] and unpublished data). By contrast, immunohistochemical studies show that c-mip is not expressed in glomerular proliferative diseases such as mesangiocapillary or extracapillary glomerulopathy [36], albeit low levels of transcript are identified by quantitative PCR. These observations suggest that c-mip is a marker of primary podocyte diseases without inflammatory proliferative lesions.

Functional consequences of c-mip induction in podocytes.

To clarify the potential effects of c-mip in podocytes, we generated transgenic mice in which a single copy of human c-mip is inserted in the HPRT locus by homologous recombination. The expression of c-mip is driven by the nephrin promoter, allowing selective expression in podocytes. Transgenic mice develop proreinuria a few days after birth and progress to nephrotic range in a few weeks. Morphological analysis shows only minimal glomerular lesions in the form of podocyte effacement and fusion of foot processes, while FSGS lesions occur lastly [36]. To investigate the mechanisms by which c-mip alters podocyte function and induces proteinuria, we used several approaches including the identification of c-mip partners by yeast-two hybrid screening and generation of inducible and stable c-mip transfectant podocyte cell lines. Among the partners, we found that Fyn, a major podocyte Src kinase, interacts *in vitro* and in *vivo* with c-mip through its PH domain [35]. In physiological conditions, Fyn binds and activates nephrin and N-WASP (*Wiskott Aldrich syndrome protein*), thus playing a key role in proximal signaling and cytoskeleton remodeling. It was recently shown that Nck interacts with and promotes Fyn-mediated phosphorylation of nephrin, a central component of the slit diaphragm

playing a crucial role in signal transduction and podocyte integrity [37, 38]. It is likely that phosphorylation of Fyn at Tyr 417 is required for Nck-Fyn interaction. Nephrin phosphorylation induces the recruitment of p85/p110 heterodimer of PI3 kinase (PI3K), through the interaction of nephrin SH2 domain with p85, the regulatory subunit of PI3K, leading to activation of the p110 catalytic subunit and generation of phosphoinositides [39]. This step initiates a signaling cascade including the activation of serine threonine kinase Akt and the recruitment of podocin and CD2-associated protein (CD2AP). The c-mip-Fyn interaction is merely negative, c-mip preventing Fyn phosphorylation at Tyr 417, which is required for its interaction with nephrin and N-WASP [32]. This results in inactivation of key proximal signaling events, including nephrin, PI3K, Akt and N-WASP phosphorylation, which is demonstrated both in stably transfected podocytes and *in vivo* in transgenic mice (Fig 2). Interestingly, inactivation of nephrin and Akt is confirmed in kidney biopsies from patients with INS, highlighting the role of proximal signaling alterations in the pathophysiology of podocyte disorders [32]. The inability of Fyn to phosphorylate nephrin might facilitate the interaction of the latter with □-arrestin2 and its subsequent endocytosis and degradation [40]. Indeed, nephrin expression is commonly decreased in c-mip transgenic mice as well as in INS [32]. However, its downregulation is not only observed in MCNS/FSGS but also in other primary nephrotic syndromes such as membranous nephropathy [41].

C-mip displays pro-apoptotic functions by p53-independent mechanisms

Podocyte survival is regulated by extracellular and intracellular signals. Nephrin and vascular endothelial growth factor (VEGF) recruit the PI3K-Akt signaling pathway, activating NF-□B and Wilm's tumor transcription factors, which are potent inhibitors of podocyte apoptosis [42]. Mutation of nephrin abrogates the antiapoptotic effect of VEGF, suggesting that nephrin is required for VEGF signaling [42]. CD2AP is an adapter protein anchoring nephrin to actin cytoskeleton and inhibits p38 mitogen–activated protein kinase (p38 MAPK) through PI3K-mediatedAkt activation [43, 44]. CD2AP-deficient mice develop nephrotic syndrome and renal failure caused by glomerulosclerosis [45]. Inhibition of PI3K pathway plays a determinant role in apoptosis occurring in CD2AP-deficient podocytes [39]. Although p38 MAPK is activated in glomeruli in many proteinuric renal diseases and in diabetic nephropathy, a greater increase is observed in inflammatory, proliferative forms such as severe lupus nephritis and vasculitis [46-49]. The increased p38 MAPK activation in glomeruli may be related to local cytokine production. In the podocyte, p38 MAPK can promote apoptosis by several mechanisms including increased production of reactive oxygen species and activation of Src homology-2 domain-containing phosphatase-1 (SHP-1) [49]. SHP-1 can be activated by PKC□, a substrate for caspase-3 [50]. Likewise, TNF-

□-induced podocyte apoptosis has been associated *in vitro* with p38 MAPK activation [43].

Apoptosis induction by c-mip could be the result of several non-exclusive mechanisms. Firstly, we found that c-mip upregulation induces *in vitro* and *in vivo* downregulation of Akt, an upstream activator of NF-□B and WT1 [32]. Inactivation of Akt is at least partly due to downregulation of PI3K, since c-mip interacts with the p85 regulatory subunit and inhibits the release of the p110 catalytic subunit that is required for PI3K activation ([51] and unpublished data). Secondly, c-mip activates p38 MAPK and increases the abundance of death-associated protein kinase (DAPK) [35, 51]. DAPK promotes apoptosis by disrupting integrin-mediated cell adhesion, altering cytoskeleton, which ultimately results in loss of extracellular matrix–dependent survival signals [52]. Alteration of the actin network induces the production of reactive oxygen species from mitochondria, leading to apoptosis [53]. Thirdly, It has been shown that c-mip binds RelA through its leucine-rich repeat–containing C-terminal domain and may target the latter to proteasome-dependent degradation [31], [36]. Altogether, *in vitro* and *in vivo* data suggest that c-mip is a multifunctional protein and may promote apoptosis through different mechanisms.

NF-□B/c-mip cross-talk influences the pathophysiology of podocyte diseases

Inhibition of angiogenesis by anti-VEGF and VEGF-receptor tyrosine kinase inhibitors (VEGFR TKIs) is believed as a major axis of oncology therapy. Because this ligand-receptor system is fully expressed and plays a prominent functional role in glomeruli, some on-target side effects are expected [54-56]. Data derived from several thousands of patients treated with anti-VEGF therapy show that anti-VEGF ligands (Bevacizumab and VEGF trap) may induce thrombotic microangiopathy (TMA), whereas VEGFR TKIs, such as sorafenib and sunitinib, are more frequently associated with heavy proteinuria [57, 58]. Histological analysis of renal biopsies of patients with nephrotic syndrome occurring under VEGFR TKI therapy shows minimal change and /or focal segmental sclerosis lesions (MC/FSG) without TMA-like lesions [58]. We found that c–mip abundance is highly increased in the podocytes of MC/FSG biopsies, whereas its expression is undetectable in TMA glomeruli [58]. On the other hand, RelA is upregulated in TMA glomeruli both in podocytes and endothelial cells, while it is dramatically reduced in MC/FSG lesions. These observations suggest that c-mip and RelA are mutually antagonistic (Fig 3). Indeed, we provide evidence that RelA binds to the c-mip promoter *in vivo* and represses its transcription, whereas RelA knockdown releases this inhibition [58]. Downregulation of RelA by VEGFR TKI such as sorafenib, also reported by other authors [59] may release the transcriptional activation of c-mip as observed in sorafenib-treated podocytes [58]. Altogether, these results may account for c-mip downregulation in

the podocytes of patients with TMA. Conversely, RTKI-mediated NF-□B inhibition promotes c-mip expression and reproduces an experimental human model of MCNS/FSGS-like lesions. Inasmuch that NF-kB is constitutively active in the podocyte, it is not surprising that c-mip expression is much lower in physiological conditions.

The mechanism by which c-mip expression is increased in podocytes of patients with INS relapse is unclear. Although c-mip interferes with RelA stability and may contribute to NF-kB downregulation, the initiating event leading to c-mip induction is unknown. Whether this mechanism involves NF-□B inhibition, which is amplified by c-mip as suggested by VEGFR TKI, or another podocyte signaling disorder induced by a circulating factor remains to be clarified.

Research on permeability factors

Resistance to therapy occurs in 50% of FSGS and 10% of MCNS patients despite various immunosuppressive treatments (cyclosporine, cyclophosphamide, high-dose steroids) [60, 61]. Such patients often progress to renal failure, requiring dialysis and transplantation. Recurrent disease in the transplant accounts in 30-50% of patients with FSGS, often within the first hours or days following engraftment [60]. This percentage is as high as 80% for the second graft if recurrence caused the loss of the first one [62, 63]. The number of years to achieve end-stage renal failure predicts the risk of recurrence after transplantation: 50% if end-stage renal failure is attained in less than 3 years and 10-20% if this period is longer [64, 65].

Given the short time between engraftment and disease recurrence, one may exclude the generation of an immune response against any component of the allogeneic glomerular filtration barrier, but a circulating factor endowed with pathogenic properties across the glomerular structure has been postulated [66]. The absence of inflammatory lesions and immune complex deposits within the glomeruli, as well as the rapid relapses following renal transplantation, support the extra-renal origin of a circulating factor. This concept is also supported by the transmission of transient nephrotic syndrome in a new born from a mother exhibiting INS relapse [67]. Indirect evidence comes from observations showing that nephrotic syndrome disappears when an MCNS or an FSGS kidney is transplanted into an INS-free patient [68, 69]. In addition to clinical observations, plasma replacement performed in patients with FSGS who escape drug therapy has been successful [70, 71]. Moreover, immediate recurrence after transplantation has been successfully treated by plasma exchange or plasma immunoadsorption techniques [72-74]. Another line of evidence suggesting secretion of a permeability factor comes from experiments showing that systemic infusion of supernatants of cultured PBMC or T cells from MCNS relapse

induces proteinuria in rats [75-77]. Immunochemical analysis of affinity column eluate fractions inducing proteinuria suggested that the molecular weight of the permeability factor is below 150 kDa [72]. Identification of a permeability factor remains a serious challenge although there is little doubt about its immune origin. Several candidates, such as cardiotrophin-like cytokine-1 (CLC-1) and soluble urokinase-type plasminogen activator receptor (suPAR), have been proposed and some of them are yet being discussed [65, 78, 79]. For now, few data related to CLC-1 are available.

SuPAR is released from cleavage of the urokinase-type plasminogen activator receptor (uPAR). High suPAR serum levels have been initially reported in most patients with primary FSGS, the highest concentrations found in FSGS patients who recur after renal transplantation. The pathophysiological relevance of these findings was uncovered when it was demonstrated that circulating suPAR binds to and activates □3 integrin, which anchors podocyte to the glomerular basement membrane, resulting in foot process effacement and nephrotic proteinuria. This interaction was prevented by a blocking antibody specific to uPAR or by a □3 integrin inhibitor. These clinical and experimental data have been recently challenged by several reports, which make highly questionable the use of this molecule as a biomarker for FSGS recurrence. First, suPAR is highly secreted in broad conditions such as infections, inflammation, cancers, cardiovascular diseases and diabetes and is not correlated with proteinuria [80]. In a large survey in children, suPAR levels were found not significantly different between FSGS, non-FSGS glomerular diseases and healthy controls, whereas higher levels were rather found in non-glomerular diseases (hypoplasia, obstructive nephropathy or cortical necrosis) versus FSGS [81]. Interestingly, serum suPAR levels were found inversely correlated with glomerular filtration rate but no correlation with the degree of proteinuria was found [81-83]. In another recent study, it was found that serum suPAR levels do not differentiate between FSGS (with or without recurrence), membranous nephropathy, diabetic nephropathy and IgA nephropathy, among others, but a negative correlation with GFR has also been noticed [84]. By contrast, it seems that urinary suPAR excretion could predict the risk of recurrence [84]. Nonetheless, given the low number of patients analyzed (five patients), this data requires to be confirmed. Although the role of suPAR and its derivative forms in FSGS remains to be clarified, compelling evidence suggests that high serum suPAR levels are significantly correlated with systemic inflammation and renal failure, so that suPAR cannot be used presently as a biomarker of FSGS recurrence. Lastly, it has been demonstrated that suPAR does not bind *in vitro* and *in vivo* to protein-A column, which is successfully used to deplete the putative circulating factor from patients with FSGS recurrence [85].

The mechanisms by which Rituximab, an anti-CD20 monoclonal antibody usually used for the treatment of lymphoma, induces remission in post-transplant recurrence has been recently revisited [86]. The efficacy of Rituximab was initially attributed to interference with B cell function and/or T cell-B cell cooperation. However, it has been reported that Rituximab, besides its original target, also binds to sphingomyelin phosphodiesterase acid-like 3 (SMPDL-3b), which was found basally expressed in the podocytes [86]. Incubation of podocytes with serum from recurrent FSGS patients induces SMPDL-3b downregulation, as well as loss of stress fibers, which is prevented by Rituximab.

The presence of permeability factors in circulation, the beneficial role of Rituximab, and the expression of c-mip by the lymphocyte-podocyte axis in pathologic conditions, might constitute different sides of the same conundrum. The current state of investigations is nonetheless far from establishing a mechanistic connexion between these three elements.

Conclusion and perspectives

Our understanding of pathophysiology of podocyte disorders in INS has considerably evolved during this last decade. The cytokine era has given way to some unknown molecules discovered by several groups using distinct approaches. It is likely that new genes relevant to INS disease will be identified in the future. For now, CD80 and Angptl4 define two different mechanisms of proteinuria. Regardless of initial event, both proteins are produced by the podocytes, which currently may be considered as an actor and not only as target of the disease.

Consistent data derived from animal models, human diseases, as well as experimental investigations support a role of c-mip in lymphocyte and podocyte disorders occurring in INS relapse. These data suggest that c-mip is at the crossroads of proximal signaling events and cytoskeletal organization, ultimately leading to regulation of cell morphology and survival. Therefore, c-mip represents a crucial research target to understanding INS immunopathogenesis. While in normal mature podocytes, basal c-mip expression is very low, the constitutive knockdown is lethal, suggesting that c-mip plays a critical role during the embryonic development (unpublished data). The recent generation of conditional c-mip knockdown in mice will be helpful to clarify c-mip function.

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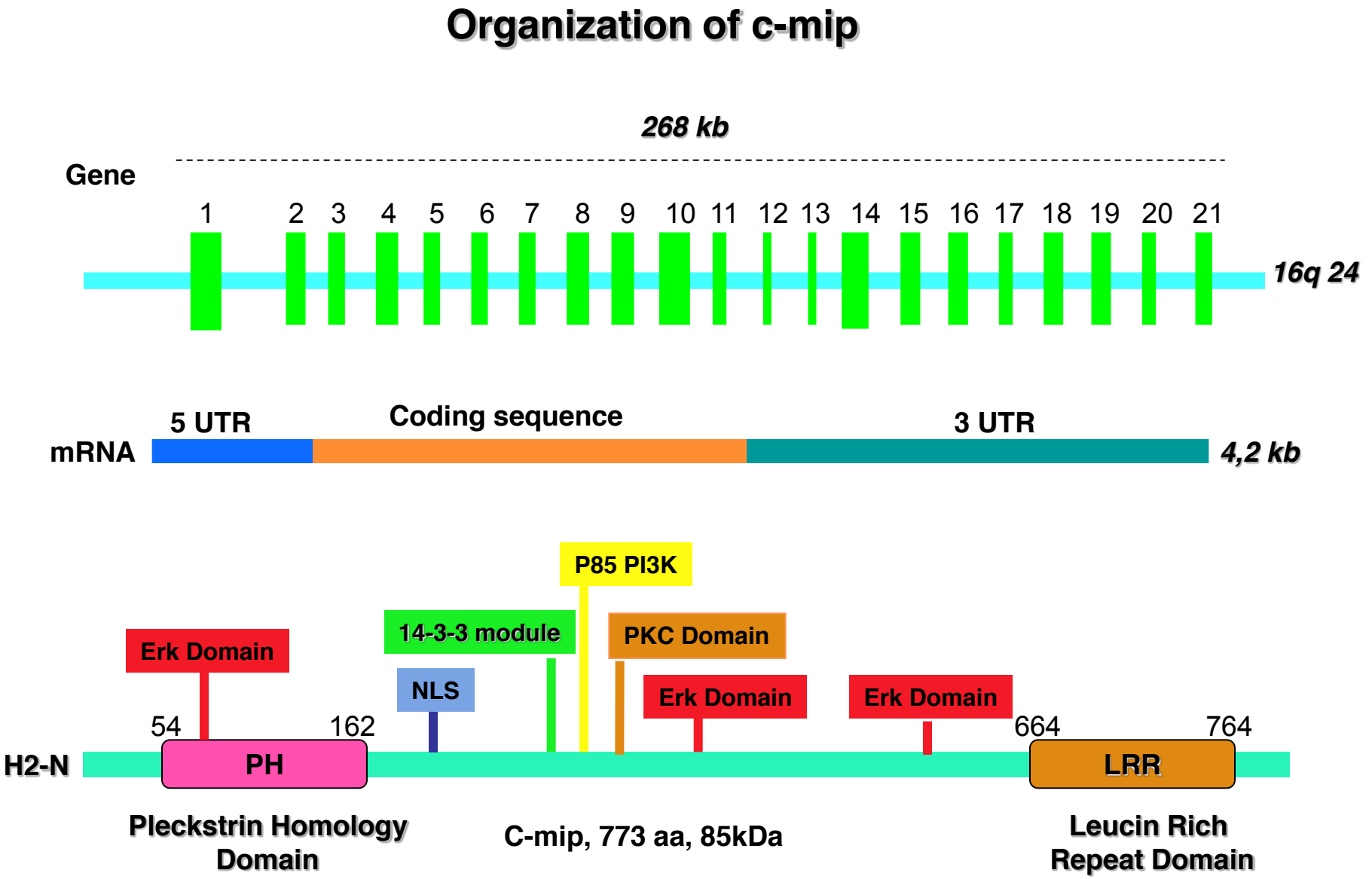
Legends

Figure 1. Structure organization of c-mip. The gene c-mip contains 22 exons spanning 270 Kb. It encodes a messenger RNA of 4.2kb consisting of 434 bp-5'UTR, 2,200 bp-coding sequence and 1600 bp-3'UTR. The protein c-mip is original in that it contains two domains usually not found within the same protein, the Pleckstrin-homogy domain (PH) and LRR domain, as well as an SH3-like domain and many docking sites for signaling molecules.

Figure 2. Current understanding of nephrin signaling. In basal conditions, clustering of nephrin in lipid rafts induces Fyn phosphorylation at tyrosine 417, leading to its conformational change in active form. The amount of active Fyn is amplified upon its interaction with Nck. Then, Fyn phosphorylates nephrin at multiple residues within the cytoplasmic domain, which subsequently recruits several proteins including podocin, CD2AP and synaptopodin. Fyn binds to and phosphorylates N-Wasp. These diverse interactions initiated by Fyn preserve cytoskeleton organization. Phosphorylation of nephrin induces the recruitment of PI3 kinase, which then activates Akt and promotes podocyte survival through activation of NF-□B and WT1. The expression of c-mip is barely detected in physiological conditions. However, in some pathological situations, c-mip abundance is increased and interferes with Fyn activity and nephrin (and likely other podocyte receptors) signaling.

Figure 3. **Cross-talk c-mip/NF-□B in anti-VEGF-mediated glomerular diseases.** Thrombotic microangiopathy (TMA) is a frequent complication of anti-VEGF ligand therapy (Bevacizumab and VEGF trap), whereas minimal change nephropathy/focal segmental glomerulosclerosis (MCN/FSG) are mostly observed following receptor tyrosine kinase inhibitor (RTKI) treatment. Glomerular TMA is associated with an upregulation of NF-□B that is a strong repressor of c-mip. By contrast, RTKI such as Sorafenib inhibits NF-□B, leading to increased c-mip abundance and cytoskeleton disorganization, which subsequently induces nephrotic proteinuria with MCN/FSG-like histological lesions.

Figure


Organization of c-mip
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16q 24
5 UTR
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P85 PI3K
14-3-3 module
PKC Domain
Erk Domain
NLS
Erk Domain
Erk Domain
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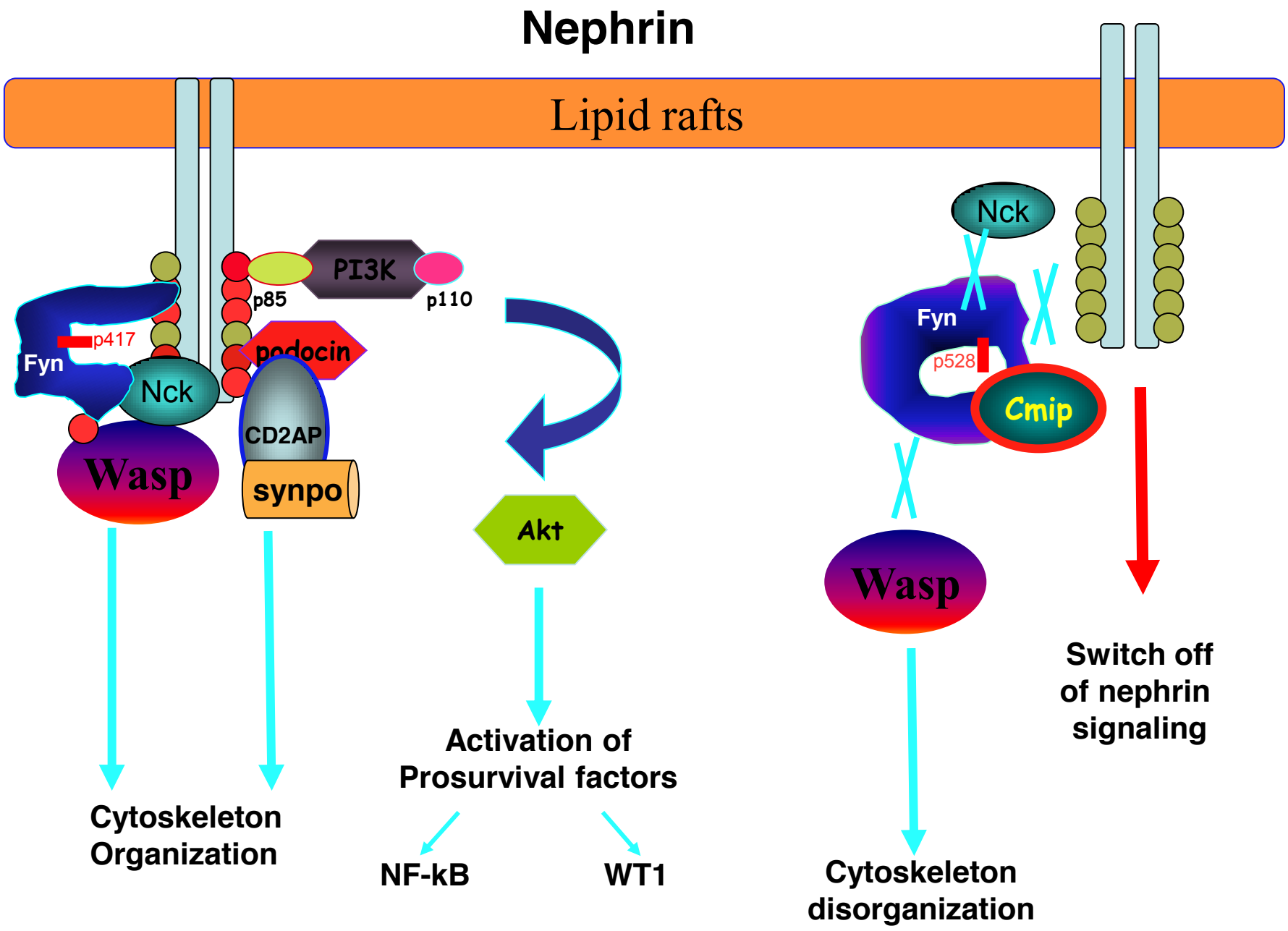
Pleckstrin Homology Domain
C-mip, 773 aa, 85kDa
Leucin Rich Repeat Domain

Figure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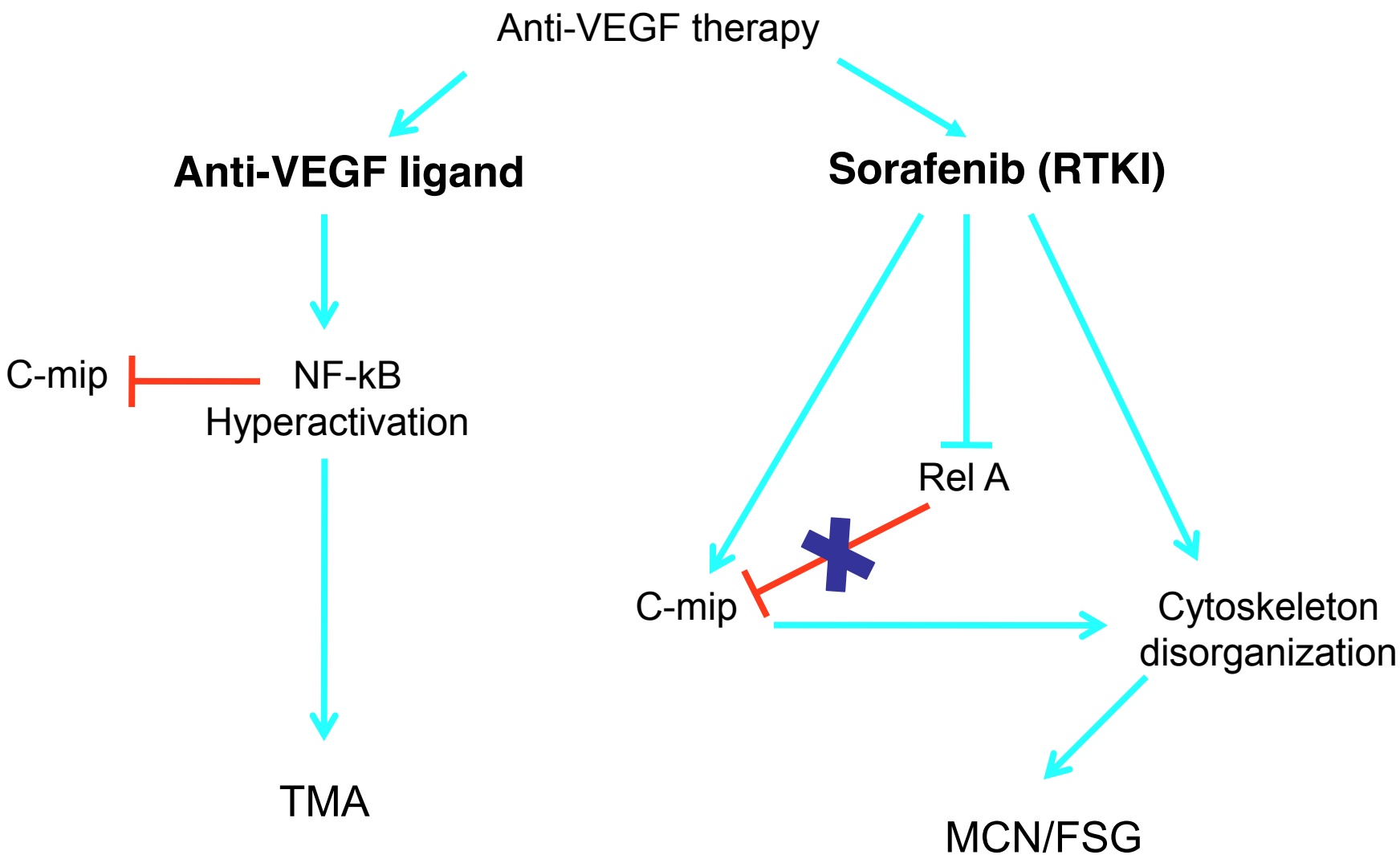
disorganization

Figure


Anti-VEGF therapy
Anti-VEGF ligand
Sorafenib (RTKI)
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Hyperactivation
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Rel A
C-mip
Cytoskeleton
disorganization
MCN/FSG

*Authors' Response to Reviewers' Comments
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Ref : Ms. No. SSIM-D-13-00043

Dear Sirs,

We have completed the revision of the manuscript. We would like to thank you for the helpful comments. As underlined in your letter, we deleted some paragraphs related to c-mip studies and included new sections on CD80, suPAR and angiopoietin-like 4.

We hope that this revision will meet the expectations of the editorial Board and the referees.

Sincerely yours,

D Sahali, M.D., Ph.D.
INSERM U 955